Background:

Autoimmune manifestations along with recurrent infections, allergies and malignancies are among the main signs of primary immunodeficiencies.

Methods:

We present a 4-year-old male, who was admitted to hospital with complaints of morning stiffness, joint pain and swelling, refusal to walk, low-grade fever, gait and coordination disturbance, delayed motor development.

At age of 1 year gait disturbance was noted and ataxia was diagnosed. He was under observation of neurologists with the diagnosis of ataxia and muscle hypotonia syndromes. Family history was non-contributory.

Results:

Ocular telangiectasia, "coffee with milk" spots and hypopigmentation on the skin, flexor-extensor contracture of a knee, movement restriction in a hip, elbow joints and cervical spine, ataxia, dysarthria, severe muscle hypotonia were revealed at physical examination. Concentration of serum immunoglobulins A and E, and the number of CD3, CD4, CD8, CD19, CD16/56 lymphocytes were decreased. An alpha-fetoprotein level in serum was increased. Infectious arthritis and osteomyelitis were excluded. Signs of bilateral koksitis and knee bursitis were revealed during ultrasound examination. The diagnosis of Ataxia Telangiectasia and Juvenile Idiopathic Arthritis was set.

Regular methotrexate therapy resulted in alleviation of arthritis, but 3 months later the first episode of pneumonia was detected. Coughing, nasal discharge, continuous crackles in lungs lasted for a long time. IV immunoglobulin replacement therapy was started.

Conclusions:

Arthritis may be one of the manifestations of PID. Our case demonstrated association of Juvenile Idiopathic Arthritis with Ataxia Telangiectasia. The treatment of autoimmune diseases associated with PID is a great challenge and should be very well thought.
RARE AUTOINFLAMMATORY SYNDROME – CANDLE SYNDROME

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Background:

Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated Temperature - CANDLE Syndrome is a very rare autoinflammatory disease was discovered in the past few decades. This inherited autosomal recessive syndrome, the first clinical symptoms of which appear in infancy.

To study the condition of cellular and humoral immunity in patient with Candle syndrome. Girl, was born in 2011; from the first months had diarrhea, respiratory infection and recurrent daily fever. Later, appearance of purpuric skin rash was observed that started from low extremes and then covered the whole body, especially the face. In age of 2, lipodystrophy on the some parts of the body, arthralgia, low height and weight, developmental delays, hepatosplenomegaly, lymphadenopathy and chronic anemia developed.

Methods:

The methods used include the examination of the white blood cells and lymphocytes, CD3 +, CD4 +, CD8 + lymphocyte populations, the ratio CD4/CD8 by flow cytometry and genetic analysis.

Results:

Patient with Candle syndrome had significantly reduced number of CD3 +, CD4 + lymphocyte in peripheral blood. The quantity of CD8+ T cells had changed to overly higher numbers; IRI level was 3 times below the norm. These factors were the most reliable indicators of immunodeficiency and imbalance in lymphocyte subpopulations. Moreover, the low levels of IgG and IgA were also detected. PSMB3, PSMB4, PSMB8, PSMB9 specific genes mutations had not been revealed during the genetic examination of the patient.

Conclusions:

It was identified that important changes in cellular and humoral immunity in patient with Candle syndrome could lead to the activation of severe inflammation.
AUTOIMMUNITY

ESID7-0037

AUTOIMMUNE DISEASES IN PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY

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Background:

Patients with PID have dysregulation of immune processes, which can result in an increased susceptibility to infectious diseases, autoimmune disorders, and malignancies.

The aim: To evaluate the autoimmune diseases in patients with primary immunoglobulin deficiency.

Methods:

Method: Data was collected on 38 patients receiving IVIG infusion at our department from January 2016 to December 2016. From a total of 38 PID patients, 34 were diagnosed with CVID, 2 with Selective IgG Deficiency, one with Selective IgA Deficiency and one with HyperIgM syndrome. Descriptive statistics was used.

Results:

Results: 38 patients (21 males, 17 females) received IVIG infusions for their hypogammaglobulinemia. Almost 40% (n = 15) experienced autoimmune diseases: 4 pts with rheumatoid arthritis, and other more with autoimmune thrombocytopenic purpura, systemic lupus erythematosus and concomitant Sjogren's syndrome, Behcet's disease, systemic vasculitis, primary biliary cirrhosis (PBC), Basedow Graves Disease. We have 5 pts diagnosed with gluten enteropathy. Of the rheumatoid arthritis patients, three are also known with CVID and one with hiperIgM syndrome. All of these patients were already know with autoimmune pathology when their immunodeficiency was diagnosed. In 80% of the pts with autoimmune diseases and ID, the first diagnosed was autoimmune disease, which was found in older pts (mean aged 45 vs 34 in pts without autoimmunity).

Conclusions:

Conclusion. A wide variety of autoimmune diseases are found in patients with PID. There is no general tissue or organ restriction, nor is there a gender or age predominance like that seen in autoimmune diseases affecting the general population.
AUTOIMMUNITY

ESID7-0070

COMMON VARIABLE IMMUNODEFICIENCY (CVID) COMPLICATED WITH GRANULOCYTIC-LYMPHOCYTIC INTERSTITIAL LUNG DISEASE (GLILD) IN CHILDREN

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Background:

GLILD is CVID complication, often noted in patients with systemic lymphadenopathy, splenomegaly, autoimmunity (thrombocytopenia, leukopenia, hemolytic anemia). Regular immunoglobulins' substitution and symptomatic therapy controlled autoimmunity and infections, but no inhibited GLILD development. Immunosuppressive therapy is suggested in symptomatic patients. In CVID children, GLILD showed mild, unspecific even no symptoms, so diagnosis is based on lungs examination with HRCT, MRI.

Methods:

Diagnosis of GLILD was based histochemistry and lymphocytes T and B analysis in lungs' biopsies.

Results:

Within our patients (2 boys and girl), in 2 the level of IgG was low, despite of regular substitution (0.5 g/kg b.w.) suggesting protein loss or trapping. All patients demonstrated autoimmunity, systemic lymphadenopathy and splenomegaly. After GLILD diagnosis, steroids were used for therapy, but only in girl it stabilised process, in 2 boys immunosuppression was necessary. In one boy therapy was stopped due to deep thrombocytopenia and leukopenia. Now, he developed lung fibrosis and progressing insufficiency. Third boy was treated with steroids without prolonged effect, after azathioprine symptoms of pancreatitis occurred. Followed this, MMF was introduced with weak response. Patient's status was good, without symptoms of lung disease, but due to poor prognosis, shortage of therapy, procedure of allogeneic HSCT was performed with resolving of GLILD.

Conclusions:

Based on course of disease of our patients, immunosuppressive therapy of GLILD, despite of mild symptoms, and HSCT as curable procedure, are suggested.
RelB deficiency causes combined immune deficiency with autoimmune manifestations. It is unclear whether hematopoietic stem cell transplantation (HSCT) can alter or reverse autoimmune manifestations.

Methods:

The patient underwent HSCT from a 10/10 matched unrelated donor after full myeloablative conditioning regimen. Patient information was derived from the Canadian Centre for Primary Immunodeficiency Registry. Follow-up period was 4.5 years.

Results:

Prior to HSCT, the patient suffered recurrent otitis media and pneumoniae, disseminated HSV, adenoviral infections and failure to thrive. He also had a prolonged episode of eczema gangrenosum as well as arthritis in his hips and knees. Immune work up revealed profound B and T cell impairment with low T cell responses to mitogens, poor response to specific antibodies, dysplastic thymus and low T cell excision circles values.

He underwent HSCT at 2 years of age and showed rapid engraftment. Post-transplant, he had one infection with Staphylococcus aureus and HSV1 superficial skin infection. He developed grade I skin GVHD on day +13 which was reversed after a pulse of methylprednisolone. Eighteen months post-transplant, he developed polyarthritis of his ankles, knees, wrists and left hip, which responded well to steroid joint injections and NSAIDs. Concomitantly, he developed ashy dermatosis on his neck, chest and buttocks. He is currently 4.5 years post-transplant and has full donor chimerism, with evidence of full immune reconstitution.

Conclusions:

HSCT in RelB deficient patients does not completely reverse the autoimmune manifestations. While HSCT resulted in full immune reconstitution, it does not reverse the joint and skin manifestations.
AUTOIMMUNITY

ESID7-0083

NOVEL PATHOGENIC COMPLEMENT FACTOR ANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS ASSOCIATED THROMBOTIC MICROANGIOPATHY

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Background:

Thrombotic Microangiopathy (TMA) is the triad of microangiopathic hemolytic anemia, thrombocytopenia, and organ dysfunction caused by abnormalities in vascular endothelium with resultant thrombosis. TMA is seen in up to four percent of patients with systemic lupus erythematosus (SLE) and can be autoantibody mediated. One of the hallmark antibody mediated TMAs is atypical hemolytic uremic syndrome (aHUS). In aHUS, antibodies to complement factor H (CFH) inhibit normal protein function leading to endothelial damage and increased alternative complement pathway activation, resulting in membrane attack complex formation and cytotoxicity. We hypothesized that aHUS/SLE patients generate additional complement factor antibodies that drive disease through complement dysregulation.

Methods:

We sequenced the plasmablast antibody repertoires of patients with TMA (resembling the aHUS phenotype) and SLE using a DNA barcoding method that sequenced the cognate heavy- and light-chain pairs of antibodies expressed by individual blood plasmablasts. The data sets were bioinformatically analyzed to generate phylogenetic trees that identified clonal families of antibodies sharing heavy- and light-chain VJ sequences. Representative antibodies were expressed and their binding properties analyzed by complement factor ELISAs and a complement factor antigen array developed in our laboratory. Functional properties were assessed by a terminal complement complex (TCC) ELISA.

Results:

Recombinant antibodies expressed from SLE/aHUS patients positively reacted with CFH by ELISA and CFH peptides by array. When added to healthy control serum, recombinant antibodies induced formation of TCC, an indicator of complement activation.

Conclusions:

We identified novel complement factor antibodies that may drive TMA in SLE and contribute to SLE pathogenesis.
Background:

Caspase recruitment domain family, member 11 (CARD11) is a scaffold protein that is a part of the signalosome complex (CBM), which is essential for regulating NF-kB in lymphoid immune cells, acting as regulator of lymphocyte activation, survival and proliferation. Both loss and gain of function (GOF) mutations of CARD11 have been described. Inactivation of CARD11 causes severe combined immunodeficiency, whereas GOF mutation leads to constitutive activation of NF-kB.

Methods:

Whole exome sequencing (WES) followed by Sanger confirmation were performed on a patient with combined immunodeficiency. The genetic variant impact on cell signaling and the resultant immune function were studied.

Results:

The patient, now 31 years old, suffered since early childhood from repeated microbial infections and oral thrush. Over the years, she had developed multiple autoimmune disorders including skin necrotizing granulomatous inflammation, lichen sclerosis, psoriasis and colitis. She also suffered since early age multi-organ atopy. WES performed on the patient identified a novel mutation in CARD11 which was confirmed by Sanger sequencing, but was not found in healthy individuals. CARD11 deficient cells demonstrated altered NF-kB pathway, leading to reduced secretion of IL-2 and IFNg, resulting in poor in vitro T cell responses to mitogens and antigens.

Conclusions:

We describe a novel genotype and phenotype of CARD11 deficiency encompassing combined immunodeficiency as well as extensive autoimmune and atopic features.
Background:

The primary immunodeficiency disorders (PID) including commun variable immunodeficiency (CVID) remains underdiagnosed in adults due to the diversity of clinical signs especially when there is no significant history of infection in childhood. We report a case with a fatal outcome.

Methods:

62 year old woman with history of angina pectoris hospitalised in 2011 for severe confusion, hyponatremia and weight loss. Immunological, infectious, metabolic and angiotensine conversion enzyme (ACE) were normal. Lyme was positive. Thoracoabdominal CT scan (TA-CT scan): aorto-pulmonary adenopathies. Lumbar puncture: lymphocytic meningitis+ positive Borelia. ACE 1.42 u/l. Magnetic resonance imaging (MRI): bi temporal hypersignal FLAIR with contrast enhancement. Positrons emission tomography FDG Scan: negative. Treatment with ACYCLOVIR then CEFTRIAXONE showed real improvement.

Results:

2013: panuveitis with erythematous skin lesions. ECA 135 u/l with Immunoglobuline G 4 g/l. LP: negative. MRI: stable without contrast enhancement. Accessory salivary gland biopsy (ASGB): sarcoidosis granuloma. TA-CT scan: Thoracic adenopathies, splenomegaly and splenic nodules. Sarcoidosis was performed. Corticoides + Methotrexate + immunoglobuline replacement therapy started. Later, a low grade marginal zone lymphoma was confirmed with therapeutic abstention. 2015: severe asthenia with reactivation of Epstein barr virus treated with GANCICLOVIR. ASGB: Sjögren disease. Confirmation of non viral cryoglobulinemia. Late 2015, acute onset of left ventricular oedema suspecting a cardiac amyloidosis, positive in ASGB, not confirmed on MRI. The rapid deterioration of cardiac and renal functions required admission in intensive care unit, dialysis and renal biopsy confirming multiple thrombi of cryoglobulinemia. Patient died rapidly before initiating adapted treatment.

Conclusions:

The analysis of the case is in favor of PID responsible for the granulomatous disease (sarcoidosis like), Sjögren and low grade lymphoma initiating an amyloidosis and cryoglobulinemia which was fatal due to its late diagnosis.
THE IgG AND IgM ISOTYPES OF ANTI-ANNEXIN A5 ANTIBODIES ARE NOT ASSOCIATED WITH VITAMIN D LEVELS IN PATIENTS WITH PRIMARY ANTIPHOSPHOLIPID SYNDROME

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Background:

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of antiphospholipid antibodies (Abs) and recurrent thromboses and/or pregnancy losses. In the absence of additional diseases, APS is designated as primary PAPS. Vitamin D has immunomodulatory roles and although there are several reports that analyze the association of vitamin D with clinical and/or serological features of PAPS, no reports that investigate the association between anti-annexin A5 Abs and vitamin D are available. Therefore, our aim was to analyze the association between the IgG and the IgM isotypes of anti-annexin A5 antibodies with vitamin D levels in PAPS patients.

Methods:

This study included 70 (8 male and 62 female) PAPS patients. The mean age of the analyzed PAPS patients was 45.97 ± 12.72. Vitamin D concentrations were measured by immunochemiluminescence assay. Anticardiolipin, anti-B2gpl and anti-annexin A5 antibodies (IgG and IgM isotypes) were measured by ELISA.

Results:

An inverse correlation between vitamin D concentrations and the age of analyzed PAPS patients (r = -0.237, P = 0.048) was observed. Median (25th - 75th) vitamin D value was 48.80 (37.48 - 68.46). Vitamin D insufficiency (25 – 75 nmol/l) was observed in 54/70 (77.14%) of PAPS patients, while vitamin D deficiency (≤ 25 nmol/l) was noticed in 4/70 (5.71%) of patients. Non-significantly increased IgM anti-annexin A5 levels were observed in 3.07% of PAPS patients with vitamin D insufficiency.

Conclusions:

The IgG and IgM isotypes of anti-annexin A5 Abs were not associated with vitamin D levels in PAPS.
Background:

Common variable immunodeficiency (CVID) is one of the most prevalent symptomatic primary immunodeficiencies (PIDs) which manifest a wide clinical variability such as autoimmunity, as well as T cell and B cell abnormality.

Methods:

In this study a total of 72 patients with CVID was enrolled. Patients were evaluated for clinical manifestation and classified according to the presence or absence of autoimmune disease. We measured regulatory T cells (Tregs), and B-cell subsets using flow cytometry in patients.

Results:

Twenty-nine patients (40.3%) have shown at least one autoimmune manifestation. Autoimmune cytopenias and autoimmune gastrointestinal diseases were the most common. A significant association was detected between the autoimmunity and presence of hepatomegaly and splenomegaly. Among CVID patients, 38.5% and 79.3% presented a defect in Tregs and switched memory B-cell, respectively, whereas 69.0% present expansion of CD21^{low} B cell.

Conclusions:

Autoimmunity may be the first clinical manifestation of CVID, thus routine screening of immunoglobulins is suggested for patients with autoimmunity.
AUTOIMMUNITY

ESID7-0127

A NOVEL EXON-SKIPPING FOXP3 VARIANT PRESENTING WITH AUTOIMMUNE DIABETES MELLITUS RESOLVING AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION DESPITE SELECTIVE TREG ENGRAFTMENT

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Background:

IPEX-syndrome caused by hemizygous FOXP3 variants leads to destructive organ-specific autoimmunity and can result in insulin-dependent diabetes mellitus (IDDM) that usually persists after otherwise curative allogeneic hematopoietic stem cell transplantation (HSCT).

Methods:

We describe a four-year-old boy with mild but typical IPEX symptoms including eczema, autoimmune hepatitis and IDDM.

Results:

Sanger sequencing of FOXP3 revealed the novel hemizygous c.816+2T>A variant affecting the splice-donor-site of exon 7. Analysis of cDNA and protein confirmed the expression of FOXP3 protein lacking exon 7 (FOXP3Δ7) that lies within the leucine zipper domain. RNAseq revealed that FOXP3Δ7 leads to differential expression of multiple but not all FOXP3 regulated genes in stimulated patient T cells. The patient underwent HSCT from a matched unrelated donor after myeloablative conditioning which led to resolution of all symptoms including his IDDM. After initial complete donor engraftment, nearly complete autologous reconstitution occurred with only regulatory T cells (Tregs) of donor origin persisting (85% donor in sorted CD4⁺CD25⁺CD127lowFOXP3hi cells). At 3 years of follow-up the patient remains disease-free with normal blood glucose levels. Autoantibody levels decreased but persisted after transplantation.

Conclusions:

In conclusion, FOXP3Δ7 impacts the T cell transcriptome and leads to IPEX. Remission of IDDM is possible after timely HSCT even with selective engraftment of donor Tregs and despite persistence of autoantibodies. Whether this implies mostly cellular autoimmunity as cause of the IDDM in IPEX and whether selective Treg engraftment can induce a long-term remission remains to be investigated.
Background:

Introduction: XIAP deficiency (XLP2) is an immune dysregulation disorder characterised by a high risk of hemophagocytic lymphohistiocytosis (HLH). Invariant NKT (iNKT) cells can be decreased in some, but not all patients. Genotype-phenotype correlations in XIAP deficiency have not been clearly defined.

Methods:

Results:

Case presentation: An 8-year old boy, with a history of splenomegaly, presented with fever for 18 days, vomiting and diarrhea. His family history was notable for a brother who died at 4 months of age with the clinical diagnosis of haemophagocytic lymphohistiocytosis (HLH) and a 15-year old asymptomatic brother with splenomegaly. The proband had a Coombs positive anemia, hyperferritinaemia, elevated liver enzymes, and positive IgM and IgG antibodies to Epstein Barr virus. Hematophagocytosis was not found on bone marrow aspiration and perforin expression was normal. Lymphocyte immunophenotyping showed elevated CD8+HLADR+ cells (95%). iNKT cells were undetectable during hospitalization and increased to 45 iNKT cells per million CD3+ cells. The patient was managed with immunoglobulin replacement therapy and dexamethasone with good effect; the lymphoproliferation was significantly decreased and his clinical condition was improved.

The asymptomatic brother had normal values of T-, B-, NK- and iNKT- lymphocytes.

Genetic analysis of both siblings revealed a hemizygous nonsense mutation in XIAP (c.1336G>T= p.Glu446Ter), encoding the X-linked inhibitor of apoptosis.

Conclusions:

Conclusion: We present two brothers with the same mutation in XIAP, but different manifestations and laboratory findings. This report broadens the scope of the clinical presentation of XLP2 and highlights the need for a better understanding the pathophysiology of XLP2.
A PATIENT OF AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME HAVING INTRACRANIAL HEMORRHAGE DUE TO THROMBOCYTOPENIA

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Background:

Autoimmune lymphoproliferative syndrome (ALPS) is a rare genetic disorder of the immune system that affects both children and adults. In ALPS, unusually high numbers of lymphocytes accumulate in the lymph nodes, liver, and spleen, which can lead to enlargement of these organs. ALPS cause numerous autoimmune problems such as anemia, thrombocytopenia, and neutropenia. Here, a rare patient of ALPS having Intracranial hemorrhage due to thrombocytopenia is presented.

Methods:

14-year-old girl with ALPS presented with deterioration in general condition and convulsion. Other than first-degree cousin marriage, nothing remarkable was in family history. Past medical history begins with persistent moniliasis, recurrent bronchitis and dermatophyte infections at 6 months. At five years of age, she suffered from epistaxis, hematuria and pneumonia. When she was 8-year-old, she had first intracranial hemorrhage and evaluated for lymphadenopathy, splenomegaly and thrombocytopenia.

Results:

Abdominal and thorax CT demonstrated paraaortical lymphadenopathy and bronchiectasis. Bone marrow aspiration was normal. Laboratory evaluation showed platelet: 33.100 /mm³, IgG:3259 mg/dl, positive direct Coombs test, and >7.86% double negative T (CD4-CD8-) cells. With these clinical and laboratory findings, ALPS was diagnosed. When admitted last time, she was unconscious and pupils were fixed dilated. CBC revealed that platelet was 23.600 /mm³. PT, aPTT and INR were normal. Chest X-ray was normal. Eye examination indicated intracranial hemorrhage. Although she was given platelet and erythrocyte suspensions plus IVIG, she died of active bleeding.

Conclusions:

Although the cause of death could be intracranial hemorrhage in ALPS, it should draw our attention to see fatality if the platelet count was 23.600/mm³.
SUCCESSFUL ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR GRISCELLI SYNDROME TYPE 2 MANIFESTED AS LONG-TERM CNS AND LUNG INJURY

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Background:

Griscelli syndrome type 2 (GS2) is a rare disease with typical combination of hypopigmentation and haemophagocytic lymphohistiocytosis (HLH). It is a life-threatening disease caused by mutations in RAB27A gene and may be treated by allogeneic hematopoietic stem cell transplantation (alloHSCT).

Methods:

We present a case with unusual clinical presentation, novel genetic mutation and successful alloHSCT.

Results:

A 5-year-old boy presented with an acute demyelinating encephalomyelitis. Several months later infiltrations in the lungs, enlarged mediastinal lymph nodes and granulomatous dermatitis appeared. Diagnostic work-up was unspecific. Due to recurrent CNS and lung attacks the patient has been steroid-dependent for 4 years. Attempts to taper steroids or acute upper respiratory infection deterioration of lung and CNS symptoms. At the age of 9 he developed classical HLH symptoms. Abnormal degranulation assay suggested primary HLH. Breakpoint PCR revealed a complex structural rearrangement at the 5’UTR of RAB27A. Reduced RAB27A transcription and expression was confirmed by RT-PCR and Western blot analyses. The patient has never had any sign of hypopigmentation. AlloHSCT from HLA-identical healthy sibling was performed using fludarabine and busulfan based reduced intensity conditioning. Acute and chronic skin graft-versus-host disease was successfully managed with steroids, extracorporeal photopheresis and psoralen with UVA radiation. The patient is full chimera, steroid-free and in an improving clinical condition.

Conclusions:

GS2 can present as isolated CNS and lung involvement without hypopigmentation. If no mutations are found in the exons, mutations in non-coding regions should be pursued by whole genome sequencing. AlloHSCT should be offered to these patients.
Background:

Clinical picture of complex T-cell primary immunodeficiencies is highly variable and a subgroup of these children present with autoimmune phenomena, mostly cytopenias.

Methods:

We describe two cases of children affected by complex T-cell primary immunodeficiency syndromes – ataxia telangiectasia (AT) and DiGeorge syndrome (DGS), treated for steroid resistant autoimmune hemolytic anemia and thrombocytopenia (Evans syndrome). Both patients present with complete IgA deficiency and IgG antibody deficiency.

Results:

The case with AT showed class switch recombination defect with high IgM profile and IgM anti-erythrocyte antibody. The case with DGS presented with severe hemolytic anemia but normal IgG and IgM levels. Positive direct antiglobulin test and immunoradiometric assay confirmed red blood cell bound IgG. Both patient had very low naïve T cell (CD3+CD4+CD45RA+) numbers (0.8% and 5.5% of CD4+ T lymphocytes) in peripheral blood. Rituximab (anti-CD20) was considered for treatment in both patients and showed excellent and durable clinical effect after 2-4 doses.

Conclusions:

Rituximab together with intravenous immunoglobulin replacement appears to be a well-tolerated and long lasting therapy for Evans syndrome patients with primary immunodeficiency.
AUTOIMMUNITY

ESID7-0203

AUTOIMMUNITY AS THE FIRST MANIFESTATION OF COMMON VARIABLE IMMUNODEFICIENCY

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Background:

Common Variable immunodeficiency (CVID) is characterized by deficient immunoglobulin production, with recurrent respiratory and gastrointestinal infections.

Autoimmune diseases occur in approximately 20%; the age at diagnosis of autoimmunity is variable and may precede the diagnosis of primary immunodeficiency.

Methods:

Clinical case:

Male of 23 years of age, begins in November 2013, with fever, jaundice, palpitations, fatigue and dyspnea. Diagnosis of autoimmune haemolityc anaemia. 11 months later epistaxis is added, with generalized petechiae, and the diagnosis of Evans Syndrome is integrated.

2014 presents pulmonary thromboembolism, pulmonary arterial hypertension and alveolar hemorrhage, initiates study protocol, concluding Antiphospholipid Syndrome, added to the immunosuppressive treatment, use of anticoagulant.

In parallel, the patient had recurrent sinopulmonary infectious: 2 events of Pneumonia that ameliorated ventilatory mechanical support, as well as recurrent rhinosinusitis, the study protocol was started, with immunoglobulin determination, IgG, IgA and IgM, 2 standard deviations below normal values, CD 19 more than 2% and establishing the diagnosis of CVID.

Results:

Autoimmune diseases occur in up to 20% of patients with CVID; hematologic diseases are the most common, in particular the combination of hemolytic anaemia and immune thrombocytopenic purpura (Evans syndrome) is present in up to 5-8% of the cases. It has not been estimated percentage of patients with CVID, who present more than one autoimmune disease. Antiphospholipid syndrome is not commonly observed in patients with CVID.

Conclusions:

Autoimmune diseases could be the first manifestation of Common Variable Immunodeficiency, and we most try to search for more than one autoimmune diseases in these patients, because that could affect their prognosis.
Background:
Gain-of-function mutations in STAT3 (Signal Transducer and Activator of Transcription 3) gene are responsible of the Autoimmune Disease Multisystem Infantile-Onset 1 (ADMIO-1) syndrome.

Methods:
A 7-year-old patient presented with Hirschsprung disease, multiple alimentary allergies, eczematous dermatitis, pulmonary interstitial disease, growth retardation, autoimmune poly-endocrinopathy and hepatomegaly.

Results:
Exome sequencing revealed a novel heterozygous mutation of STAT3 gene. Flow cytometry analysis of p-STAT3 on PBMC and EBV-immortalized B-cells after stimulation with IFN-α showed increased level of basal STAT3-phosphorylation compared to the control. Analysis of IL2RA, IL10, SOCS1 and SOCS3 mRNAs, that are strictly regulated by STAT3, by Real-Time PCR showed increased expression in unstimulated cells from patient, whereas under stimulation with IL-6 and IL-10 the patient response was similar to the control. Moreover, Western Blot analysis of STAT3 protein in EBV cells showed an increased amount of total STAT3 protein, and detectable levels of pSTAT3 in unstimulated cells.
Finally, functional studies by chromatin immunoprecipitation (ChIP) assay on EBV cells showed STAT3 binding to promoter DNA in unstimulated cells.

Conclusions:
These results suggest that this novel STAT3 mutation is associated with gain of function activation and STAT3-regulated genes paving the way for the use of JAK/STAT pathway inhibitors for treatment of patients with ADMIO1 syndrome.
Background:
Mutations in the lipopolysaccharide-responsive beige-like anchor (LRBA) gene were identified as the cause of an autoimmunity and immunodeficiency syndrome often resulting in a multi-organ immune dysregulative disease with symptoms beginning during the early childhood. A broad spectrum of clinical manifestations without reliable predictive prognostic markers has been reported. Allogeneic hematopoietic stem cell transplantation (alloHSCT) has been performed in a few severely affected patients with complete remission of autoimmunity and immunodeficiency. A lack of immunosuppressive capacity of patients’ Tregs has been described as a result of a disturbed interplay between LRBA and the CTLA-4 protein. However, the detailed pathomechanism underlying the variability of the disease symptoms remain unclear yet.

Methods:
In this study, we analysed peripheral T- and B-lymphocytes of genetically confirmed LRBA-deficient patients upon antigen stimulation. Using freshly isolated PBMCs, cells were stimulated with antigens for 20hrs. Subsequently, multicolour surface and intracellular staining was performed with PBMCs from patients and healthy controls (n=4). Inflammatory cytokines were analysed out of cell culture supernatants using a multiplex cytometric bead array protocol.

Results:
Our preliminary data show a reduced chemokine receptor expression (CxCr5) on patients’ B-cells supporting the hypothesis of a chemokine receptor mediated restriction of B-cell migration to the secondary lymphoid tissue in LRBA deficiency.

Conclusions:
Further evaluation of patients' B cells in a larger cohort of patients with LRBA-deficiency syndrome is indicated.
AUTOIMMUNITY

ESID7-0251

AGED AIRE-DEFICIENT MICE HARBOUR NEUTRALIZING AUTOANTIBODIES TO IL-17A BUT LACK SUSCEPTIBILITY FOR OROPHARYNGEAL CANDIDIASIS

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Background:

AIRE-deficiency in humans causes a severe autoimmune disease named APECED characterized by diagnostic triad including chronic mucocutaneous candidiasis (CMC), hypoparathyroidism and Addison’s disease. CMC is associated with neutralizing autoantibodies towards Th17 cytokines that are essential in protection against Candida infection. The mouse model of APECED differs from the human disease considerably: it lacks endocrine autoimmunity and candidiasis, is milder and displays remarkably different set of autoantibodies. However, aged AIRE-deficient mice develop neutralizing autoantibodies towards IL-17A. The aim of this study was to test whether auto-autoantibodies to IL-17A and treatment with anti-IL-22 lead to increased susceptibility for oropharyngeal candidiasis in infected mice.

Methods:

Oropharyngeal candidiasis model was applied using the protocol developed by S.L. Gaffen (University of Pittsburgh).

Results:

Cortisone-treated and Candida-infected mice lost body weight and developed fungal patches on the tongue and buccal mucosa. Neither 1-1.5 years old AIRE-deficient mice nor the mice treated intraperitoneally with anti-IL22, isolated from APECED patients, showed any clinical sign of Candida infection. The yeast was readily controlled by upregulated Th17 cytokines as detected by quantitative RT-PCR from mRNA isolated from the tongues of the tested animals. Only a small fraction of mice showed moderately increased CFU of C. albicans from their tongue tissue on day 6 post infection.

Conclusions:

In conclusion, the autoantibody level in AIRE-deficient mice was either too low to severely impair the protection against CMC. Alternatively, the antibodies are able to confer susceptibility only in combination with decreased Th17 cytokine secretion as described in APECED patients.
AUTOIMMUNITY

ESID7-0277

CVID CASE REPORT WITH STROKE
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Background:
Common variable immunodeficiency (CVID) is a heterogeneous disorder characterized by hypogamaglobulineia, variable defects in cellular immunity, recurrent infections, autoimmunity, granulomatous disease and susceptibility to cancer. We report a case of CVID with CNS manifestations and stroke.

Methods:

Results:
Case presentation: We present a 6y/o boy from consanguineous parents referred to immunology office because of refractory hemolytic anemia. He is known case of autoimmune hemolytic anemia since he was 3.5 y/o under treatment with IVIG and corticosteroid. Studying his medical documents revealed recurrent sinopulmonary infections, one episode of Pneumocystis jiroveci Pneumonia (PCP) and stroke event at 4 years of age with presentation of right eye blinking, left side hemiparesia and stuttering. Neuroimaging demonstrated normal blood flow and hypercoagulable state evaluations were negative that was suggestive of vasculitis. After a while he suffered from epilepsia partialis continua that Acute Disseminated Encephalomyelitis was diagnosed based on cortical and subcortical hyperintensitis in MRI. Immunological evaluations were performed showing neutropenia, low level of IgA, normal levels of IgM and IgG after receiving IVIG, decreased antibody titers to tetanus toxin, decreased CD4 T-cells in blood flowcytometry and abnormal T-cell proliferative responses to mitogens and antigens. We noticed inadequate increase in IgG level (560 mg/dl) despite receiving IVIG that was suggestive of CVID or Hyper IgM Syndrome (HIGMS). In genetic study, mutation wasn’t detected in HIGMS genes including CD40L, CD40, AID and UNG. We are waiting for ADA2 and NFKB1 genes sequencing results.

Conclusions:
We should consider association of autoimmunity and immunodeficiency. Unusual location like CNS, multiple organ involvement and age younger than usual are some clues.
AUTOIMMUNITY

ESID7-0280

PROPOSAL: Sic-reg.org – A PROSPECTIVE MULTICENTER REGISTRY STUDY FOR SEVERE IMMUNE CYTOPENIAS TO HARMONIZE DIAGNOSTIC STEPS, STRATIFY TREATMENT, AND OBSERVE THEIR NATURAL COURSE

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Background:

Autoimmune manifestations belong to the clinical spectrum of primary immune deficiencies and dysregulation disorders (PIDs) that may severely compromise the quality and expectancy of life. Immune cytopenias occur most frequently in B or T cell deficiencies, and patients with an underlying PID are at an least 120-fold increased risk to develop cytopenia as compared to the general population (Fischer et al., JACI 2017). Among a national pediatric cohort of chronic ITP, many additional or other diagnoses (in >30% of patients, including CTLA4 haploinsufficiency, SLE, Fanconi anemia) were identified, and responses to various standard treatment approaches were highly variable (Sipurzynski et al., 2016), suggesting different underlying mechanisms. Thus, the awareness and the diagnostic power of underlying disorders need to be increased to improve the clinical management of patients with severe immune cytopenias.

Methods:

The proposed ESID level 2 registry study should assist at the clinical level and allow translational research to identify phenotypic, functional, and epigenetic biomarkers for prognostic and therapeutic risk stratification. Panels for the diagnostic work-up will include hematological and immunological parameters and enable the exclusion of many differential diagnoses.

Results:

Biomarker analyses should allow early management stratification according to patterns reminiscent of known cytopenia-related PIDs even if a molecular diagnosis is not obtained (not needed or unavailable) or pending. Treatment guidelines will be derived from international standard recommendations and regularly complemented with data on newly available approaches. The natural course of the disease will be monitored.

Conclusions:

Data sets will be compatible with similar national pediatric and adult guidelines or registries.
AUTOIMMUNITY

ESID7-0294

CLINICAL PHENOTYPIC DIVERSITY IN PATIENTS WITH HOMOZYGOUS AND COMPOUND HETEROZYGOUS VARIANTS IN THE GENE CODING FOR LPS-RESPONSIVE BEIGE-LIKE ANCHOR (LRBA) PROTEIN.

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Background:

Patients with LPS-responsive beige-like anchor protein (LRBA) deficiency have CTLA4 loss and immune dysregulation leading to clinical features of immune deficiency and autoimmune insults of end organs including bone marrow, brain, lungs, liver and gut.

Methods:

We share the clinical spectrum of our cohort of 7 patients (Male/Female:2/5; median age 17 years; range 5-24yrs). We evaluated the patients by immunophenotyping, imaging and extended genetic testing.

Results:

Our patients share a wide ethnic ancestry: Europe, Brazil, Azerbaijan, punjab and african american. Four patients have significant brain lesions and one of them also has spinal involvement. Four patients have significant autoimmune GI disease. Two patients presented with autoinflammatory diseases and organomegaly and one of them with lipodystrophy improved on leptin and sirolimus. One patient developed Burkitts lymphoma; following chemotherapy he presented with nonmalignant lymphocytic brain lesions that led to diagnosis of LRBA deficiency. One patient with Sickle Cell Disease underwent extended genetic testing as she developed autoimmune hemolytic anemia associated with generalized lymphadenopathy at age 16. Treatment regimens include corticosteroids and steroid sparing measures: MMF (n=2), plaquenil (n=1), sirolimus (n=3) and abatacept (n=3).
Conclusions:

Clinical diagnosis of LRBA deficiency remains challenging due to their phenotypic diversity. Cellular protein assays for LRBA and CTLA4 should be utilized to validate the LRBA deficiency. Genetic screen for modifier genes that may account for variable clinical features is desirable to elucidate phenotype-genotype associations and determine optimal treatment. As SNPs of LRBA are common in some ethnic populations, frequency of a variant in a given ethnic group should be noted.
Autoimmunity

ESID7-0301

Role of Glycoxidatively Modified IgG in the Immuno-pathology of Rheumatoid Arthritis

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Background:

Glycation and oxidation are inextricably linked post translation modification (PTMs), resulting in glycoxidation of macromolecules. Protein glycoxidation is reported to play a significant role in the pathogenesis of several diseases including rheumatoid arthritis (RA). A prudent role of reactive oxygen species (ROS) has been found in tissue damage and inflammation associated with RA. Joints of RA patients also have deposition of advance glycation end products (AGEs), contributing to pathology directly or through the release of ROS. Immunoglobulin G (IgG) is susceptible to glycation due to high content of arginine and lysine residues. This study has probed the role of glycoxidatively modified IgG in the immuno-pathology of RA.

Methods:

Human IgG was modified with methylglyoxal (MG), a highly reactive dicarbonyl compound which is mainly derived from triose intermediates of glycolysis. The modified IgG (MG-IgG) was characterized by various techniques like UV-Vis, fluorescence, CD, LC/MS, MALDI spectroscopy besides scanning and transmission electron microscopy. Antigen-antibody interactions were evaluated by ELISA and gel-shift assay.

Results:

Structural integrity and aromatic microenvironment of the IgG was found to be disturbed upon modification. MG exposed the hydrophobic pockets of the protein, reduced its β pleated sheet content and affected tertiary conformation. Amorphous aggregates were observed in electron microscopy in the modified IgG. MG-IgG presented neo-epitopes on the protein surface leading to an aggressive immune response. Auto-antibodies derived from RA patients exhibited strong affinity towards MG-IgG in comparison to the unmodified counterpart.

Conclusions:

The glycoxidatively modified IgG has been discussed as a putative antigen for the production of auto-antibodies in RA.
Background:

The most common type of chronic neutropenia in pediatric patients is chronic benign neutropenia, also known as primary autoimmune neutropenia, usually lasting over 6 months and often presenting after a viral illness. However it can be an early sign of complex immune dysregulation.

Our goal is to investigate possible immune mediated mechanisms underlining chronic neutropenia in order to address the correct diagnosis and therapeutic strategy.

Methods:

Among the patients who refer to our Haematology/Oncology Unit, we selected twenty-five patients evaluated for chronic neutropenia. These patients were further characterized throughout clinical, immunological, haematological and genetic investigations.

Results:

An early genetic analysis of patients with neonatal onset and severe neutropenia revealed the presence of ELANE gene mutations in four patients.

A deeper bone marrow evaluation showed defects of cellularity in five patients: one of them was a Fanconi Anemia, one was a Schwachman Diamond Syndrome and three need further haematological investigation.

The immunological analysis of the remaining sixteen patients allowed the identification of four autoimmune neutropenia cases and two with autoimmune cytopenia (mean age at diagnosis 11 months, mean neutrophil count 0.5*10^9/L). The other ten patients showed different combination of neutropenia and leucopenia, bone marrow alterations and multiorgan autoimmunity (mean age at diagnosis 5.9 years, mean neutrophils count 0.7*10^9/L).

Conclusions:

To identify possible mechanisms underlining chronic neutropenia we are performing a deeper characterization of last 16 patients through immunophenotype analysis and the study of a gene panel by NGS methods. We expect to identify new subgroups of immunedysregulation disorders “hidden behind simple neutropenia”.
The Multiple Autoimmunity and Immunodeficiency (MAID) Clinic was established to provide care for patients with profound autoimmunity, often secondary to a primary immune defect. The clinic is multidisciplinary with rheumatologists and immunologists working collaboratively. We endeavored to review the experience in the MAID clinic to characterize clinical patterns and outcomes.

Patients seen in the MAID clinic from its inception in 2009-2012 were identified through the hospital’s administrative scheduling program. Medical records were reviewed for demographic and clinical data.

13 males and 16 females with a mean age of 11.8 years were studied. On average, patients presented with 2 (range:0-9) known autoimmune diagnoses, the most common being colitis (27%), thyroiditis (24%), lymphadenopathy (21%), and cytopenias (17%). The most frequent infections were mucocutaneous (52%) and sinopulmonary (55%). Positive autoantibodies were found in a majority of patients (52%), while abnormal lymphocyte subtypes (38%), immunoglobulins (48%), and vaccine responses (31%) were frequently noted. Identified genetic defects included IPEX (n=2), STAT1 GOF (n=1), C4 (n=1) and perforin (n=1) deficiencies. Patients were more likely to have a genetic diagnosis, if they had consistent follow-up (2.8 vs. 0.6 years of follow-up). After MAID consultation, treatment changes were recommended in 52% of patients.

The MAID clinic offers a collaborative model for the management of patients with autoimmunity and immunodeficiency. Consultation in the MAID clinic often resulted in new treatment recommendations. These findings also highlight the importance of a long-term follow-up for patients with immune dysregulation as genetic conditions are frequently identified over the course of treatment.
AUTOIMMUNITY

ESID7-0322

STRATIFICATION OF SCID/CID IN A COHORT OF 156 CHILDREN TRANSPLANTED AT BRESCIA CHILDREN’S HOSPITAL: HSCT CHARACTERISTICS AND AUTOIMMUNITY MANIFESTATIONS

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Background:

SCID are the most severe PID, characterized by impaired T and B lymphocyte function, normally leading to death within the first year of life without HSCT.

CID are an heterogeneous group of inherited immune disorders with impaired T cell development or function and increased susceptibility to infections and/or immune dysregulation; the presentation is beyond the first year of life.

Methods:

In the Pediatric BMT Unit of Brescia 156 PID were transplanted between 1990 and 2016. Median age at transplant was 19 months (range 2–156 months).

Results:

We treated 115 SCID (OS 71%) and 41 CID (OS 66%): 16 leaky SCID(OS 66%), 17 Omenn Syndrome (OS 72%), 7 HLA class II deficiency (OS:43%), 1 PNP(OS:100%).

- 15/16 leaky SCID received HSCT from HPC-M (5 HLA-ID, 8 MUD, 2 Haplo) and 1/16 from HPC-A (1Haplo).

- 11/17 of the Omenn Syndrome received transplantation from HPC-M:(3HLA-ID, 6MUD, 2Haplo); 3/17 from HPC-A (3 HLA-ID) and 3 from HPC-M+HPC-A (3Haplo).

- 5/7 HLA class II deficiency received HSCT from HPC-M (3HLA-ID, 1MUD, 1Haplo) and 2/7 from HPC-A (2MUD).

- PNP patient received HSCT from MUD HPC-M.

In our group of transplanted PID the prevalence of autoimmunity events was of 25% (39/156), most of them were disthyroidism and autoimmune haemolitic anaemia.

Conclusions:

HSCT is the best therapeutic approach for SCID and also for CID. Surprisingly the overall survival post-BMT of SCID and CID children as well the incidence of autoimmune complications are similar, since the latter are older, has residual immunity and continous infections.
AUTOIMMUNITY

ESID7-0343

EVALUATION OF RECENT THYMIC EMIGRANTS (RTEs) AND DIFFERENT PHENOTYPE CORRELATION IN A COHORT OF 44 PATIENTS AFFECTED BY DEL22 SYNDROME.
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Background:
Children with Del22 have a significant risk of recurrent and severe infections and an increased risk of developing autoimmune complications that might prove important for their clinical management. To date, the mechanism by which autoimmunity occurs is unclear and it is probable that it is multifactorial. Currently, there are no immunological markers to identify or predict a higher risk of developing severe infections and autoimmune manifestations in patients with Del22 syndrome. Recent studies point to the pivotal role of RTEs in the regulation of T effector function, homeostasis and trafficking. The aim of this study is to describe the clinical and immune aspects of a population of patients affected by Del22 syndrome and to explore whether RTEs have a pathogenic role in the characterization of different clinical phenotypes.

Methods:
We evaluated a cohort of 44 Del22 syndrome patients referred to the Pediatric Immunology Department of Anna Meyer Children’s Hospital of Florence between January 2013 and January 2017. For different clinical subgroup of Del22SP we performed a complete immune characterization.

Results:
Our data confirm that the prevalence of autoimmune disease in the Del22 syndrome cohort is high. The most common autoimmune disease identified was hypothyroidism.

Conclusions:
Our study suggests that low percentage values of RTEs might indicate a major risk for autoimmune disease in Del22 patients, suggesting a crucial role of RTEs in the process of immune tolerance.
Background:

To analyze the incidence and spectrum of manifestations in Primary Antibody Deficiencies (PAD), diagnosed in adults during the years 1980 – 2015.

Methods:

Two groups of patients were included in this study: sixty-seven (67) cases of Common Variable Immune Deficiency (CVID) and fifty-three (53) cases of Selective IgA Deficiency (IgA-D). Most cases were hospitalized for a variety of clinical symptoms.

Results:

Among the 67 CVID cases, 11 (16.4 %) presented with Idiopathic Thrombocytopenic Purpura (ITP), which preceded patients with the diagnosis of the immune deficiency in most cases. Four of them had in addition Autoimmune Hemolytic Anemia (AHA). Patients with ITP in spite of immunoglobulin therapy had often recurrences and 3 of them had to undergo splenectomy. Out of 53 inpatients with IgA-D, autoimmune disease presented as follows: eight (16.9%) had Systemic Lupus Erythematosus (SLE) with high titers of antinuclear (ANA) and anti-dsDNA autoantibodies, one had Primary Biliary Cirrhosis (PBC) with positive anti-mitochondrial antibodies (AMA), one Crohn’s Disease (CD) with positive anti-smooth muscle antibodies (ASMA), one ITP and one Autoimmune Hemolytic Anemia (AHA).

Conclusions:

The incidence of autoimmune disease is frequent in adult patients with PAD but its pattern differs significantly as in CVID predominate autoimmune cytopenias, whereas rheumatic autoimmune disease, SLE especially, appears more frequently in IgA-D patients.
MULTIPLE SYSTEMIC AUTO-IMMUNITY IN COMMON VARIABLE IMMUNODEFICIENCY (CVID): A PARADIGMATIC CASE

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Background:

Autoimmune diseases and lymphoid malignancy are well described complications associated with CVID. However, few studies relate CVID with adrenocorticotropic hormonal (ACTH) deficiency, autoimmune or genetic triggered.

Methods:

Case

Results:

16 year old girl diagnosed with CVID at the age of 6, following recurrent lower respiratory infections associated with bronchiectasis. A panhypogammaglobulinemia (IgG 336mg/dL, IgA 23,7mg/dL, IgM 19mg/dL) and poor response to vaccines was documented. Immunoglobulin substitution therapy and prophylactic antibiotics were started with good clinical response from an infectious point of view. At age 9 she had a mild manifestation of vitiligo, self-limited. At 11 years, Hodgkin lymphoma with lymphocytic predominance, limited to an axillary lymph node, was diagnosed. She underwent successful treatment with rituximab and is currently in complete remission. Short stature, delayed puberty and fatigue at the age of 14 suggested pituitary insufficiency. Further investigation confirmed secondary adrenal insufficiency and thickening of the stalk of the pituitary gland in the brain MRI was interpreted as a possible lymphocytic hypophysitis. Other autoimmune investigation revealed positive anti-thyroglobulin and anti-peroxidase titer, with normal thyroid function. She is now being treated with hydrocortisone (0,3mg/kg/day) and growth hormone is being considered. The history of infections in the last year under steroids is remarkable for a sepsis due to Staphylococcus aureus.

Conclusions:

This case represents the diversity of complications of CVID. We highlight the association with autoimmune hypophysitis, rarely described, demonstrating the important interactions between immune and endocrineal systems. Steroid treatment in CVID, even in low doses, and its possible role in invasive disease is also debated.
NADPH OXIDASE 2 DERIVED REACTIVE OXYGEN SPECIES PROMOTE CD8+ T CELL EFFECOR FUNCTION IN MURINE MODEL OF CHRONIC GRANULOMATOUS DISEASE

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Background:

Reactive oxygen species (ROS) are a group of highly reactive molecules produced mostly from the phagocyte NADPH oxidase complex (NOX2), are involved in phagocytic host defense, and have been proposed as a signal transducer for cytotoxic T cell activation.

Methods:

In order to examine the role of NOX2 in cytotoxic T lymphocyte (CTL) activation, a CTL dependent type 1 diabetes model [non obese diabetic (NOD)] system was used. NOD mice were bred with NCF1 mutated mice (Ncf1<sup>m1J</sup>) in order to abrogate ROS production. CD8+ T cells were isolated from splenic tissue and stimulated with anti-CD3/CD28. Intracellular cytokine responses and mRNA production were quantified.

Results:

Genetic ablation of NOX2 causing poor ROS production in CD8+ T cells of NOD- Ncf1<sup>m1J</sup> mice and pharmacologic inhibition of ROS with apocynin in CD8+T cells from NOD mice exhibit significantly suppressed T-bet resulting in reduced production of IFNg, granzymeB, and TNFa, following stimulation with anti-CD3/CD28 that was H<sub>2</sub>O<sub>2</sub> dependent. These defects were isolated to proximal T cell signaling in which genetic or pharmacologic ablation of NOX2 caused failure in deactivating tumor suppressor complex (TSC)1/2 compromising downstream mammalian target of rapamycin (mTOR) expression.

Conclusions:

In this murine model of type 1 diabetes, NOX 2 plays a non-redundant role in T cell receptor – mediated CTL effector function. NOX2-derived H<sub>2</sub>O<sub>2</sub> inhibits TSC1/2 activity and thus promotes mTOR function during CTL activation, leading to an enhanced production of T-bet, which boosts CTL effector function and cytolytic activity.
AUTOIMMUNITY

ESID7-0419

REVISITING REGULATORY T CELLS IN TYPE 1 DIABETES
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Background:

Type 1 Diabetes (T1D) is an autoimmune condition where insulin-producing beta cells are killed by T cells. Unregulated control of T cell-mediated pathology has been linked, in part, to paucity of T regulatory cells (Tregs). There is conflicting evidence whether Treg paucity is linked to developmental or peripheral homeostatic perturbation. Recently we documented that newly developed (ND) Tregs, thymic resident Tregs and recent thymic emigrant (RTE) Tregs could be identified on the basis of RAG-GFP expression. Furthermore, we showed that the cytokine and costimulatory pathways required for the homeostasis of the individual populations was distinct. Here, using the RAG-GFP reporter system and the well-described T1D model- the non-obese diabetic (NOD) mouse, we revisit Treg biology in normal versus T1D-prone mice.

Methods:

FVB-RAG-GFP mice were backcrossed to NOD and NOD-µMTKO mice for 20 generations. Flow cytometry analysis was employed to determine the frequencies, absolute numbers, and presence of homeostatic markers of precursor Tregs, ND Tregs, thymic resident Tregs and RTE Tregs in primary and secondary lymphoid tissue.

Results:

Preliminary evidence suggests mature Tregs are enhanced in NOD thymus with respect to control mice. Although precursor, ND, resident and RTE Tregs are readily detectable, their numbers and homeostatic proliferation may be influenced by the absence of B cells, depending on the tissue examined and the strain of mouse investigated.

Conclusions:

B cells may play an unappreciated role in the homeostasis of defined Treg populations. Future investigations on the B cell-Treg relationship in T1D-setting, particular the role of cytokines will be informative.
FAMILIAL THROMBOCYTOPENIA IN THREE MALE SIBLINGS
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Background:

Familial thrombocytopenia is uncommon. Specific molecular defects have been identified in some families. We describe three male siblings with thrombocytopenia and other immune related findings. The parents are healthy and non-related.

Methods:

Clinical and investigative findings were obtained from the brothers and their parents.

Results:

Case 1:A 15 year old male presented with spontaneous ecchymotic patches and oral mucosal bleeding. Platelet count - 1000/mm³, mild hepatomegaly on ultrasonography and IgA was raised. As the response to IV methyl-prednisolone was poor, IVIG, prednisolone and azathioprine were used. He has had recurrent RT infections and as his latest platelet counts are suboptimal Rituximab is being considered. Case 2:A 19 year old male was found to have thrombocytopenia whilst being investigated for a large scalp haematoma aged 2 years. He has been treated with oral prednisolone and needed pulse IV dexamethasone. Presently he is off steroids and platelet count is 50000/mm³. Serum IgM is reduced and IgA is raised. He has chronic bilateral lower limb eczema, an atrio-fascicular accessory pathway and gets recurrent RT infections. Case 3:A 12 year old male had fever, cervical lymphadenopathy and hepatosplenomegaly aged 3 years. He then developed AIHA and thrombocytopenia. The thrombocytopenia persisted and was treated with prednisolone and cyclosporine. Aged 9 years, he developed SLE and a year later, class IV lupus nephritis was found on renal biopsy.

Conclusions:

An AR or XR genetic cause is likely in this family. The identification of the exact molecular defect may help with selecting appropriate medications to target abnormal immune pathways.
NEW STAT1 MUTATION IN SUMOYLATION SITE CONFFERS GAIN OF FUNCTION AND COMBINED IMMUNODEFICIENCY RESPONSIVE TO RUXOLITINIB

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Background:

Gain of function STAT1 mutations in the coiled coil and DNA binding domains are reported to be due to delayed de-phosphorylation and underlie autosomal dominant chronic mucocutaneous candidiasis/combined immunodeficiency.

Methods:

We report a case of combined immunodeficiency in a 10 year old boy due to a novel mutation in the C-terminal region of STAT1 that is predicted to alter sumoylation of STAT1, thereby increasing phosphorylation by a novel mechanism and leading to gain of function.

Results:

Features both of autoimmunity and immunodeficiency improved after commencing treatment with ruxolitinib, a janus kinase 1/2 inhibitor, and functional testing after treatment confirmed reversal of the STAT1 hyperphosphorylation.

Conclusions:

A mutation in the C-terminal region of STAT1 can lead to gain of function and associated combined immunodeficiency. Janus kinase inhibitors may represent a treatment option for such patients, particularly in the absence of a suitable haematopoietic stem cell donor.
CHRONIC MUCOCUTANEOUS CANDIDIASIS DUE TO HETEROZYGOUS MUTATION IN AIRE; A CASE REPORT.

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Background:

Mutations in AIRE typically cause a rare autosomal recessive inherited disorder known as autoimmune polyendocrinopathy syndrome type 1 (APS-1) associated with multi-organ autoimmunity and can present with chronic mucocutaneous candidiasis. Recently, mono-allelic mutations in the first plant homeodomain (PHD1)- zinc finger of AIRE have been linked with a milder phenotype and incomplete penetrance.

Methods:

We report the case of a 9 year old boy with chronic mucocutaneous candidiasis from early childhood, but no evidence of organ specific autoimmunity otherwise.

Results:

A heterozygous mutation (c.901G.A; p.V301M) was found in AIRE, along with low level anti-IL-17 antibodies. A sequence variant in STAT1 was also detected, but no change in STAT1 activity noted on functional testing.

Conclusions:

Heterozygous mutations in the PHD1 domain of AIRE can lead to disease and should be considered in the differential of chronic mucocutaneous candidiasis.
AUTOIMMUNITY

ESID7-0445

SJÖGREN'S SYNDROME IN A PATIENT WITH INTERLEUKIN 12 RECEPTOR BETA 1 (IL12Rβ1) DEFICIENCY

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Background:

IL12Rβ1 deficiency is a primary immunodeficiency resulting in susceptibility to infection by poorly pathogenic mycobacteria and severe infection caused by Salmonella spp. IL12Rβ1 is a component of both the receptor for interleukin 12 (IL-12) and interleukin 23 (IL-23). Genetic studies and evidence derived from mouse disease models suggest the implication of IL-12 and IL-23 in development of autoimmunity, including Sjögren's syndrome (SS).

Methods:

Detection of Bacillus of Calmette Guérin (BCG) by culture after lymphadenectomy in left axilla. Flow cytometry of PBMC: staining for IL12Rβ1 and intracellular staining to test for STAT4-phosphorylation after stimulation with IL-12. Sanger sequencing of the IL12RB1 gene.

Results:

We present the case of a 50-year-old male with IL12Rβ1 deficiency due to compound heterozygosity (c.1623_1624delGC(pGln542Stop) and c.1791+2T>C(donor splice site)), who - two years after an infection with BCG - presented with recurrent fever and sicca-syndrome. No indication of an infectious origin of these symptoms could be found. On the basis of fulfilled American-European consensus classification criteria for SS, including a positive minor salivary gland biopsy, we made the diagnosis of SS.

Conclusions:

Apart from persistent antigenic stimulation, which may drive autoimmune inflammation in primary immunodeficiency, evidence on the pathogenic role of IL-12 and IL-23 in SS suggests, that the same immunological mechanism may underlie both defense against infection and the maintenance of tolerance. To our knowledge, this is the first report of a case of autoimmunity in form of SS in a patient with a primary immunodeficiency and one of the rare cases of manifested autoimmunity in case of IL12Rβ1 deficiency.
AUTOIMMUNITY

ESID7-0449

NEUTRALISING AUTO-ANTIBODIES AGAINST IL-6 IN HUMANS
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Background:

IL-6 is a pleiotropic cytokine, which is rapidly produced in response to tissue damage and infection and plays an important role in innate and adaptive immunity. Various primary immunodeficiencies (PIDs) have been described that are associated either with defects in IL-6 signalling or IL-6 production, including STAT3 deficiency and Toll-like receptor signalling pathway defects. Three patients with high titer neutralizing antibodies to IL-6 have been reported so far, all presenting with severe bacterial infections. In all patients, serum C-reactive protein levels where not elevated during infections, suggesting impaired acute phase responses (Puel et al., 2008; Nanki et al., 2013).

Methods:

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Results:

We identified high titre serum IgG antibodies to IL-6 in ten patients with histories of severe and/or recurrent infections, in one patient suffering from multiple sclerosis, without obvious history of infections and also in one healthy control (Fig 1). Antibodies were found to be inhibitory as shown by their capacity to block IL-6 induced STAT-3 phosphorylation in hepatoma cells (Fig 2).
Conclusions:

Patients presented with a range of infections including gram+ bacteria, mycobacteria, fungi and viruses. Our data show that anti IL-6 serum antibodies may be associated with a wider range of infections than previously suggested. The role of anti-IL-6 auto-antibodies in disease susceptibility still needs to be further elucidated.
FAMILIAL AUTOIMMUNITY AND RECURRENT INFECTIONS LINKED TO A NOVEL HOMOZYGOUS NFkB2 MUTATION

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Background:

We report a family of three siblings of consanguineous descent from Morocco. A 37 year-old woman who’s medical history includes universal alopecia, oral lichen plan, hashimoto’s thyroiditis, listeria meningitis, herpes labialis, and a fulminant auto-immune hepatitis at the age of 15 followed by a liver transplant. On clinical examination, lingual ulcers, whitlow on fingers, diffuse folliculitis and onychomycosis are found. Her younger brother, aged 33, reports patchy alopecia, oral ulcers, recurrent pneumonias, giardiasis, anemia and thrombocytopenia. The third sibling, a 42 year old man, has a similar albeit less severe phenotype with recurrent severe oral herpetic. Their mother died in childbirth, and their father subsequently had two children with the mother’s sister that are reported to be healthy.

Methods:

Immunological and genetic work-up was performed for the first two siblings. IgG levels were low, while IgA and IgM levels were in the normal range. Response to polysaccharide vaccine was poor. Profound B-cell lymphopenia with an increase in CD21(low)CD38(low)% was found. The T-cell compartment was characterized by an excess in activated CD8+ HLA-DR+. In addition, a reduction in peripheral T-regulators was noted for the sister.

Results:

A rare homozygous missense variant in exon 11 of NFkB2 (R311C) was disclosed.

Conclusions:

Based on previous reports, a mutation in this gene produces a phenotype compatible with the ones we present here. This is to our knowledge the first homozygous NFKB2 mutation linked to a primary immunodeficiency with auto-immunity. Functional tests are ongoing.
PHENOTYPE OF STAT3-GAIN OF FUNCTION MUTATION IN FEMALE PATIENT FROM THE CZECH REPUBLIC

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Background:

STAT3 (signal transducer and activator of transcription 3) gain of function mutations lead to immune dysregulation with prominent autoimmunity and immunodeficiency due to regulatory T cell aberration and impaired cytokine signaling resulting in defected STAT5 and STAT1 signaling.

Methods:

We present a case of 21 years -old female patient with failure to thrive, growth and sexual maturation retardation since the patients childhood. At 17 years of age the patient measured 125.6cm (-6.6 SD) and weighed 19.7kg (-10.5 SD), with bone age of 9 years. Psychomotoric development of the patient was slower. She suffered from subsequent recurrent upper and lower respiratory tract infections, which required repeated admissions to hospital and treatment with antibiotics. Lung damage with bronchiectasias, pulmonary hypertension on echocardiography and restrictive lung disease on a lung function test was confirmed at the age of 18 years. Patient requires long-term oxygenotherapy.

Results:

Hypogammaglobulinaemia, low post-vaccination antibodies, disturbed cellular immunity with normal to low B-lymphocytes, low T-lymphocytes and high activated HLA-DR+ T-lymphocytes, enlarged peripheral lymph nodes and significant splenomegaly is present. Since 12 years of age anemia and thrombocytopenia with positive PAT 4+ and free Abs in NAT and enzymatic test, with normal bone marrow. From 16 to 18 years of age, the patient developed several episodes of exacerbation of the autoimmune cytopenia.

Conclusions:

Newly whole exome sequencing (WES) was performed and mutation c.2144C>T, leading to substitution of leucine for proline in position 715 of the STAT3 protein was detected. No other mutations in genes explaining the phenotype, with focus on growth retardation, were found.
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FAS, PI3KCD AND TNFRSF13B AS DISEASE CAUSING-MUTATIONS: APOPTOSIS THROUGH DEATH RECEPTOR GENE EXPRESSION SIGNATURES AND SHARED DOWNREGULATED GENES IN PID PATIENTS

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Background:

TNF and TNF receptor superfamily members are important components of several signalling pathways involved mainly in apoptosis and inflammation. A plethora of diseases – ranging from cancer, chronic inflammatory diseases and neurodegeneration to rare conditions, such as primary immunodeficiencies (PIDs) – show disturbances in these pathways.

Methods:

To identify critical targets, compare and shed light on the molecular physiopathology of three specific PIDs, we performed an array containing 44 assays to genes associated with death receptor mediated apoptosis in five patients. Despite causing distinct pathologies, mutations in FAS (2 related patients), PIK3CD (1 patient) and TNFRSF13B (2 patients) share some clinical manifestations in common, particularly lymphadenopathy and organomegaly.

Results:

The high prevalence of downregulated genes found for all patients clearly suggests an apoptosis failure. Regardless a specific patient gene expression signature and considering a −2.0-fold change cutoff, four targets were constant: CASP2, IKBKB, TNFRSF1A and TNFRSF25.

Conclusions:

CASP2 upregulation causes apoptosis; also, it is involved in cell signalling, greatly inducing MAPK14 and NFkB activation. The protein encoded by IKBKB phosphorylates the inhibitor in the inhibitor/NFkB complex, causing dissociation of the inhibitor and NFkB activation. TNF triggers TNFRSF1A, a main death receptor which plays a role in cell survival, apoptosis, and inflammation. TNFRSF25 has been shown to stimulate NFkB activity and regulate cell apoptosis, including lymphocyte homeostasis. Knockout studies suggested its role in thymic self-reactive T cell removal. Identifying critical targets in PIDs is instrumental not only to predict clinical manifestations and develop new therapies but also to clarify autoimmunity and lymphomagenesis molecular physiopathology.
Background:

The actin-related protein-2/3 (ARP2/3) complex is composed of 7 subunits and is involved in actin polymerization, cellular motility and endocytosis. ARPC1 (actin-related complex 1) is one of its regulatory subunits and is present in two isoforms (ARPC1A and ARPC1B) that interact with nucleation promoting factors (NPFs), such as WAS family proteins. Recently, a child with combined immunodeficiency, severe inflammation and allergies with mutation in ARPC1B was described. We now report a second patient with ARPC1B deficiency.

Methods:

Flow cytometry was used to immunophenotype PBL subsets and lymphoproliferation to PHA. Skin, lymph node and GI biopsies and GIT endoscopies were performed. Whole exome sequencing (WES) and linkage analysis were performed in the patient and relatives.
Results:

The patient is a 3-year-old boy, fourth child born for consanguineous parents; 2 siblings had died earlier in life. Shortly after birth he developed severe eczema and later on, chronic bloody diarrhea, sinopulmonary and cutaneous infections. Persistent anemia and thrombocytopenia with Auto-Ab were documented. Skin biopsies suggested erythrodermia, while LN biopsy showed mixed follicular hyperplasia and GIT biopsies and endoscopies revealed chronic inflammation. Immunophenotyping of PBLs showed low CD4+ T cells with normal B and NK cells and monocytosis, CD21low B and transitional B cells were expanded, and naïve, central and effector memory CD4+ T cells and naïve CD8+ T cells were decreased; TCRgd+ T cells were increased. T cells did not proliferate to PHA. WES and linkage analysis identified a homozygous mutation in ARPC1B (p.W86X).

Conclusions:

To our knowledge this is the second patient with ARPC1B deficiency.
AN ACTIVE SYSTEMIC LUPUS ERYTHEMATOSUS CASE WITH COINCIDING RHABDOMYOLYSIS WHO DRAMATICALLY BENEFITS FROM THERAPEUTIC PLASMA EXCHANGE: A CASE REPORT AND LITERATURE REVIEW

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Background:

Systemic lupus erythematosus (SLE) is a chronic connective tissue disease with autoimmune nature. Circulating immune complexes, autoantibodies have been implicated in the pathogenesis. Therapeutic plasma exchange (TPE) is an extracorporeal treatment technique designed for removal of pathogenic substances from the circulation. Data on use of TPE in SLE patients is scarce and conflicting. Rhabdomyolysis is a syndrome of various etiology, characterized by muscle necrosis and release of intramuscular components. Herein we present a case of complicated lupus coinciding with rhabdomyolysis who dramatically benefits from TPE.

Methods:

A 23-year-old female with history of SLE was admitted. She sought medical care with severe breathlessness, generalized edema and decreased amount of urine. On admission, her physical examination revealed fever, tachycardia, tachypnea, hypertension, hypoxemia, pretibial edema, and crackles in the lung. She had anemia, and high creatinine levels. Urinalysis revealed 20-30 red and white blood cells per high-power field. Serum complement was significantly decreased. Many autoantibodies were found positive. During ongoing treatment, the patient was diagnosed with rhabdomyolysis. As the patient had congestive heart failure, administration of fluids could worsen pulmonary edema. She was faced with a high risk of permanent nephropathy due to myoglobin’s tubular toxicity and circulating immune complexes. TPE was considered to be the most rational approach. Patient received TPE treatment for 10 days.

Results:

Within weeks, urine output increased up to 2500-3000 ml/day. Serum creatinine levels decreased. She didn’t need hemodialysis. Hypoxemia resolved. Follow-up echocardiography improved. Pleural and pericardial effusion completely disappeared.

Conclusions:

TPE is an option that should be kept in mind in selected SLE cases. We recommend being more courageous to begin TPE in the presence of an additional medical problem which is foreseen to benefit from TPE.
Background:

Spondyloarthritis (SpA) are a group of inflammatory joint diseases which cause chronic, progressive, axial inflammation of spine and sacroiliac joints. Diagnostic criteria for SpA are clinical symptoms, radiology, MRI or ultrasound following the Assessment of SpondyloArthritis international Society (ASAS) criteria. Although similar to other inflammatory joint diseases such as rheumatoid arthritis (RA) and psoriatic arthritis (PsA), SpA has a considerably different pathology.

Our aim is to test the presence of antibodies to oxidative post-translationally modified collagen type II (oxPTM-CII) in serum samples from SpA patients.

Methods:

Collagen type II (CII) was exposed to various reactive oxidants and analysed by SDS-PAGE. Binding to native and oxPTM-CII was evaluated by ELISA and Western Blotting. We used sera from 67 patients with SpA. Control subjects included: 54 patients with psoriatic arthritis; 60 subjects with early RA (ERA); 49 subjects with undifferentiated arthritis (UA); 19 patients with fibromyalgia (FM) and 70 healthy subjects (HC). A selection of strong binders were further evaluated by western blot and competitive ELISA.

Results:

Reactivity to oxPTM-CII was observed in 72% of SpA samples, in 33% of PsA samples and in 35% of UA samples, binding was stronger to oxPTM-CII. Binding of sera from SpA was similar to ERA samples. Sensitivity and specificity of binding to oxPTM-CII in SpA samples were 72% and 95.7% respectively. SpA samples bound to a range of molecular weights between 150 and 25 displaying a different binding pattern compared to ERA.

Conclusions:

Antibody to oxPTM-CII may have the potential to become biomarker for SpA diagnosis.
CHARACTERIZATION OF AUTOANTIBODY TARGETS IN IPEX SYNDROME USING PROTEOME-WIDE ARRAYS

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Background:

Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) is a monogenic autoimmunity syndrome caused by mutations in the FOXP3 gene. The disease is associated with a severe functional defect of thymic-derived T regulatory (Treg) cells, one of the main subsets maintaining peripheral immune tolerance. Affected children develop multiple autoimmune manifestations from early age, including enteropathy, dermatitis, and type 1 diabetes. Autoantibodies targeting the intestinal protein Harmonin (USH1C) are present in patients with enteritis and represent a valuable diagnostic marker, since they are not detectable in patients with enteropathy and wild type FOXP3.

Methods:

We aimed at further exploring the autoantibody repertoire in IPEX, also beyond those associated with enteropathy. Microarrays containing thousands of purified full-length human proteins enabled us to investigate a large proportion of the human proteome in parallel. We studied sera from 15 patients with IPEX and healthy controls.

Results:

In the proteome-wide screen we replicated the detection of known autoantibodies and further identified several new major immune targets, including a Harmonin-interacting protein, type 1 interferons and a striking multitude of nuclear receptors.
Conclusions:

Using an unbiased proteome approach, we have identified novel autoantigens in IPEX, a model disease for impaired peripheral T cell tolerance. Future studies of patients with defects in other checkpoints of B and T cell tolerance will address the question, whether specific patterns of autoantigens emerge that reflect specific pathways of disease pathogenesis. Such studies in genetically defined model diseases promise insights also for more common autoimmune conditions.
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PHENOTYPING THE MYELOID COMPARTMENT OF GERMLINE SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 (STAT3) GAIN OF FUNCTION PATIENTS

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Background:

STAT3 is a member of the Signal Transducer and Activator of Transcription family of transcription factors and is essential to immune responses. Heterozygous germline mutations in STAT3 can cause gain of function (GOF) resulting in immune dysregulation and deficiency. Clinical and laboratory phenotypes of GOF mutations are diverse; altered TH17-T-reg balance may be linked to autoimmunity whereas reduced memory B-cell switching results in hypogammaglobinaemia. To date, research has primarily focused on the lymphoid compartment; the effects on myeloid cells are relatively unknown. STAT3 is linked to dendritic cell (DC) development in the mouse. STAT3 KO mice are deficient in DCs and are unresponsive to Flt3L-driven DC expansion.

Methods:

We analysed blood from healthy controls and 3 different STAT3 activating mutations by flow cytometry to profile the full complement of DCs and monocytes as we recently described using single cell RNA-sequencing. We quantitated absolute cell numbers using TruCount for CD14+ and CD16+ monocytes, cDC1, cDC2, plasmacytoid DC (pDC) and our newly discovered AXL+SIGLEC6+ DCs (ASDCs), a functionally and transcriptionally distinct population previously contaminating pDCs.

Results:

cDC1, cDC2 and CD14+ monocytes were present at similar numbers to healthy controls. However, ASDCs were markedly reduced with slight reduction in pDCs compared to controls. CD16+ monocytes appeared to be slightly elevated although CD14+ monocytes were unaffected.

Conclusions:

We demonstrate the profile of blood DCs and monocytes in STAT3 GOF patients. Further studies on the consequences of STAT3 GOF mutations on the immune function of blood mononuclear phagocytes are being pursued.
DISCRIMINATION AMONG ACQUIRED AND MONOGENIC SISTEMIC AUTOIMMUNE DISORDERS: INTERFERON SCORE, STAT1 AND STAT3 PHOSPHORYLATION AS DIAGNOSTIC TOOLS

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Background:

Type I Interferonopathies are an heterogeneous group of disorders including both monogenic and acquired diseases. We have tried to distinguish the two forms by mean of inflammation/autoimmunity signal transduction pathways analysis.

Methods:

We have evaluated STAT1 and STAT3 activities and IFN Score in patients with Aicardi-Goutières Syndrome (AGS), genetically determined Systemic Lupus Erythematosus (SLE) and SAVI as monogenic forms, and patients with acquired SLE or SLE-like and patients with suspected interferonopathies of unknown etiology.

Results:

All patient’s primary cell showed a significative increase in activation and expression of STAT1 as compared to normal donors. A constitutive STAT3 activation is present only in primary cells from acquired disease. Activated cultured patients’ cells of hematopoietic origin showed alterations in STAT1 signaling and expression only in cell lines derived from genetically determined diseases. Similarly, gene expression induced by type I IFN via STAT1 is altered in all patients in primary cells, while only in the genetically determined diseases we demonstrated a constitutive activation giving a high IFN score.

Conclusions:

Although these diseases share a high production of type-I IFN, they differ in pathogenesis and we observed differences in cell lines behaviours: monogenic type-I interferonopathies are intrinsically predisposed to increased activation of the type-I IFN, while cells derived from patients with acquired disease show similar behavior to healthy controls indicating that the impairment occurs only in vivo and depends on the milieu in which the cells are located. We can consider these assays useful in differential diagnosis of patients with suspected Interferonopathies and in therapeutic effects monitoring.
Impact of PTPN22*R620W autoimmune-associated variant on human cytotoxic lymphocyte function

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Background:

PTPN22 is a cytoplasmic non-receptor tyrosine phosphatase expressed in hematopoietic cells and implicated in regulation of antigen receptor signaling in T cells as well as Toll-like receptor and type I IFN receptor signaling in myeloid cells. A single-nucleotide polymorphism (SNP) in PTPN22 (c.1858C>T, p.R620W; rs2476601) is associated with several human autoimmune diseases, including rheumatoid arthritis, type-1 diabetes and systemic lupus erythematosus. The amino acid substitution disrupts PTPN22 interaction with key cell signaling mediators such as CSK and TRAF3.

Methods:

Fluorescence-activated cell sorting of several different subsets of lymphocytes from buffy coats of healthy donors from Karolinska Huddinge Hospital followed by westernblot. PBMCs from genotyped donors carrying the three different allele combinations (Risk/Risk, Protective/Risk and Protective/Protective) used for functional assays.

Results:

We have evaluated PTPN22 expression levels in different cytotoxic T cell and NK cell subsets. Interestingly, PTPN22 expression was significantly regulated in such subsets at both the transcript and protein level. Therefore, in an effort to understand how PTPN22 may regulate cytotoxic lymphocyte function, we are evaluating cell subset responses relative to PTPN22 genotype.

Conclusions:

Significance will be presented and discussed
EPIDEMIOLOGICAL AND CLINICAL FEATURES OF PATIENTS WITH PRIMARY ANTIBODY DEFICIENCY DISORDERS IN THE EAST OF TURKEY

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Background:

Introduction: Primary antibody deficiency (PAD), the most common primary immunodeficiency disorder, represents a heterogeneous spectrum of conditions caused by a defect in any critical stage of B cell development and is characterized by impaired production of normal amounts of antigen-specific antibodies. This retrospective study aimed to describe the demographic and clinical features of patients diagnosed with primary antibody deficiency.

Methods:

Methods: The medical records of pediatric patients who were diagnosed as primary antibody deficiency were reviewed. Patients were diagnosed as primary antibody deficiency based on the ESID diagnostic criteria.

Results:

Results: A total number of 60 patients with PAD were identified; 39 patients (65%) were males and median age is 5 (1-18) years old, median diagnosis age is 3 (6 mo-14 yr) years. Twenty-one patients (35%) diagnosed with transient hypogammaglobulinemia of infancy, thirteen (21.7%) with selective IgA deficiency, five (8.3%) with congenital agammaglobulinemia, five (8.3%) with selective IgM deficiency, five (8.3%) with unclassified hypogammaglobulinemia, four (6.7%) with IgG subclass deficiency, two (3.3%) with severe combined immune deficiency, two (3.3%) with common variable immunodeficiency, two (3.3%) with hyper IgM syndrome, one with pneumococcal vaccine responsiveness, respectively. Eleven patients (18.3%) had consanguinity and six patients (10%) had family history. The most common complaint is frequent respiratory tract infections. Four patients had dermatitis in addition to infection. Three patients had hematopoietic stem cell transplantation and 23 patients (38.3%) received intravenous immunoglobulins treatment. Three patients were died during the follow up. The demographic of patients and clinical features were summarized in table 1.

Conclusions:

Conclusion: Our results indicated that the patients who diagnosed with PAD have frequently respiratory tract infections and have consanguinity and family history.
Background:

Activation of various compartments of the immune system has been described in common variable deficiency disorders (CVID), however little is known about granulocyte function and activation in patients suffering from CVID.

Methods:

We determined granulocyte activation markers in 46 CVID patients (25 females, 21 males aged 22-82 years) and compared them with 28 healthy controls (19 females, 9 males, aged 22-78 years). All CVID patients were in a stable state without apparent acute infections. Plasma levels of elastase, myeloperoxidase and neutrophil gelatinase-associated lipocalin NGAL were determined by ELISA. The expression of granulocyte activation markers CD11b, CD62L were determined by flow cytometry on CD15+ cells. In patients on intravenous immunoglobulin (IVIG) treatment (n=26) the determination was performed prior to IVIG infusion. The results were compared by mean of Mann-Whitney rank-sum test.

Results:

Plasma levels of elastase, myeloperoxidase and NGAL were markedly increased in CVID patients compared to healthy controls (p<0.001 in all cases). Similar significances were obtained when the levels of serological activation markers were divided by the absolute granulocytes count in the time of determination. This difference remained significant also after CVID patients with CRP >10 mg/l (n= 7) were excluded.

Mean fluorescence intensity (MFI) of granulocyte activation marker CD62L was significantly increased in CVID patients (p= 0.01), however there was no difference in MFI of CD11b (p=0.10).

Conclusions:

All these data point to activation of granulocytes in CVID patients, even in patients without apparent acute exacerbation of infection.

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DIFFERENT ALLERGIC MANIFESTATIONS IN A CASE WITH DOCK8 DEFICIENCY

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Background:

The autosomal recessive form of hyperimmunoglobulin E (AR-HIES) syndrome is an uncommon type of primary immunodeficiency disorders caused by deletion of the dedicator of cytokinesis 8 (DOCK8) gene. It is distinguished by elevation of the level of serum IgE antibody, atopic dermatitis, and recurring viral and bacterial infections.

Methods:

Case Presentation: A 7-year-old girl, the only child of the related parents, was admitted to the hospital. She has been suffering from recurrent episodes of cough and dyspnea from the age of 18 months and has been under treatment with inhaled corticosteroid and as-needed short acting beta-agonist. Nevertheless, she has experienced several attacks of asthma that has led to emergency department visit and hospitalizations. Severe, recalcitrant and intensifying atopic dermatitis has started from early months of life. Milk protein allergy was documented by skin prick test. She was admitted to the hospital at the age of 4 because of eczema herpeticum and distributed herpetic lesions with eye involvement. She has also experienced diffuse intractable wart and recurrent pneumonia.

Results:

Laboratory evaluation showed normal IgE (87 IU/L) and low IgM (<5mg/dl) level. None of other laboratory examinations including flow cytometry showed significant abnormal findings. According to the history, her DNA was extracted and genetic evaluation confirmed the diagnosis of DOCK8 deficiency type of AR-HIES. She was referred for bone marrow transplantation.

Conclusions:

Although atopic patients are disposed to bacterial and viral infections, but whenever an atopic patient presents with recurrent and intractable or unusual infection, primary immunodeficiency diseases should be considered as the differential diagnosis.
SPLENECTOMY IN PATIENT WITH CVID IN AZERBAIJAN
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Background:
CVID is the most common type of primary immunodeficiency characterized by hypogammaglobulinemia with recurrent bacterial infections. Usually there are decrease IgG and IgA or all three types IgG, IgA and IgM antibody levels.

Objective: Last years we detected 85 patients with PID, 20 of them had CVID and only one had also hemolytic-anemia, which leads to splenomegaly and resulted in splenectomy.

Methods:
Immune system was examined by immune cells phenotyping in peripheral blood, level of serum IgM, IgG, IgA, IgE by ELISA, phagocytic activity by NBT, CIC by photometric method.

Results:
Boy was born from the second pregnancy and not related parents. All vaccinations were made without complication. In age of one iron deficiency anemia has been detected, in age of 2 child had repeated respiratory infectious. At the age of 4 he was ill with chickenpox, stable anemia (Hb-64g/l) and splenomegaly was detected at examination. Autoimmune hemolytic anemia and splenomegaly was diagnosed. Thalassemia was not detected.

On immunological examination it was determined a significant decrease in the level of serum IgA, IgM, IgG and IgE antibodies. Total number of T-lymphocytes, T-helper and B-lymphocytes were normal, number of T-suppressor cells was increased. Severe anemia persisted for many years. At the age 8 the splenectomy was made. The child took antibiotics and fluconozolium for two years after surgery and still receive IVIG each 4 weeks during last 3 years. Now he is at age 15. During last years Hb level is around 136-145 q/l.

Conclusions:
Splenectomy and replacement IVIG therapy improved the condition of our patient.
B CELL

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SIGNIFICANCE OF B-CELL SUBSETS IN PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY AND UNCLASSIFIED ANTIBODY DEFICIENCY

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Background:

Our aim is to evaluate B-cell subsets in CVID and unclassified antibody deficiency cases, and additionally, to investigate their association with treatment requirement.

Methods:

The study included 25 CVID cases, 41 unclassified antibody deficiency cases and 36 healthy individuals who were aged between 4-18 years old.

Results:

The absolute count of total memory B cells and switched memory B cells were lower in CVID cases compared to the control group. Additionally, absolute count of IgM memory B cells in 4-10 years old age group, and absolute count of plasmablasts in 10-18 years old age group were lower in CVID cases compared to both control and unclassified antibody deficiency groups. Unclassified antibody deficiency group was categorized based on IVIG replacement therapy; accordingly, the percentage of switched memory B cells was significantly lower in the IVIG-receiving group compared to the control group, whereas there was no significant difference between IVIG-receiving group and CVID group. Regarding the comparison of non-IVIG replacement group and CVID group, absolute count of total memory B cells, IgM memory B cells, and switched memory B cells were significantly lower in the CVID group.

Conclusions:

B-lymphocyte subsets in unclassified antibody deficiency cases who do not require IVIG replacement were rather similar with the healthy group; on the other hand, percentage of switched memory B cells in unclassified antibody deficiency cases who require IVIG replacement was not different from that of the CVID cases; therefore, these cases should be monitored in terms of CVID development.
Background:

Fatigue is increased in common variable immunodeficiency (CVID). However, the prevalence of patient-reported fatigue is unknown, and the association between fatigue and immunoglobulin-G treatment (IgGT) wear-off is not well understood. We aimed to measure patient-reported fatigue prevalence and identify the effects of IgGT on fatigue.

Methods:

Data from 916 patients with CVID who responded to the 2013 immunodeficiency treatment survey and had answers on the fatigue/wear-off effect questions were analyzed. Demographics, co-morbidities, IgGT route and dose, quality of life (QoL), perceived health, and disability were compared between fatigued and non-fatigued.

Results:

The overall prevalence of fatigue was 77.5% in patients with CVID. Patients receiving IVIG had higher prevalence of fatigue compared to SCIG (83.9% vs 68.84%, P<0.001). Females had higher prevalence of fatigue compared to males in both IVIG (86.63% vs 75.97%, p=0.004) and SCIG (71.39% vs 57.33% P=0.017). In SCIG, receiving weekly infusion, and a higher total monthly dose was associated with less fatigue. Patients on IVIG at dosage >600 mg/kg month were more likely to report "no fatigue" for treatment <4 weeks versus patients on dosage <600 mg/kg month p<0.001). Fatigued patients reported lower physical QoL scores compared to non-fatigued, and fatigue was associated with disability and unemployment.

Conclusions:

Fatigue is associated with IgGT wear-off effect which is more profound in patients receiving IVIG, and patients receiving their IgGRT at longer intervals or lower doses. Further studies to correlate fatigue severity with IgG levels are warranted to help identify the impact of IgG wear-off effect on fatigue.
B CELL

ESID7-0086

B-Cell RECEPTOR REPERTOIRE SEQUENCING (BCR RepSeq)) IN PATIENTS WITH PRIMARY IMMUNODEFICIENCY

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Background:

Many patients with primary immunodeficiencies (PIDs) manifest with defects in antibody production. However, the severity of the underlying immunodeficiency is difficult to determine using existing diagnostic approaches. Functional properties of B-cells are determined by their B-cell receptor (BCR). In PID patients, BCR diversification is often diminished, leading to an altered BCR repertoire. Here, we explore high-throughput BCR repertoire sequencing for the assessment of B-cell function in PID patients.

Methods:

BCR heavy chain transcripts were PCR amplified from RNA extracted from lysed peripheral blood B cells. The protocol captures all isotypes, and incorporates unique molecular identifiers to allow subsequent removal of PCR artefacts. Samples were multiplexed, and sequenced on the Illumina MiSeq.

Results:

So far, we have analysed 5 healthy control and 7 PID samples (Hyper-IgE syndrome (STAT3), ADA2 deficiency [n=2], PIK3R1 deficiency, CVID, Hypogammaglobulinemia [n=2]). Compared with controls, patient BCR repertoires generally presented more naïve B-cell characteristics, including longer CDR3 lengths and lower V-gene mutation rates. Combining several BCR repertoire properties using principal component analysis showed healthy control and patient samples to form distinct clusters. While the healthy controls clustered densely, the patients showed more variability. Interestingly, patients with a more severe phenotype localised further away from healthy control samples compared with less affected patients.

Conclusions:

We demonstrate that BCR repertoires from PID patients are different from those of healthy controls. BCR repertoire properties seem to correlate with the severity of the underlying immunodeficiency suggesting that this method allows to assess global B-cell function, and may therefore aid in the treatment decision process.
ISOLATED DECREASED SERUM-IGM AS INCIDENTAL FINDING: A DIFFICULT DILEMMA

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Background:

Isolated decreased serum-IgM has been associated with severe/recurrent infections, atopy and autoimmunity. The reported high prevalence of clinical problems in IgM-deficient patients may reflect the skewed tertiary centre population studied. Also, many papers on IgM-deficiency have included patients with more abnormalities than just IgM-deficiency. We studied selective IgM-deficiency in a large teaching hospital in ’s-Hertogenbosch, the Netherlands (encachment area 350,000).

Methods:

All decreased serum-IgM levels (1-July-2005 to 23-March-2016; n=8049 IgM<0.4g/l; n=2067 truly solitary [IgG+IgA normal/IgM<age-matched reference]) were studied retrospectively.

Results:

349/2036(17%) adults and 10/31(32%) children had primary isolated decreased serum-IgM (slgMdef), proven persistent in 42/349(12%) adults and 3/10(30%) children; their medical charts were reviewed. 10/42(24%) adults and 2/3(66%) children were analyzed for potential immunodeficiency. Of the 32/42(76%) adults who were analyzed for other problems, 7/32(22%) had a history of symptoms that could be related to antibody deficiency; 18/25(72%) patients without such symptoms remained asymptomatic during follow-up; 7/25(28%) developed symptoms that could be related to antibody deficiency (mainly infectious problems). Only 2 adults and 1 child had proven true slgMdef (IgG-subclasses/vaccination responses determined+normal).

Conclusions:

In many medical protocols serum-IgM is determined routinely, leading to incidental findings of decreased serum-IgM. Only a few are for certain true slgMdef; in most patients follow-up, IgG-subclasses/vaccination responses were lacking. So, on the one hand decreased serum-IgM is often probably of little significance. But on the other hand, 42% of primary, persistent cases had a history of symptoms that could be related to antibody deficiency. A larger cohort of true slgMdef patients is needed to fully explore the clinical consequences.
Background:

Primary immunodeficiency diseases are a group of disorders that result from a variety of defects of the immune system. Primary antibody deficiencies (PAD) are the most common forms of these disorders. Occurrence of recurrent infections, autoimmune diseases, cancers and lymphoproliferative disorders is higher in PAD patients. Chronicity of these diseases, delayed diagnosis, inadequate treatment, and treatments side effects may affect Quality of life (QOL) of PAD patients. Evaluating QOL is important for patient care, understanding the burden of these diseases and finding patients major health problems. We investigated the QOL in a group of PAD patients undergoing regular follow-up and treatment at the Children's Medical Center Hospital in Tehran, Iran.

Methods:

Seventy patients with a diagnosis of PAD in 2 age groups (younger and older than 18 years) were included. Quality of life was measured using PedsQL and SF-36 questionnaires. Correlation of demographic, clinical and immunological parameters with QOL scores were assessed and patients' scores were compared with the normal population, using non-parametric tests of SPSS software.

Results:

Patients expressed significantly reduced scores in some mental and physical components. Patients with longer follow-up periods had higher scores in mental components but physical components scores were still low. There was no significant correlation between sex, age and disease types with scores.

Conclusions:

PAD patients had significantly lower scores in mental and physical components compared to normal population. By early diagnosis and long-term follow-up periods, we may be able to prevent complications and help patients have a better quality of life.
B CELL

ESID7-0126

TOLL-LIKE RECEPTOR 2 AND 4 IN COMMON VARIABLE IMMUNE DEFICIENCY

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Background:

Common Variable Immunodeficiency (CVID) is the most common clinically primary antibody deficiency, characterized by hypogammaglobulinemia and increased susceptibility to recurrent bacterial infections. Since Toll-Like Receptors (TLRs) play important role in maturation and differentiation of B-cell, defect in TLRs could be involved in the pathogenesis of CVID. In this sense, we evaluated the profile of TLR2 and TLR4 and their signaling in CVID patients.

Methods:

Peripheral Blood Mononuclear Cells (PBMCs) of CVID patients and controls were isolated and stimulated with/without lipopolysaccharide (LPS) and lipoteichoic acid (LTA). Flow cytometry was used for assessing the protein expression of TLR2 and TLR4. We retrieved patients’ data about their response to Pneumovax-23 and B-cell subtypes.

Results:

We found the significant elevated protein expression of TLR2 and higher ligand responsiveness for TLR2 and TLR4. There was a significant correlation between low number of end stage B-cells and TLR2 expression furthermore TLR2 was associated with hypo response to the vaccine.

Conclusions:

According to the correlation of low number of end stage B-cells and poor vaccine response with higher expression of TLR2 we hypothesize that there is a functional defect in this receptor and/or it’s downstream in PBMCs of CVID patients.
ANTI-IgA ANTIBODIES IN PATIENTS WITH PRIMARY ANTIBODY DEFICIENCY RECEIVING INTRAVENOUS IMMUNOGLOBULIN

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Background:

Immunoglobulin replacement therapy is an effective route of management for both infectious and non-infectious complications in predominantly antibody deficiency (PAD). Trace levels of IgA (ranged from 0.4 to 2500 mg/ml), which exist in all immunoglobulin products, could lead to an increased susceptibility for adverse reactions in PAD patients. Furthermore, the exact mechanism which stimulates the anti-IgA antibody production in PAD is still unknown. The aim of this study was to evaluate IgG anti-IgA antibodies in PAD patients receiving intravenous immunoglobulin (IVIG) and its predisposing factors.

Methods:

Available patients with confirmed diagnosis of PAD, who underwent regular IVIG replacement therapy in our center, were enrolled in the study. Control group included 24 healthy individuals as the negative control and eight symptomatic patients with IgA deficiency as the positive control groups. IgG anti-IgA antibody level was measured by ELISA method.

Results:

A significant difference was observed between Anti-IgA level of common variable immunodeficiency (CVID) and other PAD groups (p=0.02). Moreover, six CVID patients were seropositive for the IgG anti-IgA antibody, with higher susceptibility to the adverse reactions (p<0.001). IgG anti-IgA level has a negative relationship with serum IgA level (r=-0.06) and IVIG treatment duration (r=-0.006).

Conclusions:

Our data suggested that there was a significant association between anti-IgA antibody presence and adverse reactions, especially in CVID patients with higher susceptibility of producing this constitutional antibody.
B CELL

ESID7-0146

PRESCRIPTION OF IMMUNOGLOBULIN REPLACEMENT THERAPY FOR PATIENTS WITH NON-CLASSICAL AND SECONDARY ANTIBODY DEFICIENCY: AN ANALYSIS OF PRACTICE IN THE UNITED KINGDOM & REPUBLIC OF IRELAND.

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Background:

Immunologists are increasingly being asked to assess patients with non-classical and secondary antibody deficiency to determine their potential need for immunoglobulin replacement therapy (IGRT). Immunoglobulin is a limited, expensive resource and no clear guidance exists for this broad patient group. The purpose of this survey is to establish what factors influence the decision to commence IGRT in adult patients, when diagnostic criteria for primary antibody deficiency are not fulfilled.

Methods:

Under the auspices of the United Kingdom Primary Immunodeficiency Network (UKPIN) a study group was established which circulated an online questionnaire to the consultant body across the UK and Ireland. Results provided a snapshot of the current clinical practice of 71% of consultant immunologists, from 30 centres.

Results:

In order of importance, factors which influence the decision to commence IGRT include: number of hospital admissions with infection, serum IgG level, bronchiectasis, radiologically proven pneumonia, number of positive sputum cultures, number of antibiotic courses and results of immunisation studies. The commonest test vaccine used was Pneumovax 23 with measurement of serotype specific responses at 4 weeks, with a threshold of 0.35μg/ml in 2/3 of serotypes measured. 86% of patients are treated with a trial of prophylactic antibiotics prior to consideration of IGRT. Efficacy of IGRT trial is assessed at between 6-12 months.

Conclusions:

There was consistency in clinical practice using a combination of clinical history, evidence of infections and vaccination testing for diagnosis. However, there was some variation in the implementation of this practice, particularly in vaccine choice and assessment of response to vaccination.
IgM AND IgA ANTI-PNEUMOCOCCAL CAPSULAR POLYSACCHARIDES AS PROGNOSTIC TOOL FOR COMMON VARIABLE IMMUNODEFICIENCY: A LONGITUDINAL STUDY

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Background:

The clinical spectrum of CVID ranges from a poorly symptomatic form to severe phenotypes. Due to high prognosis heterogeneity, prognostic factors are required.

Methods:

We evaluated the anti-polysaccharide IgA and IgM responses on 75 CVID in a longitudinal study over a 6-year period. Patients were immunized with the 23-valent pneumococcal polysaccharide vaccine (Pneumovax®). During the follow up, concomitant CVID-related conditions, immunoglobulin serum levels, respiratory infections and outcome were recorded.

Results:

As expected, CVID patient had lower IgM/IgA response than HD. For CVID, three immunological phenotypes were identified by post-vaccination IgM and IgA levels: IgM/IgA responders, IgM-only responders and non-responders. CVID non-responders and IgM-only responders developed more frequently respiratory infections, gastro enteric symptoms, and autoimmune manifestation in comparison to IgM/IgA responders. Malignancies were found more frequently in the non-responders and IgM-only responders groups in comparison to IgM/IgA responders. Eleven patients died during the study time. Survival analysis according to the IgM/IgA responder status showed that the 6-years estimated survival for non-responders vs IgM-only vs IgM/IgA responders was respectively after after two year: 93%, 87% and 100%; after 4 years: 87%, 80% and 100%; after 6 years: 83%, 80% and 100%. Interestingly, in our series only two deaths were due to infective complications: five were consequent to malignancies, one to autoimmune cytopenias and three to not-CVID related conditions.

Conclusions:

Even if patients could not raise the protective humoral level, in CVID the antipolysaccharide IgA and IgM responses could represent a prognostic factor, individuating patients with less immunological impairment, low incidence of comorbidities and better survival.
Background:
Congenital agammaglobulinaemia generally presents with recurrent infections in childhood. We assessed clinical characteristics of these patients who have lived into their adulthood.

Methods:
We reviewed clinical notes of all our current adult patients diagnosed with congenital agammaglobulinaemia.

Results:
There were a total of 13 male patients with mean age of 38 years old (age range 21-52 years old), median age of diagnosis was less than one year old (range: 6 months to 5 years old). All have no B-cells. Five patients had genetic tests and they showed Bruton’s tyrosine kinase (BTK) gene mutation. Two other patients had BTK protein analysis and they lacked of BTK protein expression. All patients had normal total CD3 (CD4 and CD8) lymphocyte counts and normal T lymphocyte proliferation to either phytohaemaglutinin or poked weed mitogen. Common clinical features were chest infection and rhinosinusitis. However, no patient had severe pneumonia needed hospitalisation. Different comorbidities have been noticed even with same BTK gene mutations. Eleven patients had bronchiectasis, meanwhile eight patients had chronic sinus disease. All patients have been treated with immunoglobulin replacement with or without antibiotic prophylaxis. All patients, except one, had normal liver function. Eight patients had thyroid function tests and they were normal.

Conclusions:
Generally, all our patients were stable with immunoglobulin replacement therapy with or without prophylactic antibiotics. However, a significant number of patients have developed bronchiectasis, in spite of early diagnosis and treatment. Autoimmune disorders are not common in these patients comparing with common variable immunodeficiency.
B CELL

ESID7-0163

B CELL LYMPHOMA IN PAKISTAN
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Background:

To study the pattern of small B cell lymphomas in Pakistan.

Methods:

This descriptive study was carried out at the Shaukat Khanum Hospital and 1721 cases of Non-Hodgkins Lymphoma (NHL) diagnosed during a period of ve years (1998-2002) and classed according to REAL/WHO classification. The antibodies used included Leukocyte Common Antigen (LCA), Pan B (CD20, CD79a), Pan T (UCHL-1), Bcl 2, Mib 1 (Ki 67) and Cyclin D1 (Dako, Denmark)

Results:

Out of the 1721 NHL cases, only 140 (8.1%) could be categorized as small B-cell NHL. The study group comprised small lymphocytic lymphoma/chronic lymphocytic leukemia (58 cases; 41.4%) followed by follicular lymphoma (46 cases; 32.9%), mantle cell lymphoma (15 cases; 10.7%), extra nodal marginal zone B cell lymphoma of MALT type (15 cases; 10.7%), lymphoplasmacytic lymphoma (5 cases; 3.6%) and splenic marginal zone B-cell lymphoma (1 case; 0.7%). No case of nodal marginal zone lymphoma was diagnosed.

Conclusions:

Small B-cell NHL was more common in males; with male to female ratio of 2.1. Majority of the small B-cell NHL were nodal at presentation with a nodal to extranodal ratio of 3.4.
B CELL

ESID7-0175

TACI (TRANS-MEMBRANE ACTIVATOR AND CALCIUM-MODULATING CYCLOPHILIN LIGAND INTERACTOR - TNFRSF13B) GEN MUTATION: OUR CASES AND EXPERIENCES
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Background:
TNFRSF13B mutations, which encodes B-cell receptor transmembrane activator and calcium modulator ligand interactor (TACI) were found to be associated with hypogammaglobulinemia. Compound heterozygotes and homozygotes have been identified, but in the majority of cases, simple heterozygous variants are present. In this study we aimed to evaluate the patients diagnosed with common variable immunodeficiency(CVID)/unclassified hypogammaglobulinemia(UCH) found to have TACI mutation.

Methods:
Clinical characteristics, laboratory data of 21 CVID/UCH patients with TACI mutation and complications developed during follow up were evaluated retrospectively between 2003-2016.

Results:
Male/female ratio was 16/5. Initial presenting symptoms of all cases were infections. Current age, age at diagnosis, symptom onset age, and follow up time are respectively 8.2±3.9, 4.6±3.4, 1.8±2.6, 2.1±1.6 years. 66.7% of cases followed with CVID, 33.3% with UCH. Complications (bronchiectasis, asthma, chronic otitis) were present in 42.8% of the cases. Autoimmunity, splenomegaly, lymphoproliferation and malignancy were not observed. IgG, M and A levels were 538±236; 61±41.5; 45.6±57.0 mg/dl respectively. In one UCH and in two CVID cases, naïve B cells were high, nonswitch and switch memory B cells were low, in other cases values were normal. Both of the cases with homozygous mutations were in CVID group. The most common mutation was found to be p.C172Y (19%).

Conclusions:
B cell regulation is affected at different levels in this patients. Autoimmunity, lymphoproliferation, malignancy may develop but not commonly seen in childhood. Identification of patients and members of family carrying mutations, and follow up will be useful to determine its potential impact on clinical course.
EVALUATION OF COMMON VARIABLE IMMUNE DEFICIENCY PATIENTS

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Background:

Common variable immune deficiency (CVID) is the most frequent symptomatic primary immune deficiency in adults. Current estimates suggest a prevalence of approximately one in 25,000 in the general population. Patients frequently become symptomatic later in life. Careful clinical history, however, may reveal symptoms in some patients dating back to early childhood. The disorder is characterized by recurrent and/or severe infections, autoimmunity, malignancy and allergic disorders. Here, clinical and laboratory findings of CVID patients followed-up by our clinic were assessed in this study.

Methods:

Clinical as well as laboratory findings such as immunoglobulins (Ig), IgG subgroups, flow cytometry, specific antibody responses and isohemagglutinin titers from our 10 CVID patients were evaluated.

Results:

There were 10 patients, 6 male and 4 female, and mean age was 13 years. Different clinical pictures were observed in our CVID patients. One patient had Potter syndrome, one had recurrent meningoencephalitis, one with bronchiectasis and atopic dermatitis, three with asthma, one with recurrent pneumonia, one with bronchiectasis, one with bronchiectasis and asthma, and one patient had recurrent sinusitis and nephrolithiasis. Consequently, asthma in 40%, bronchiectasis in 30%, recurrent meningoencephalitis in 10%, atopic dermatitis in 10%, Potter syndrome in 10%, and recurrent sinusitis was observed in 10% of the patients.

Conclusions:

Our findings indicate that variability in clinical picture and evaluation by different specialists cause delay in the diagnosis of CVID.
ICF SYNDROME: CLINICAL, IMMUNOLOGICAL AND CYTOGENETIC ANALYSIS OF SEVEN CASES

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Background:

Immunodeficiency, centromeric instability, facial dysmorphism (ICF) syndrome is a rare autosomal-recessive disease, has four types due to mutations in DNMT3B, ZBTB24, CDCA7 and HELLS genes. Clinical features are recurrent, prolonged respiratory, skin and digestive system infections, facial dysmorphism including hypertelorism, flat nasal bridge, epicanthal folds, low-set ears, protrusion of tongue, mild micrognathia. In laboratory findings, there are reduced immunoglobulin levels, rearrangements in the vicinity of the centromeres (the juxtacentromeric heterochromatin) of chromosomes 1, 16, or 9.

Methods:

In this study clinical, immunologic, cytogenetic characteristics of ICF syndrome patients referred to Immunology Clinic in last five years were retrospectively analysed.

Results:

Patients' median age of onset of symptoms and age at diagnosis was 2 months (range 0 to 8) and 5 years (range 0.25 to 13), respectively. All suffered from frequent respiratory tract infections. More than half (4/7) were given IVIG treatment before diagnosis for antibody deficiency. Patient 1(P1) and P2 had scoliosis, mandibularankylosis and developmental dysplasia of hip respectively. In two patients (P2,P6), structural abnormalities of nervous system were found in cranial MRI, such as subcortical nodular heterotopies, cerebellar tonsillar ectopic, ectopic neurohypophysis, cortical focal atrophy. P7 had hepatic mass requiring biopsy.

Conclusions:

In addition to facial dysmorphism and immunodeficiency; skeletal deformities and congenital central nervous system anomalies may associate ICF syndrome. Also gastrointestinal tract and hepatobiliary system could be affected.
ASSOCIATION OF FREIBURG CLASSIFICATION AND CLINICAL MANIFESTATIONS IN ADULTS WITH COMMON VARIABLE IMMUNODEFICIENCY

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Background:

Patients with common variable immunodeficiency (CVID) comprises a heterogeneous group with different causes of hypogammaglobulinemia predisposing to recurrent infections, a higher incidence of autoimmunity, and malignancy.

Objective: to establish association with Freiburg classification and the clinical manifestations in adults with CVID.

Methods:

We studied 18 patients from a cohort, with a diagnosis of CVD, of the Immunodeficiency Clinic of the Specialties Hospital. Flow cytometry was performed with determination of lymphocyte subpopulations with absolute and relative numbers, including: CD19, CD27 + IgM-IgD-, CD21 low. Patients were grouped according to the classification of Freiburg into 3 types: IA, IB, II.

Results:

7 male and 11 female, current average age of 42 years. 8 with hematological autoimmune diseases, 7 with IDCV-associated enteropathy. 1 with chronic myeloid leukemia and 3 with predominance of infectious processes.

According to the classification of Freiburg, 4 patients with group IA, 4 IB and 10 with group II.

75% of patients with IA and IB groups had hematological autoimmunity diseases compared to 20% of those in group II. 40% of patients in group II had predominance of infections and 30% IDCV-associated enteropathy.

4 patients died, 2 due to massive lower gastrointestinal bleeding, 2 due to septic shock.

Conclusions:

The main utility of classifications, according to B cell subtypes, is to be able to find a marker, which identifies the phenotype of the disease. In this study, patients with less than 0.4% of CD27+ IgM-IgD- were associated with hematological autoimmunity, and those with more than 0.4% with enteropathy and predominance of infectious processes.
GASTRIC AND DUODENAL PATHOLOGICAL FINDINGS IN AN ITALIAN COHORT OF 58 CVID PATIENTS: A CROSS-SECTIONAL STUDY

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Background:

Clinical manifestations of CVID, the most frequent symptomatic primary immunodeficiency in adulthood, include infections, inflammatory and autoimmune diseases, and malignancies. Also, the gastrointestinal tract is involved. In this cross-sectional study we assessed the main gastric and duodenal pathological findings in a cohort of adult CVID patients.

Methods:

58 patients (26 males, mean age 46.2±14.9 years) diagnosed with CVID according to the ESID diagnostic criteria underwent upper endoscopy. Gastric antrum and descending duodenal biopsies were collected in each patient, irrespectively of the macroscopic findings and the gastrointestinal symptoms reported. A cut-off level of 25 lymphocytes per 100 enterocytes was chosen to define an increase of the intraepithelial lymphocytes in the duodenal biopsies.

Results:

The main pathological findings were: a) chronic active gastritis (55%), associated in 8 cases (25% of the gastritis) with the Helicobacter pylori infection, b) autoimmune atrophic gastritis (5%) and, c) chronic duodenitis with increased intraepithelial lymphocytes and lack of plasma cells (46%). Intestinal metaplasia (IM) of the gastric antrum was reported in 5 patients (8.6%). In one patient IM was associated with the H. pylori infection and persisted after the pathogen’s eradication. In two patients IM was associated with autoimmune atrophic gastritis. Giardia lamblia was detected in the duodenum samples in 3 cases (5%). An antrum undifferentiated gastric adenocarcinoma was found in a 58-year-old woman.

Conclusions:

In our cohort of patients, according to previous reports, gastric and duodenal pathological findings are common and affect prognostic outcome. A strict endoscopic follow-up is required in CVID patients irrespectively of the gastrointestinal symptoms.
Active phosphoinositide 3-kinase δ syndrome (APDS) is a combined immunodeficiency resulting from a gain-of-function mutation of PIK3CD, the gene encoding the catalytic subunit of PIK3Kδ which is expressed predominantly in leukocytes.

Methods:
We describe the first case of PIK3CD gene mutation in Korea.

Results:
A 4-year-old boy, who was diagnosed with common variable immunodeficiency at the age of 21-months from other hospital, was referred to our outpatient clinic for further evaluation of persistent cervical lymph node enlargement, hematochezia and increased serum IgM level. In laboratory examination, complete blood count showed white blood cell 7,910/µL, hemoglobin 10.9 g/dL and platelets 122 x 10^3/µL. Levels of serum immunoglobulin IgG, A, M were 332 mg/dL (normal range; 441–1,135 mg/dL), <1 mg/dL (normal range; 22–159 mg/dL), 569 mg/dL (normal range; 47–200 mg/dL) respectively. He was finally diagnosed with active phosphoinositide 3-kinase δ syndrome (APDS) with PIK3CD gene mutation (NM_005026.3:c.3061G>A (p.Glu1021Lys), heterozygous) confirmed through Next-Generation Sequencing (NGS).

Conclusions:
In this study, we report the first case in South Korea characterized by PIK3CD gene mutation, previously diagnosed as unidentified primary immunodeficiency with idiopathic lymph node enlargement, splenomegaly, protein-losing enteropathy, and decreased serum IgG and IgA with increased IgM level.
IDENTIFICATION OF HETEROZYGOUS NFKB1 VARIANTS IN CVID COHORT USING TARGETED NEXT GENERATION SEQUENCING

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Background:

CVID is characterized by recurrent bacterial infections of respiratory tract. It is the most prevalent symptomatic antibody deficiency syndrome. NFKB1 encodes the transcription-factor precursor p105 protein which can undergo cotranslational processing by the 26S proteasome to produce the active subunit p50 (canonical NF-κB pathway).

Methods:

Using targeted next generation sequencing, we identified five heterozygous novel NFKB1 mutations in a cohort of 180 patients with CVID.

Results:

Two novel heterozygous NFKB1 truncated mutations detected in two affected brothers from non-consanguineous parents and one unrelated CVID patient (p.Ser338Leufs*94; p.S302Ffs*7). These mutations are predicted to lead to non-functional proteins, which might undergo rapid decay, thus resulting to p50 haploinsufficiency. Another CVID patient harbored a NFKB1 frameshift mutation (p.Ile567Asnfs*6), which is predicted to lead to aberrant p50 or loss of p105. In two further patients we identified NFKB1 heterozygous splice-donor-site mutation (c.1210+1G>A) causing in-frame skipping of exon 12 and a point mutation with unknown effect.

Conclusions:

Heterozygous NFKB1 mutations seem to be prevalent in CVID patients and may cause monogenic CVID with very different clinical manifestations and onset.
PERITONITIS, PNEUMONIA AND PLEURAL EFFUSION AS A PRESENTATION OF X-LINKED AGAMMAGLOBULINEMIA

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Background:

X-linked agammaglobulinemia (XLA) is a rare primary immunodeficiency caused by defects in the Bruton tyrosine kinase (BTK) gene, characterized by impaired early B-cell development, defective antibody production, and increased susceptibility to bacterial infections at an early age. Some XLA patients show atypical presentations, with most reports concentrating on the diagnosis at a relatively old age. They presented with infections at late age or with unusual pathogens; however, other atypical manifestations have only rarely been reported.

Methods:

Iranian patients with congenital agammaglobulinemia were included for genetic studies and parameters such as clinical and immunologic phenotypes were evaluated in these patients.

Results:

During this evaluation we found a 4 year old boy whom was referred to our hospital with full clinical picture of peritonitis. At primary care physicians consulted with clinical immunologists to visit the patient. Past medical history was suggestive of recurrent sinopulmonary infections and pleural effusion. Tonsils and adenoids were absent. The clinical findings were enough to think of predisposing immunodeficiency disorder for current presentation. Furthermore, serum immunoglobulins and percentage of B cells were profoundly low. At this phase the patient was neutropenic too. According to multi-organ infection, we cancelled the operation and treated him with antibiotics and Intravenous Immunoglobulin, and chest tube drainage. The patient recovered uneventfully without laparotomy surgery. Thereafter, mutation analysis confirmed that the patient had BTK missense mutation within SH1 domain (Hemizygous, c.1856C>T p.619).

Conclusions:

Overwhelming multiorgan infections should be considered as the possibility of underlying primary immunodeficiency disorder.
ONE PATIENT OF CVID WITH UNUSUAL PRESENTATIONS

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Background: CVID is the most common primary immunodeficiency after Selective IgA deficiency. According to the statistics of the registry bank of PID in Iran, CVID is the most common disease among humoral immunodeficiencies, which includes 45% of primary immunodeficiency in Iran. CVID is a heterogenous disease with unknown ethology and characterized by antibody decrease as well as the decrease in number of, and function of T lymphocytes. The clinical manifestations of CVID are repeated bacterial infections, autoimmunity, and malignancy. Methods:

A 33 years old man that have recurrent and repetitive aphtus oral lesion with odinophagy from 13 years ago. He had go to endoscopy that shows result nonspecific and report just a chronic gastritis. also he had several BMD result in recent last year that show osteoporoses with low T scor.. He also had several rheumatologic study whithout any possitive result. He treated as behcet Disease with corticosteroid that have not any effect on oral lesion. To study the reason of recurrent relapse of oral lesion show candida albikans that treated with Itraconazol. The imonological finding for recurrent lesion did not have specific result just low decrease in CH50 and PHA. on the way there was not any net diagnose for his immundificensy disease and they need more study special team working. The patient with probably diagnosis of CV ID had treated with IgIg that response to it and he get prophylaxis treatment as AB, itraconazol and acyclovir. He trended to trans sexuality and is under the hormontheraoy.

Results:

lab tests were Ch50:80%(LOW), PHA:2.7(Low), LTT:3.7(LOW), Ig level:low

Conclusions:

CVID with T cell abnormality associated with unusual presentations.
NUCLEAR FACTOR KAPPA B1 (NFkB1) MUTATION IN A PATIENT WITH COMMON VARIABLE IMMUNODEFICIENCY.

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Background:

The NFKB1 signal pathway is crucial in the regulation of the natural and adaptive immune response. In recent years it appears that mutations in NFkB1 have been identified as the cause of common variable Immunodeficiency (CVID). Here we present a CVID patient with the new mutation identified in NFkB1 gene.

Methods:

Case Report

Results:

A six-years-old girl referred us with recurrent upper respiratory tract infections until three years old. She was born from non-consanguineous parents and she didn't have any siblings. No pathological finding was found on physical examination. In laboratory tests; Hemoglobin: 12.1 g/dl, white blood cell count: 10700/mm³, absolute lymphocyte count: 7000/mm³, absolute neutrophil count: 2800/mm³, platelet count: 243,000/mm³, IgG: 281 mg/dl, IgM: 33.5 mg/dl, IgA: 13.1 mg/dl were detected. Tetanus, H. influenza B, diphtheria and hepatitis B vaccine responses were positive. The lymphocyte subsets were normal, T cell proliferation response was low. Intravenous immunoglobuline therapy was initiated due to recurrence of infections despite prophylactic antibiotic administration. There were no mutations in TACI, ICOS, BAFF-R, CD19 genes. Preimplantation genetic testing was planned and c.1477G>T (p.Glu493) heterozygous mutation on NFkB1 gene was detected, which was previously not determined. The mother, who had history of recurrent upper respiratory infections and IgA deficiency, also had the same mutation.

Conclusions:

NFkB mutations that were identified in 2001 as the cause of CVID can manifest with various clinical presentations such as immunodeficiency and immunodisregulation. NFkB1 mutations should be considered in CVID patients.
Background:

We report the case of a 8 year old boy affected by XLA (X-linked agammaglobulinemia), diagnosed at birth (maternal family history positive to XLA). Intravenous immunoglobulin replacement therapy was administered up to the age of 18 months when subcutaneous immunoglobulin (SClG) 20% were started (100 mg/kg each week) resulting in a constant IgG through level of ~ 900 mg/dl. Sporadic infections of upper airways requiring antibiotic therapy were described, while normal growth was reported and no hospitalization was necessary for severe infections.

Methods:

Since the age of 6 he developed poorer compliance to therapy due to the frequency of infusions. He was therefore switched on recombinant human hyaluronidase-facilitated SCIg (fSCIg). Ramp up with 3 progressively increased doses (from 2.5 gr up to 10 gr) of immunoglobulin 10% associated with hyaluronidase was performed in 30 days.

Results:

Injection site reaction occurred following only the first infusion and characterized by pain (while increasing infusion speed) redness and swelling (~ 4 cm) declined over a period of 5 hours. After 3 months of fSCIg (10 gr every 4 weeks) no adverse reactions, and no infections were described. IgG through level was slightly increased (960 mg/dl). The higher quality of life due to fewer needle punctures and longer infusion intervals resulted in a complete compliance to the therapy from both the patient and his caregivers.

Conclusions:

Our experience with the youngest, to our knowledge, Italian patient on fSCIg confirms it as an excellent treatment option, particularly when compliance to lifelong therapy is reduced.
LATE XLA DIAGNOSIS IN A PATIENT WITH SEVERE UVEITIS
V. Moschese¹, S. Graziani², M. Sgrulletti¹, S. Corrente¹, G. Di Matteo², S. Di Cesare², V. Giovinazzo¹, M. Borzi³, C. Loredana¹
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Background:

X-Linked Agammaglobulinemia (XLA) is a primary immunodeficiency (PID) caused by mutations of BTK gene, which causes an arrest in the early differentiation of B cells. XLA is characterized by low or absent circulating mature B cells (CD19+ < 2%) and consequent profound hypogammaglobulinemia. XLA patients typically suffer from recurrent or persistent infections, mainly by encapsulated microorganisms, occurring after the first year of life. Intermittent reports in literature have described autoimmune/inflammatory manifestations in XLA.

Methods:

We report the case of a 50 years old patient with a history of recurrent respiratory infections since childhood and chronic diarrhea since he was a teen. Family history and diagnostic workup excluded Inflammatory Bowel Disease (IBD) and Coeliac Disease. At 40 years he developed severe uveitis, treated with corticosteroids.

Results:

Due to the clinical history, an immunological workup was performed which revealed a severe hypogammaglobulinemia (IgG 302 mg/dl, IgA 35 mg/dl, IgM 34 mg/dl), deficient antibody response to S. Pneumoniae (16.13 mg/dl) and absent CD19+ cells (0.2%). The identification of a mutation in exon 2 of BTK gene (c.32T>C with substitution p.L11P) allowed a definitive diagnosis of XLA.

Conclusions:

Autoimmune/inflammatory diseases, such as arthritis, cytopenias and IBD, have been described in XLA patients. This is the first report of uveitis in a patient who received a late diagnosis of XLA. Further studies are needed to clarify the impact of BTK mutations on autoimmune/inflammatory conditions. Improving awareness and early diagnosis of PID is important to initiate timely interventions and improve clinical outcome and quality of life of these patients.
B CELL

ESID7-0319

A DEFECT OF MEMORY CELL POOL: ANY LINK WITH HUMAN GROWTH?

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Background:

Primary Immunodeficiency (PID) patients may present an heterogeneous clinical phenotype with an increased incidence of recurrent infections, autoimmune disorders, allergic diseases and neoplasia. Due to this heterogeneity, sometimes, a complex and timely workup is required.

Methods:

We report the case of a 5 years old child with a history of recurrent respiratory infections (mainly otitis with chronic otorrhea), severe failure to thrive (<3 SD) and language delay.

Results:

Coeliac disease, Inflammatory Bowel diseases (IBD), Cystic Fibrosis and allergy have been excluded. Genetic analysis for SHOX, PTPN11 and HRAS was negative. The immunological investigation revealed low levels of IgM (43.9 mg/dl) with normal values of IgA (47.10 mg/dl) and IgG (750 mg/dl) and of IgG subclasses. Peripheral B cells were decreased (CD19 6.1%; 183/uL), as well as switched memory B cells (CD19+CD27+IgD⁻:2.5%). Also a memory T cell defect was identified in the CD4 compartment where Effector Memory T helper cells were reduced (CD3+CD4+CD27⁻CD45RA⁻ 1.6%). Notably, low specific antibody response to Tetanus (0.03 UI/ml), Measles (<5 UI/ml) and S. Pneumoniae (6.29 mg/dl) was observed. A booster of pneumococcal polysaccharide vaccine elicited a transient antibody response (150.68 mg/dl) 1 months after vaccination, with a dramatic decrease (5.37 mg/dl) 7 months after the booster.

Conclusions:

The immunological workup, performed in our patient, indicates a PID due to a defect of memory cell pool with abnormal orchestrated B-T cell responses to pathogens. Further immunological and genetic investigations, including next generation sequencing (NGS), are needed to understand the pathogenic mechanism and its impact on human growth and immunity.
B CELL

ESID7-0333

SERUM FREE LIGHT CHAINS AS A POSSIBLE DIAGNOSTIC TOOL IN PRIMARY HYPOGAMMAGLOBULINEMIA: A MULTICENTRIC STUDY ON 344 CVID PATIENTS
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Background:

Serum free light chains (sFLC) are widely used as a prognostic marker in B cells lymphoproliferative disorders and as a marker of disease activity in many autoimmune conditions. Differential diagnosis between CVID and lymphoproliferative disorders may be challenging in adult patients. We recently suggested a role of sFLC in the diagnostic work-up of CVID, in a small single-center cohort of adult patients.

Methods:

sFLC levels were determined in 344 adult patients with CVID (according to ESID-PAGID criteria), enrolled in 5 different Italian referral centers for PID. B cell phenotype was available, according to EUROCLASS Study, of a subgroup of 120 patients.

Results:

In 76.3% of our patients a reduction of either kappa or lambda light chains or both was observed; 49.7% showed a decrease of both k and l chains, 24.5% presented a reduction of k chain only and 2.1% presented a reduction of l chain only. According to our previous study, we defined “κλ+” “κλ−” and “κ−λ” as “CVID-like patterns”. Among the subgroup analysis, 47.5% of patients with CVID-like pattern presented SmB cells <2% (vs. 19% in normal FLC patients). Moreover, CD21lo cells resulted increased in 35% of CVID-like pattern patients (vs. 14.3%).

Conclusions:

a CVID-like sFLC pattern is present in more than 75% of our CVID cohort. This confirms our previous data and the promising diagnostic value of sFLC in the initial work-up of primary hypogammaglobulinemia. The subgroup analysis suggests the need for further investigations on the relationship between a CVID-like pattern and the impairment of B cell function.
GOOD'S SYNDROME. SERIES OF FOUR CASES.

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Background:

Good’s syndrome is a rare association of thymoma with immunodeficiency. Clinical manifestations include compressive symptoms secondary to thymoma and recurrent sinopulmonary infections, among others.

Methods:

Case 1. Male, 42 years old, antecedent of allergic rhinitis and chronic rhinosinusitis. Thymoma type A from the World Health Organization (WHO) classification, IgG 40mg/dl, CD4/CD8 0.51 ratio and CD19 absent. Thymectomy was performed and gammaglobulin replacement with favorable clinical evolution.

Case 2. A 47-year-old male with a history of chronic sinusitis, tinea corporis with delayed evolution and chronic cough. Thymoma AB. After thymectomy, she presented urinary sepsis with positive blood culture for Morganella morgagni. It was identified IgG 180mg/dl, CD4/CD8 ratio 0.8 and CD19 absent; she received gammaglobulin with good response.

Case 3. Female, 78 years old. Antecedent of pneumonia, oral candidiasis, oral lichen planus and bronchiectasis. Thymoma AB, IgG 530mg/dl, CD4/CD8 ratio 0.8 and CD19: 2 cells/ml. Favorable evolution after gammaglobulin replacement.

Case 4. Female, 55 years old, chronic cough and dyspnea. Thymoma B1, received 8 chemotherapy sessions and subsequent thymectomy. One year later, pure red cell aplasia was diagnosed. He had chronic cough, nocturnal diaphoresis and weight loss, began antituberculous for suspicion of pulmonary tuberculosis, with unfavorable evolution. Bronchiectasis and IgG 300mg/dl was identified. He received replacement therapy with immunoglobulin, he died of pulmonary sepsis.

Results:

Good's syndrome has a mortality of 45% to 10 years, the timely administration of gammaglobulin, will impact on prognosis of the patient.

Conclusions:

Good's syndrome is a diagnosis that should be considered, in adults patients with thymoma.
RECESSIVE AGAMAGLOBULINEMIA – IS SUBSTITUTION IMMUNOGLOBULIN TREATMENT ENOUGH?

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Background:

Recessive agammaglobulinemias are very rare Primary Immunodeficiencies characterized by total absence of B lymphocytes. Scarce clinical information is available regarding clinical evolution and outcome.

Methods:

We present two cases of recessive agamaglobulinemia with very unfavorable evolution despite correct immunoglobulin substitution therapy.

Results:

Case 1: A girl with chronic diarrhea was diagnosed with a recessive agammaglobulinemia (µ-chain mutation) in the first year of life. Substitutive therapy with IV IgG was performed with adequate through levels. At age 13 she presented an Enteroviral meningoencephalitis with progressive neurologic deterioration. Failure-to-thrive, hypotrophy of the limbs, pubertal delay and psychomotor delay became evident. She died from an Enterobacter cloacae septic shock at 18 years of age.

Case 2: A girl with recessive agamaglobulinemia was diagnosed at 5 years of age and begun IV IgG replacement therapy. She presented a chronic bilateral otitis and underwent surgery. A slight developmental delay became progressively evident. At age 12 years she presented a tremor that increased progressively with an extrapyramidal syndrome and dementia. High dose IgG was given without beneficial effect. CSF was normal in several lumbar punctures with negative Enteroviral PCR. At present she is 18 years old and became completely dependent, with severe cerebral atrophy.

Conclusions:

These two patients presented a dismal evolution in spite of adequate substitution IgG therapy. Apparently a much more severe course is to be expected than in XLA, as suggested by other single case reports in the literature. An international survey is mandatory to confirm these findings as other treatment options may have to be considered.
**Background:**

Congenital agammaglobulinemia is characterized by a lack of B lymphocyte maturation predisposing to early onset recurrent bacterial infections. The objective of this study is to describe the clinical and outcome characteristics of patients with agammaglobulinemia.

**Methods:**

We retrospectively analyze medical records of patients with agammaglobulinemia followed in the pediatric immune-hematology unit, Tunis between 2000 and 2016.

**Results:**

Forty patients were collected, the median age at onset of symptoms was 6.5 months. The average age at diagnosis was 3 years. The infectious symptomatology was mainly respiratory. Thirteen patients had a pneumonitis leading to bronchiectasis in the third of the cases. Hypotrophy was observed in half of the cases. Short stature was present in 30% of patients. One patient had autoimmune cytopenia. One patient developed Hodgkin disease and one developed bronchoalveolar carcinoma. All patients had an absence of lymphocytes B and low IgG value (mean = 1.8g/l). T cell number and function were normal in all the cases. All patients received regular venous immunoglobulins leading to reduced frequency of infections in most patients. Four patients died (10%). The causes of death were septicemia with severe hypotrophy in three patients and bronchoalveolar carcinoma in one patient. Among the survivors, the quality of life was bad in only 5 patients.

**Conclusions:** Congenital agammaglobulinemia is often responsible for recurrent bacterial infections of the respiratory and digestive tracts. Regular substitution of immunoglobulins often leads to a favorable outcome. However, the disease could be fatal by the risk of cancer and severe infections.
IDENTIFICATION OF A PNEUMOCOCCAL POLYSACCHARIDE ANTIBODY DEFICIENCY TO CONJUGATE VACCINE-INDUCED IMMUNITY (PCV-SPAD) PREDOMINANTLY IN 2-5 YEAR OLD CHILDREN

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Background:

Through review of a population of patients with recurrent respiratory infections compared to controls, we have previously shown a population of patients, aged 2-5 years of age, who responded poorly to vaccination with conjugated polysaccharide vaccine (PCV). These patients displayed a suboptimal protective serotype response, both at 0.5 mg/ml and 1.3 mg/ml, which mimicked the unimmunized cohort of patients aged 2-20y with similar clinical profiles of infection.

Methods:

Database review

Results:

We further show that this lack of responsiveness is not serotype-specific, as all of the PCV-included serotypes were similarly low. We found that the mean concentrations of the PCV serotypes (4, 6b, 9v, 14, 18c, 19f, 23f) in these immunized 2-5 y olds with recurrent infections were similar to comparable patients aged 2-20y who had not been immunized. Interestingly, in patients who were older than 5 years of age, regardless of infection or immunization history, these same serotype responses were notably higher, warranting further investigation into this cohort to delineate characteristics of infectious susceptibility of this population.

Conclusions:

Based on these observations, we posit a novel form of immune deficiency, referred to as Specific Antibody Deficiency to conjugated-pneumococcal challenge (PCV-SPAD) which should be differentiated from the more commonly understood Specific Antibody Deficiency to polysaccharide-pneumococcal challenge (PPV-SPAD). This likely represents a recognizable entity for clinicians, and may be a result of delayed immune maturation or may continue further into adulthood. This distinction should allow us to better categorize pneumococcal responsiveness in patients based on age, clinical history, and prior vaccination history.
BACKGROUND:

The response to individual pneumococcal serotypes after vaccination with the conjugate pneumococcal vaccines is variable. Individual serotypes in the vaccine may fail to induce a significant increase in antibody concentration; we define this as specific antibody deficiency to conjugate pneumococcal vaccine serotypes (PCV-SPAD). Several severity levels of PCV-SPAD can be identified, both in healthy children and, in a higher proportion, in children with recurrent infections.

METHODS:

We evaluated if the assessment of memory B cells would help to identify children in whom the presence of PCV-SPAD is associated with increased susceptibility to infections.

Antibody-mediated immunity including memory B cells was evaluated in 62 controls and in 35 patients with recurrent infections without immunoglobulin deficiencies or other known debilitating conditions. Total memory (CD19^+CD27^+), class-switched memory (CD27^+IgD^-), and IgM memory (CD27^-IgM^+) B cells were assessed.

RESULTS:

Only switched memory B cells were found to be lower as a group in patients with recurrent infections, many of whom also had lower responses to conjugate polysaccharides.

CONCLUSIONS:

The specificity and significance of this observation is under active investigation. We postulate that the low class-switched memory B cell numbers may either contribute to low antibody production or may be a co-factor generally increasing susceptibility to recurrent infections in some patients.
TREATMENT OF CLINICALLY RESISTANT GIARDIASIS WITH FACILITATED SUBCUTANEOUS IMMUNOGLOBULIN AND ANTI-PROTOZOAL THERAPY IN A PATIENT WITH AGAMMAGLOBULINEMIA; A CASE REPORT.

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Background:

Treatment of giardiasis resistant to first line therapy (metronidazole) is a challenge, particularly in patients who are immunocompromised.

Methods:

We report a case of 17 year old with agammaglobulinemia who developed clinically nitroimidazole-resistant giardiasis with secondary protein losing enteropathy.

Results:

The patient failed multiple courses of metronidazole, and was unable to maintain his serum immunoglobulin levels in the therapeutic range with his regular intravenous immunoglobulin therapy. A switch to high dose facilitated subcutaneous immunoglobulin (HyQvia) and combination second line anti/protozoal therapy (albendazole, tinidazole, nitazoxanide) led to clinical resolution and pathogen clearance.

Conclusions:

High-dose, facilitated subcutaneous immunoglobulin may be a treatment option for difficult to treat giardiasis in patients with hypogammaglobulinemia.
Background:

The X-linked lymphoproliferative disease is characterized by recurrent lymphoma, EBV-driven HLH, and dysgammaglobulinemia. Currently, HSCT is the only available definitive therapy.

Methods:

We report discordant long-term immune reconstitution following HSCT in 3 siblings with XLP, two of whom received matched sibling transplants from a sister who is a disease carrier, while the other received a matched unrelated transplant.

Results:

At 7 years post reduced intensity transplant, all had 100% donor chimerism, none developed severe GVHD, and all were off immunosuppression. The two with the carrier donor have SAP expression in around 60-65% of T cells. However, they have poor B cell reconstitution and inability to discontinue immunoglobulin replacement, along with T-cell lymphopenia and poor T cell proliferative responses to mitogens and antigens. This is in marked contrast to that of the other sibling who received a matched unrelated HSCT. He has excellent B and T-cell immune reconstitution, and is currently off immunoglobulin replacement. The asymptomatic female sibling donor has normal lymphocytes and immunoglobulins, with non-skewed bimodal SAP expression. Further studies, including evaluation of T-follicular helper cells, are currently underway to understand the reason for poor immune reconstitution.

Conclusions:

HSCT from female XLP carriers could lead to poor immune reconstitution. Further studies are warranted to understand the role of the SAP-deficient donor immune fraction in long-term immune reconstitution. This report could have clinical implications for using carriers as donors in other X-linked immune defects.
CONSTITUTIONAL MISMATCH REPAIR DEFICIENCY (CMMRD) & PRIMARY IMMUNODEFICIENCY (PID): HYPOGAMMAGLOBULINEMIA OR INCREASED TRANSITIONAL B-CELLS, A TWO WAY ROAD FOR PID DIAGNOSIS

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Background:

It is well known that patients with Constitutional MisMatch Repair Deficiency (Constitutional MisMatch Repair Deficiency, CMMRD), OMIM #276300) suffer from an autosomal dominant disease which confers elevated risk to develop recurrent infections, hypogammaglobulinemia, tumors in infancy-mostly hematological-and CNS. This group of diseases are caused by biallelic inactivating mutations in mismatch repair system. The aim is to characterize their clinical, immunological and molecular findings.

Methods:

We identified 4 cases in three families (three consanguineous unrelated families). Clinical and lab data were collected from probands and parents. Immunoglobulins, T and B-cell subsets were studied in deep. Targeted NGS sequencing of CMMRD genes was performed (MLH1, MSH2, MSH6 & PMS2) confirmed disease causing mutations. In all tissue samples, both healthy and tumoral ones, microsatellites instability, immunohistochemistry from repairing MMR proteins. Radiosensitivity defect is showed in Figure 1

Results:

All patients showed a combination of café au lait spots, hypogammaglobulinemia and/or increased transitional B-cells.

<table>
<thead>
<tr>
<th>Age (y) at onset</th>
<th>Café au lait spots</th>
<th>Tumor</th>
<th>IgG, A, M (mg/dL)</th>
<th>B-cell subsets</th>
<th>DNA repair defect &amp; radiosensitivity</th>
<th>Gene</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>Yes</td>
<td>Lymphoblastic Lymphoma</td>
<td>IgG 99, IgA 9, IgM 18</td>
<td>Low CD19+ cells/ul (CD19 + CD38+++ IgM+++): 91% (HD 2.7%)</td>
<td>Yes</td>
<td>MLH1 (unresponsive to chemotherapy)</td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>Diagnosis</td>
<td>Treatments</td>
<td>Laboratory Results</td>
<td>Outcome</td>
<td></td>
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<tr>
<td>6</td>
<td>Nephroblastoma</td>
<td>WNR</td>
<td>Increased transitional B-cells</td>
<td>Complete remission after chemotherapy</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Lymphoblastic Lymphoma (x2), Burkitt Lymphoma</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Alive (after HSCT)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Acute lymphoblastic Leukemia, Glioblastoma</td>
<td>IgG 106, IgA 17, IgM 24</td>
<td>Yes</td>
<td>Alive (active disease, treatment ongoing)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions:**

- The graphs illustrate the induction of γ-H2AX at varying times post-irradiation (10 Gy) with different treatments.
- The control group shows minimal γ-H2AX induction, whereas treated groups exhibit significant increases, especially in the MLH1 and LIGIV groups.

- The data suggest that treatments targeting DNA repair mechanisms are effective in enhancing γ-H2AX induction, indicative of increased DNA damage and repair activity.
The presence of café au lait spots, hypogammaglobulinemia or increased transitional B-cells points out to CMMRD in addition to primary immunodeficiency
Report of a Case of Primary Immunodeficiency With Novel Homozygous Mucosa-associated Lymphoid Tissue 1 (MALT1) Mutation

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Background:

Introduction: Immunodeficiency 12 (IMD12) is a rare primary immunodeficiency in infancy with recurrent bacterial and fungal infections, bronchiectasis and growth delay.

Methods:

We describe a male infant with novel MALT1 mutation.

Results:

Case Presentation: A 6 m/o male infant was admitted because of chronic diarrhea and failure to thrive. He had history of repeated pneumonia and draining otitis media and also severe and generalized dermatitis. The parents were first relative. Laboratory work up showed: WBC: 21300 (41.1 % neutrophil, 50 % lymphocyte, 5.9% eosinophil), hemoglobin: 10.4, platelet: 384000. In immunologic work up had IgG: 253 mg/dl, IgM: 10 mg/dl, IgA: 34 mg/dl, IgE: 480 IU/ml and poor antibody responses to protein antigens. Flowcytometry showed normal enumeration of T, B and NK cells. Specific IgE test for foods revealed high titers of specific IgE to multiple food allergens. The patient was treated for atopic dermatitis and elimination diet for foods was advised. Also monthly intravenous IVIG and prophylactic co-trimoxazole was started.

Whole exome sequencing (WES) and analysis of genomic DNA detected novel homozygous mutation in MALT1 gene (NM_173844: exon13: c.T1705C) leads to amino acid change (p. W569R). In follow up the case had partial improvement of dermatitis and diarrhea but his respiratory distress was continued. High resolution CT scan of lung revealed bilateral lung fibrosis. The patient was referred for stem cell transplantation.

Conclusions:

Conclusion: MALT1 deficiency should be suspected in every infant with severe dermatitis and repeated infections especially when associated with lung fibrosis or bronchiectasis. WES can help for early diagnosis.
Follicular T Cells from Common Variable Immunodeficiency Patients Are Skewed towards a Th1 Phenotype

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Background:
Follicular helper CD4+CXCR5+ T cells (Tfh) provide B cell help and induce durable antibody responses. Circulating cTfh with different helper capabilities can be identified: cTfh1(CXCR3+CCR6-), cTfh2(CXCR3−CCR6−) and cTfh17(CXCR3−CCR6+). Alterations in Tfh function and/or distribution have been associated with autoimmunity, infectious diseases and monogenic immunodeficiencies. Common variable immunodeficiency (CVID) is a primary immunodeficiency characterized by defective B cell differentiation and low levels of antibody production.

Methods:
We studied in 34 CVID patients, by flow cytometry, effector CD4+CXCR5+ cTfh cell subsets (cTfh1, cTfh2, cTfh17 and cTfh17.1) and IFNg or IL-17A producing cTfh cells in peripheral blood samples from CVID patients stimulated with PMA and ionomycin.

Results:
We found a higher percentage of cTfh1 cells in the smB- subgroup compared to controls (31% vs. 23%;p<0.05) and smB+CVID patients (31% vs. 21%;p<0.01) accompanied by a higher percentage of IFNg producing cTfh (37% vs. 13%;p<0.01) in CVID patients compared to controls. Interestingly, patients with the highest percentage of cTfh1 cells were included within the smB- subgroup and had the lowest immunoglobulin levels at diagnosis (IgG<40 mg/dl). Although the percentage of cTfh17 cells was significantly decreased in smB−CVID patients compared to controls (15% vs. 23%;p<0.01), no differences were found in IL-17A production. We also found an increased percentage of CXCR3+CCR6+ cTfh17.1 (a population analogous to the recently described pathogenic Th17.1) in the smB−CVID group compared to controls (9% vs. 4%;p< 0.05).

Conclusions:
The lower capacity of cTfh1 cells to help B cells to produce immunoglobulins could influence B cell fate and compromise B cell compartment in smB-CVID patients.
Background:

Primary antibody deficiencies (PADs) are characterized by increased susceptibility to infections. Data on antibiotic prophylaxis in preventing acute exacerbations in PADs are lacking.

Methods:

We performed a randomized placebo-controlled double-blind trial to determine if azithromycin (250 mg 3 time/week for 3 consecutive days) decrease the frequency of exacerbation in PADs with Chronic Obstructive Pulmonary Disease (COPD) on IgG therapy. A total of 130 PADs patients were screened; 89 were randomized: 44 received azithromycin and 45 received placebo for 24 months in addition to their usual care.

Results:

A total of 130 PADs patients were screened; 89 were randomized: 44 received azithromycin and 45 received placebo for 24 months in addition to their usual care. A life-table analysis showed that the risk of acute COPD exacerbations was reduced in azithromycin group (P<0.0001). The rate of 2-year of follow-up was 72% in the azithromycin group and 75% in the placebo group. The median time to the first COPD exacerbation was 126±192 days among participants receiving azithromycin and 60±150 days in placebo group (P<0.026). The frequency of exacerbations per patient-year was 3.3 in azithromycin group while 4.58 per patient-year in placebo group (HR 1.8, 95% CI, 1.0 to 3.2). The hospitalization rate per patient-year was lower in the azithromycin group than in placebo group (0.16 vs 0.36, HR 2.031, 95% CI 0.93-4.42, p 0.039). Quality of life, assessed by SF-36 and St. George’s Respiratory Questionnaire, was also recorded in the two groups.

Conclusions:

Azithromycin decreased the frequency of COPD exacerbations and improved Quality of Life in PADs.
PRENATAL DIAGNOSIS OF PIDs: AN EMERGING EXPERIENCE IN EGYPT

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Background:

Primary Immunodeficiency disorders are a heterogeneous group of diseases affecting the immune system and result in life threatening conditions unless urgently treated with immune reconstitution. Although, stem cell transplantation has proved to be a successful treatment for many PID disorders, this treatment option is hindered by many obstacles in developing countries. The advances achieved in the past few years in unraveling the genetic defect behind many PIDs allowed genetic counselling and prenatal diagnosis (PND) to become an important part of the management. This is of special importance in populations with high prevalence of PIDs and yet limited resources.

Objectives: To present the results and impact of genetic counselling and PND on the families and as part of the management plan from the largest primary immunodeficiency center in Egypt.

Methods:

Based on the molecular diagnosis of index cases, genetic counselling was offered for families with affected children. Nine families came for PND. Targeted Sanger sequencing for 5 different genes (RAG1&2, NCF2, NCF1, IL-10Rβ) were performed in Chorionic Villous samples (CVS) based on the previous diagnosis of the index cases.

Results:

Five fetuses were either normal or carriers and families decided to continue the pregnancy. Diagnosis was confirmed after birth. In five pregnancies molecular diagnosis of the CVS proved the fetuses to be diseased and HLA matching with family members were tested for possibility of SCT.

Conclusions:

In spite of the genetic heterogeneity behind PIDs, genetic counselling plays a critical role in the management and future decisions of the affected families.
EXOME RE-ANALYSIS AND COMPLIMENTARY TESTING IDENTIFY NOVEL MUTATIONS FOR IMMUNODEFICIENCY DISORDERS

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Background:

The advent of next-generation sequencing technologies, especially the introduction of whole exome sequencing has provided an opportunity to screen a patient’s entire exome to establish genetic diagnosis. The utility of WES to identify novel genes and variants causative of Mendelian disorders has been clearly demonstrated in recent years. For primary immune disorders (PID), there are at least a few dozen new genes are being discovered each year.

Methods:

We re-analyzed patients' WES data with initial negative results for new clinical phenotype and/or in novel genes that have been recently discovered to cause PID. In addition, knowing the limitation of the WES, we were able to utilize a few complimentary testing strategies, such as, deletion-duplication analysis, epigenetic testing and Sanger rescue sequencing to identify mutations that help to reach a definitive diagnosis in patients that were tested to be “negative” or “inclusive”.

Results:

We were able to establish new diagnosis in a good number of patients with PID. For example, we have a patient with a suspected clinical diagnosis of CVID, but the initial WES yielded no positive findings. Two years later, physician requested analysis for CECR1 gene, which was recently report in patients with recurrent infections and antibody deficiency without vasculitis, which have been the feature in patients ADA2 deficiency in the earlier reports. Re-analysis in this patient resulted in the finding of a nonsense mutation, additional rescue sequencing find another non-coding likely pathogenic variant.

Conclusions:

Periodic re-analyses of WES data and complementary testing helped to establish definitive diagnosis in patients with PID.
TIMELY REMOVAL OF ANTI-CPS ANTIBODIES IS IMPORTANT FOR ACCURATE MEASUREMENT OF PNEUMOCOCCAL CAPSULAR POLYSACCHARIDE IgM

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Background:

Capsular polysaccharide (CPS) antibodies are produced in response to natural infection and vaccination but have little protective properties. Removal of these antibodies is crucial for accurate determination of specific anti-pneumococcal capsular polysaccharide antibodies (PCP). We assessed the incubation times required for interaction between anti-CPS and CPS and its effect on the measurement of PCP IgM.

Methods:

Serum samples from 20 healthy individuals were incubated in reaction buffer +/- CPS for times indicated and run in the VaccZyme™ PCP IgA and PCP IgM ELISA assays (The Binding Site Group Limited, UK).

Results:

The median change in concentration of PCP IgM following 2 hours incubation with CPS was significantly higher than when incubated without CPS, 15% (range 4 to 27, n=18) vs 3% (range -4 to 20, n=16; P<0.001). This median change in the presence of CPS was significantly greater than observed with PCP IgA, 5% (range -44 to 18, n=17) vs PCP IgM 15% (range 4 to 27, n=18; p<0.001). Assessment of incubation times showed a significantly lower median change in concentration of PCP IgM between 1-2 hour than after 0-1 hours, 1% (range -5 to 9, n=20) vs 7% (range -7 to 17, n=20, p<0.05).

Conclusions:

The requirement to remove non-specific CPS antibodies is greater for PCP IgM than PCP IgA. The reaction to remove anti-CPS occurs during the first hour of incubation. We recommend an incubation time of at least 1 hour with CPS for the accurate measurement of PCP specific IgM.
THERE IS A LACK OF CORRELATION BETWEEN ANTIBODY RESPONSES TO VACCINATION IgG SUBCLASS CONCENTRATIONS

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Background:

It has been suggested that the concentration of serum polyclonal IgG subclass (IgGSc) may correlate with the concentrations of vaccine-specific antibodies (VR). We hypothesised that they would provide independent B cell information and designed the present study to compare the concentrations of three IgGSc to the concentration of antibodies produced in response to vaccination.

Methods:

Using a multiplex assay, concentrations of IgGSc 1-3 and antigen specific antibodies against tetanus (TET), diphtheria, Haemophilus influenzae type b and 12 pneumococcal (PN) serotypes (PN1, PN3, PN4, PN5, PN6B, PN7F, PN9V, PN14, PN18C, PN19A, PN19F and PN23F) were obtained from 277 adults (140:185 M:F, median 57 years, range 18–91, serum IgG >6g/L) referred to Queen Elizabeth Hospital, Birmingham, UK. Cut-off values were the lower limits of published normal ranges, or medical decision points.

Results:

Agreement between IgGSc and VR ranged from 19-88% with only 2/45 comparisons (4.4%) reaching statistical significance: IgG1 vs. PN19F p=0.0005 and IgG2 vs. TET p=0.02, with 68% and 72% agreement, respectively. Spearman’s correlations between IgGSc and VRs were: IgG1, very weak (-0.05-0.17); IgG2, very weak-moderate (0.04-0.50); IgG3, very weak-weak (0.04-0.30), p<0.0001-0.52.

Conclusions:

Concentrations of IgGSc and VR correlate poorly. IgGSc measurements do not accurately predict VR and therefore will not always classify the patients the same. Measurement of both markers will provide clinicians with independent information about the immune status of the individual that may influence diagnosis, treatment and monitoring decisions.
A NOVEL GERMLINE GAIN-OF-FUNCTION VARIANT IN PIK3CD

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Background:

The primary immunodeficiency (PID) activated phosphoinositide 3-kinase δ syndrome (APDS, also known as p110δ-activating mutation causing senescent T-cells, lymphadenopathy, and immunodeficiency; PASLI disease) is caused by heterozygous gain-of-function (GOF) variants in the gene PIK3CD that encodes the p110δ subunit of PI3Kδ. The predominant clinical phenotypes of APDS patients are primary antibody deficiency often with polyclonal raised IgM, recurrent sinopulmonary infections with encapsulated bacteria, bronchiectasis, benign lymphadenopathy, lymphoma and autoimmunity. We report a patient with a clinical phenotype of APDS, and a family history suggestive of an autosomal dominant PID, in whom a novel GOF variant in PIK3CD was identified.

Methods:

A 56-year-old woman of Anglo-European origin with childhood onset bacterial sinopulmonary infections, bronchiectasis, and a father who had had common variable immunodeficiency, was investigated using a targeted PID gene panel. PI3Kδ over-activity was assessed by intracellular expression of pAKT in T cells at baseline and with anti-CD3/CD28 stimulation.

Results:

Immunological investigations revealed panhypogammaglobulinaemia (IgG 3.7g/l, IgA <0.07g/l, IgM 0.3g/l) and increased terminal differentiated CD8+ T cells (32%). Genetic analysis identified the novel variant PIK3CD NM_005026.3:c.1213C>T:p.(Arg405Cys) (ExAC MAF = 0, Polyphen2 = 0.982, SIFT = 0, GERP = 5.35). This variant resides in the C2 domain of the p110δ, impairing binding to the regulatory p85α subunit of PI3Kδ. pAKT levels were increased in patient T cells compared with control.
Conclusions:

Four *PIK3CD* GOF pathogenic variants causing APDS have been described to date. *PIK3CD* c.1213C>T:p.(Arg405Cys) should be considered as a fifth cause of APDS.
Assessment of antibody deficiency includes response to challenge immunisation. Poor vaccine response is a criterion for CVID. The polysaccharide vaccine Pneumovax is used to assess T cell independent response in patients, and poor pneumococcal antibody response 4-6 weeks after vaccination is consistent with specific antibody deficiency and CVID.

Methods:

We audited all patients receiving Pneumovax test vaccination between 2014-2016 who had serotype specific pneumococcal antibodies (SSPN), pneumococcal IgG, and pneumococcal IgG2 tested at 3 time points: Initial clinic visit, day of vaccination, and post-vaccination.

Results:

66 patients were included in the study, 65%(43) female. On average they received test vaccination 4 months after initial clinic visit, and 88%(58) had post-vaccination bloods taken at exactly 4 weeks. There was good correlation between pneumococcal IgG and IgG2 (r=0.93). There was also good correlation between pneumococcal antibody results taken at initial clinic visit and on the day of vaccination for IgG (r=0.78), IgG2 (r=0.76) and SSPN (r=0.88). Post vaccination there was a significant increase in all three measures (p≤ 0.01). Post-vaccination responses decreased with age. A total pneumococcal IgG of < 50 mg/L corresponds to on average 3/12 SSPN serotypes reaching adequate levels (>0.35 mcg/ml).

Conclusions:

There is little value added in performing pneumococcal IgG2 in addition to IgG. There is no need to repeat pneumococcal antibody testing at the time of vaccination. Post vaccine responses decrease with age (raising the question of age-related reference ranges). A total pneumococcal IgG <50 mg/L corresponds to protective SSPN antibodies of approximately 25%.
DIAGNOSTICS

ESID7-0089

PATHOGENIC GENETIC VARIANTS IDENTIFIED BY TARGETED NEXT-GENERATION SEQUENCING IN SOME UNDEFINED PRIMARY IMMUNODEFICIENCY CASES (IZMIR EXPERIENCE)
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Background:
Primary immunodeficiency (PID) disorders are a heterogeneous group of inherited diseases caused by a variety of monogenic immune defects. Thus far, mutations in more than 250 different genes causing PIDs have been described. Diagnosis can be costly and time consuming because of genetic and phenotypic heterogeneity of these disorders. Next Generation Sequencing (NGS) has recently become a popular tool for gene identification and molecular diagnosis of human diseases. Here we present the utility of targeted next generation sequencing (TNGS) of a comprehensive primary immunodeficiency panel.

Methods:
TNGS workflow based on an Ion AmpliSeq™ Primary Immune Deficiency Research Panel was designed for sequencing 266 PID genes on Ion S5™ Sequencer. Twenty nine pediatric patients with PIDs were analyzed and identified for probable disease-causing mutations.

Results:
Pathogenic variants were detected in 17 (%58) of 29 patients. These variations included hemizygous mutations in BTK, heterozygous mutations in CTLA4, PIK3CD, STAT1, AIRE and TTC37, and homozygous mutations in CD40LG, LRBA, PNP, ORAI1, IL12RB1, RAG1, IFNGR1 and ZAP70.

Conclusions:
By means of TNGS we diagnosed most of our cases definitely and arranged their therapeutic regimens.
GRID - A NEW CLINICAL DIAGNOSTIC TEST TO DIAGNOSE PATIENTS WITH PRIMARY IMMUNE DISORDERS (PID)

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Background:

Primary Immune disorders affect 15,000 new patients every year in Europe. Genetic tests are usually performed on a single or very limited number of genes leaving the majority of patients without a genetic diagnosis.

Methods:

We developed and validated a new clinical diagnostic platform called GRID, Genomics of Rare Immune Disorders, to screen in parallel 277 genes, including 2015 IUIS genes, known to be causative of Primary Immune disorders. The system is flexible and can rapidly incorporate new genes including the expected 2017 IUIS update.

Results:

We performed a formal validation using DNA samples from PID patients with known (n=186) and unknown (n=93) genetic aetiologies resulting in 96.5% coverage of the 3Mb target region at 20X fold or higher. We obtained 100% sensitivity using a wide range of single nucleotide variants (SNVs), insertions and deletions (INDELs) and copy number variations (CNVs), including multi-exons or multi-gene deletions.

Our Clinical Multi Disciplinary Team evaluate the sequencing results and the clinical synopsis of each patient and assign pathogenicity and contribution to phenotype to causal variant(s) following the latest Guidelines.

One example of the effectiveness of this platform in cohort screening is represented by a patient initially diagnosed with X-linked agammaglobulinemia due to a missense variant found in the BTK gene with severe inflammatory bowel disease. GRID results identified two additional compound heterozygous variants in IL17RC, potentially driving the altered phenotype. Conclusions:

Due to lowering costs of Next Generation Sequencing technologies, screening platforms like this may become the necessary standard of care for PID.
PRIMARY IMMUNODEFICIENCIES IN VILNIUS UNIVERSITY HOSPITAL SANTARISKIU KLINIKOS

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Background:

Primary immunodeficiencies are inherited disorders of immune system predisposing affected subjects to an increased rate and severity of infection, immune dysregulation with autoimmune disease and aberrant inflammatory responses as well as malignancies. Primary immunodeficiency can occur at any age. Any compartment of the immune system can be affected serving as a base of primary immunodeficiency and accordingly classified.

Methods:

Statistical data presenting patients from Vilnius University Hospital, Santariskių Klinikos, Primary Immunodeficiencies Competence Center analyzed according age, gender and disease.

Results:

Institutional Primary Immunodeficiencies data base was launched in Vilnius University Hospital Santariskių Klinikos in 2012. Forty-seven adults and twenty-five children with primary immunodeficiency are registered at this moment in this data base, 14 of them have genetically confirmed diagnosis. Five children with immunodeficiency are dead. According to the gender 52.2 % (35/67) are female and 47.8 (32/67) are men. The average of age is 30.8 years. Common variable immunodeficiency (17), selective IgA deficiency (13) and C1 esterase deficiency (8) are the most common diseases. Five hematopoietic stem cell transplantations were performed. Twenty adults and four children are treated with substitutional immunoglobulin therapy in a case of antibody deficiency.

Conclusions:

Seventy-two primary immunodeficiency patients are registered in the Primary Immunodeficiencies data base and one fifth of them have genetically confirmed diagnosis. All patients are followed-up and treated according to their disease recommendations.
CAUSES OF LOW NEONATAL T-CELL RECEPTOR EXCISION CIRCLES (TRECS): A SYSTEMATIC REVIEW

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Background:

The amount of research addressing TREC screening in newborns in order to identify immunodeficiency has increased dramatically in recent years, making it challenging for clinicians and researchers alike to have an overview over all the recent progress in the field.

Methods:

In this Systematic Review we aim to provide a systematic overview of studies describing patients with low TRECs and the associated diseases, which range from genetically caused Severe Combined Immunodeficiency to idiopathic lymphopenia. We aim to provide clinicians with a guide on what diseases to assess when evaluating neonates with low TRECs and aid them in the management of such patients until a diagnosis is made. Furthermore, we would like to highlight the need for further research into the origins of lymphopenia at birth.

Results:

The most common genetic cause for low TRECs was 22q11.2 deletion syndrome followed by Interleukin-2 receptor gamma (IL2RG) and adenosine deaminase (ADA) deficiency. Furthermore, 12 syndromes were associated with low TRECs at birth as were diseases with no apparent genetic cause. We recommend cutoff values and screening algorithms as proposed in large prior studies.

Conclusions:

Based on the published data we can encourage screening for specific genetic mutations associated with Primary Immunodeficiencies (PID). Furthermore, we provide a list of syndromes and other diseases that should be excluded in patients with low TRECs. During the assessment of patients, we recommend stringent management. Finally, our research reveals the very high percentage of patients left undiagnosed after a positive TREC screening, highlighting the much-needed research in the field of PIDs.
DIAGNOSTICS

ESID7-0107

NOVEL TACI VARIANT IN COMMON VARIABLE IMMUNODEFICIENCY

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Background:

Transmembrane activator and calcium-modulating cyclophilin ligand interactor (TACI) controls differentiation of long-lived plasma cells. Around 10% of patients with Common Variable Immunodeficiency (CVID) carry TACI variants that impair TACI function and are likely to contribute to autoimmune manifestations. However, 1–2% of normal individuals carry these variants with no signs of CVID. Mice lacking TACI (TACI−/−) exhibit defective long-lived antibody responses to antigen, enlarged spleens and lymph nodes, expansion of peripheral B-cell population and develop autoimmunity.

Methods:

Sequencing of TACI encoding gene (TNFRSF13B) was performed in a cohort of 38 unrelated Portuguese patients with CVID, together with phenotype characterization of circulating B and T-cell subsets by flow cytometry and clinical data collection.

Results:

We identified one patient compound heterozygous for the c.204insA mutation and the missense variant, F185C. The variant F185C is predicted to be damaging (SIFT, PolyPhen) and to our knowledge it has never been previously identified in CVID patients nor in European healthy cohorts. The 49 years-old patient has psoriasis and evidence of lymphoid hyperplasia as well as a familial history of autoimmunity (Type 1 Diabetes and SLE) and lymphoma. Notably about half of the circulating B-cells featured an unswitched memory phenotype (CD27+IgD+IgM+), an expansion not observed in CVID patients solely carrier of c.204insA or other TACI variants. There was also increased frequency of T follicular helper cells in blood.

Conclusions:

A novel TACI variant was identified in a CVID patient. Ongoing studies and data from progeny will allow to further understand this phenotype resembling that of the TACI−/− mouse model.
A NOVEL MUTATION OF THE WAS GENE IN A COLOMBIAN FAMILY WITH WISKOTT-ALDRICH SYNDROME AND DIFFERENT PHENOTYPIC DISEASE.

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Background:

Wiskott-Aldrich syndrome (WAS) is a rare X-linked disorder characterized by microtrombocytopenia, eczema, autoimmune phenomena, recurrent infections, secondary to immunodeficiency and increased incidence of malignancy. WAS gene has been linked to the region Xp11.23, encoding a 502-aminoacids intracellular protein expressed exclusively in the cytoplasm of hematopoietic cells. A widespectrum of the WAS gene mutations have been identified causing a variety of clinical phenotypes, ranging from isolated thrombocytopenia to severe WAS.

Methods:

Case Report

Results:

In the present report, is described a Colombian family with two cousins affected with unreported Wiskott-Aldrich syndrome genotype with a phenotype composed of eczema, thrombocytopenia, and normal platelet volume. However, our patients does not have humoral defect but cellular immune defects have been presented with T cell function diminished as evidenced by a poor response to mitogens. We found a novel mutation likely pathogenic by sequence analysis of genomic DNA with a Duplication (1 bp) in exon 12. This duplication creates a frame shift starting at codon Glu488. The new reading frame ends in a STOP codon 6 positions.

Conclusions:

The sequencing mutation analysis is helpful for the diagnosis of novel mutations in WAS patients and also expanding the spectrum of WASP mutations for carrier detection and symptomatic diagnosis. This case report illustrates the importance of having a high index of suspicion despite normal platelet volume, as well as adding to the growing number of known mutations associated with WAS.
OUTCOMES OF NEXT-GENERATION TARGETED GENE PANEL DNA SEQUENCING FOR PATIENTS WITH PRIMARY IMMUNODEFICIENCIES

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Background:

Targeted gene panels (TGP) and whole exome sequencing (WES) are the two most commonly used NGS techniques used to identify genetic causes of primary immunodeficiencies (PIDs). There is no standard first-line genomic test for the diagnosis of PIDs. We have previously shown that WES achieved a genetic diagnosis in 54% of patients with PIDs and now present a comparison study using TGP sequencing.

Methods:

Genomic DNA was isolated from 334 individuals with clinical and laboratory evidence of PIDs. Automated library and template preparation were performed using the Ion Chef and the PIDv2 targeted gene panel of 264 genes associated with PID (ThermoFisher). Sequencing was performed on the Ion Torrent S5 System with an average depth of ~392x. The average reagent cost was $190 per sample.

Results:

TGP sequencing identified a genetic diagnosis in 219 (66%) of 334 patients. These include 120 missense variants, 28 nonsense mutations, 10 splice-site mutations, 37 frameshift mutations, two non-frameshift deletions, and 22 large deletions encompassing at least one exon of a gene. Eight of the 219 diagnosed individuals (4%) had more than one pathogenic mutation. We identified two mutations that satisfied clinical criteria for a pathogenic variant, but had no discernable biologic effect upon functional testing.

Conclusions:

TGP sequencing achieved a higher diagnostic yield than WES at a lower cost due to increased depth of coverage. TGPs may serve as an effective first genomic test for patients with PIDs. Functional validation remains necessary for minimizing false positive genetic findings.
DETECTION OF HYPOGAMMAGLOBULINAEMIA OVER A THREE YEAR PERIOD USING A LABORATORY INFORMATION MANAGEMENT SYSTEM

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Background:
Extrapolated data from the UK Primary Immunodeficiency Registry comparing NHS Tayside to other UK regions suggests an under-detection of primary and secondary antibody deficiency disorders.

Methods:
All IgG results over a 3-year period measured through Ninewells Hospital Dundee were identified using CliniSys Laboratory Information Management System (LIMS). The clinical details of all patients with IgG values <4.0g/L were reviewed. If no explanation for the low IgG was apparent requesting clinicians were contacted and asked to repeat the IgG and refer to Immunology for further assessment.

Results:
113 patients with an IgG <4.0g/L had an underlying plasma cell dyscrasia or lymphoproliferative disease and were excluded from further review. 18 paediatric results were within an age-appropriate reference range and were also excluded. 41 patients had an IgG <4.0g/L of uncertain aetiology. Requesting clinicians were contacted and the patients reviewed subsequently by a Consultant Immunologist.

Conclusions:
Data extraction using LIMS was a useful means of detection of hypogammaglobulinaemia. It may be a useful adjunct in the earlier recognition of immunodeficiency.
DIAGNOSTICS

ESID7-0275

HYPER-IGE SYNDROME WITH THE MUTATION C.1699A>G IN STAT3 GENE - CASE REPORT
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²Hospital Clínico Universitario Virgen de la Arrixaca, Servicio de Aislados, Murcia, Spain

Background:

Hyper IgE syndrome (HIES) is a rare primary immunodeficiency characterized by elevated levels of IgE, chronic eczema, and recurrent infections mainly by Staphylococcus aureus and a variety of connective tissue and skeletal abnormalities. The syndrome has autosomal dominant (AD) and recessive forms (AR). AD-HIES is mainly due to STAT3 mutations and AR-HIES to mutations in DOCK8 or Tyk2.

Methods:

We report a case of a 14 month-old girl with bilateral recurrent otitis, severe atopic dermatitis, laterocervical abscess (culture reported growth of Staphylococcus aureus) that required surgical excision and epiphysiolysis of distal femur after mild trauma. The patient showed a slightly coarse facial appearance with low implantation ears. No dental abnormalities was observed. No family history of recurrent infections or consanguinity.

Results:

Laboratory results showed increased level of total IgE (14700 IU/ml). No other abnormalities were observed in humoral and cellular immunity. The score based on US National Institutes of Health system for the diagnosis of HIES-AD was 44, classifying the diagnosis as probable. Direct sequencing of STAT3 gene was performed and revealed a heterozygous mutation (c.1699A>G; p.Asn567Asp).

Conclusions:

This mutation causes a change of one amino acid, which may affect the folding properties and dimer stability of the protein and may be the main cause of the patient's syndrome. The mutation was not observed in the parents, indicating de novo occurrence. The early diagnosis of patients with suspected Hyper-IgE syndrome allows a close follow-up, prompt treatment and prevention of associated complications and the possibility of genetic counseling.
A NATIONWIDE STUDY OF SEVERE AND PROTRACTED DIARRHOEA IN PATIENTS WITH PRIMARY IMMUNODEFICIENCY DISEASES

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Background:

Diarrhoea lasting longer than 14 days and failing to respond to conventional management is defined as severe and protracted diarrhea (SD). We investigated the prevalence, pathogens and prognosis of SD in primary immunodeficiency diseases (PIDs)

Methods:

During 2003-2015, PIDs patients reaching the SD received reactive oxygen species (ROS), IL-10 signaling function and candidate genetic analysis for PIDs predisposing inflammatory bowel disease.

Results:

Among 246 patients with predominantly paediatric-onset PIDs, 21 [Btk (six), IL2RG (four), WASP, CD40L, gp91 (three each), gp47, RAG2 (one each)] and five [CVID (four), SCID (one)] without mutations had SD before prophylactic treatment. Detectable pathogens included pseudomonas, salmonella (six each), E. coli, cytomegalovirus, coxsackie virus and cryptosporidium (one each), all of whom improved after a mean 17 days of antibiotics and/or IVIG treatment. Seven (7/26; 27.0%) patients died [respiratory failure (four), lymphoma, sepsis and intracranial haemorrhage (one each)]. The patients with WAS, CGD and CD40L and SD had a higher mortality rate than those without. Another five males with mutant XIAP, STAT1, FOXP3 (one each) and STAT3 (two) had undetectable-pathogenic refractory diarrhoea (RD) that persisted >21 days despite aggressive antibiotic/steroid treatment, resulting in a significantly higher mortality rate (p=0.0278). For the patients with RD without anti-inflammatory optimization, those with mutant XIAP and FOXP3 died of Crohn's-like colitis and electrolyte exhaustion in awaiting transplantation, while transplantation cured the STAT1 patient.

Conclusions:

In our cohort, 10.6% (n=26) had SD and 2.0% (n=5) with XIAP, STAT1, FOXP3 and STAT3 mutations presenting as RD had higher mortality (2/5=40%) and needed early anti-inflammatory interventions.
NOVEL MUTATIONS IN DIFFERENT GENES FOR PRIMARY IMMUNODEFICIENCY DISEASES IN A SINGLE-CENTER COHORT FROM NORTH-INDIA

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2National Defense Medical College- Tokorozawa, Pediatrics, Saitama, Japan
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4University of Hong Kong, Pediatrics and Adolescent Medicine, Hong Kong, Hong Kong S.A.R.
5Duke University Medical Centre, Medicine and Biochemistry, Durham- North Carolina, USA

Background:

Primary Immunodeficiency Diseases (PIDs) are grossly unrecognized and undiagnosed in India due to lack of awareness and dearth of laboratory amenities. Identification of genetic mutations is an integral part of management of PID patients. It helps in confirmation of the diagnosis and is also critical for prenatal diagnosis and genetic counselling.

Methods:

Mutation studies were performed at Kazusa DNA Research Institute, Chiba, Japan, Dept of Pediatrics and Adolescent Medicine, Queen Mary Hospital, University of Hong Kong, Hong Kong and at our centre. Records were retrieved from the files of primary immunodeficiency clinic at our institute. The main objective is to analyse the spectrum of novel mutations in primary immunodeficiency diseases.

Results:

Genetic mutations were detected in 152 patients suspected to have various PIDs (Table 1). Mutation in BTK gene was the most common (22.6%) followed by that in CYBB gene (17.6%). Mutation in WAS gene was in 14.9% of the patients. Novel mutations were detected in 34 patients (22.3%).

Table 1. Novel mutations in various PIDs

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Gene &amp; Disease</th>
<th>No. of Novel mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BTK (XLA)</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>CYBB (X-CGD)</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>WAS</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>STAT1 GOF (CMC)</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>RAG1 (AR-SCID)</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>DCLRE1C (AR-SCID)</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>IL7RA (AR-SCID)</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>ADA (AR-SCID)</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>IL2RG (AR-SCID)</td>
<td>3</td>
</tr>
</tbody>
</table>

DIAGNOSTICS

ESID7-0287
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Gene &amp; Disease</th>
<th>No. of Novel mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>PRF1 (HLH)</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>ELA2 (Cyclic neutropenia)</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>CD40LG (X-HIGM)</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>FASLG (ALPS)</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>STAT-3 (AD-HIES)</td>
<td>2</td>
</tr>
</tbody>
</table>

**Conclusions:**

Identification of novel mutations extends the spectrum of genotypic heterogeneity in PIDs and would help in defining disease-related mutations.
Background:

X-linked agammaglobulinemia (XLA) or Bruton disease is a primary immunodeficiency caused by Bruton's tyrosine kinase (BTK) gene defect. XLA patients have very low B lymphocytes and profound deficiency in all immunoglobulin isotypes. We report here the clinical, immunological and molecular profile of XLA Algerian patient.

Methods:

The patient explored for suspicion of primary immunodeficiency, is a boy aged of 4 years old, his brother was dead at 6 years old without specific diagnosis.

Biological investigation included:

- Measurement of serum IgG, IgA and IgM levels by nephelometry.

- Lymphocyte immunophenotyping T, B and NK cells by flow cytometry.

- Genetic analysis by direct sequencing of BTK gene.

Results:

Onset of symptoms appeared during the first year of life. Main clinical manifestations were recurrent cutaneous abscesses, pulmonary infections and autoimmune hemolytic anemia. Immunological profile was consistent with agammaglobulinemia (B <2%, decreased IgG, IgA and IgM). Genetic analysis identified a new mutation: W395X. This nonsense mutation affects the SH1 domain of BTK. Patient’s mother is heterozygous carrier for this mutation.

Conclusions:

We characterized here a novel BTK mutation in XLA patient with auto-immune features.
AN UK NATIONAL SURVEY FOR THE USE OF CALCULATED GLOBULIN IN THE DIAGNOSIS OF ANTIBODY DEFICIENCY

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Background:

The morbidity and mortality of primary and secondary antibody deficiencies is often exacerbated by a significant diagnostic delay. Calculated globulin (CG), which is derived from the difference between total protein and albumin results, reflects immunoglobulin serum levels. From 2014, CG has been used by the 13 Welsh Biochemistry Laboratories as screening test for antibody deficiency in the Welsh population. A value of CG < 18g/L was identified as cut-off and reports on levels below this had an electronic comment added to the report.

Methods:

A survey was carried out via the National audit group using a questionnaire to determine current practice with respect to the use of calculated globulin (CG) across UK biochemistry laboratories. The CG Audit Questionnaire was developed as an electronic Survey Monkey tool to enable electronic data capture across the UK and consisted of three sections exploring: the methodology employed for total protein, albumin and globulin calculation; the workload and the tests results obtained in the previous 12 months; the outcomes in the laboratories where an automated comment suggesting further investigations in case of low CG had already been introduced.

Results:

The report describes the results derived from this survey with for the first time a comprehensive overview of the use of CG screening for antibody deficiency across the UK with details of methodology and the numbers of low CG levels.

Conclusions:

The information may help inform the introduction of screening for antibody deficiency more widely with the aim of shortening diagnostic delay and reducing morbidity and mortality.
MUTATIONS IN PIK3R1 CAN LEAD TO APDS2, SHORT SYNDROME OR A COMBINATION OF THE TWO

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Background:

Mutations in PIK3R1 gene have been associated to two different conditions: a primary immunodeficiency, called APDS2, of recent description and SHORT syndrome. 47 patients with APDS2 have been reported to date, only one of them sharing both PIK3R1-related phenotypes.

Our objective is to describe two more patients affected by APDS2 and SHORT syndrome.

Methods:

Next generation sequencing was performed by the use of a PID-related genes panel of our own design. The intracellular content of p-Akt and p-S6 was measured by using the PhosFlow System (BD Biosciences).

Results:

Patient 1 is a 10-year-old male who presents, among others, recurrent respiratory infections, lymphoproliferative episodes, congenital cardiopathy, hearing loss, growth impairment and reduced subcutaneous fat. His immunological study and infections history were compatible with APDS2, while phenotypically he fulfilled criteria of SHORT syndrome. The mutation c.1425+1G>A (NM_181523) in the gene PIK3R1 was detected.

Patient 2 is a male who presented with recurrent respiratory infections since birth, congenital cardiopathy, hearing loss and growth impairment. At four years of age he started to have lymphoproliferative episodes. At 20 years old, he developed a non-Hodgkin lymphoma. Six months after, he developed a classic Hodgkin lymphoma. He died at the age of 26 because a recurrence of the first malignancy. Immunologically he fulfilled criteria of APDS2 while his phenotype was consistent with SHORT syndrome. The mutation c.1425+1G>T (NM_181523) in the gene PIK3R1 was detected.

Conclusions:
Patients with mutations in PIK3R1 should be evaluated by clinical immunologists and geneticists as they can have two phenotypes in association with one genotype.
DIAGNOSTICS

ESID7-0313

BIG DELETIONS IN COMMON PID-RELATED GENES AND ITS DETECTION FOR MOLECULAR DIAGNOSIS

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Background:

Primary immunodeficiencies (PID) are usually caused by punctual mutations, although big deletions and complex chromosomal rearrangements have also been described. Deletions can happen because of recombination events with high homologous sequences as pseudogenes.

Our objective is to look over PID-related genes that tend to suffer big deletions, to explain the reason why it happens and to show detection approaches through real cases.

Methods:

CGH array of the patients and a healthy control (Promega) has been performed through the platform KaryoArray®v3.0 (8x60K, Agilent). MLPAs have been performed with probes of our own design or with commercial ones, using in both cases other commercial reagents and protocol (MRCHolland). RT-PCR (Retro-transcriptase Polymerase Chain Reaction) of NCF1 gene was performed and set up in our lab.

Results:

DCLRE1C gene has an increased rate of deletions because of the existence of a pseudogene which shares high homology with it in some of its exons. NCF1 gene also shows complete or partial deletions of the gene as a common mechanism of disease. The reason is the existence of two homologous pseudogenes. Big sporadic deletions in other genes, as DOCK8, CTLA4, ACP5 or CYBB have been found. MLPA and, in some cases, CGH array have been performed to diagnose affected individuals and carriers in multiple kindreds.

Conclusions:

PID are genetic diseases that can be mediated by the presence of a deletion affecting a gene or part of it. Diagnostic methods to detect reduction of genic dosage are indispensable in molecular diagnosis laboratories.
Background:

Down syndrome (trisomy 21) is a congenital condition characterized by multiple malformations, some incompatible with life. Most of the patients diagnosed with Down syndrome have an increased risk of developing autoimmune diseases as well as severe infections because of the associated immunodeficiency.

Methods:

We present the case of a 8 year old female patient, previously known with Down syndrome and idiopathic juvenile arthritis, which was admitted in our clinic for fever, papular erythematous rash, mouth sores, and a lack of appetite.

Results:

Workup revealed marked leukopenia, elevated inflammatory markers, and a coagulase-negative staphylococcus isolated in the blood culture. Based on clinical and laboratory data, the diagnosis was: severe sepsis with coagulase-negative staphylococcus and pancytopenia in a child with Down syndrome and idiopathic juvenile arthritis. Complex treatment with a broad spectrum antibiotic, human immunoglobulins, intravenous fluid therapy and symptomatic medication is established. Evolution was favorable with resolution of symptoms and normalization of workup after 7 days of treatment.

Conclusions:

Children with Down syndrome have a higher risk of developing severe infections, sometimes with saprophytic microorganisms. It is well known that Down syndrome predisposes to severe infections, especially when the patient has an associated immunodeficiency (idiopathic juvenile arthritis). In this particular case, the association between Down syndrome and juvenile arthritis favored the onset of a severe infection with coagulase-negative staphylococcus.
DIAGNOSTICS

ESID7-0325

A NEW APPROACH TO USE hCAP-18 IN BLOOD PLASMA FOR DIAGNOSIS AND PROGNOSIS OF BONE-MARROW FAILURE DISORDERS
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³Karolinska Institutet, Department of Women's and Children's Health, Stockholm, Sweden

Background:

Different underlying causes of neutropenia may have similar initial clinical presentation, especially in pediatric patients, and early establishment of a correct diagnosis can be difficult. The congenital bone marrow failure disorders (cBMFDs) are a clinically related but heterogeneous group of disorders. For a number of cBMFDs the bone-marrow neutrophil production is negatively affected wherefore these patients present with various degrees of neutropenia, and are at a high risk for contracting severe infections and, importantly, also for developing leukemia. We have previously demonstrated that the neutrophils of patients with severe congenital neutropenia (SCN) are deficient in the antimicrobial peptide LL-37 and its pro-protein hCAP-18 Reduced blood plasma hCAP-18 levels was used to discriminate SCN from autoimmune and idiopathic neutropenia. HYPOTHESIS Blood plasma hCAP-18, which stems from the bone marrow where it is produced during neutrophil differentiation, reflects bone-marrow myelopoietic activity. AIM To facilitate and improve diagnosis of congenital bone marrow failure disorders.

Methods:

Analysis of blood plasma hCAP-18 levels by Western blot and ELISA from patients with different forms of bone marrow failure conditions. Diagnosis established by means of clinical manifestations, family history, laboratory findings, imaging, histological, and if available, genetic findings.

Results:

For those bone-marrow failure disorders so far tested in which the plasma level of hCAP-18 was low, the myelopoiensis was found to be defective.

Conclusions:

Blood plasma hCAP-18 levels could potentially be used as a simple complementary tool in assessment of bone-marrow myelopoietic activity in congenital bone marrow failure disorders.
NEWBORN SCREENING FOR SCID IN TUSCANY-A 3-YEAR PILOT TREC AND KREC SCREENING STUDY

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¹AOU Meyer, Immunology, Florence, Italy

Background:

Severe combined immunodeficiency (SCID) is a group of severe disease which affect immune system. Infants with SCID are healthy at birth but die of recurrent severe infection in infancy unless adequate therapy is provided. SCID meet the following criteria necessary to be included in a neonatal screening program using RT-PCR of DBS for TREC and KREC.

Methods:

Newborn screening for SCID, characterized by T and/or B cell lymphopenia, was carried out in a pilot program in Tuscany, a 3-year period, encompassing 18,981 children. T cell receptor excision circles (TREC) and kappa-deleting recombination excision circles (KREC) were measured simultaneously using RT-PCR on DNA extracted from dried blood spots.

Results:

None abnormality of TREC values has been found so far. Low expression of KREC has been found 4 times: 2 cases were false positive, one patient was affected by Bruton disease and in the last case patient presented absent IgA and IgM and absent CD19. No genetic mutations of BTK,RAG1,RAG2,TACI,ICOS were found. KREC on peripheral blood sample were absent.

Conclusions:

Replacement treatment was therefore started with IVIG to prevent life-threatening infections. Routine testing was periodically carried out. At the age of 3 months we noticed a sudden recovery of the levels of IgM, IgA and CD19+cells which led to a complete normalization of the values. IVIG treatment was therefore suspended. TREC and KREC quantification are useful screening tests for severe T and B cell immunodeficiency and implementation of these tests in whole country is highly important to identify SCIDs before clinical presentations.
SPECIFIC ANTIBODY DEFICIENCY IN A MEXICAN PATIENT
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Background:

2 year old male patient, no consanguinity reported, with background of pharyngitis and recurrent sinusitis treated with multiple antibiotics. No background of ear infections or pneumonia. He also had events of cough and wheezing events. No cultures were taken.

Is assessed by pediatric otolaryngology for an event of 2 months of evolution with purulent mucus secretion and retronasal discharge, with partial response to antibiotics, falling to suspend these ones.

Methods:

Test Fot specific IgG for 23 pneumococcal serotypes, which results in levels lower than 1.3 mcg / ml, 22 of the 23 serotypes. It also has IgG 466mg / dl, rest of immunoglobulins are normal. Pneumococcal polysaccharide vaccine of 23 serotypes was applied

Results:

The Patient had little response to the previously studied serotypes, so a specific antibody deficiency was diagnosed. It is currently managed with monthly intravenous gammaglobulin and prophylactic azithromycin with adequate response to treatment.

Conclusions:

Specific antibody deficiency should be suspected in patients with persisting sinus and pharyngeal infections despite adequate antimicrobial therapy with normal IgG levels.

To our knowledge, this is the first reported case of this immunodeficiency in our country
DIAGNOSTICS

ESID7-0369

HAE CAUSED BY A NOVEL DEEP INTRONIC MUTATION IN SERPING1 INTRODUCING A CRYPTIC EXON

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²Central European Institute of Technology, Medical Genomics, Brno, Czech Republic
³Faculty of Medicine- Masaryk University- St.Anne’s Faculty Hospital, Department of Clinical Immunology and Allergology, Brno, Czech Republic

Background:

More than 500 mutations have been described in SERPING1 gene as causative for hereditary angioedema (HAE) so far. The gene shows a wide mutation spectrum and also 20-25% of de novo mutations. Typically, the molecular analysis of SERPING1 gene begins with amplification and direct sequencing of exon and exon/intron boundaries. In case no protein function affecting mutation is found, screening of large copy number variations follows.

Methods:

Sanger sequencing, mRNA capillary electrophoresis, minigene analysis.

Results:

The above mentioned procedure failed to uncover a disease causing mutation in a family whose affected members showed typical clinical and laboratory features of HAE. Therefore C1-INH function impairing defect located in untranslated (UTR) or intronic parts of the gene was suspected. UTR sequencing did not reveal any substantial change, thus mRNA splicing was analysed. RNA extracted from peripheral blood mononuclear cells was tested by capillary electrophoresis. Samples of affected patients showed a small amount of variant transcript prolonged by 89 bp, which was not observed in healthy controls. Consequent sequencing of intron 6 revealed a novel splicing mutation c.1029+384 A>G, which co-segregated with the disease occurrence in the family. Functional analysis of a minigene construct confirmed the mutation specific aberrant splicing.

Conclusions:

Our findings indicate that the conventional procedure of molecular analysis might be insufficient especially in the view of genetic instability of SERPING1 gene. When the causative mutation is not found in the coding regions, RNA based mutation screening should be applied.

Supported by AZV grant 16-3441A and specific research grant MUNI/A/1183/2015.
DIAGNOSTICS

ESID7-0374

WHOLE GENOME SEQUENCING AND REVISITING THE EXOME DATA ARE ADDING UP THE SOLVE-RATE FOR PIDD


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5Oslo University Hospital, Section of Clinical Immunology and Infectious Diseases, Oslo, Norway
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8Telemark Hospital, Department of Medical Genetics, Skien, Norway

Background:

Primary immunodeficiency diseases (PIDDs) are clinically and genetically heterogeneous disorders. Whole exome sequencing (WES) techniques have been proven successful in detecting molecular causes of disease as they detect both disease causing gene variants in known PIDD genes, as well as novel disease genes, and oligogenic causes of phenotype variability. In a previous WES study of a large PIDD cohort, we established a likely molecular diagnosis in 40% of the 278 families, describing 14 novel disease genes, variants in unexpected genes, mosaicism, and copy number variants undetected by conventional testing (JACIJan2017).

Methods:

To investigate the remaining 60% of cases, plus 5 newly clinical diagnosed/WES tested individuals, we applied whole genome sequencing (WGS) to a subset of patients. Various strategies were employed based on assumed inheritance, disease family segregation and available samples. One quartet, 9 trios, 6 duos and 6 singletons genomes have been sequenced on HiSeqX (Illumina).

Results:

As of March 2017, we have performed ~50 WGS with >30x coverage. Extended genetic testing by WGS combined with revisiting the original WES data revealed a monogenic basis for several of the previously unsolved cases. We have detected 2 novel PIDD genes, and further characterized 2 disease related duplications on the X-chromosome, one involving the DKC1 promoter, and the other a MED12 insertion on another chromosome.

Conclusions:
The cost and turn around time of WGS precludes its application as a first tier clinical test, but our findings show the potential of a genome wide approach to delineate the molecular causes of the patients’ various phenotypes.
CLINICAL AND MOLECULAR CHARACTERISATION OF CZECH HEREDITARY ANGIOEDEMA PATIENTS

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Background:
Hereditary angioedema (HAE) due to C1 inhibitor deficiency is a genetically and clinically heterogeneous disease with a complex pathophysiology. Essentially, defects in the SERPING1 gene may lead to reduced level (HAE type I) or impaired function (HAE type II) of C1 inhibitor.

Methods:
A cohort of 161 Czech HAE patients from 76 families was subjected to the SERPING1 gene molecular analysis. Medical records of the patients were reviewed for disease onset, frequency and severity of edema attacks, laboratory values and treatment modalities.

Results:
Out of 52 unique mutations detected in 76 unrelated HAE patients, the most prevalent were missense (41%) and frameshift defects (29%), followed by aberrant splicing (12%), large deletions (17%) and nonsense mutations (5%). Ten unrelated patients (13%) suffered from HAE type II, all of them carrying the missense mutation affecting Arg466. No defect in SERPING1 coding regions has been found in two families. In these cases, the search for the disease causing mutation continues by analysis of regulatory and intronic regions using specific approaches to detect RNA splicing defects. All missense mutations were assessed using SIFT, Polyphen2 and CADD score, most of them predicted as damaging or probably damaging by at least one of these bio-informatic tools.

Conclusions:
Despite the relatively high number of patients, unambiguous correlation between the genotype and phenotype could not be determined.

This project was supported by MZd grant 16-3441A and specific research MSMT grant MUNI/A/1183/2015.
DEVELOPMENT OF A NOVEL EPIGENETIC IMMUNE CELL QUANTIFICATION METHOD FOR PID NEWBORN SCREENING

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Background:

We present a novel DNA-based epigenetic immune cell quantification method for absolute quantification of multiple immune cell phenotypes in dried blood spot (DBS) samples.

Methods:

We identified cell type-specific DNA regions of de-methylation in purified T-, B-, NK-, monocyte and granulocyte cells. Using specific qPCR assays we show that these regions exhibit complete de-methylation in their respective cell phenotype while methylated in all other cell types tested. We devised a method for absolute and relative quantification of the different immune cell types in clinical samples like fresh and frozen blood, tissue and dried blood spots. Using these methods, we demonstrate >95% concordance with flow cytometric analyses of fresh blood samples from healthy subjects and HIV patients.

Results:

Due to the stability of the analyte (DNA) and the low sample input requirements, epigenetic immune cell counting is uniquely suitable for the analysis of archived samples as well as samples of limited availability. We therefore tested 250 dried blood spot (“Guthrie card”) samples from healthy newborns and 30 patients with Primary Immunodeficiencies (PID) – both SCID and non-SCID forms. Using our epigenetic immune cell counting method we were able to correctly identify 29/30 PID patients.

Conclusions:

We propose application of epigenetic immune cell quantification for expanded PID screening of newborns.
SCREENING FOR IMMUNODEFICIENCY AMONG CHILDREN WITH METHYL MALONIC OR PROPIONIC ACIDEMIAS
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Background:
An association has been suspected between organic acidemia and immunodeficiency. We sought to evaluate B, T and natural killer (NK) cells in children with methylmalonic (MMA) or propionic acidemia (PA).

Methods:
We enrolled 23 MMA and 7 PA patients. Subjects were evaluated for warning signs for immunodeficiency and immunodeficiency related score (IDR), together with complete blood count, serum immunoglobulins, and Candida intradermal testing. All patients underwent lymphocyte subsets enumeration: CD3+, CD4+ and CD8+T cells, CD19+ and CD27+ B cells and CD3-CD56+ NK cells.

Results:
Patients’ ages ranged from 6-86 months (mean: 37.93 ± 23.2 SD months). Recurrent chest infections were the most commonly encountered (50%), followed by gastroenteritis (36.7%). Patients had average duration of infection around 6 days, none had IDR score above 5 and none fulfilled 2 or more of the warning signs for immunodeficiency. BCG scar was found in 24/26 (92.3%) of vaccinated patients. Candida test was positive in all the tested patients (14/14). Out of the 30 patients, 3 had neutropenia, 1 had lymphopenia, 17 had low absolute CD19+ counts, 5 had low absolute CD27+ counts, 14 had low CD3+ counts, 9 had low CD4+ counts, 8 had low CD8+ counts, 12 had decreased CD4/CD8 ratio and 8 had low CD56+ counts. All patients had normal serum immunoglobulins.

Conclusions:
Although our patients had variable decrease in their lymphocyte subsets, yet, their immunoglobulins and candida test were normal. Further studies with specific antibody response and lymphocyte proliferation in relation to residual enzyme activity may provide more conclusive results.
PNEUMOCYSTIS CARINII PNEUMONIA IN AN INFANT WITH NEUTROPenia AND Bronchiectasis

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Background:
Neutropenia can be either acquired or congenital. A variety of syndromes include neutropenia and abnormalities in T, B, or natural killer cell function and make patients more susceptible to infectious complications. One condition is the hyper-IgM syndrome, in which a defective class-switch recombination results in normal or increased levels of serum IgM associated with deficiency of IgG, IgA, and IgE and poor antibody function. CD40 ligand (CD40L) deficiency is the most common form of hyper-IgM syndrome.

Methods:
A 6-year-old boy with a history of neutropenia since the first years of life, respiratory infections and bronchiectasis was admitted with cough, fever and severe hypoxemia. The most important pulmonary sign was the presence of rales in both lungs; laboratorial results showed 6140 leukocytes/µl with 290 neutrophils, and 222 mg/dL of C-reactive-protein; chest x-ray showed bilateral multifocal infiltrate. Based on the hypothesis of Pneumocystis carinii pneumonia Sulfamethoxazole/Trimethoprim and Filgastrim were instituted with good clinical response; infection by such agent was confirmed.

Results:
This child with neutropenia has been followed up in a Hematology Consultation and autoimmune and congenital neutropenia were already excluded; immunoglobulin assay: low IgG and IgA, normal IgM (144 mg/dL). Although the serum IgM assay was normal, a CD40L assay was performed, obtaining a low value, which allowed the diagnosis of hyper-IgM syndrome.

Conclusions:
Neutropenia may occur in any of the primary immunodeficiency disorders. This case of pneumonia with severe hypoxemia in a child with neutropenia, with normal IgM, shows the need for a high index of clinical suspicion for CD40L deficiency.
SUCCESSFUL BONE MARROW TRANSPLANTATION IN A BRAZILIAN 4 MONTH-OLD MALE INFANT WITH OMENN SYNDROME WITHOUT BCG VACCINE.

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Background:

Omenn syndrome is a rare disease.

Methods:

Immunophenotyping by flow cytometry.

Results:

EEOS was admitted with 54 days of life. Her 36-week gestation progressed to vaginal delivery with meconial amniotic fluid, circular cord. He was born on 05/08/2015 with 2465g, edema of the face, hands and feet, petechiae, erythroderma, generalized lamellar skin peeling, mild respiratory distress, hepatosplenomegaly, adenomegaly and jaundice. ORh + blood group, hemoglobin 17.8 g / dL; 20,600 leukocytes / mm³; 121,000 platelets / mm³; total bilirubin (BT) 11.1 direct (BD) 0.9 and Indirect (BI) 10.2. He evolves with worsening of thrombocytopenia (25,400 / mm³), eosinophilia (between 14 and 43%), leukocytosis (53,800 / mm³) and anemia. In 50 days he received 3 transfusions of red blood cells, presented four episodes of infection (Klebsiella sp, Staphylococcus hominis and haemolyticus). Exams ( 14 / 09/2015): AST 375 U / l, ALT 177 U / l, LB: 6.69, BD: 3.75 and γGT 174 U / l; IgM: 17.8 mg / dl; IgG: 280 mg / dl; IgA: 26 mg / dl ; IgE: 210 IU / ml. At admission weighed 2200g, erythroderma and skin desquamation, hepatosplenomegaly, with IgM <16.8 mg / dl (15-191); IgA <less than 6.0 mg / dl (2.8-58); IgG: 150 mg / dl (282-940) and IgE 6710 IU / ml (<15). He required gamma globulin replacement, intubation, inotropic support, two paracenteses of ascitic fluid, Sulfamethoxazole-Trimetropim, Methylprednisolone and Meropenem for Klebsiella pneumoniae.

Conclusions:

Diagnosed as T-B-NK+ SCID, Omenn syndrome, this child underwent successful bone marrow haploidentic transplantation with excellent motor development.
DIAGNOSTICS

ESID7-0418

MEASURING PNEUMOCOCCAL SEROTYPE SPECIFIC IgG ANTIBODY TITRES COMPARED WITH WHOLE 23-VALENT PNEUMOCOCCAL POLYSACCHARIDE VACCINE IgG ANTIBODY TITRES

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Background:

*Streptococcus pneumoniae* is a major cause of morbidity and mortality. Assessing responses to pneumococcal (Pn) vaccination is important when assessing immunogenicity. Testing for IgG-specific pneumococcal antibodies can either be against individual serotypes or against the combined 23 serotypes found in the Pneumovax-23® vaccine (PPV-23).

Methods:

Pn serotype-specific IgG antibodies from an HIV-infected cohort at University Hospitals Birmingham (N=153) were tested pre- and post- Pn vaccination using a multiplexed Luminex assay (University of Birmingham). This measured IgG antibodies to 12 Pn serotypes (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) and the PPV-23® vaccine.

Results:

Significant correlations were detected between PPV-23® vaccine IgG titres and all 12 Pn-specific IgG titres across 1537 patient serum samples (all p<0.001). However, these correlations were only weak to moderate in strength (Spearman coefficients ranging from 0.19-0.46). Furthermore, a ROC curve analysis for the prediction of patients with protective levels (0.35 µg/ml- WHO) on 8 out of 12 Pn serotypes returned an area under the curve of 0.68 (95% CI: 0.66 – 0.71, p<0.001) against whole PPV-23.

Patients with high concentrations against whole PPV-23® have significant variation in their individual serotype antibody concentrations.

Conclusions:

Whole PPV-23® antibodies do not show the breadth and diversity of serotype-specific antibodies. There are only weak to moderate correlations with no predominant serotypes identified. Clinically, there is no cross-protection between serotypes, so if whole PPV-23® cannot predict pan serotype protection, these data highlight the importance of serotype testing.
ACUTE NEUROLOGICAL DEFICITS AND RESPIRATORY ILLNESS IN A 7 MONTH OLD PATIENT: ATYPICAL PRESENTATION OF ZAP-70 IMMUNE DEFICIENCY

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Background:

The clinical heterogeneity of ZAP-70 deficiency is well established. Patients are at risk of exaggerated inflammation and autoimmunity. With the typical normal absolute lymphocyte count (ALC), diagnosis can be challenging.

Methods:

We describe a previously healthy 7-month-old patient who presented with acute flaccid paralysis (AFP) and respiratory distress.

Results:

A pronounced left-sided neurological deficit was evident (weakness of the left arm and leg, left-sided areflexia) and her nasopharyngeal aspirate was positive on nucleic acid amplification testing (NAAT) for enterovirus or rhinovirus. Other initial laboratory investigations were normal including ALC (9.6 x 10⁹/L). AFP was considered given the clinical picture, possibility of an enteroviral infection and MRI suggestion of cervical spinal cord inflammation. Her respiratory status deteriorated and she required mechanical ventilation. She was treated with high dose immunosuppression (IV methylprednisolone 30mg/kg/day x 5 days and IVIG 0.5g/kg/day x 4 days). Because of her symptomatology and her Mennonite ancestry, an underlying immune deficiency was considered. Workup revealed almost absent CD8 but normal quantification of CD4, CD19 and NK cells. ZAP-70 protein expression was absent on flow cytometry (T and NK cells). Further laboratory investigations revealed that rhinovirus (not enterovirus) was positive from bronchoalveolar lavage (BAL). Once immunodeficiency was confirmed, a second BAL looking for opportunistic pathogens revealed Pneumocystis jiroveci on NAAT. ZAP-70 homozygous pathogenic mutation was confirmed. HSCT has been successfully performed after gradual recovering over several weeks.

Conclusions:

To our knowledge, this is the first reported case of ZAP-70 deficiency presenting with acute neurological deficits that appear to be related to autoimmunity.
A CASE OF COMMON VARIABLE IMMUNODEFICIENCY WITH CONCOMITANT CMV ILEITIS AND AMYLOIDOSIS

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Background:
Common variable immunodeficiency (CVID) is a primary immunodeficiency syndrome characterized by hypogammaglobulinemia, deterioration of antibody production and recurrent infections. In addition to the increased frequency of viral infections such as CMV, secondary amyloidosis is also a complication that can be seen in patients with CVID. We present a case with CVID which was later diagnosed with CMV and amyloidosis during the follow-up.

Methods:

Results:

45-years-old male patient with a history of sinopulmonary infections in the past fifteen years had been followed up for bronchiectasis for four years. During the follow-up period, immunological evaluations led to diagnosis of CVID and intravenous immunoglobulin (IVIG) treatment with a dose of 600mg/kg was initiated for once every three weeks. After 15 months of IVIG treatment, the patient was admitted with complaints of cough, dyspnea and diarrhea. Examination revealed poor general condition, BP:80/60 mmHg, pulse rate:120/min, SaO₂:84%, dry tongue, rales in the left lung, increased intestinal sounds. Laboratory demonstrated leukocytosis, elevated sedimentation, low potassium and sodium and also proteinuria of 2520mg/day. In addition to fluid and electrolyte replacement, IVIG treatment (600mg/kg/week) was initiated. Antibiotherapy was initiated for pneumonia. After colonoscopy for diarrhea, CMV ileitis and amyloidosis were detected on biopsy. AntiCMV-IgM resulted negative though CMV-PCR was positive and gancyclovir treatment was initiated. Persistence of proteinuria, aggravation of chronic lung disease and emergence of cardiogenic shock culminated in exitus on the 14th day of hospitalization.

Conclusions:

When proteinuria is detected in patients with CVID, amyloidosis should be deemed. When diagnosing infections such as CMV ileitis, even if the serologic tests are negative, the diagnosis should be confirmed by PCR and, if necessary, biopsies.
Background:

Thymomas are rare mediastinal tumors. These tumors are often asymptomatic, yet may also present with symptoms such as lichen planus and hypogammaglobulinemia. Lichen planus is a papulosquamous disease that involves the skin and mucous membranes. Good syndrome (GS), a rare disease of the immune system, is composed of hypogammaglobulinemia and thymomas and is often seen in the fourth and fifth decades of life. In this paper, we present a case of GS in a 49-years-old male patient.

Methods:

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Results:

The patient with a history of thymectomy and radiotherapy treatment for thymoma about 14 years ago applied to our polyclinic with complaints of frequent infections, intra-oral and genital lesions. Patient had multiple hospitalizations with diagnosis of pneumonia and sinusitis. On physical examination, there were ulcerated white plaques partly covering the tongue, palate and oral mucosa and two ulcerated lesions on the penis. Laboratory tests revealed IgG: 6.14 g/L (7.51-15.6) and CD19(+)B cell: 1.3% (7.0-23.0). The biopsies of white plaques in the mouth and ulcerated lesions on the penis were evaluated and considered as lichen planus. Medical history, clinical findings and laboratory results were found to be consistent with GS. Due to persistent hypogammaglobulinaemia, intravenous immunoglobulin therapy was initiated at 600 mg/kg and continued every three weeks.

Conclusions:

In adults, there are many clinical manifestations accompanied by immunodeficiency. Early diagnosis of immunodeficiencies can increase the quality of life and reduce the morbidity and mortality. In approximately 10% of the cases with thymoma, hypogammaglobulinemia may also be present. Patients with thymoma should be evaluated for immunodeficiency before and after thymectomy.
PATIENT WITH ACTIVATED PHOSPHOINOSITIDE 3-KINASE δ SYNDROME

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Background:
Activated phosphoinositide 3-kinase d syndrome (APDS) is a recently described combined immunodeficiency resulting from gain-of-function autosomal dominant mutations in PIK3CD, the gene encoding the catalytic subunit of phosphoinositide 3-kinase d (PI3Kd). The immunological presentation of patients affected with APDS may include lymphopenia, elevated IgM serum levels, low IgG serum levels, viral infections, lymph node enlargement and elevated risk to develop lymphomas.

Methods:
A male born to unrelated Spanish parents, came to our attention at 24 months of age due to recurrent respiratory infections, chronic infection by EBV, lymph node enlargement and poor growth. Immunological showed hypogammaglobulinemia with elevated IgM serum levels (IgG 44 mg/dl, IgA 0 mg/dl, IgM 370 mg/dl) and reduction of B cells (38 cells/µl).

In June of 2015, “Trusight one” was performed for different genes, including PIK3R1 in Vall d’Hebron Hospital (Dc Roger Colobran).

Results:
Whole exome analysis revealed a heterozygous G-to-A mutation (chr5 c.1425 +1G>A) at a splice donor site at the PIK3R1 gene. The PIK3R1 splice site mutation causes skipping of an exon, corresponding to loss of amino acid residues 434–475 in the inter-SH2 domain. Sanger sequencing confirmed that the heterozygous mutation was present in the patient but not in his healthy siblings or parents, indicating that it was a de novo mutation.

Conclusions:
The patient present a hiperactivation of protein AKT
THE EUROFLOW APPROACH TO FLOW CYTOMETRY IN PATIENTS WITH PRIMARY IMMUNODEFICIENCIES

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Background:

In this evolving field of PID, we decided with the EuroFlow consortium to develop a PID orientation tube that facilitates fast, standardized and validated immunophenotypic diagnosis of lymphoid PID which also allows therapeutic monitoring and full exchange of data between centers. Our aim was to develop a tool that would be universal for all lymphoid PIDs and offer high sensitivity to disclose a lymphoid PID (without a need for specificity to diagnose particular PID) and to guide and prioritized further diagnostic modalities and clinical management.

Methods:

While the tube composition was central to our effort, it has been defined after several cycles of evaluation in a multicenter setting, equally important was the pre-analytical part (sample preparation and instrument setup) the software analysis and the interpretation. This concept has been tested on 85 healthy controls and 97 PID patients with defined genetic defects.

Results:

Absence (or strong reduction) of lymphocyte subsets generally results from a defect in production or survival and it is frequently an immunological basis of the most severe PIDs that present a clinical emergency. Disturbed distribution of lymphocyte subsets rather reflects a defect in activation, maturation or differentiation. These two layers were integrated by generating a multidimensional view based on a reference data set in Infinicyt software.

Conclusions:

The major advantage of the EuroFlow approach is that data can be fully exchanged between different laboratories, which is especially a great advantage for the PID field, with generally a low number of cases per center.
DIAGNOSTICS

ESID7-0447

SERUM IGG ANTIBODY RESPONSE TO PREVIOUS CONTACT WITH COMMON PATHOGENS AND THE ASSESSMENT OF ANTIBODY FAILURE IN CVID

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Background:

An early diagnosis of CVID leading to timely initiation of immunoglobulin treatment depends on assessment of IgG antibody failure, especially in patients that present with autoimmune or auto-inflammatory disease and lack a prolonged history of infections. Diagnostic vaccination could be inappropriate under certain circumstances, e.g. when a prompt decision on initiation of immunoglobulin replacement therapy is needed.

Methods:

In the present study we addressed the question whether examination of IgG antibodies to previous infections/immunizations/exposure to common pathogens may suffice to rule out antibody failure in such patients. We examined serum IgG antibodies against thirteen common pathogens (staphylococcus enterotoxins and hemolysins, streptolysin O, streptococcal DNAse B, 23-valent pneumococcal and Hib capsular polysaccharide, measles, mumps, rubella and VZV) in untreated CVID patients (n=34; age 41.5 [29.3-59] years; serum IgG 166 [94-316] mg/dl, median [IQR]) and immunocompetent age-matched controls (n=1619).

Results:

The percentage of individuals with detectable antibody titers against the thirteen antigens was 54 to 95% in the controls, and 9 to 53% in the patients (only 12% of the patients had no detectable IgG-antibodies at all). All IgG antibody titers were significantly decreased in the patients as a group. An antibody score was calculated using a combined assessment of all 13 IgG antibody measurements; 94% of the patients had a low antibody score as compared to 0.1% of the controls.

Conclusions:

Examination of IgG antibodies to common pathogens enabled the detection of antibody failure in CVID with a sensitivity and positive predictive value of 94.1% and a specificity and negative predictive value of 99.9%.
DIAGNOSTICS

ESID7-0482

FUNCTIONAL ASSESSMENT OF DOCK8 (DEDICATOR OF CYTOKINESIS 8)

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Background:

Autosomal recessive DOCK8 deficiency results in Hyper IgE syndrome (HIES), a combined T- and B-cells defect encompassing a range of clinical symptoms including recurrent respiratory tract infection, viral and staphylococcal skin infection, mucocutaneous candidiasis, eczema and food allergy. Immunological features including elevated eosinophil count and IgE levels, lymphopenia with reduced numbers of CD4 and CD8 lymphocytes, B-cells and NK cells are also seen in other immunodeficiencies and are not diagnostic. Currently diagnosis depends on confirmation of a causative genetic mutation with or without analysis of DOCK8 protein levels. With more widely available exon and genome genetic screening, variants of unknown significance present a challenge for diagnosis particularly where protein expression is preserved. Therefore, a robust functional assay that reliably tests DOCK8 protein function is required.

Methods:

Immortalised B-cell lines (Lymphoplasmacytic Cell Lines) were utilised from two individuals harbouring DOCK8 homozygous mutations and healthy controls.

Cell lines were firstly examined for DOCK8 protein expression via western blot. DOCK8 missense mutation (E750X), and DOCK8 deletion (991_1040del) demonstrate reduced protein expression in comparison to healthy controls.

Utilising our panel of lymphoplasmacytic cell lines we examined DOCK8 dependent and independent activation of STAT3, with a view to developing a method to identify functional defects in DOCK8.

Results:

LCL’s harbouring DOCK8 mutations demonstrated reduced ability to phosphorylate STAT3 when challenged with CpG, signalling via TLR9 and DOCK8, providing the basis for a functional assessment of DOCK8.

Conclusions:

Preliminary data obtained in this study requires further analysis to develop a robust DOCK8 functional assay.
GRANULOMATOUS AND INTERSTITIAL LUNG DISEASE IN CHILDREN WITH PRIMARY IMMUNODEFICIENCIES

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Background:

Primary immunodeficiency diseases (PIDs) predispose the affected children to recurrent, severe and opportunistic pulmonary infections as well as non-infectious complications, resulting from chronic inflammatory response, post-infectious sequelae and structural changes, immune dysregulation, autoimmunity, lymphoproliferation and, finally, intensive therapy including immunosuppression and chemotherapy.

Methods:

Evaluation of clinical manifestations, radiological features and immunological correlations of chronic lung disease in antibody production defects, such as common variable immunodeficiency and agammaglobulinemia, in combined immunodeficiencies e.g. ataxia-telangiectasia and hyper-IgE syndrome, in severe combined immunodeficiencies as well as in chronic granulomatous disease.

Results:

The radiological evaluation of the respiratory tract of different PIDs demonstrates a variety of pulmonary findings, such as ground glass opacities, intralobular lines, irregular interlobular septal thickening, paraseptal or irregular emphysema, patchy atelectasis as well as subpleural or disseminated pulmonary nodules and granulomas. Aberrations of the immune response predisposing to interstitial and granulomatous lung disease in pediatric PIDs are heterogeneous. They most frequently comprise defective development of switched memory B cells, impaired B-cell help due to follicular B cell dysfunction, as well as abnormalities within the T-cell compartment, such as reduced number of naive T cells, central memory T helper cells along with activation of NK and NKT cells and gamma/delta T cells.

Conclusions:

The response to immunoglobulin and antibiotic therapy as well as the outcome is variable, frequently associated with progressive restrictive lung disease, hypoxia and exercise intolerance. Chronic lung disease is the most common and early complication of pediatric PIDs which significantly contributes to the morbidity and mortality of affected children.
A New Mutation in Human TYK-2 Deficiency in a case report
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Background:

A defect in TYK-2 protein is one of the rarest immune deficiencies that are inherited as a recessive autosomal. The intracellular protein TYK-2 is one of the members of the JAK family (TYK-2, JAK-1, 2, 3), which causes the activation of STST4 and ultimately the production of IFN-y. TYK-2 protein mutation leads to a disorder in IFN-Y production. This study offers a report on the recognition and analysis of the immune system of a little boy with TYK-2 defect among MSMD patients.

Methods:

The patient is a 7-year-old boy who was affected with the auxiliary LAP in the injection area of BCG vaccine. He was referred to the Immunology, Asthma and Allergy Research Institute (IAARI) and completed screening test for primary Immune deficiency. Lymphocyte Transformation Test (LTT) was conducted with BCG mitogen and antigen and IFNY and IL12 cytokines along with the patient’s genetic study were done.

Results:

The patient was normal with regard to all humoral and cellular immunity tests. Furthermore, LLT with mitogen was normal, but no response was received against BCG antigen. IFN-Y and IL12 cytokine assessments were carried out, which indicated that the patient had problem producing IFN-Y and the genetic response was a defect in TYK-2 in exon7

Conclusions:

A defect in TYK-2 protein and the disorder in the production of IFN-Y can cause virus infections and intracellular bacteria such as salmonella, BCG and Candida. Early diagnosis and treatment with antibiotics and antifungal along with IFN-Y treatment and probably HSCT (Hematopoietic stem-cell transplantation) can help control the disease.
USE OF SUBCUTANEOUS IMMUNOGLOBULIN IN IMMUNE DEFICIENCIES

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Background:

Children with immune deficiencies are prone to bacterial infections affecting the respiratory tract and gastrointestinal canal. To prevent or alleviate infections, replacement therapy with IgG is needed, usually on a lifelong basis. The IgG can be administered intramuscularly, intravenously, or subcutaneously. Subcutaneous IgG therapy, using small portable pumps for once-per-week self infusions, has shown many advantages compared with the two other routes of administration.

Methods:

The demographic findings, clinical and laboratory findings, subcutaneous immunoglobulin dosage and dose frequency, infusion time, area and methods, adverse events and frequency of infections were obtained from patient files and recorded.

Results:

13 patients (10 male, 3 female) aged between 3 and 16 years who were treated with subcutaneous immunoglobulin were enrolled. Subcutaneous immunoglobulin was administered at a dose of 0.03-0.43 gr/kg/dose with one-two week intervals. In a 15 months follow-up period, no acute severe bacterial infection was observed. Local adverse reaction was reported in only 4 of 110 infusion (3.6%). No serious adverse events were reported. All 13 patients were willing to continue IgG replacement therapy by subcutaneous administration.

Conclusions:

The subcutaneous route is attractive to many patients because of a reduced incidence of systemic adverse events, flexibility in scheduling and its comparative ease of administration, at home or in a clinic. Ig replacement therapy by subcutaneous route is an efficient, safe and easy option which is eligible for individual administration.
Background:

Common variable immunodeficiency (CVID) is the most frequently symptomatic primary immunodeficiency (1/25000/50000). 20-60% patients present gastrointestinal manifestations. This study describes the gastrointestinal disorders prevalence and their characteristics in CVID patients that we follow-up in our CVID consultation.

Methods:

This is an observational and retrospective study in which there were analyzed the presence of gastrointestinal manifestations in 36 CVID’s patients diagnosed in the last 20 years, by the ESID established criteria. There were considered the etiology of the digestive disorders (infectious, inflammatory or neoplastic) and the affected organ.

Results:

36 patients were diagnosed. The mean age at diagnosis was 26.28 (+/-19) with male predominance (69.4%, 25 cases). The onset clinic in everyone was respiratory infections. 61.1% (22 cases) presented gastrointestinal clinic, 72.7% (16 cases) were confirmed by biopsy. 30.6% (11 cases) presented hepatic affectation (7 HCV cases, 1 varicella hepatitis, 1 granulomatosis and 2 hepatotoxicity). 54.5% (6 cases) of them were confirmed by biopsy.

Conclusions:

The digestive affection was very frequent in our series (22 cases, 61.1%), according to other published series. The main etiology was infectious (37.2%), and the most related agent was G. lamblia. The differential diagnosis must be done with inflammatory, infoproliferative and neoplastic disorders. Within inflammatory manifestations, we have to highlight the spue-like affectation. 50% biopsied patients showed nodular lymphoid hyperplasia (NHL), which can progress to intestinal lymphoma.

The gastrointestinal manifestations are a common reason to consult in IDCV patients. Although the infectious is the most common, alternative etiologies should be considered in patients with non-affiliated chronic diarrhoea or abdominal pain.
Novel STAT-1 gain-of-function mutation in the SH2 domain in autosomal chronic mucocutaneous candidiasis and bacterial and virus infections

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Background:
Chronic mucocutaneous candidiasis (CMC) is characterized by persistent or recurrent disease of the nails, skin, oral, or genital mucosae caused by Candida albicans. Heterozygous dominant gain-of-function (GOF) mutation in signal transducer and activator of transcription 1 (STAT1) causing impaired STAT1 dephosphorylation (1,2). The hypermorphic mutations are also associated with an increased risk of autoimmune disorders, arterial aneurysms, and squamous cell cancers (3).

Methods:
Mutation of STAT1 gene (NM_007315.3) was analyzed by bi-directional sequencing. Complete immunological studies were performed.

Results:
The boy was born full-term to non-consanguineous parents and since the first weeks of life presented recurrent episodes of oral candidiasis. At 2-month of age he suffered a bronchopneumonia. Epiglottitis by Candida albicans, pneumonia by Rhinovirus and Parainfluenza IV and gastroesophagitis by cytomegalovirus required new Hospital admission at 22-months. At this moment fluconazole and cotrimoxazole prophylactic treatment was initiated. During the last 3-years he presented several episodes of fever, oral aphthae and respiratory infections. Until this time he has not presented autoimmune disorders. Growth and development were appropriate for age. He had no family history of severe infections or immunodeficiency. Immunological study revealed discrete T-CD4+ lymphopenia, depletion of NK-cells and progressive decrease of serum IgA levels. Screening of autoantibodies was only positive for anti-smooth muscle. De novo p.N658H monoallelic mutation was detected in exon 22 (SH2 domain).

Conclusions:
We describe the dominant GOF mutation in STAT1 in patient with persistent oral thrush, recurrent viral infection, low level of T-CD4+ and NK, and time-dependent decline in IgA serum level. More studies are carried out to evaluate the effect of this new mutation in this patient.
Detection of Sp110 deficiency by flow-cytometry and application to screening patients for veno-occlusive disease with immunodeficiency

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Background:

Mutations in Sp110 are the underlying cause of veno-occlusive disease with immunodeficiency (VODI), a often fatal, combined and difficult to treat primary immunodeficiency. Several factors make establishing the diagnosis of VODI challenging: 1) Current screening strategies to identify severe combined immunodeficiency are based on measuring T cell receptor excision circles (TREC). This approach will fail to identify VODI patients because the disease is not associated with severe T cell lymphopenia at birth; 2) The SP110 gene contains 17 exons, making it a challenge for Sanger sequencing. Recent next generation (NGS) platforms that examine the sequence of all 17 exons are available in very few laboratories only. Since early treatment is critically important for survival of patients with VODI, broadly usable diagnostic tools are needed to detect Sp110 protein deficiency.

Methods:

The establishment of an intracellular monoclonal antibody-staining, that can be analysed with flow cytometry and is performed on freshly isolated peripheral blood mononuclear cells (PBMCs) from patients and healthy individuals.

Results:

Specificity of the Assay could also be shown consistently with reconstitution experiments in knockout Jurkats and in primary patient T cells. Blinded Identification of a patient out of six Healthy individuals was reliable.

Conclusions:

Here we report a novel flow cytometric assay that is easily applicable in diagnostic laboratories and might thus become a standard assay in the work-up of suspected VODI cases. SP110 expression can be detected in PBMCs from shipped blood. In addition, the assay will facilitate to study the biological functions of SP110, which are currently still poorly understood.
E-POSTER DISCUSSION 1: CELLULAR THERAPY

ESID7-0072

COMBINED IMMUNODEFICIENCY AND PROGRESSIVE LYMPHOPROLIFERATIVE DISEASES OF ACTIVATED PI3Kδ SYNDROME RESCUED BY HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background:

Activated PI3Kδ syndrome (APDS) is a recently described primary immunodeficiency syndrome characterized by recurrent respiratory infections, lymphoid hyperplasia, and herpesviridae infections. This is caused by germ line gain-of-function mutations of PIK3CD which lead to lymphocytes hyperactivation and senescence. Because of limited reported cases, its long-term prognosis and clinical manifestations were not fully understood. Hematopoietic stem cell transplantation (HSCT) would be a choice for treating its progressive immunodeficiency and accompanied malignancy, but outcomes and appropriate procedures remain undefined.

Methods:

We reviewed our patient registry and performed questionnaires to collaborating facilities in Japan and Taiwan.

Results:

We identified 22 patients of APDS. Patients had broad spectrum of clinical manifestations from milder cases such as who presented a few infectious episodes or only mild lymphadenopathy, to recurrent severe infections and life-threatening lymphoproliferation. Frequent opportunistic infections and low T-cell receptor excision circles revealed this syndrome as a combined immunodeficiency. Thirty-year overall survival was 84.8% but events, including death, HSCT and massive lymphoproliferation, were frequent till adolescence. Eight patients underwent HSCT due to recurrent infections or uncontrollable lymphoproliferation. All conditioning regimen were fludarabine-based reduced intensity conditioning (RIC). Eventually all cases achieved donor engraftment but two cases rejected the first HSCT and three cases developed poor graft function. Two cases deceased after engraftment due to regimen related toxicity or sepsis at late period.

Conclusions:

APDS showed variable clinical manifestations and laboratory findings but progressive combined immunodeficiency and massive lymphoproliferation would become life-threatening. Fludarabine-based RIC-HSCT could be curative although frequent complications and engraftment failures were remarkable.
EFFECT OF EXTRACORPOREAL PHOTOPHERESIS ON THYMOPOIESIS IN A COHORT OF PAEDIATRIC PATIENTS WITH ACUTE GRAFT-VERSUS-HOST DISEASE

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²Great North Childrens’ Hospital, Paediatric Haematopoietic Stem Cell Unit, Newcastle, United Kingdom

Background:

Acute graft-versus-host disease (aGvHD), as well as corticosteroids and other non-selective immunosuppressive agents, cause thymic damage and negatively affect T-lymphocyte reconstitution following haematopoietic stem cell transplant, which is essential for a successful clinical outcome. Extracorporeal photopheresis (ECP) is an alternative immune-modulating therapy that could, by reducing aGvHD and allowing weaning of other immunosuppression, facilitate thymic recovery and restoration of normal thymopoiesis.

Methods:

We report the effect of ECP on thymopoiesis in 4 paediatric patients with corticosteroid-refractory aGvHD, measured by sequential monitoring of CD3⁺CD4⁺CD45RA⁺CD31⁺ naïve T-lymphocytes using flow cytometry and T-cell receptor excision circles (TRECs) using PCR.

Results:

All patients, with grade II-IV aGvHD, demonstrated a highly abnormal T-cell receptor (TCR) repertoire prior to ECP treatment measured by TCR DNA spectratyping, indicative of reduced thymic output. Three of four patients showed improved thymopoiesis with increased absolute numbers of naïve T-lymphocytes (Figure 1) and TRECs with ECP treatment. This was an accompanied by a reduction in activated CD3⁺CD4⁺HLA-DR⁺ lymphocytes, clinical improvement and reduction in concurrent immunosuppression in all patients.
Conclusions:

Although more data are required, these are the first prospective data to suggest that ECP may allow restoration of thymic function and abet adaptive immunoreconstitution in at least some paediatric patients with aGVHD, which is important when considering treatment of patients with primary immunodeficiency, in whom the purpose of treatment is to restore normal immunity.
E-POSTER DISCUSSION 1: CELLULAR THERAPY

ESID7-0079

COMPARISON OF QUALITY OF LIFE IN IL7Rα, RAG & ARTEMIS AND ADA SCID

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Background:

Quality of life (QoL) data post-HSCT for SCID are scarce, with 1 study reporting poor function in survivors compared to healthy controls1. However, treatment and genotype differences may confound the analyses.

Methods:

We assessed QoL of SCID survivors according to genetic diagnosis and quality of immunoreconstitution. Patients >2 years post-HSCT and families answered the PedsQL v4.0 questionnaires. Mean scores were compared with published UK norms.

Results:

Responses according to SCID genotypes were IL7Ra (10/14, 71%), Adenosine Deaminase (ADA) (12/16, 75%) and Artemis & RAG SCID (11/16, 69%) patients/parents. Median time post-HSCT was 11 years (range 2-27). QoL scores were normal for IL-7Ra patients and parents. ADA patients had significantly lower QoL except in the emotional domain. Parents of ADA SCID reported significantly lower QoL in all domains. Artemis & RAG SCID patients reported significantly lower QoL in 2 domains. However, subgroup analysis showed Artemis and RAG SCID patient and family without on-going medical issues reported normal QoL.

Conclusions:

QoL for IL7Ra SCID and those without on-going medical issues were normal. ADA SCID patients and families reported lower QoL, confirming previous studies.
<table>
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<th>Parent Report</th>
<th>UK Norms Mean</th>
<th>IL7Ra SCID Mean (p-value)</th>
<th>ADA SCID Mean (p-value)</th>
<th>Artemis &amp; RAG 1/2 SCID Mean (p-value)</th>
<th>On-going medical issues Mean (p-value)</th>
<th>No on-going medical issues Mean (p-value)</th>
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<td>Total</td>
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E-POSTER DISCUSSION 1: CELLULAR THERAPY

ESID7-0087

COMPARISON OF LONG-TERM T LYMPHOCYTE COUNT AND CD4 NAÏVE COUNT IN IL7Rα/RAG & ARTEMIS/ADA SCID: A COHORT STUDY

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Background:
Long-term thymopoiesis for specific SCID genotypes, in relation to conditioning, is poorly documented. We analysed thymopoiesis in post-transplant IL7Rα/RAG & Artemis/ADA SCID patients, with particular emphasis on outcome with/out conditioning.

Methods:
A longitudinal study of CD3+ and CD4+CD45RA+ naïve T-lymphocyte count performed for 15/18 IL7Rα, 5/13 RAG, 5/8 Artemis and 16/19 ADA SCID survivors transplanted between 1987-2012. Multi-level mixed modelling analysis compared trend of changes in T-lymphocytes and CD4+ naïve cells.

Results:
Median age (range) was: IL7Rα 14 years (4 – 27); Artemis/RAG 10 years, (2 – 18); ADA 11.5 years (3 – 25). Longitudinal analysis of CD3+ lymphocyte count showed no difference in overall trend of CD3+ lymphocyte numbers between conditioned versus unconditioned recipients for IL7R SCID (p = 0.92) and ADA SCID (p = 0.28) and after 5 years post-HSCT for Artemis and RAG SCID. Conditioned recipients of ADA and Artemis & RAG SCID had higher CD4+ naïve cell counts (p = 0.04 and p =0.04, respectively). CD4+ naïve lymphocytes in unconditioned recipients was persistently < 500 cells/µl post-transplantation. CD4+ naïve cell counts was non-significantly higher in conditioned IL7Rα SCID recipients, p = 0.45 (Figure).

Conclusions:
Conditioning resulted in improved longitudinal thymopoiesis in ADA, Artemis & RAG SCID. Sustained CD3+ outputs are seen after 20 years post-HSCT for IL7Rα SCID, RAG & Artemis and ADA SCID.
HEMATOPOIETIC STEM CELL TRANSPLANTATION RESULTS OF NIK DEFECTS

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Background:

NFκB signaling pathway is essential in regulating a variety of immune responses. NFκB inducing kinase (NIK) is the principal component of non-canonical NFκB pathway, transducing signals from TNF receptor superfamily members such as BAFFR, CD40, LTβR and RANK. Homozygous mutation in NIK gene leads to a combined immunodeficiency, presenting with recurrent bacterial, viral and Cryptosporidium infections.

Methods:

Here, we present three cases bearing NIK mutations, two of whom underwent HSCT successfully.

Results:
Conclusions:

Loss-of-function mutations in NIK result in defective non-canonical NFκB signaling, which demonstrate as combined immunodeficiency with aberrations in functions of B-, T- and NK-cell lineages. Early diagnosis and prompt HSCT are crucial for survival of these patients.
E-POSTER DISCUSSION 1: CELLULAR THERAPY

ESID7-0181

HLA-MISMATCHED DONOR HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN WITH PRIMARY IMMUNE DEFICIENCIES: COMPARATIVE ANALYSIS OF VARIOUS APPROACHES ACROSS THE UK


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Background:

Haploidentical/mismatched unrelated donor grafts are an alternative for patients with primary immunodeficiency (PID) who lack an HLA-matched donor. Various strategies are sought to mitigate risks of graft versus host disease (GvHD)/rejection associated with such transplants.

Methods:

We surveyed results of 103 PID patients who received 107 haploidentical/ mMUD grafts. Patients were divided into: Group 1: TCRαβ+/CD19+ depleted (n=25 cases; 84% received ATG/Alemtuzumab), Group 2: CD34+ selected with T cell add-back (n=17), Group 3: Unmanipulated marrow (n=28). Group 2,3 received Alemtuzumab. Group 4: Cord (n=36; 81% no serotherapy). Conditioning included Treosulphan/Fludarabine±Thiotepa; 57% or Fludarabine/Melphalan; 28%

Results:

Two-year overall survival of the entire cohort was 79% with stable disease-lineage chimerism above 20% in all patients in groups 1 & 2 and 95%, 77% in Groups 3,4, respectively. Transplant related mortality (TRM) was 21.5%; viral infections was cause of death in initial 100 days (70%). There was no difference between groups regarding viral reactivation, graft loss ,TRM. Systemic aGvHD was not encountered in Group 1, but was significantly higher in Group 4 (22%) versus Group 2, 3 (12%, 14%, respectively). None of the Group 1 patients had cGvHD while it was recorded in 25-30% of patients in the other groups. Continuation of immunosuppressive therapy beyond 1 year post-HSCT was significantly higher in Group 4 (17%) while 0-8% of patients in the other groups.

Conclusions:

TCRαβ+/CD19+ depleted grafts show comparable rates of engraftment, survival, disease amelioration but lower incidence of systemic or cGvHD over other strategies of graft manipulation. TCRαβ+/CD19+ depleted haploidentical/mMUD HSCT is a suitable alternative when matched donor is not available for PID patients.
E-POSTER DISCUSSION 1: CELLULAR THERAPY

ESID7-0437

RISK FACTORS, TREATMENT AND IMMUNE DYSREGULATION IN AUTOIMMUNE CYTOPENIA FOLLOWING ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC PATIENTS – A SINGLE CENTER RETROSPECTIVE ANALYSIS

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Background:

Autoimmune cytopenia (AIC) is a relatively rare but serious complication after hematopoietic stem cell transplantation (SCT). The aim of this retrospective study is to evaluate incidence, potential risk factors, current treatment strategies and outcome in our center and to explore the immune dysregulation predisposing to AIC.

Methods:

In this study, we evaluated incidence, outcome, potential risk factors and current treatment strategies. A nested matched case-control study was performed to search for biomarkers associated with AIC.

Results:

Of 531 consecutive SCTs at our center between 2000 and 2016, 27 were complicated by the development of AIC (cumulative incidence 5.2%) after a median of 5 months post-SCT. AIHA (48%) and Evans syndrome (33%) were the most common AICs. We identified non-malignant disease, alemtuzumab serotherapy pre-SCT and CMV reactivation as independently associated risk factors. The cytokine profile of patients appeared to skew towards a more pronounced Th2 response compared to controls. First-line treatment or a wait-and-see approach led to resolution of AIC in 33% of cases. Second and subsequent-line therapies with rituximab (n = 16), bortezomib (n = 7) or sirolimus (n = 3) eventually led to resolution of AIC in 44%, 57% and 100% of cases, respectively. AIC is a relatively rare complication of SCT.

Conclusions:

In conclusion, we identified CMV reactivation post-SCT as a new clinical risk factor for the development of AIC. The cytokine profile of patients appears to favor a Th2 response. Rituximab, bortezomib and sirolimus are promising second-line treatment modalities.
CLINICAL IMPROVEMENT IN SCLEROSING CHOLANGITIS AFTER SUCCESSFUL HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN PRIMARY IMMUNODEFICIENCIES

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²King’s College Hospital, Paediatric Liver Centre for Hepatology- Gastroenterology and Nutrition & Institute of Liver Studies, London, United Kingdom
³University of Kobe, Medical School, Kobe, Japan

Background:
Liver involvement leading to sclerosing cholangitis (SC) can occur in patients with combined immunodeficiencies and is often associated with chronic cryptosporidium infection¹. SC can progress to biliary cirrhosis, end-stage liver disease and/or cholangiocarcinoma²,³. The aim is to present the clinical improvement of SC following successful haemopoietic stem cell transplantation (HSCT).

Methods:
The patients were identified from databases at 2 centres in London: Paediatric Liver Centre at King’s College Hospital and Great Ormond Street Hospital for Children. Clinical, radiological and histological information were compared pre and post- HSCT.

Results:
Four patients with PID and histologically confirmed, cryptosporidium associated SC pre-transplant were identified. The underlying diseases were CD40 ligand deficiency, cartilage-hair hypoplasia, Wiskott Aldrich Syndrome and DOCK 8 deficiency. They all underwent successful non-myeloablative unrelated donor HSCT. The age ranged between 10 to 17 years. There was an initial significant deterioration in liver function tests (LFTs) post-HSCT in three patients but later all showed reversion to normal (3) or nearly normal (1) LFTs. There was marked improvement in the liver histopathology in the three biopsied after HSCT. However, all continue to have some residual dilatation of the extrahepatic biliary system on ultrasound but with no intrahepatic ductal changes on magnetic resonance cholangio-pancreatography.

Conclusions:
HSCT may considerably improve and even revert SC in children with PIDs, suggesting that establishing immune competence and clearing chronic infection can control of this life-threatening complication.
CORRECTION OF BOTH IMMUNODEFICIENCY AND HYPOPARATHYROIDISM BY THYMUS TRANSPLANTATION IN A CASE OF COMPLETE DIDGEORGE SYNDROME

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¹Great Ormond Street Institute of Child Health, Immunology, London, United Kingdom
²Universitatsklinik fur Kinder und Jugendmedizin, Immunology, Ulm, Germany
³Great Ormond Street Hospital, Pathology, London, United Kingdom
⁴Great Ormond Street Hospital, Haematology, London, United Kingdom

Background:

Thymus transplantation corrects the immunodeficiency in complete DiGeorge syndrome (DGS) but patients continue to suffer from other features including hypoparathyroidism¹. We report a child with DGS who achieved both immunological reconstitution and correction of hypoparathyroidism following thymus transplantation.

Methods:

A female child with complete DGS caused by poorly controlled maternal diabetes during gestation underwent thymus transplant from a male donor. She had presented with neonatal hypocalcaemia, had absent T cells, skeletal abnormalities and a mild cardiac defect. She was infected with respiratory syncytial virus (RSV), rotavirus and human herpes virus 6 (HHV6) prior to transplantation.

Results:

Four weeks after transplantation she developed mesangio-proliferative glomerulonephritis treated with corticosteroids and Eculizumab with a rapid resolution of the problem. Biopsy from the site of transplantation, taken after 3 months, in addition to showing thymic epithelium, showed a nodule of endocrine tissue which stained for parathormone (PTH) and was shown by XY-FISH to be of male origin. Blood PTH levels were very low/undetectable prior to transplantation but rose to normal by 7 months and remain so after 22 months. She has stopped calcium and calcitriol treatment and maintains normal plasma calcium levels. Latest T cell counts show CD3 1193, CD4 530, CD8 610 x10⁶/L with 54% and 34% naïve CD4/CD8 cells respectively. She has cleared RSV and rotavirus, suppressed HHV6 levels and controlled a later-acquired EBV infection.

Conclusions:

Parathyroid tissue, likely ectopic in the donor thymus, was co-transplanted with thymus and has resulted in correction of the hypoparathyroidism.

SEARCHING DIFFERENTIAL DIAGNOSIS MARKERS BETWEEN SEVERE SEPSIS AND HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH)

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⁴BioCruces Health Research Institute. Ikerbasque- Basque Foundation for Science, Immunopathology Group, Bilbao, Spain
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⁷Hospital Universitario Cruces. BioCruces Health Research Institute. University of the Basque Country, Paediatric Service. Pediatric Oncology Group, Bilbao, Spain

Background:

HLH diagnostic criteria are fulfilled in many septic patients but the treatment decision is different. Aim: To compare data from septic and HLH patients in order to find biomarkers for differential diagnosis.

Methods:

Revision of clinical features of septic and HLH patients. sCD25 and sCD163 serum concentration was determined by ELISA in septic patients and healthy donors. sCD25 serum concentration was reported in 23 HLH cases in Spain.

Results:

Sixty-three septic patients were included (42 males/21 females. Median age: 61.9 years-old) from 2010 to 2015. Transfusion center provided 60 healthy donors’ samples (37 males/23 females. Median age: 51 years-old). Data from 23 HLH patients (13 males/10 females. Median age: 1.23 years-old) were included. Diagnostic clinical data in septic patients: 30/47-fever, 34/49-anemia, 17/53-thrombopenia, 27/53-coagulopathy. sCD25 revealed significantly increased levels in sepsis when compared to healthy donors. No differences were found between septic patients who died and survivors. sCD25 median concentration in 11 HLH patients (14,550 pg/ml) was higher than in septic patients (4072 pg/ml). sCD25 results were expressed in U/ml in 12 HLH patients and sCD25>2,400 U/ml in 7. Results expressed in U/ml or pg/ml cannot be compared due to the lack of measurement standardization and conversion formula.

Conclusions:

sCD163 and sCD25 are increased in severe sepsis. sCD25 measurement units and cutoff levels as HLH diagnostic criteria should be considered carefully. Lack of formula to convert the concentration values among ELISA tests brands could misinterpret study results. More specific diagnostic criteria are needed for differential diagnosis between sepsis and HLH.
E-POSTER DISCUSSION 10: CLINICAL PID II

ESID7-0284

A HUMAN CTL-BASED FUNCTIONAL ASSAY FOR FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Background:

Familial hemophagocytic lymphohistiocytosis (FHL) is a fatal syndrome of immune dysregulation and hyper-inflammation caused by a defect in granule-dependent cytotoxic pathway, the major immune effector mechanism employed by cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. A defective cytotoxic activity of CTLs and NK cells is one of the hallmark findings of FHL.

To assess the pathological significance of a mutation in FHL gene, comprehensive functional analysis including protein expression, lysosomal degranulation, and cytotoxicity of human CTLs is advantageous. However, the functional studies are often hampered by limited availability of patient material and there has been no simple and versatile assay system established to date.

Methods:

With Herpesvirus Saimiri, we established immortalized FHL2 and FHL3 human CTL lines. Subsequently, we evaluated if these cell lines can restore their cytolytic function by transiently expressing cDNA constructs.

Results:

Established FHL model CTL lines showed reduced protein expression and impaired cytotoxicity. Their functional defects could be recovered by transiently expressing wild type cDNA constructs, but not by that of pathological mutants.

Conclusions:

Using the established human FHL model cell lines, we could evaluate the functional significance of a FHL gene mutation on protein expression, cytolytic granule degranulation and cytotoxicity. The system is theoretically applicable to other types of FHL. Moreover, these human CTL cell lines can be used for the screening of new drugs and therapeutic targets for inventing new strategies of FHL treatment.
DEFICIENCY OF CD70 IS RESPONSIBLE OF A CASE OF CHRONIC ACTIVE EBV (CAEBV) INFECTION PRESENTING AS PERIODIC FEVER

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Background:

Chronic active EBV (CAEBV) infection is a rare condition associated with severe lymphoproliferation. Aim of this study was to describe the clinical course and the genetic characterization of a patient with CAEBV presenting as a periodic fever.

Methods:

In a patient with CAEBV infection a whole exome sequencing (WES) approach was undertaken and variants were prioritized with a custom pipeline to identify the genetic cause of his condition, validated through Sanger Sequencing.

Results:

The patient, son of consanguineous parents, after a mononucleosis, started to present episodes of fever with tonsillitis and adenitis, with good response to steroids. In the following months recurrent respiratory infections and episodes of cheratitis were observed. A progressive reduction of immunoglobulin levels with an increase of CD20+ cells were also detected. EBV PCR revealed 25000 copies for 100000 leucocytes. A lymphnode’s biopsy was consistent with CAEBV. The patients underwent stem cell transplantation with a complete normalization of clinical and immunological features. Among variants identified through WES analysis of the patients and his parents, a homozygous mutation of the CD70 gene appeared to fit the expected recessive model of inheritance. The variantc.163-2A>G affects the exon2AG-acceptor splice site of the CD70 gene (NM_001252) thus leading to a deficiency in CD70, as confirmed by cell surface analysis of PHA-T cell blasts and EBV-transformed lymphoblastoid cell lines both derived from the patient. Mutations of CD70, the ligand of CD27, have been recently reported to be associated to similar clinical pictures.

Conclusions:

We describe the case of a newly identified genetic cause of CAEBV.
ONCOLOGIC COMPLICATIONS IN CHILDREN WITH PRIMARY IMMUNODEFICIENCY (PID): SINGLE CENTER EXPERIENCE.

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Background:

Oncologic complications make a significant impact on morbidity and mortality in PID patients.

Objective: To evaluate the incidence of various malignant complications in children with PID.

Methods:

We retrospectively analyzed 350 PID patients aged from 2 months to 21 years (median 6 years), followed in our Center between years 2012 and 2016.

Results:

43 patients (13 female and 30 male) developed the following oncologic complications: lymphoma (34), leukemia (7), solid tumors (ganglioglioma, medulloblastoma, mielosarkoma and hepatocellular carcinoma (4). Among lymphomas there were five Hodgkin's, five T-cell, six Burkitt's and eighteen diffuse large B-cell lymphomas. Two or more oncological complications were observed in 5 patients. Median age of debut of malignant complications was 8.4 years.

Patients with highest rate of oncological complications belonged to the group of DNA repair defects (n=29) and combined PID (n=8).

Interestingly, in 25 patients PID diagnosis was verified only after the development of malignancy.

Conclusions:

Our study demonstrates high incidence of oncological complications in patients with PID with the highest prevalence in the group with chromosomal breakage syndromes - up to 67%. Early diagnosis of PID and oncological awareness is important for monitoring of these patients.
Background:

Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders characterized by impairments in communication and social interaction, stereotyped interests and repetitive behaviors. Current researches suggest a significant role for immunodysregulation in the pathophysiology of ASD, with the existence of autoantibodies directed against neuronal proteins and elevated production of anti-phospholipid antibodies (aPL) in ASD patients compared to typically developing controls and children with developmental delays.

Aim: To assess levels of aPL in ASD patients and in their mothers

Methods:

We assessed levels of Lupus anticoagulant (LAC) and anticardiolipin antibodies IgM and IgG in a cohort of 60 ASD children and young adults (50 males and 10 females), aged 2-34 years (mean 11.6±6.6SD) and in their mothers. Inclusion criteria were the presence of any dysimmune or autoimmune illness in the family. Exclusion criteria were any infectious disease, oncological history in the subject or immunomodulant therapy during or previously the procedure. APL positivity was confirmed according to the International guidelines.

Results:

We found an ApL positivity in 10 patients (17%) and in 7 (12%) ASD patients’ mothers. Both LAC and IgG anticardiolipin antibodies were found to be positive in 2 ASD patients and in 2 mothers; LAC positivity only was found in 7 ASD and 4 mothers while IgG anticardiolipin positivity was found in 1 ASD patients and in 1 mother. Two ASD patients and their mothers (3%) showed both LAC and anticardiolipin positivity.

Conclusions:

These results suggesting possible biomarkers for evaluating pathogenic mechanisms and possible targeted treatments in ASD.
THE ICATIBANT OUTCOME SURVEY: MORE THAN 4,500 ICATIBANT-TREATED ATTACKS IN PATIENTS WITH TYPE I OR II HEREDITARY ANGIOEDEMA

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Background:

Icatibant is a bradykinin B2 receptor antagonist used to treat attacks of hereditary angioedema due to C1-inhibitor deficiency (C1-INH-HAE) in adults. The Icatibant Outcome Survey (IOS; NCT01034969), an international observational study, monitors safety and effectiveness of icatibant in a real-world setting. Here we report data from >4,500 icatibant-treated attacks in patients with type I/II C1-INH-HAE.

Methods:

Patient characteristics and icatibant-treatment outcomes were recorded at clinic visits from 52 centers across 12 countries. Descriptive retrospective analyses were performed on data collected from July 2009–February 2017.

Results:

Icatibant was used to treat 4,541 angioedema attacks in 517 patients with type I/II C1-INH-HAE. Mean age at enrollment was 40.7 years (range 16.5–81.8), and 57.8% of patients were female. Proportions of very mild/mild, moderate, and severe/very severe attacks were 9.5%, 36.3%, and 54.2%, respectively (N=3,859 attacks with severity assessed). Of attacks with anatomical location data (N=4,386), 58.4% of attacks affected the abdomen, 44.9% affected the skin, and 5.4% affected the larynx. Most icatibant injections were self-administered (N=3,457/3,837 attacks; 90.1%). Median time to icatibant administration was 1.5 hours (N=2,067 attacks). Median time to symptom resolution was 6.5 hours (N=1,932 attacks). Median attack duration was 9.5 hours (N=1,647 attacks). Of 4,541 icatibant-treated attacks, 396 (8.7%) also were treated with C1-INH rescue therapy. Icatibant was well tolerated, with no unexpected safety outcomes.
Conclusions:

IOS has accumulated a large database of patients with C1-INH-HAE, providing insight into the characteristics of this rare disease. Treatment outcomes and safety of icatibant remain consistent with those from the Phase III studies.
ESID7-0413

PREVALENCE, OUTCOMES AND TREATMENT STRATEGIES FOR AUTOIMMUNITY AND HYPERINFLAMMATION IN PATIENTS WITH RAG DEFICIENCY


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Background:

Hypomorphic variants in recombinase activating gene (RAG) cause a spectrum of immune pathology including profound inflammation and autoimmunity.

Methods:

We compiled the largest cohort to date of 50 RAG deficient patients with hypomorphic mutations and non-infectious pathology.

Results:

The most common autoimmune features were cytopenias (80%), inflammatory skin disorders (38%), granulomas (28%) and neuropathies (12%). Cytopenias were the earliest observed autoimmune complication with median ages of onset 23, 24, and 42 months for autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP) and autoimmune neutropenia (AN), respectively. Cytopenias preceded the clinical diagnosis of immunodeficiency and molecular diagnosis of RAG deficiency with median ages of 33 and 62 months, respectively. Cytopenia occurred after infection or live viral vaccination in 9 patients (18%). Two or more autoimmune complications were documented in 32 patients (64%). A total of 15 patients (30%) were deceased at a median age of 10.25 years, mostly secondary to multi-organ failure and/or sepsis (60%). 34 patients (68%) had received hematopoietic stem cell transplant (HSCT) (median of 6.0 years). Autoimmunity was a secondary indication for transplantation in 48% of cases. The outcome of treatment strategies for autoimmune cytopenias was guarded with limited responses to first-line IVIG and steroids (20.8% for AIHA, 21.4% for ITP, and 0%
for AN), often incomplete or temporizing responses to second-line biologics, and a frequent need to progress to HSCT (50% for AIHA, 50% for ITP, and 64% for AN).

Conclusions:

Hypomorphic RAG deficiency may present with a wide spectrum of treatment-refractory autoimmune and inflammatory complications that may warrant HSCT.
HPV-DRIVEN WARTS AND INTRAEPITHELIAL NEOPLASIA POST-HSCT IN DCML/MonoMAC SYNDROME

Background:
Patients with DCML/MonoMAC syndrome due to GATA2 mutations suffer from HPV-driven warts and anogenital intraepithelial neoplasias. The outcome of HPV-driven disease following haematopoietic stem cell transplant (HSCT) in this cohort is not well known.

Methods:
Review of outcomes post-HSCT on HPV-driven warts and intraepithelial neoplasias in the DCML/MonoMAC patients.

Results:
5/6 patients with DCML/MonoMAC had identified mutations in GATA2. All 6 had palmo-plantar warts with no spontaneous resolution or treatment responses pre-HSCT and one partial response to acitretin. 4/6 patients had HPV+ cervical and vaginal-intraepithelial neoplasia (CIN/VIN), 2/6 had anal intraepithelial-neoplasia (AIN). 3/6 patients underwent HSCT, 1/6 is referred for HSCT, 2/6 died of infections before the genetic diagnosis. Of all 3 patients who have undergone HSCT with reduced intensity conditioning, 2/3 had AIN/CIN/VIN pre-HSCT and one had palmo-plantar warts only. One patient developed recurrent anogenital warts and CIN grade-3 despite good reconstitution and full donor chimerism following HSCT 12 years ago. Another patient, also with good immune reconstitution and full donor chimerism, has persistent HPV+ vulval lesions 3.5 years post-HSCT despite extensive surgery for VIN/AIN early post-HSCT. The third patient, with mixed chimerism, has reduced but persistent palmo-plantar warts 3 years post-HSCT. One patient received Gardasil given 6 years post-HSCT but showed no improvement.

Conclusions:
We observed variable outcomes post-HSCT. Complete resolution of warts and intraepithelial neoplasias was not observed. Gardasil vaccination failed to induce clinical improvement in one vaccinated patient. Longer follow-ups and larger patient cohorts are necessary to determine whether current HSCT protocols can effectively eradicate HPV-disease in DCML/MonoMAC syndrome.
LONG-TERM FOLLOW-UP OF IRANIAN LRBA DEFICIENCY COHORT

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Background:

LPS-responsive beige-like anchor protein (LRBA) deficiency is a combined immunodeficiency caused by mutation in LRBA gene. The patients have a variety of clinical symptoms including hypogammaglobulinemia, recurrent infections, autoimmunity and enteropathy.

Methods:

A total of 17 LRBA-deficient patients were enrolled in this cohort study. For all patients’ demographic information, clinical records and laboratory data were collected.

Results:

Hypogammaglobulinemia were reported in 82.4%, CD4+ T cell deficiency in 29.4% and CD19+ B cell deficiency in 64.7% patients. All patients have history of infectious complications; pneumonia was the most common occurring infection. A history of lymphoproliferative disorders was observed in 82.3%, enteropathy in 76.5%, and autoimmunity 76.5% of patients.

Conclusions:

LRBA deficiency has a very broad and variable phenotype and should be considered especially in children with early onset hypogammaglobulinemia, severe autoimmunity and recurrent respiratory tract infections.
E-POSTER DISCUSSION 2: IMMUNE DYSREGULATION DISORDERS

ESID7-0211

NATURAL KILLER CELL MATURATIONAL AND FUNCTIONAL ALTERATIONS IN CTLA-4 HAPLOINSUFFICIENCY

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Background:

CTLA-4 haploinsufficiency represents a complex clinical and immunological disorder. Viral infections, as well as EBV-associated lymphomas have been described in affected patients. While T and B cells have been studied in CTLA-4 haploinsufficiency, data on Natural Killer (NK) cells from these patients are lacking.

Methods:

Maturational and functional evaluation of NK cells from affected patients and healthy controls were performed.

Results:

The evaluation of the expression pattern of activating and inhibitory NK cell receptors, on human NK cells (both CD56bright and CD56dull subsets) allowed us to discriminate NK cell subsets based on their maturational and functional status. While the expression of activating NK cell receptors (NCRs, NKG2D, NKG2C), CD57,CD62L was similar to the healthy controls, CXCR1 and KIRs presented a different pattern of expression when compared to healthy controls. Of interest, CTLA-4 mutated NK cells showed an important expansion of the immature CD56bright CD16-/int subset, which represents the NK subset that produces high levels of IFN-γ when adequately stimulated. Thus we then investigated functionally CTLA-4 mutated NK cells. While we did not observe degranulation defects in the mutated NK cells, we found an important defect in IFN-γ production upon IL-12 and IL18 stimulation in the CTLA-4 mutated NK cells when compared to healthy controls, in contrast with the expansion of the CD56bright CD16-/int subset observed in CTLA-4 mutated NK cells.

Conclusions:

Our data show for the first time functional and maturational alterations in CTLA-4 mutated NK cells. Further studies are ongoing to better characterize NK cells in CTLA-4 haploinsufficiency.
Background:

Evans syndrome (ES) is a rare autoimmune disorder of children whose underlying context is unknown. A collaborative national network aims to propose a clinical classification of children with ES, based on our strategy for searching genetic etiologies.

Methods:

The clinical course of patients diagnosed between 1981 and 2015 presenting with non-selected early-onset ES was reviewed and preliminary genetic studies of those patients were conducted by direct Sanger sequencing, target gene panel NGS-sequencing or whole exome sequencing.
**Results:**

The clinical course of 155 children with early onset of ES from 26 centers revealed additional immune manifestations suggesting an underlying immune dysregulation in 78% of them. Preliminary genetic studies identified a disease-causing genetic defect for 15 patients in 6 genes: 1 del 22q11, 4 TNFRSF6 gene, 5 cytotoxic-T lymphocyte antigen-4 gene, 2 STAT3, 2 lipopolysaccharide-responsive beige-like anchor and 1 somatic mutation in KRAS. Systemic lupus erythematosus was diagnosed during the course in 13 children. Presenting features as lymphoproliferation, hypogammaglobulinemia or pulmonary manifestations were significantly associated with genetic defects. With a median follow-up from initial diagnosis of 7.8 years (0.1-28.8), 71% of children required one or more than one second-line immune treatments and 10% died.

**Conclusions:**

Childhood ES could be secondary to an underlying immune defect in 78% of cases. Some major clinical manifestations suggest a known genetic defect, and should prompt paediatricians to perform genetic screening, starting with the growing pool of genes involved in PIDs with autoimmunity. This will have direct consequences on patient’s management.
E-POSTER DISCUSSION 2: IMMUNE DYSREGULATION DISORDERS

ESID7-0316

ANALYSIS OF GUT MICROBIOTA IN PRIMARY IMMUNODEFICIENCIES AND IMMUNE DYSREGULATION SYNDROMES

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Background:

The immune system plays a fundamental role in the maintenance of a mutualistic host-intestinal microbiota relationship. Moreover, the gut microbiota composition is involved in the development and regulation of the immune system. Autoimmune enteropathy is a pivotal feature in many primary immunodeficiencies (PID) and congenital immune dysregulation syndromes. These diseases are rapidly fatal, unless treated by allogeneic stem cell transplantation (HSCT). We investigated whether an altered homeostasis of the intestinal tract may influence the disease phenotype and clinical course.

Methods:

We collected whole blood and faecal samples of thirty-two patients with severe combined immunodeficiency and congenital immune dysregulations syndromes. Six patients underwent HSCT and were monitored over the treatment. Immunophenotypic analysis was performed on blood samples of patients enrolled, at diagnosis, before and at different times after HSCT. Microbiota composition of faecal samples was analyzed by Next-Generation Sequencing (NGS). Faecal short chain fatty acid (SCFAs) were quantified by UPLC/MS.

Results:
In patients we observed an alteration of gut microbiota compared with healthy subjects and an inverted correlation among biodiversity and severity of intestinal clinical manifestations. Treatment with HSCT induce a modification of gut flora with a recover of a normal composition in patients with disease resolution and a complete disruption of biodiversity in subjects with no successful treatment.

Conclusions:

Dysbiosis appears to be common feature of these patients, as consequence of their genetic defect and/or clinical status requiring pharmacological prophylaxis. Preliminary results indicate a correlation between gut microbiota and HSCT outcome, suggesting the suitability of the microbiome as a biomarker.
FUNCTIONAL ASPLENIA AND SPECIFIC POLYSACCHARIDE ANTIBODY DEFICIENCY IN A GIRL WITH STING-ASSOCIATED VASCULOPATHY WITH ONSET IN INFANCY (SAVI) PRESENTING WITH RECURRENT PNEUMOCOCCAL SEPSIS

Background:

STING-associated vasculopathy with onset in infancy (SAVI) is a disease caused by gain-of-function mutations in the gene TMEM173. TMEM173 encodes an important adaptor protein called stimulator of IFN genes (STING). GOF mutations cause increased transcription of IFNβ and cause autoinflammation. Clinically, patients present with a syndrome with lupus-like vasculitis and vascular damage affecting fingers, toes, ears, and nose and with interstitial lung disease. Some patients suffer severe infections.

Methods:

Here we present a case report of a now 10-year-old girl diagnosed with SAVI including description of immunodeficiency assessment.

Results:

A girl with SAVI presented at age 3 months with pneumococcal sepsis and suffered a second episode of pneumococcal sepsis at age 6 years. Subsequent evaluation for immunodeficiency's associated with invasive pneumococcal disease was performed. Total immunoglobulin levels were normal. Assessment of vaccination responses showed specific polysaccharide antibody deficiency (pneumococcal serum antibody titer 28 days post vaccination with 23-valent pneumococcal polysaccharide vaccine > 1 microg/mL in 0 of 4 serotypes tested). Blood smear evaluation revealed the presence of Howell Jolly bodies consistent with functional asplenia. The patient was started on subcutaneous immunoglobulin treatment and antibiotic prophylaxis to prevent further infections. In addition, she was started on ruxolitinib to treat the inflammatory syndrome.

Conclusions:

This case report demonstrates how vasculitis associates with SAVI may result in splenic dysfunction and immunodeficiency. Functional asplenia has previously been described associated with vasculitis.
in patients with systemic lupus erythematosus. Clinicians caring for patients with SAVI should include immunodeficiency assessment in their patient evaluation.
E-POSTER DISCUSSION 2: IMMUNE DYSREGULATION DISORDERS

ESID7-0461

GENERATION OF FUNCTIONAL NEUTROPHILS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS AS A MODELING PLATFORM FOR AUTOINFLAMMATORY DISEASES

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Background:

The continuous generation of myeloid cells and neutrophils is of great interest to address basic research questions and to study cell function in health and disease. Primary human neutrophils present with very short ex vivo life span which significantly hampers in vitro studies. Thus, efficient and continuous generation of mature neutrophils from human pluripotent stem cells (hPSCs) using xeno-product free differentiation models is of particular interest.

Methods:

Here, we show efficient generation of mature neutrophils from human induced pluripotent stem cells (hiPSCs) free from murine stromal cells. We established and optimized an embryoid body (EB)-based differentiation protocol for hiPSCs generated from peripheral blood-derived (PB) CD34+ HSCs. Additionally, we differentiated PB-derived CD34+ HSCs into mature neutrophils and compared different culture conditions. We compared these cells phenotypically with neutrophils derived from cord blood of neonates and determined the neutrophil phenotype using flow cytometry and cell morphology as well as functional assays such as phagocytosis and reactive oxygen species (ROS).

Results:

HiPSC-derived granulocytes can be identified clearly by their morphology and the segmented nucleus. Surface markers such as CD66b and CD11b could be detected on mature cells. Functional features such as phagocytosis, ROS-production after LPS-stimulation and cytokine secretion were found in line between neutrophils from hiPSCs and peripheral blood.

Conclusions:

Hereby, we propose a disease-modeling platform that enables the continuous generation of patient-specific cells of the innate immune system for fundamental research questions targeting inflammation and pathophysiological features of autoimmune diseases.
NEUTROPENIA IN PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY: A RARE EVENT ASSOCIATED WITH SEVERE OUTCOME.
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Background:
Common variable immunodeficiency (CVID) is characterized by recurrent infections and defective immunoglobulin production. Neutropenia is a rare event during CVID.

Methods:
The French DEFI study enrolled patients with primary hypogammaglobulinemia. Patients with neutropenia (< 1.5*10⁹/L) were retrospectively analyzed.

Results:
From 2004 through 2010, a total of 473 CVID patients were enrolled (followed-up until June 2015). Among 473 patients with CVID, 16 patients displayed neutropenia. Sex ratio (male/female) was 10/6. Median age was 27 [0-59] at the diagnosis of neutropenia. Four CVIDs were familial forms, others were sporadic. The most frequent symptoms, except infections, were autoimmune cytopenia (75%). Ten patients have lymphoproliferation. Two patients were in the infection only group and the others belonged to one or several other groups. The mean level of IgG was 2.1g/l[0.35-4.4]. Most patients presented increased numbers of CD21low CD38low B-cell as already described in CVID autoimmune cytopenia group. Neutropenia was considered autoimmune in 10 cases. We completed by a large panel of gene involved in primary immune deficiencies analysis for 7 patients by NGS. We didn’t found any known causing disease mutation in genes coding for PI3K-d, CTLA-4 or LRBA. Five patients died during the follow-up, which represent an increased percentage of death compared to the whole DEFI group (31.3% vs. 3.4%, P<0.05).

Conclusions:
Neutropenia is a very rare event in CVID. It is generally associated with another cytopenia and presumably autoimmune. It is also coupled with more severe hypogammaglobulinemia. It could be a prognosis factor considering the patients presentation and the rather high rate of deaths.
E-POSTER DISCUSSION 3: CLINICAL PID I

ESID7-0111

PARODONTAL DISEASE IN PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY (CVID)
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Background:

CVID is the most frequent symptomatic primary immunodeficiency of the adulthood. CVID patients are susceptible to many oral diseases. The aim of our study was to evaluate and characterize incidence, diagnosis and possible treatment of parodontal disease in these patients.

Methods:

We enrolled 30 patients with CVID and 30 healthy controls in a prospective randomized controlled trial. We collected data about patient’s history and physical examination of the oral cavity. For each patient we registered indexes of periodontal disease (DMFT: Decayed-Missing-Filled-Teeth, PCR: plaque-control-record, BOP: bleeding-on-probing) and measure of stimulated and not stimulated salivary flow.

Results:

The most frequent oral disease detected was Recurrent aphthous stomatitis (63% of CVID patients vs 3.3% of healthy people), characterized by burning red areas, bubbles and small open ulcers. Herpes labialis was another frequent disease (43% of CVID patients vs 13.3% of healthy people) with swollen and reddened vesicles cluster on lips. Angular cheilitis, an inflammatory disease that affects corners of the mouth, often bilaterally, was reported in 26% of CVID patients vs 10% healthy people. Oral candidosis (20% of CVID patients) was the most common fungal infection of the mouth in these patients. More rare was the detection of Periodontitis (5-10% of CVID patients).

Conclusions:

CVID patients, compared to healthy people, show a higher rate of oro-dental manifestations, such as recurrent aphthous stomatitis, herpes labialis, oropharyngeal candidiasis, gingivitis and early onset aggressive periodontitis. These complications can negatively affect quality of life, therefore, dental preventive treatments through inclusion of these patients in a follow-up program, are needed.
E-POSTER DISCUSSION 3: CLINICAL PID I

ESID7-0115

EVALUATION OF HEALTH-RELATED QUALITY OF LIFE IN COMMON VARIABLE IMMUNODEFICIENCY USING CVID_QoL QUESTIONNAIRE: IMPACT OF CLINICAL, IMMUNOLOGICAL AND THERAPY-RELATED FACTORS ON THE BURDEN OF DISEASE

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Background:

CVID_QoL is the first validated disease-specific tool to assess health related Quality of Life (HRQoL) Common Variable Immunodeficiency. The instrument includes a CVID_QoL global score and three dimensions: emotional-functioning (EF), relational-functioning (RF), and gastrointestinal and skin symptoms (GSS). This is the first study assessing the impact of clinical, immunological and therapeutical factors on HRQoL in CVID patients by CVID_QoL questionnaire.

Methods:

154 adults CVID completed the questionnaire. To avoid bias, patients who received a diagnosis of malignancy in the preceding year and subjects who were diagnosed as CVID less than 6 months before were excluded. Therapy setting, clinical and immunological data were collected.

Results:

Age was related to CVID_QoL and its dimensions EF and RF. To be female, taking more than two medications/day, having diagnosis of cancer, having an unexplained-persistent entheropathy, having an autoimmune complication, to be underweight or to be admitted in hospital for infection and to be affected by other chronic disease were factors associated to higher CVID_QoL score. CVID_QoL and EF were directly related with the number of infections in the year preceding the study. The experience of pneumonia, of relapsing episodes of diarrhea (≥ 4 for year), sinusitis, and bronchitis (≥ 2 for year) was associate to more severe CVID_QoL scores. No difference were observed between patients receiving SCIG, IVIG or both; no correlation was found between IgG trough level or Igs serum level at diagnosis and CVID_QoL scores.

Conclusions:

This study provides the impact of immunological, clinical and therapeutical factors on the burden of disease in patient with CVID.
E-POSTER DISCUSSION 3: CLINICAL PID I

ESID7-0130

BCG COMPLICATIONS IN PATIENTS WITH PRIMARY IMMUNODEFICIENCIES UNDERGOING STEM CELL THERAPY/THYMIC TRANSPLANT

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4MHRA, Paediatric Unit, London, United Kingdom

Background:

BCG vaccination is contraindicated in immunocompromised children. However as it is given at birth, many children with primary immunodeficiencies (PID) are vaccinated prior to diagnosis with PID. There are currently no guidelines for the management or prevention of BCG disease in these children.

The aim of this study was to evaluate the outcome following BCG vaccination in children with PID in the pre- and post-transplant period.

Methods:

Retrospective case-note review of all BCG vaccinated PID patients at Great Ormond Street Hospital from 2003-2015.

Results:

Of 397 PID patients, 48 children (27 males) with mean age 5.84±1.39 (0-48 months) had received BCG. 35/48 had SCID. 38/41 were vaccinated within 28 days of birth. 34/48 received HSCT, 6/48 had gene therapy and 2/48 thymic transplant. 23/48 developed clinically active BCG disease. Children who had received BCG but had no evidence of clinically active disease were managed with 2 or 3 drug prophylactic regimens in our cohort.

BCG induced immune reconstitution syndrome (IRS) and BCG reactivation post HSCT occurred in 9/42 patients. Overall mortality was 6.25%; however, no deaths were directly associated with BCG complications.

Conclusions:

2/3 of our cohort patients presented with BCG disease pre-transplant highlighting that BCG vaccination causes significant morbidity in patients with PID requiring long term treatment with multiple antibiotics before and after transplantation. The vast majority of patients with no evidence of BCG disease received antimycobacterial prophylaxis. Neonatal screening for PID in targeted populations, in conjunction with delayed BCG vaccination, should be considered to protect these vulnerable infants.
E-POSTER DISCUSSION 3: CLINICAL PID I

DISTINGUISHING CLINICAL CHARACTERISTICS OF CVID WITH GASTROINTESTINAL MANIFESTATIONS: AN OVERVIEW OF THE USIDNET DATA.

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Background:

Gastrointestinal symptoms are common in CVID patients and often severe. We report clinical characteristics of CVID patients in the USIDNET registry with GI conditions.

Methods:

The USIDNET Registry was queried for information about CVID and related GI conditions.

Results:

704/1570 (45%) CVID patients in the USIDNET registry have had a GI condition (GI+). The most common are: reflux (15%) diarrhea (10% chronic, 10% intermittent), abdominal Pain (10%), IBD (Crohn's, UC and other colitis, enteritis) (10%), liver disease (8%), constipation (5%). The most common infectious organisms in the GI+ group were C. Difficile, Giardia, Salmonella, CMV, Hepatitis, H. Pylori, Pseudomonas, Rotavirus, EBV, H. Influenza. The most common autoimmune manifestations in the GI+ group were thyroiditis, cytopenias, DM1, uveitis, Sjogrens, SLE, Raynauds, vasculitis, sarcoid and rheumatoid arthritis.

When compared with CVID without GI condition (GI-), there was no significant difference in gender or race between GI+ and GI- (58% female 42% male, ~80% Caucasian) or age of diagnosis (29.2 yrs vs 30.6 yrs respectively.) The GI+ group showed younger age of symptom onset (17.7 yrs vs 22.6 yrs), longer time from symptom onset to diagnosis (9.5 vs 7.7 yrs), and younger age of death (41 yrs vs 48 yrs). Reports of autoimmune disease were higher in the GI+ group (12.9% vs 4.6%). The GI+ group was more likely to have received antibiotic (42% vs 19%), anti-inflammatory (35% vs 21%) and or immunosuppression (25% vs 17%).

Conclusions:

GI conditions in CVID are common. Earlier diagnosis and targeted treatments require a better understanding of the disease spectrum.
E-POSTER DISCUSSION 3: CLINICAL PID I

ESID7-0348

GENERATION OF A SCORING TOOL TO GUIDE SCREENING FOR GATA2 DEFICIENCY IN PATIENTS WITH ASSOCIATED CLINICAL MANIFESTATIONS.
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Background:
Heterozygous germline mutations in GATA2 are associated with a plethora of clinical manifestation. The limited knowledge amongst adult caretakers, its diverse presentation, and potential late-onset, account for delayed diagnosis. Also prevalence is poorly circumscribed.

Objectives:
To generate a scoring tool to guide screening, to evaluate the prevalence in specific patient populations, and to increase awareness of GATA2 deficiency and associated morbidities in specialties outside primary immunodeficiency.

Methods:
A weighted scoring tool was developed using clinical and laboratory parameters based on reported associations with GATA2 deficiency ranging from 0-29 points.

Results:
122 adult patients (111 MDS, 9 chronic myelo-monocytic leukemia, 1 aplastic anemia, and 1 myelofibrosis patient) were included. Median age at diagnosis was 67y (range 16-88, 14 were diagnosed before age 50). In 17 AML developed, 28 underwent hematopoietic stem cell transplantation (HSCT). At the time of analysis 30 had deceased. Monocytopenia was observed at least once in 91 patients (75%) and was present in >50%, >75% and >95% of measurements in respectively 20 (16%), 9 (7%), and 2 (2%) patients. Median GATA2-score in the 122 patients was 5 (range 0 – 20) with top 10% scoring ranging from 13-20 points. GATA2-score was significantly higher in patients diagnosed with MDS before the age of 50, who developed AML or underwent HSCT (p<0.01 Mann-Whitney-U-test). Sequencing results are currently anticipated to enable further validation.

Conclusions:
Preliminary results demonstrate a wide range of scores amongst adult, mostly MDS patients and correlation with sequencing information is awaited to validate this GATA2-scoring tool.
E-POSTER DISCUSSION 3: CLINICAL PID I

ESID7-0435

ANALYSIS OF GENETIC DATA FROM THE UKPID REGISTRY 2017
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Background:

Over 300 genes are already known to cause Primary Immune deficiency (PID). Genetic discovery is increasing through the work of the NIHR Bioresource (BRIDGE) and 100,000 genomes projects. The UKPID Registry contains data on over 4000 PID patients from 37 UK immunology centres, including details of genetic mutations, many of whom have been entered into these national genotyping projects.

Methods:

Data for current patients in the UK registry were analysed for known (clinical laboratory confirmed) genetic defects. Additional breakdown of findings for those individuals over and under 18 years of age and correlation with clinical diagnoses is given.

Results:

1069 of 4426 patients on the UKPID registry have a recorded genetic mutation. The highest single disorder with genetic information available is X-linked agammaglobulinaemia with Btk mutations. (See Table 1).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of Patients</th>
<th>Estimated Prevalence/100K Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Btk (x-linked)</td>
<td>173</td>
<td>0.26</td>
</tr>
<tr>
<td>Del 22q11.2</td>
<td>85</td>
<td>0.13</td>
</tr>
<tr>
<td>GP91-phox(CYBB)</td>
<td>59</td>
<td>0.09</td>
</tr>
<tr>
<td>ATM</td>
<td>49</td>
<td>0.07</td>
</tr>
<tr>
<td>CD40L (CD154)</td>
<td>47</td>
<td>0.07</td>
</tr>
<tr>
<td>WASP (x-linked)</td>
<td>46</td>
<td>0.07</td>
</tr>
<tr>
<td>Gamma C (x-linked)</td>
<td>39</td>
<td>0.06</td>
</tr>
<tr>
<td>STAT3</td>
<td>37</td>
<td>0.06</td>
</tr>
<tr>
<td>ADA</td>
<td>28</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table 1. 10 most prevalent genetic defects in UK cohort.
Conclusions:

Estimable prevalence of genetic diagnoses in the UK cohort are lower for some diagnoses than the expected case load, however, data is skewed towards those patients with an extreme phenotype, or confirmation has been sought as part of BMT or gene therapy workup. A more complete analysis of the genotyped data confirms the more prevalent findings currently expected in CVID from other cohorts i.e. TACI, NFKB2 and ICOS.
Background:

Immunoglobulin therapy for X-Linked Agammaglobulinaemia (XLA) is composed almost entirely of IgG, and lacks the IgA/IgM isotypes. The subsequent risk of recurrent sinopulmonary infections and the development of bronchiectasis remains a major concern for patients and clinicians alike. However, modern data detailing incidence and progression of bronchiectasis in XLA is lacking.

Methods:

We performed a retrospective case note analysis of 14 XLA (4 children, 10 adult) patients managed at our centre.

Results:

8/14 XLA patients have developed bronchiectasis. Patients with bronchiectasis are older (32.11 vs 21.59 years) and had a higher median age of diagnosis (7.0 vs 2.64 years). The median age of diagnosis for the complete group is 3.3 years which has improved over time. Mean IgG trough levels pre-bronchiectasis are comparable to those patients with no current lung disease (7.74 vs 7.75 g/L). Median pre-bronchiectasis infection incidence, however, was higher compared to those patients with no current lung disease (0.84 vs 0.61/year). 3 patients required radical surgery (2 pneumonectomies, 1 lung transplant). A further 3 have radiological progression of their bronchiectasis. First recorded FEV1 Z scores for patients who would later develop bronchiectasis were lower than those patients with no current lung disease (-2.06 vs -0.27). This lung function has remained stable with the last recorded median FEV1 Z scores being -2.10 and -0.92.

Conclusions:

The limitations with current therapy may mean bronchiectasis is inevitable for some patients. New strategies including newborn screening may offer promise to future patients, but more data are first needed to fully evaluate current management.
PARTIAL JAK1 DEFICIENCY PREDISPOSES TO MYCOBACTERIAL INFECTION BUT DOES NOT AFFECT VIRAL SUSCEPTIBILITY

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Background:

We recently identified loss of function mutations in Janus Kinase 1 (JAK1) as a novel cause of Mendelian susceptibility to mycobacterial diseases in a patient with immunodeficiency, atypical mycobacterial infections and urothelial carcinoma. JAK1 belongs to a family of tyrosine kinases that mediates signaling from different cytokine receptors, showing a broad defect in multiple immune response pathways.

Methods:

We investigated the role of JAK1 in (1) myeloid cells during mycobacterial infection in vitro, using a JAK1-deficient THP1 cell line generated using short-hairpin RNA technology. (2) Fibroblasts of the patient with JAK1 loss of function mutations to test antiviral response in vitro.

Results:

JAK1 deficient cells exhibit reduced STAT1 phosphorylation following IFNγ stimulation and reduced induction of expression of interferon-regulated genes, demonstrating loss of JAK1 function.

Following infection with BCG and Salmonella, JAK1-deficient cells showed reduced clearance of intracellular bacteria compared to control. Apoptosis and acidification capacity of the phagosomal compartment, mechanisms that promote mycobacterial killing, were reduced in the knock down cells after IFNγ stimulation.

We observed suppression of viral infection after IFNα and IFNγ stimulation in both patient and control fibroblasts. These results are consistent with the lack of clinically severe viral infections in our patient, suggesting that partial JAK1 activity may be sufficient to develop antiviral immunity.

Conclusions:
We have shown that JAK1 deficiency impairs immune cell function through the IFNγ pathway and demonstrate a critical role in host defence against mycobacterial infection. This should be considered during the administration of biologic drugs affecting the JAK1/STAT1 pathway.
THYMIC EPITHELIAL CELLS REQUIRE P53 TO SUPPORT THEIR LONG-TERM FUNCTION IN THYMOPOIESIS IN MICE

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Background:

Thymic epithelial cells (TECs) provide crucial microenvironments for T-cell development and tolerance induction. As the regular function of the thymus declines with age, it is of fundamental and clinical relevance to decipher new determinants that control TEC homeostasis in vivo. Beyond its recognized tumor suppressive function, p53 controls several immunoregulatory pathways.

Methods:

To study the autonomous role of p53 in thymic epithelial functioning, we developed and analyzed mice with conditional inactivation of Trp53 in TECs (p53cKO).

Results:

We report that loss of p53 primarily disrupts the integrity of medullary TEC (mTEC) niche, a defect that spreads to the adult cortical TEC (cTEC) compartment. Mechanistically, we found that p53 controls specific and broad programs of mTEC differentiation. Apart of restraining the expression and responsiveness of RANK that is crucial for mTEC differentiation, deficiency of p53 in TECs altered multiple functional modules of the mTEC transcriptome, including tissue-restricted antigen expression. As a result, p53cKO mice presented premature defects in mTEC-dependent regulatory T cell differentiation and thymocyte maturation, which progressed to a failure in regular and regenerative thymopoiesis and peripheral T-cell homeostasis in the adulthood. Lastly, peripheral signs of altered immunological tolerance unfold in mutant mice and in immunodeficient mice that received p53cKO-derived thymocytes.

Conclusions:

Our findings position p53 as a novel molecular determinant of thymic epithelium function throughout life.
E-POSTER DISCUSSION 4: MECHANISMS OF IMMUNE DYSREGULATION I

ESID7-0140

IMPLICATION OF INTERLEUKIN 23 IN THE INFLAMMATORY EVENT OCCURRING IN MYASTHENIA GRAVIS THYMUS

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Background:

Myasthenia gravis is an autoimmune disease characterized by an inflammatory process in the thymus in which an overexpression of IL17 related genes and dysfunctions in Treg and Th17 cells have been reported (Gradolatto et al., 2014). Furthermore, differentiation of T-cells towards a pathogenic Th17 phenotype is regulated by a group of cytokines such as Interleukin 6, 21, 23 (IL-6, IL-21, IL-23) and TGF-β3 (Lee et al., 2012).

Methods:

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Results:

Here, we investigate the level of expression of the cytokines involved in Th17 differentiation in AChR+ MG thymuses. We found that IL-6, IL-21, IL-23 and TGF-β3 are significantly overexpressed. Nevertheless, only IL-23 remain overexpressed in periphery. Additionally, we observed that MG thymic epithelial cells (TEC) overexpress IL-23. Consequently we analyze the factors that can induce IL-23 overexpression in normal TEC cultures. We found that Poly(I:C) induce the expression of IL-23 through IFN type 1 (overexpressed in MG thymuses). While interferon type 2 or LPS had no effect. More, expression of IL-23 in TEC is also stimulated by IL-17.

Conclusions:

Our results show that overexpression of IL-17 in MG thymuses is probably due to the overexpression of the cytokines that favor the pathogenic Th17 phenotype, especially by IL-23. We also showed that deregulation of IL-23 expression by TEC is induce by IFN type 1 and IL-17. Hence, in AChR+ MG thymuses, differentiation of Th17 cells is maintained by a pro-inflammatory microenvironment lead by IL-23. This creates an inflammatory loop between IL-23 expression by TEC and IL-17 producing cells explaining the chronic inflammation in MG thymuses.
Utilizing novel 14-colour flow cytometry techniques to gain new insights into the pathogenesis of Aspergillus nidulans infection in chronic granulomatous disease (CGD)

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Background:

Invasive aspergillosis is the commonest cause of death in CGD. Aspergillus nidulans accounts for a third of cases and has a higher case-fatality rate than A. fumigatus. Little is known about disease pathogenesis or why A. nidulans demonstrates a unique predilection for CGD patients.

Methods:

CGD (CYBB/C57BL6) and WT (C57BL6) mice were infected with A. fumigatus or A. nidulans by intratracheal instillation of 5x10⁴ conidia. Pulmonary immune response was investigated at set time points following infection using 14-colour FACS-analysis of lung digestes, multiplex bead assays and histology.

Results:

Excessive and sustained neutrophil recruitment was demonstrated in CGD mice within 24hrs of A. fumigatus (p<0.0001) and A. nidulans infection (p<0.01). CGD mice, in contrast to WT mice, failed to recruit eosinophils following A. fumigatus (p<0.0001). Delayed recruitment was observed with A. nidulans (p<0.001 at d3 p.i.). Almost complete loss of alveolar macrophages was seen in CGD mice following infection (p<0.001 and p<0.01 at d7 p.i. for A. fumigatus and A. nidulans respectively). CD3+ cells were both relatively (p<0.01) and absolutely (p<0.0001) reduced in uninfected CGD mice compared with WT. Robust T-cell recruitment was demonstrated following infection. Exaggerated and sustained IL-1α and IL-1β production was demonstrated during both infections with levels increasing 100-fold by d17 p.i. (p<0.0001 both cytokines). Significant differences in TNF-α, IFN-γ, IL-17, IL-10 and IL-12 were seen between the two infections.

Conclusions:

Utilizing advances in multi-colour flow cytometry has facilitated an in-depth characterisation of the pulmonary innate response to A. nidulans infection in a murine model of CGD.
NEUTRALIZING AUTOANTIBODIES AGAINST IL-6 ARE A RARE RISK FACTOR FOR PYOGENIC BACTERIAL INFECTIONS

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Background:

Interleukin-6 (IL-6) is among the most important mediators of the acute-phase response and is released early in the course of bacterial infections. Patients with impaired IL-6 production as in inherited IRAK-4- or MyD88-deficiency or impaired IL-6Rα-STAT3-dependent signaling are at increased risk for severe pyogenic bacterial infections (PBIs) and in particular for staphylococcal skin infections. Neutralizing autoantibodies against IL-6 (anti-IL-6-nAAbs) have been previously described in three patients suffering from severe bacterial infections caused by S. aureus, S. intermedius and E. coli.

Methods:

In our study, we determined the prevalence of anti-IL-6-nAAbs in a cohort of patients with bacterial infections (n = 348), a cohort of patients with autoimmune diseases (n = 564), a cohort of patients with mycobacterial infections (n = 366) and three cohorts of individuals without any PBI (n = 988). All patients and individuals were investigated for specific anti-IL-6-AAbs by using an anti-human-IL-6-IgG-antibody ELISA. The neutralizing capacity was determined by quantifying the STAT3-phosphorylation in human CD4+ T-cells as well as in murine Ba/F3-gp130 and Ba/F3-gp130/IL-6Rα cells after stimulation with IL-6. Binding sites were subsequently examined by epitope mapping.
Results:

We identified a further patient with anti-IL-6-nAAbs and the first who developed a life-threatening pneumonia caused by *S. pneumoniae*. In addition we discovered anti-IL-6-nAAbs in two adults with autoimmune diseases, in one obese adolescent without PBlS as well as in three mothers with premature rupture of membranes in the last trimester of pregnancy.

Conclusions:

We assume that anti-IL-6-nAAbs are a risk factor for PBlS yet with incomplete penetrance.
E-POSTER DISCUSSION 4: MECHANISMS OF IMMUNE DYSREGULATION I

ESID7-0278

THE ROLE OF ACTIVATING TMEM173/STING MUTATIONS IN THE CONTROL OF THE ADAPTIVE IMMUNE RESPONSE

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Background:

The recognition of microbial nucleic acids is a major mechanism by which the immune system detects pathogens. The cGAS–STING pathway is a component of the innate immune system that functions to detect the presence of cytosolic DNA and trigger expression of inflammatory genes. DNA is normally found in the nucleus of the cell. Localization of DNA to the cytosol is associated with viral infection. Upon binding to DNA, cGAS generates 2′3′-cGAMP as an endogenous ligand to activate the stimulator of interferon gene, STING. Activation of STING can lead to its exit from the ER into the secretory pathway, leading to the phosphorylation of IFN regulatory factor 3 (IRF3) to allow for the production of type I interferons (IFNα and IFNβ).

Methods:

We performed proliferation assay, ISG signature and confocal microscopy on patient’s cells with activating TMEM173/STING mutations with exacerbated inflammation and autoimmunity.

Results:

This mutation led to the Golgi localization of STING, and to uncontrolled type-I IFN production by lymphocytes. Strikingly, we observed an in vitro proliferation defect of the patients’ primary T cells. This proliferative defect is independent of the type-I IFN receptor signaling.

Conclusions:

This is suggesting that STING activation controls lymphocyte proliferation. To better understand if there are other genetic events implied in the IFN-I production, we generated EBV transformed B-cell lines (B-EBV) from STING mutated patients and created MBD21 (cGAS), TMEM173 and TBK1 knockouts using CRISPR-Cas 9 technology. These tools will allow us, to decipher the molecular mechanisms by which STING is controlling lymphocyte proliferation and possibly self-tolerance.
LENTIVIRAL VECTOR-MEDIATED GENE COMPLEMENTATION OF PATIENT MONOCYTES AS A TOOL TO DISSECT THE ROLE OF ADA2 IN INFLAMMATION

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Background:

Patients with combined absence of interleukin-17 receptor A (IL-17RA) and adenosine deaminase 2 (ADA2) have been described to present with recurrent fungal mucocutaneous infections, chronic inflammation, vasculitis, and high levels of pro-inflammatory cytokine production. IL-17RA deficiency is responsible of chronic mucocutaneous candidiasis, while ADA2 deficiency (DADA2) results in a recently described autoinflammatory disease characterized by vasculopathy, systemic inflammation with livedoid skin rash, CNS involvement and immunodeficiency. The pathogenesis of DADA2 is still poorly understood.

We postulated that the chronic inflammation and the pro-inflammatory cytokine profile observed in our IL-17RA/DADA2-deficient patient's monocytes are the consequence of ADA2 deficiency. Whether or not the absence of IL-17 signaling modifies the phenotype of DADA2 in our patient remains to be investigated.

Methods:

We set out to test these notions by performing gene complementation of patient's cells using lentiviral-mediated gene transfer of the CECR1 and/or IL17RA coding sequences. Lentiviral vectors expressing ADA2 and IL-17RA under the transcriptional control of the human ubiquitin C promoter (UbC) were used to transduce patient's monocyte after exposure to viral-like particles containing Vpx (VLP-Vpx) to enhance the transduction efficiency.

Results:

With this approach, we achieved restoration of IL-17RA expression in ~ 30% of patient monocytes. The pro-inflammatory profile of gene-corrected and control cell populations will be assessed.

Conclusions:

These experiments may shed some light on the immunological mechanism underlying DADA2 and may elucidate the potential interplay between the IL-17 and ADA2 pathways. Moreover, these
investigations will provide a bench test for the development of gene therapy for IL-17 deficiency and DADA2.
LYMPHADENOPATHY DRIVEN BY TCR-Vγ8Vδ1 T-CELL EXPANSION IN FAS-RELATED AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS-FAS)


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Background:

Mutations in the FAS gene are linked to autoimmune lymphoproliferative syndrome (ALPS-FAS). This disease is associated with abnormally high counts of αβTCR+, CD4-CD8- double negative (DN) T-cells. Here, we report on two patients with ALPS-FAS, who respectively carried a novel (c.657delA) mutation and a previously described mutation in FAS. Whereas the DN T-cells in ALPS patients usually express αβTCRs, those isolated from our patients’ lymph nodes expressed γδTCRs. These γδT cells were highly proliferative and had a cytotoxic phenotype.

Methods:

Immunohistochemistry, flow cytometry and gene sequencing analysis were used to diagnose ALPS-FAS characterized by γδT cell infiltrates. We used next generation sequencing to study the TCR repertoire within expanded lymph nodes. Furthermore, we used in vitro assays to determine the sensitivity of Vδ1 T cells to therapeutics used in these patients.

Results:

The T cells within enlarged lymph nodes were restricted to Vγ8Vδ1-TCR usage, and had oligoclonal complementary-determining region 3 repertoires. We also established that Vδ1-T cell expansion is controlled by FAS-dependent apoptosis in vitro; this finding suggests that the massive accumulation of Vδ1-T cells in these two patients was linked to their FAS mutations. Lastly, we evidenced elevated levels of in vitro methylprednisolone, rapamycin and pyrimethamine resistance in Vδ1-T cells (relative to αβ-T cells).

Conclusions:
The discovery of FAS-dependent apoptosis in Vδ1-T cells makes the latter possible culprits for the lymphadenopathy observed in patients with FAS mutations. The cells’ rapamycin and methylprednisolone resistance should prompt clinicians to look for Vδ1-T cell proliferation in patients with treatment failure.
Mosaic ELANE mutation causing severe congenital neutropenia in an asymptomatic mother

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Background:
Severe congenital neutropenia (SCN) is an inherited disease characterized by low neutrophils count in the peripheral blood (PB) and maturation arrest in the bone marrow at the promyelocyte-myelocyte stage. Heterozygous mutations in the ELANE gene are the major cause of SCN, leading to an autosomal dominant inheritance.

Methods:
Direct sequencing of ELANE in a girl with SCN identified a heterozygous missense mutation: c.607G>C (p.G203R, formerly G174R). The same mutation was also detected in her asymptomatic mother with normal neutrophil count. We confirmed mutant variant ratio by sequence signals and measured the frequency of the mutant allele by subcloning in various cell types. We established ELANE-mutated and non-mutated induced pluripotent stem (iPS) cells from the mother’s T cells and compared granulopoiesis from these iPS cells.

Results:
In the sequence analysis of separated PB and nail tissue, the mutant variant was observed at about half frequency in CD14+ cells, CD3+ cells, CD19+ cells, CD56+ cells and CD34+ cells, and at low frequency in nail tissue. Conversely, it was not detected in neutrophils. In the subcloning analysis of extracted DNA from CD3+ cells and CD34+ cells, the mutant allele was identified in 37.5% and 38.1 %, respectively. Granulopoiesis from mutated iPS cells revealed little sensitivity to granulocyte-colony stimulating factor compared to it from non-mutated iPS cells.

Conclusions:
These results demonstrate that ELANE-mutated clones fail to develop into neutrophils. Mother’s normal neutrophil count in the PB suggests that ELANE-mutated clones do not affect non-mutated granulopoiesis, and which compensates for the absence of ELANE-mutated granulopoiesis.
E-POSTER DISCUSSION 5: LATE BREAKERS

ESID7-0502

TIPPING THE BALANCE: HOW MALT1 PROTEASE DEFICIENCY DISRUPTS IMMUNE HOMEOSTASIS

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Background:

The paracaspase MALT1 is a key regulator of canonical NF-kB activation downstream of multiple receptors in both immune and non-immune cells. In response to antigen-receptor triggering in B and T lymphocytes, MALT1 acts as part of the CARD11-Bcl10-MALT1 (CBM)-complex and promotes dual signaling outcomes via a scaffold function and via a protease function. Both MALT1 knockout (KO) and MALT1 protease-deficient (Malt1⁺/⁻PD) mice are characterized by a significant reduction in regulatory T cells, but in contrast to the rather immunodeficient phenotype of MALT1 KO mice, Malt1⁺/⁻PD animals develop a spontaneous multi-organ inflammatory pathology associated with expansion of effector T cells and lymphocytic infiltrates in various tissues. Additionally, Malt1⁺/⁻PD mice demonstrate elevated serum IgG1/IgE levels and lymphadenopathy at physiological barrier sites such as the intestine.

Methods:

Crossing to TCRtg, BCRtg and Foxp3-EGFP reporter mice, extensive FACS-based phenotyping and the use of germ-free Malt1⁺/⁻PD mice as well as ELISA-based methods were employed.

Results:

We provide evidence that T lymphocytes rather than B lymphocytes are the key drivers of the Malt1⁺/⁻PD pathology. Our data further indicates that the elevated serum IgG1 reflects uncontrolled B cell activation by intestinal microbiota, likely as a result of insufficient tolerance induction to commensal antigens and disrupted epithelial barrier functions. Consequently, we are currently addressing the effects of MALT1 protease deficiency on the non-immune compartment including epithelial cell function and barrier integrity.

Conclusions:

Overall, our data demonstrate a crucial role of the MALT1 protease for the maintenance of immune homeostasis and provide new insights into the immune alterations occurring in Malt1⁺/⁻PD mice.
NEUTROPHILS IN COMMON VARIABLE IMMUNODEFICIENCY: PLAYERS OR VICTIMS?

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Background:

Patients with CVID display an increased frequency of infections, along with/without lymphoproliferation, enteropathy and autoimmunity. Replacement treatment results in the decrease, but not in the elimination of infections, and has a rather weak effect on the management of the other clinical phenotypes. Recent evidence indicates that polymorphonuclear neutrophil (PMN) compartment participates in normal B cell differentiation and homeostasis, although its role in CVID phenotype is obscure. Our objective is to address whether CVID patients exhibit abnormalities in the PMN compartment in both resting and activation states.

Methods:

Heparinized blood samples from 23 CVID patients (male/female: 7/16, mean age: 41.3y, range: 14-66; 5 newly-diagnosed) and 14 healthy controls (male/female: 4/10, mean age: 41.5, range: 21-65) were analyzed by flow cytometry for the expression pattern of CD10, CD11b, CD11c, CD16, CD18, CD64 and CD66b, before and after isolation, as well as before and after incubation with LPS (100ng/mL).

Results:

The majority of CVID patients, regardless of their treatment status, displayed a significantly increased CD64 expression compared to controls (p<0.05, in all cases). Moreover, newly-diagnosed patients exhibited an increased expression of CD11b on resting PMN compared to controls (p=0.014), but not after isolation or incubation with LPS. Finally, no significant differences were observed regarding the other PMN markers, regardless of isolation or activation states.

Conclusions:

Our results suggest that PMN of several CVID patients, regardless of their treatment status, display a constant activation state that may affect both their capacity for further activation and the disease clinical phenotype.
E-POSTER DISCUSSION 5: LATE BREAKERS

ESID7-0522

A NOVEL HUMAN ELANE MUTATION ASSOCIATED WITH INFLAMMATORY ARTHRITIS, DEFECTIVE NETOSIS, AND RECURRENT PARVOVIRAL INFECTION

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Background:

We describe a 38-year-old woman presenting with a 2-year history of inflammatory arthritis, rash and daily fevers. She was noted to have chronic parvoviral infection with persistently detectable viral titers and a novel mutation in the ELANE gene. ELANE encodes neutrophil elastase, a neutrophil serine protease with important antimicrobial effects and is found as part of neutrophil extracellular trap (NET) complexes. Pathogenic ELANE mutations have been described in forms of hereditary neutropenia. However, our patient was never neutropenic. Because of the striking clinical scenario, we investigated this mutation functionally.

Methods:

We used electron microscopy to document NETosis in stimulated and unstimulated cells, and also performed 3D conformational mapping to determine the impact of the coding-change. Cytokine production by the patients neutrophils was also measured.

Results:

The patient’s activated neutrophils demonstrated significantly decreased ability to form NETs by scanning electron microscopy, as well as quantitative defects in neutrophil activation and neutrophil elastase activity. Three-dimensional mapping revealed that the mutation could alter protein folding and surface charge distribution, potentially perturbing protein trafficking. Finally, patient’s neutrophils demonstrated altered levels of IL-12 and IL-8 - key cytokines for antiviral immunity and neutrophil chemotaxis.

Conclusions:

This mutation impacted neutrophil function by decreasing NETosis and altering key antiviral activities of neutrophils. This is the first report of a human pathogenic ELANE mutation associated with a defect in NET-osis and a distinct syndrome of recurrent viral infection and chronic inflammation.
The crucial role of PKC-δ isoform plays in Tat-TLR4 signalling pathway to activate NF-κB and CXCL8 production

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Background:

The objective of this study was to study the role of PKC and TLR4 pathways, to determine the nature of the PKC isoforms involved and to understand their interrelation with the activation of NF-κB and CXCL8 gene product expression

Methods:

In order to investigate the role of these two PKC isoforms in the control of CXCL8 production, we chose to bypass the upstream steps of Tat-TLR4 signalling pathway activation by delivering, in the absence of Tat treatment, either PKC δ or βII isoforms directly as Wt, DN or CAT into HEK-null or HEK-TLR4 cell lines and to test their capacities to stimulate the production of CXCL8.

Results:

Here, we show that Tat-induced CXCL8 production is essentially dependent on the activation of PKC delta isoform, as shown a) by the capacity of PKC delta dominant negative, and Rottlerin, a selective PKC delta pharmacological inhibitor, to inhibit Tat-induced CXCL8 production and b) by the ability of the constitutively active isoform of PKC delta to induce CXCL8 production in a HEK cell line in the absence of Tat stimulation. The finding that comparable amounts of CXCL8 were produced following stimulation with either Tat protein, PKC-delta CAT transfection, or both, argue for the implication of one common pathway where PKC delta is activated downstream of TLR4 recruitment and leads to the activation of NF-κB.

Conclusions:

Altogether, our results underline the crucial role of PKC delta isoform in activating gene expression of CXCL8, a cytokine largely implicated in the physiopathology of HIV-1 infection
E-POSTER DISCUSSION 5: LATE BREAKERS

ESID7-0525

DEVELOPMENT OF ARRAY-BASED SCREENING PANELS FOR AUTOANTIBODY DIAGNOSTICS IN MONOGENIC AUTOIMMUNITY SYNDROMES

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Background:

Autoimmune polyendocrine syndrome type 1 (APS1) is a rare monogenic disorder caused by mutations in the AIRE gene. The disease is associated with a severe defect of central immune tolerance and features multiple autoimmune manifestations including the disease defining three hallmark components: chronic mucocutaneous candidiasis, hypoparathyroidism and adrenal failure.

Autoantibody analysis plays an important role in the diagnosis of APS1.

Methods:

In this project, we aimed to develop an antigen array that could allow for comprehensive autoantibody screening in APS1. Purified full-length human proteins were acquired from commercial sources covering a majority of the established antigens in APS1 (n>20). Proteins were deposited on coated glass slides using a microarray printer. The arrays were probed with sera from APS1-patients or controls followed by a fluorescence-labeled anti-human IgG. Different parameters were optimized, including the type of slide coating, printing settings and serum concentration. Autoantibody results were compared to that of in-house radio-ligand binding assays and commercial proteome arrays (ProtoArray®).

Results:

The custom array showed excellent results for many known APS1 autoantigens, including interferon alpha, interferon omega, interleukin 22, interleukin 17F and GAD65. The results were reproducible and consistent with that of other, independent methods.

Conclusions:

Our preliminary investigations suggest that the antigen array can be used for measuring multiple autoantibodies in parallel in APS1 with minimal sample consumption. This screening tool may facilitate in the diagnosis, prognosis and clinical follow-up of patients with APS1. The antigen panel may also be extended for covering a broader group of monogenic autoimmunity syndromes.
ESID7-0529

Genetic analysis of a cohort of 451 patients with primary antibody deficiencies

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Background:

With a prevalence of approximately 1:10,000 primary antibody deficiencies (PADs) constitute the most common symptomatic primary immunodeficiencies (PIDs). They comprise a heterogeneous group of disorders characterized by a dysfunctional antibody production. Genetic analysis plays an increasingly important role in the work-up of PAD patients and has helped to gain a better understanding of underlying molecular pathways.

Methods:

We have designed a gene panel comprising 120 genes associated with antibody deficiency. Here, we report on our experiences with targeted panel resequencing employing Agilent’s HaloPlex and Illumina’s MiSeq technologies.

Results:

In this study we have investigated 451 patients with CVID, hypogammaglobulinemia and agammaglobulinaemia. In total, we have detected 114 mutations in ADA, AICDA, BTK, CECR1, CTLA4, LRBA, NFkB1, NFkB2, RAG2, PIK3CD, PIK3R1, SEC61A1, STAT1, STAT3, TNFRSF13B and XIAP. Employing a targeted re-sequencing panel proved to be a very time- and cost-efficient, yet reliable, method for the establishment a genetic diagnosis. Overall, a genetic diagnosis could be made in up to 25% of investigated patients. Classical intrinsic B cell defects proved to be the minority of all
Conclusions:

This large genetic cohort analysis highlights once again that the primary antibody deficiencies have a diverse genetic background and genetotype-phenotype correlation is often poor, although certain clinical features may hint towards a specific group of defects. In case of negative screening results by panel sequencing further work-up including whole exome sequencing should be considered for patients with complex disease, a positive family history or early onset of disease.
MALIGNANCIES AS THE MAIN CAUSE OF DEATH IN CVID: AN ITALIAN LONG-TERM MULTICENTER STUDY

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Background:

In the last decade, CVID life expectancy increased due to improvements in surveillance, prevention and treatment of recurrent and severe infections, whilst cancer mortality did not change.

Methods:

In this large CVID Italian cohort (400 patients), we assessed cancer prevalence over a thirty years follow-up and we estimated mortality rate in hematological, gastrointestinal malignancies and in other cancers to assess the quality of the current prevention strategies.

Results:

Patients with malignancies were significantly older at CVID-diagnosis (44±2 vs 22± 12 yrs) than cancer-free CVIDs. The prevalence of hematological, gastric and other cancers were 15%, 6% and 20%, respectively. Hematological malignancies were generally non-Hodgkin’s B-cell lymphomas and often involved extra nodal sites. The overall survival in hematological cancer, gastric cancer and other malignancies group was: (1-yr) 67%, 54%, 88%; (2-yrs) 61%, 36%, 80%; (20 yrs) 61%, 27%, 29%. Treatment of CVID-associated cancer was similar to the treatment of cancers in other settings and included anti-CD20 monoclonal antibodies as a therapeutic agent in B-lymphoid malignancies. The rate of infections during chemotherapy was low, whilst the incidence of severe malabsorption in patients who underwent gastrectomy was high. Moreover, patients who died were significantly more likely to have suffered from cancer.

Conclusions:

In our cohort, cancer appeared to be the main cause of death in CVID. The prevalence of CVID-associated malignancies recorded was higher in comparison to other study and rising over the time. CVID with cancer should receive full therapy regimen, due to a risk of infection similar to the non-CVID cancer population.
Background:

FOXP3 is a key transcription factor for the maintenance of immune tolerance. FOXP3 mutations result in dysfunction of FOXP3+ regulatory Treg cells (Tregs) causing immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome, a severe early onset autoimmune disease. Both currently available treatments, pharmacological immune suppression and allogeneic hematopoietic stem transplantation, present poor long-term disease-free survival or overall survival limitations, respectively. To provide more effective treatments for IPEX patients, we are developing lentiviral-mediated FOXP3 gene transfer (LV-FOXP3) in T cells and FOXP3 gene editing in HSCs and testing these approaches in preclinical models.

Methods:

Using LV-mediated constitutive expression of FOXP3 we successfully converted IPEX patients-derived CD4+ effector T cells (Teff) into Treg-like cells (CD4LV-FOXP3 T cells) with stable suppressive capacity in vitro and in vivo (Passerini, Sci Transl Med, 2013). To edit FOXP3 gene, we used CRISPR-Cas9 protein combined with an AAV6 packaged donor DNA template for FOXP3 and deltaNGFR as marker gene in HSCs, to preserve the regulated expression of the functional FOXP3 protein.

Results:

We have now generated nominal and alloantigen-specific CD4LV-FOXP3 T cells further demonstrating the potential clinical benefit of CD4LV-FOXP3 T cells, which could be applied not only in IPEX but also be targeted to immune mediated diseases against auto-antigens. In addition, our gene editing approach effectively targeted FOXP3 in HSCs and gene-edited HSPCs could be transplanted into NSG mice and show long-term multilineage reconstitution.

Conclusions:

Our data indicate that both LV-FOXP3 and FOXP3 gene editing hold promise for the treatment of IPEX syndrome.
E-POSTER DISCUSSION 5: LATE BREAKERS

ESID7-0536

DYSFUNCTIONAL TELOMERASE IN NAÏVE CD4+ T-CELLS IN PRIMARY SJÖGREN'S SYNDROME
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Background:
Lymphopenia is a frequent finding in primary Sjögren’s syndrome (pSS) affecting mostly the CD4+T-cell population. Here we examine possible underlying defects.

Methods:
We included 47 pSS patients and 50 healthy controls (HC) in a prospective, cross-sectional study. Prevalence of T-cell subpopulations was assessed by flow cytometry according to standard surface staining protocols. Naïve CD4+T-cells were isolated by MACS technology for telomere length and T-cell receptor excision circle (TREC) assessment by real-time PCR. Telomerase activity was analyzed according to the Telomeric Repeat Amplification Protocols (TRAP).

Results:
We found lower numbers of CD4+T-cells in pSS patients compared to age matched HC (560/µl vs. 943/µl, p<0.0001). The reduced naïve subset accounted for most of this difference (203/µl vs. 429/µl, P=0.0001). The number of TRECs in naïve CD4+T-cells was already reduced in young pSS patients (58copies/ng DNA vs. 2058copies/ng DNA, p<0.0001) and was further decreased in older patients (14copies/ng DNA vs. 117copies/ng DNA, p=0.000). To test for a proliferative history we performed telomere length as well as telomerase activity analysis. Patients displayed significantly shortened telomeres compared to age-matched HC (6.5kbp vs. 7.0kbp, p=0.04). In HC shorter telomeres resulted in an elevation of telomerase activity, a finding that we could not observe in pSS patients.

Conclusions:
Our data indicate extensive replicative history of naïve CD4+T-cells in pSS resulting in premature shortening of telomeres. In contrast to HC, naïve CD4+T-cells in pSS are unable to induce telomerase activity. This may lead to the reduction of the naïve CD4+ T-cell pool resulting in CD4+T-cell lymphopenia.
E-POSTER DISCUSSION 5: LATE BREAKERS

ESID7-0540

SELETALISIB, A NOVEL, SELECTIVE PI3Kδ INHIBITOR, RESTORES DEFECTIVE PI3K SIGNALLING IN IMMUNE CELLS FROM ACTIVATED PI3Kδ SYNDROME PATIENTS (APDS)

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Background:

APDS is an immunodeficiency defined by PI3Kδ-activating mutations in genes encoding the catalytic (p110δ; disease termed APDS1) and regulatory subunits (p85α; APDS2) of PI3Kδ, resulting in hyperactive signalling. Patients suffer from lymphoproliferation, recurrent sinopulmonary infections that can lead to bronchiectasis and an increased risk of lymphoma. APDS patients have high proportions of transitional B cells and low proportions of memory B cells. The T cell compartment is characterised by increased senescent CD8⁺ T cells, and reduced naive T cells.

Methods:

Seletalisib is a potent, selective PI3Kδ inhibitor (Allen et al., JPET 2017) directly targeting the causal mechanism in APDS. Phase 1 clinical studies with seletalisib demonstrated excellent oral bioavailability (Helmer et al., J Clin Pharmacol. 2017, In press), a manageable safety and tolerability profile, displayed PK results supportive of once-daily dosing and evidence of biological effect (Helmer et al., Eur J Clin Pharm, 2017).

Results:

Levels of pAKT were found to be elevated in both APDS genotypes (Figure 1A). Seletalisib demonstrated potent inhibition of PI3K signalling in both APDS1- and APDS2-derived T cell blasts
Conclusions:

These data supported a proof-of-concept study in APDS. This study is ongoing and aims to assess the safety, tolerability and PK of seletalisib in APDS patients from 12 years of age (EudraCT2015-002900-10). The effects of dosing on clinical and cellular endpoints are being explored.
E-POSTER DISCUSSION 6: MOLECULAR ASPECTS OF B CELLS IN PID

ESID7-0088

APDS - A B CELL INTRINSIC CLASS SWITCH RECOMBINATION DEFICIENCY
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Background:

APDS1 and APDS2 patients frequently present with a hyper-IgM syndrome-like phenotype with decreased IgG and IgA but normal or elevated IgM serum levels. To investigate the disturbance of Ig class switch recombination, Sµ-Sa switch junctions were analyzed from APDS1 and APDS2 patients.

Methods:

PCR-amplification and sequence analyzes of Sµ-Sa switch recombination junctions.

Results:

The study of switch junctions revealed a trend towards the usage of microhomology in Sµ-Sa recombination junctions from APDS1 and APDS2 B lymphocytes compared with controls. This increase in microhomology was associated with a decrease in the proportion of junctions with no microhomology. In addition we observed a lower number of point mutations around the junctions in APDS1 and APDS2 deficient cells compared with controls.

Conclusions:

Our Sµ-Sa recombination junctions analysis from APDS1 and APDS2 B lymphocytes suggests a B cell intrinsic class switch recombination defect as cause for the hyper-IgM syndrome-like phenotype frequently observed in these patients, possibly associated to disturbed AID-induced DNA lesion induction.
Background:

Germline IKAROS haploinsufficiency, caused by heterozygous \textit{IKZF1} mutations, has been recently identified as a cause of common variable immunodeficiency or other forms of dysgammaglobulinemia associated with low peripheral B cells. The condition is characterized by incomplete penetrance and variable expressivity in symptomatic subjects.

Methods:

Genetic analyses encompassed whole exome sequencing and Sanger confirmation. \textit{IKZF1} transcript expression was analyzed with cDNA sequencing. IKAROS protein expression, bone marrow aspirates and blood lymphocytes were analyzed with flow cytometry.
Results:

Two siblings with infections, autoimmunity and dysgammaglobulinemia as well as their asymptomatic mother harbored a novel germline heterozygous frameshift mutation in \( IKG1 \) (p.S46Afs*14) introducing a premature stop codon near the N-terminus. Although cDNA sequencing suggested normal expression of the mutant transcript, IKAROS protein expression was about 40% lower in the \( IKG1 \) mutant subjects compared to wild type family members and healthy controls. In the asymptomatic mother, non-penetrance was also evident on a cellular level ranging from immature B cells in the bone marrow to terminally differentiated B cells in the blood. The symptomatic siblings had a variable B cell developmental block at the immature to naive mature stage, which is markedly later than the arrest observed in previously published symptomatic cases.

Conclusions:

We here report the first truncating \( IKG1 \) mutation associated with germline IKAROS haploinsufficiency as well as the first bone marrow examination in an asymptomatic \( IKG1 \) mutation carrier. Furthermore, we expand the mutational, clinical and immunophenotypical spectrum of germline IKAROS haploinsufficiency and offer novel insights in early B cell development in both manifesting and non-manifesting mutation carriers.
BRUTON'S TYROSINE KINASE REGULATES HUMAN INTERLEUKIN-10 (IL-10) PRODUCING B REGULATORY CELL (Bregs) INDUCTION AND FUNCTION

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Background:

Bruton's tyrosine kinase (BTK) plays important roles in diverse biological functions in different cell types. Besides B cells, its role has been studied, among others, in Natural Killer (NK) cells, neutrophils, monocytes, dendritic cells (DCs) and others. We have recently shown that BTK plays an important role in Toll-like receptor 9 (TLR9)-dependent DC activation in humans. B regulatory cells (Bregs) on the other hand represent a recently identified B cell subset with particular immunoregulatory properties, able to produce Interleukin-10 (IL-10). Bregs have been extensively studied in various autoimmune and immunomediated disorders. One of the major experimental approaches applied in human Breg induction involves TLR9 activation via CpG stimulation of human B cells. To date, no data are available on the potential role of BTK in the induction and function of Bregs in humans.

Methods:

We decided therefore to investigate the role of BTK in healthy controls in terms of Breg induction and IL-10 production.

Results:

Our data show that combined stimulation via CpG/TLR9 and PMA/I induces the formation of human Bregs in a valid and constant pattern, both in terms of phenotype and IL-10 production/mRNA levels induction measured by ELISA and Real-Time respectively. The addition of Ibrutinib, an irreversible BTK inhibitor, reduces the induction of Bregs and the production of IL-10 by more than 50%.

Conclusions:

These data provide the first evidence for a novel role of BTK in human TLR9-dependent Breg induction and function. Further studies are ongoing to better characterize the BTK involvement in human Breg biology.
EPSTEIN-BARR VIRUS CHRONIC INFECTION IN TWO PATIENTS WITH APDS SYNDROME: A COMBINED RISK FACTOR FOR DEVELOPING LYMPHOPROLIFERATIVE DISORDERS


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Background:

Activated PI3K Delta Syndrome (APDS) is a primary immunodeficiency disease predisposing to lymphoma. PI3K/AKT/mTOR pathway is known to be inappropriately activated in cancer and during Epstein-Barr virus (EBV) infection, leading to increased risk to develop lympho-proliferative disorders (LPDs).

Methods:

We report our experience of two APDS patients presenting with EBV chronic replication and LPDs.

Results:

The first case is a 30-year-old girl presenting since early life with recurrent sinopulmonary infections, lymphadenopathy, failure to thrive and chronic EBV infection. She had dysgammaglobulinaemia consistent with a Hyper-IgM syndrome. At 18-year-old she suffered from a Hodgkin’s lymphoma. At 24-year-old, onset of warning gastrointestinal symptoms led to an endoscopic evaluation with histological finding suggestive of IBD-like disease. Mutation in PIK3R1 (c.1425+1G>T) was identified at the age of 30. One year later, during a routinely colonoscopy a diffuse large B-cell lymphoma was diagnosed.

The second patient is a 19-year-old boy with a diagnosis of primary immunodeficiency and a hyper-IgM-like pattern. Mutation in PIK3CD (c.3061G>A) was found at 16-year-old. He presented multiple lymphadenopathies, airways infections and EBV chronic replication since childhood. At 19-year-old, deterioration of clinical conditions led us to perform a digestive endoscopy with a diagnosis for an extra nodal large cell lymphoma B.
Conclusions:

APDS patients have a high incidence of lymphoma, since childhood. Importantly, gastrointestinal LPDs localization may be silent, so clinicians should consider early and alternative diagnostic tools and involvement of a multidisciplinary team with strict follow up even without specific symptoms. Moreover, inhibiting the PI3K/AKT pathway may reduce malignancies associated with latent herpesvirus infections.
Background:

PI3Kδ signalling is required for normal immune cell development and function. Activated PI3K delta Syndrome (APDS) is a recently described cause of primary immunodeficiency, resulting from gain of function mutations affecting the p110δ catalytic- or p85α regulatory subunits. APDS leads to a combined immunodeficiency resulting in the clinical features of recurrent sino-pulmonary bacterial infections, herpesvirus infections, bronchiectasis, autoimmunity and lymphoproliferation. Respiratory tract infections secondary to *Streptococcus pneumoniae* are a frequent finding.

Methods:

We have created a conditional mouse model of APDS (p110δ^{E1020K}) to use a tool to further understand the pathophysiology of APDS.

Results:

Consistent with APDS patients, p110δ^{E1020K} mice show increased susceptibility to airway infection with *S. pneumoniae*. The time course of infection leading to pathology is extremely short, thereby lessening the likelihood of involvement of an antigen-specific immune response. Surprisingly, however, this susceptibility disappears if p110δ^{E1020K} expression was restricted to T cells or myeloid cells but is present in mice in which p110δ^{E1020K} expression is restricted to B cells. Therefore hyperactive p110δ B cells recapitulate the susceptibility to *S. pneumoniae*. Further experiments indicate that this susceptibility occurs via an antibody-independent mechanism. Following a detailed characterisation of splenic and lung B cells from *S. pneumoniae* infected mice we have identified a novel expanded population of tissue-resident IL10^+^ B cells from the PI3Kδ^{E1020K} mice.

Conclusions:

We postulate that this B cell subset has immunoregulatory properties and contributes to the susceptibility seen to *S. pneumoniae* in mice with hyperactive PI3Kδ signalling.
GAIN-OF-FUNCTION MUTATIONS IN PIK3CD RESULT IN DYSREGULATION OF BOTH T AND B CELLS AT MULTIPLE STAGES INCLUDING DEVELOPMENT, ACTIVATION AND DIFFERENTIATION

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Background:

Gain of function (GOF) mutations in PIK3CD, encoding the p110δ subunit of PI3-kinase, cause a primary immunodeficiency characterized by recurrent respiratory infections, increased susceptibility to herpes virus (EBV, CMV, VZV) infection and B-cell lymphoma. Interestingly, many of these patients also suffer from a range of autoimmune conditions.

Methods:

To better understand how over active PI3K contributes to the phenotype of these patients, we analysed the phenotype and function of lymphocyte populations in these individuals. To extend these studies in vivo, we generated a mouse model using CRISPR/Cas9 to introduce the common disease-causing mutation (E1021K) into Pik3cd. This mouse model also allowed us to determine whether changes observed in lymphocyte populations were cell intrinsic or were secondary to PI3K over activation in other cell types.

Results:

These studies, both in patients and in the mouse model, revealed a clear defect in B cell development in the presence of the GOF mutations. Further, B cells also displayed a cell intrinsic defect in isotype switching, however many other aspects of the B cell response remained intact. We also observed defects in T cell populations including altered Tfh cell differentiation and function.

Conclusions:

Over activation of PI3K results in dysregulation of T and B cell responses at multiple levels, both from cell intrinsic and extrinsic effects. These defects provide an explanation, at least in part, for the dysregulated antibody responses observed in these patients that result in both immune deficiency and autoimmunity.
ESID7-0259

CLINICAL, IMMUNOLOGICAL, CYTOGENETIC AND MOLECULAR FEATURES OF ICF PATIENTS IN SAUDI ARABIA

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Background:

Immunodeficiency, centromeric instability, and facial anomalies (ICF) syndrome is a rare, genetically heterogeneous, autosomal recessive disorder. Patients present with recurrent infections, Hypogammaglobulinemia and cytogenetic abnormality involving chromosome 1, 9, and 16. Four Types have been identified till now based on the gene affected: ICF1 (DNMT3B mutations), ICF2 (ZBTB24 mutations), ICF3 (CDCA7 mutations), and ICF4 (HELLS mutations).

Methods:

Whole exome sequence was done on all patients with Hypogammaglobulinemia following in our institution followed by cytogenetic analysis.

Results:

We identified 5 patients with ICF1 from the same family having the same mutation in DNMTB3 reported previously Kaya et al. Three new patients with ICF2 two of them are siblings carrying a novel frameshift mutation as the same time they are carrier for FLH3 mutated gene UNC13D a novel frameshift mutation and the third is having a homozygous 2-nucleotide deletion in ZBTB4 but found to have no cytogenetic abnormality. Two sibling with mutation in the HELLS gene causing ICF4. We found that many patient following as hypogammaglobulinemia diagnosed as ICF after gene analysis 11/17 (64%).

Conclusions:

Our study expands the mutation spectrum in ICF syndrome and supports the need for screening patients with hypogammaglobulinemia with cytogenetic analysis.
SELECTIVE IgA DEFICIENCY – FROM DEFECTIVE T CELL INDEPENDENT RESPONSES TO ABROGATED TRANSITIONAL B CELLS

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Background:

Selective IgA deficiency (IgAD) is the most common primary immune deficiency in the western world and an immune dysregulation syndrome with affected individuals suffering from a manifold burden of autoimmunity, atopic diseases and infections.

Methods:

We analyze IgAD T and B cell populations by FACS, phosphoflow and ELISA.

Results:

Here we show that adult IgAD individuals have both significantly reduced number and percentage of transitional B cells (CD19+CD24hiCD38hi) both in vivo and after in vitro induced expansion through TLR9-CpG stimulation. In addition, this impaired developmental defect was confined to the previously defined B regulatory cells (CD19+CD24hiCD38hiIL10+). IgAD B cells had non-significantly different pStat5 and pERK after CpG stimulation demonstrating responsiveness to CpG in IgAD. The T cell independent stimulation of isolated B cells with TLR9-CpG stimulation failed though to induce IgA production, even with the addition of exogenous IL-10. IL-10 did induce a small IgA production in PBMCs from IgAD individuals, made only long-lived responses in IgG but failed to make long-lived IgA production. The defect in B cells did not seem to affect T cells since their proportions in peripheral blood and ex vivo induced T-effector and T-regulatory cells were comparable to healthy controls regarding fractions, proliferation, induction capability and suppressive functions; pointing towards a defect restricted to B cells.

Conclusions:

Our findings demonstrate a T cell independent B cell maturation defect suggesting defective interactions between innate immunity and B cells in IgAD individuals providing hints for the development of potential therapeutic targets in autoimmunity and IgAD.
B CELL DYSREGULATION IN IKAROS DEFICIENCY ASSOCIATED WITH A NOVEL IKZF1 MUTATION

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Background:

Mutations in the transcription factor IKAROS (encoded by \textit{IKZF1}) cause an autosomal dominant form of antibody deficiencies characterized by progressive loss of B cells, low serum immunoglobulins (Ig) and increased risk of bone marrow failure and leukemia. We recently described a 13-year-old boy and his mother who initially presented with treatment-refractory idiopathic thrombocytopenia and were found to have a novel heterozygous missense mutation in the DNA-binding zinc finger 3 domain of \textit{IKZF1} (c.584A>G, p.His195Arg). Based on the findings we further examined their B cell function.

Methods:

Immunophenotyping, autoantibody testing and T- and B-cell repertoire studies were performed.

Results:

Patient and his mother have decreased levels of IgA and IgM and switched memory B cells. Patient also has low IgG and NK cells whereas the mother has total B cell lymphopenia and low naive CD4 T cells. Deep heavy chain B-cell receptor repertoire sequencing revealed clonally expanded B cells with top 10 clones accounting for 12-15\% of all clones v.s. 1.36\% in healthy family controls. In the top 10 clones we also detected reduced CDR3 length (43 v.s. 52 in controls) and skewed usage of proximal D and J genes. T-cell repertoire and bone marrow studies are underway.

Conclusions:

Despite having mild clinical symptoms, our 2 patients have a clear evidence of B cell clonality. Due to a high risk of developing bone marrow failure and leukemia, patients with IKAROS deficiency should be monitored for skewing of immune cell compartments.
Background:

Increased vitamin B12 level is one of the additional criteria for diagnosis ALPS. The exact mechanism of the increase is unknown.

Objective: To evaluate diagnostic value of vitamin B12 level for patients with ALPS.

Methods:

We analyzed vitamin B12 levels (B12l) in 20 children with ALPS (ages 4 months - 17 years, time of observation 6 months – 56 months) at various stages of the disease activity, with and without therapy with rapamycin.

Results:

13 patients had various Fas gene defects (ALPS Ia), 5 patients fulfilling ALPS criteria with unknown genetic defect (ALPS III) and 2 patients had the same mutation in CASP10 (ALPS Ila). Upon diagnosis 8 children with ALPS Ia and 2 patients with ALPS III had B12l 1500 or higher. They all had pronounced lymphoproliferation and/or significant autoimmune symptoms. After 24 month of sirolimus therapy their symptoms resolved and B12l became normal in all of them. 5 patients with ALPS Ia, 3 patients with ALPS III and 2 patients with ALPS Ila had normal B12l. Most of them had minimal lymphoproliferation and no autoimmune phenomena.

Conclusions:
In our cohort more than 30% of patients with mutations in FAS and 60% patients fulfilling ALPS criteria had a normal value of vitamin B12 before targeted therapy. Given the normalization of vitamin B12 in a group of patients with a high initial value, we assume that vitamin B12 is not only a diagnostic marker, but also can be used as a marker of disease activity.
A HOMOZYGOUS MISSENSE MUTATION IN ADENYLATE KINASE 2 PRESENTED WITH HYPOGAMMAGLOBINEMIA AND ABSENCE OF AGRANULOPOIESIS

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Background:

Adenylate kinase 2 (AK2) is a mitochondrial enzyme, and the mutated AK2 gene has been assigned to the most severe form of SCID (reticular dysgenesis); which characterized by the absence of granulocytes and almost complete deficiency of lymphocytes in peripheral blood.

Ak2 gene localized on chromosome 1, the encoded protein is an intermembrane mitochondrial enzyme, which catalyze the reversible transfer of phosphate groups between nucleoside triphosphates and monophosphates. Also, AK2 is required for the induction of unfolded protein response (URP) during B cell differentiation and the establishment of the plasma cell secretory function.

Methods:

We were reporting four cases with a much milder clinical phenotype of primary immunodeficiency than reticular dysgenesis with a newly described homozygous missense mutation in AK2 gene.

Results:

The four cases we were presenting are from two different Saudi families for consanguineous parents, three of them presented in their second half of the first year with recurrent and prolonged pulmonary infection, found to have profound hypogammaglobinemia, normal granulocytes, intact thymus and lymphoid tissues, and normal T-lymphocyte subset and mitogen stimulation response. The last patient discovered by screening for hypogammaglobinemia at the age of 3 months, done because of a previously diagnosed sibling.

Conclusions:

Up to our knowledge, we were presenting the first four cases of this recently described AK2 homozygous missense mutation associated with hypogammaglobinemia. Finally, we suggest testing for AK2 gene defect as part of an investigation for hypogammaglobinemia at least, if other causes were negative.
PRIMARY ANTIBODY DEFICIENCY AND IMMUNODYSREGULATION IN A PATIENT WITH ATAXIA PANCYTOPAenia SYNDROME DUE TO DE NOVO MUTATIONS IN SAMD9L


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Background:
We present a case of primary antibody deficiency, cytopaenias, immunedysregulation and progressive cerebellar symptoms initially presenting in infancy.

Methods:
Whole genome sequencing was used to establish a molecular diagnosis; the Nanopore MinION platform and allele specific PCR were used for phasing of variants.

Results:
Immunophenotyping in adulthood showed increased plasma cells and CD21lo-B cells and a reduced CD4:CD8 ratio.

WGS revealed three non-synonymous de novo SNVs, two of which lay in the same gene, SAMD9L. At the time of discovery, although SAMD9L had been shown to influence T-cell migration, we could not conclusively establish the pathogenicity of these variants. However, within a year dominantly inherited gain-of-function variants in SAMD9L were linked to Ataxia-Pancytopaenia syndrome with clinical features including cytopaenias, progressive cerebellar dysfunction, combined immune deficiency, susceptibility to marrow failure and myeloid leukaemia.

The de novo mutations identified here (p.Y1118C and p.R359Q) lay in the same exon but were 2,277bp apart and thus could not be phased by short reads. We therefore used the Nanopore MinION platform and obtained 28 reads (5.0-17.9kb) spanning both loci, suggesting that the mutations lay in cis. This was supported by allele-specific PCR, consistent with the variants representing a single mutational event.

Conclusions:
The patient presented represents the 1st case of Ataxia-Pancytopaenia syndrome due to de novo mutations in SAMD9L and expands the phenotype of this syndrome to include manifestations of
immunedysregulation, such as severe enteropathy and arthropathy. The study also highlights the importance of periodically re-reviewing candidate variant lists and the utility of long-read technology for phasing clustered multi-nucleotide mutations.
Background:

Recurrent bacterial sinopulmonary and cutaneous infections associated to eczema and elevated serum levels of IgE are features of the hyper-IgE syndrome (HIES), a complex primary immunodeficiency disorder. Herein, we report on the molecular basis of the novel PGM3-deficiency associated to a rare autosomal recessive HIES and the assessment of related intervention approaches.

Methods:

We screened for known hypomorphic PGM3 mutations large pedigrees of affected consanguineous families and assessed for selected patients with severe eczema the effect of an ointment of GlucNAc supplement.

Results:

We identified in consanguineous tunisian families with multiple affected individuals, who were wild type for STAT3 and DOCK8, two distinct homozygous mutations in Phosphoglucomutase 3 (PGM3). PGM3 catalyzes the reversible conversion of N-acetyl glucosamine 6P to N-acetyl glucosamine 1P required for biosynthesis of uridine diphosphate N-acetylglucosamine an essential precursor for protein glycosylation. The mutations are one in-frame deletion and one amino acid substitution, both of them permit at least some expression and translation of the variant PGM3. These hypomorphic mutations did show dose-dependent increase of enzyme activity in vitro, so we hypothetized that supplementing with an excess of substrate might improve residual enzymatic activity in vivo. Two patients suffering eczema with extremely severe itching were treated with GlucNAc supplement and results are discussed.

Conclusions:

This is a novel and rare primary immunodeficiency due to a congenital disorder of glycosylation and underlying hypomorphic PGM3 mutations. A supplementation with an excess enzyme substrate or by a compound that bypasses the block could be a therapeutic approach to improve, at least, their severe eczema.
A NOVEL DIGENIC HUMAN IMMUNODEFICIENCY IMPAIRING IFNAR1 AND IFNGR2


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Background:

Primary immunodeficiencies are most commonly monogenic disorders characterized by vulnerability to specific infectious pathogens. Patients with atypical spectra of infections may have more than one genetic defect.

Methods:

Whole exome sequencing was performed on the proband. Signaling downstream of IFNGR2 and IFNAR1 was assessed with flow cytometry, Western blotting, and qPCR. Fibroblasts were transduced with lentivirus containing either empty vector or IFNAR1 for reconstitution studies.

Results:

Whole exome sequencing of a patient with disseminated M. bovis, Streptococcus viridians bacteremia, and human cytomegalovirus (HCMV) viremia identified novel mutations in two genes regulating distinct IFN pathways. The patient has a homozygous frameshift deletion in IFNGR2, which encodes the signal transducing chain of the IFN-γ receptor, resulting in minimal protein expression and absent downstream signaling. The patient also has a homozygous deletion in IFNAR1 (IFNAR1 557Gluext*46), which encodes the IFN-α receptor signaling subunit. The mutation replaces the protein’s stop codon with 46 novel C-terminal codons. The IFNAR1 557Gluext*46 mutant protein was expressed in patient fibroblasts at a level comparable to that of wild-type IFNAR1 in control fibroblasts. Signaling in response to IFN-α stimulation was significantly impaired in the patient’s fibroblasts, evidenced by decreased STAT1/STAT2 phosphorylation, nuclear translocation of STAT1, and expression of IFN-α-stimulated genes critical for HCMV immunity. The patient fibroblasts had defective control of HCMV replication after IFN-α stimulation, which was corrected by expression of wild-type IFNAR1.

Conclusions:

This is the first example of a human mutation in IFNAR1, as well as the first digenic immunodeficiency specific to type I and type II IFNs.
E-POSTER DISCUSSION 7: GENETICS OF PID

ESID7-0138

CARD9 p.R70W, A TURKISH FOUNDER MUTATION ASSOCIATED WITH MUCOSAL AND INVASIVE FUNGAL INFECTIONS, DISRUPTS DOWNSTREAM NF-κB SIGNALING BY INHIBITING BCL10 RECRUITMENT

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Background:

Inherited CARD9 deficiency is an autosomal recessive primary immunodeficiency predisposing uniquely to chronic and invasive fungal infections in otherwise immunocompetent individuals.

Methods:

We studied eleven patients with CARD9 c.208C>T p.(R70W) mutations belonging to five families living currently in Belgium or France but originating from the same region in Turkey. They present a spectrum of both mucosal and systemic fungal infections. We performed an in vitro system to explore the biochemical mechanism of the R70W mutation, using a constitutively active CARD9 L213LI mutant, which allows dissection of downstream signaling events. We performed NF-κB luciferase reporter assays and co-immunoprecipitation experiments.

Results:

Segregation analysis of four microsatellite markers and five SNPs revealed a common haplotype of 1.03 Mb surrounding the mutation, suggesting a Turkish founder mutation. Luciferase assays showed that the constitutive NF-κB activity of the L213LI mutant was completely eliminated in a double mutant containing both R70W and L213LI mutations. Consistent with these
results, co-immunoprecipitation of CARD9 showed that the double mutant fails to pull down Bcl10 and MALT1, whereas the L213I mutant does.

Conclusions:
Identification of the R70W CARD9 mutation as a founder in patients with CMC and/or invasive fungal infections originating from Turkey implies that targeted testing of this mutation would be valuable in this specific population. The R70W mutation prevents the interaction with downstream MALT1 and Bcl10, which leads to inactivation of downstream CBM complex-dependent signaling to NF-κB.
E-POSTER DISCUSSION 7: GENETICS OF PID

ESID7-0346

THE ROLE OF AUTOPHAGY IN THE PATHOGENESIS OF STK4 DEFICIENCY
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Background:
Deficiency of serine/threonine kinase 4 (STK4) has been reported in patients with primary immunodeficiency characterized by profound lymphopenia and recurring bacterial and viral infections. Recently it was shown that phosphorylation of LC3 by STK4 is essential for autophagy in mice. In humans, previous reports showed binding of STK4 to ATG8 family proteins. However, the functionality of autophagy has yet to be determined in STK4 deficient patients.

Methods:
Whole exome sequencing was used to analyse 30 patients with clinical symptoms of ALPS but no mutations in classical ALPS genes (FAS, FASLG, CASP10). Functional validation of variants was performed on primary or transformed T-cells.

Results:
Here we report on two patients from unrelated families with consanguine background who presented with chronic lymphadenopathy, increased number of DNTs (CD3+TCRαβ+CD4-CD8-), autoimmune hemolytic anemia and highly elevated vitamin B12 levels. According to diagnostic criteria these patients were initially diagnosed with ALPS. Interestingly, WES revealed homozygous truncating STK4 mutations leading to deficiency of STK4. We detected reduced proliferation of patient PBMCs after stimulation with different lymphocyte specific activating agents. Contrary to the initial diagnosis we could not find a dysregulation of FAS-mediated apoptosis in patient derived primary T-cells. However, patient cells were significantly more susceptible to apoptosis caused by serum starvation, which indicates defective autophagy. Analysis of LC3 turnover assays will reveal the functionality of autophagic flux in STK4 deficient cells.

Conclusions:
Patients with STK4 deficiency might present with clinical symptoms resembling ALPS. Additional to defects in proliferation and chemotaxis STK4 deficient lymphocytes might also have defective autophagy.
E-POSTER DISCUSSION 7: GENETICS OF PID

ESID7-0351

PROTEIN KINASE C DELTA (PRKCD) COMPOUND HETEROZYGOUS MUTATIONS IN SIBLINGS WITH RECURRENT INFECTIONS AND AUTOIMMUNITY

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Background:

Eight children have been previously described with homozygous mutations in PRKCD gene causing recurrent infections and features of systemic lupus erythematosus.

Methods:

We report two siblings presenting with recurrent infections from infancy (a girl with haemophilus influenza septicaemia and her brother with listeria meningitis). They both had persistent bloody diarrhea and the boy developed autoimmune haemolytic anaemia aged 1 year, followed by sepsis with enterococcus faecium growing in multiple blood cultures. They both have an intermittent painful erythematos rash which faded to a hyperpigmented stain.

Results:

A targeted immunology gene panel found 2 mutations in both children’s PRKCD gene. Mum had one mutation only and Dad is not available for testing. They both had a borderline positive ANA, but have a variety of other autoantibodies detected by screening (anticardiolipin, antithyroglobulin, anti-GAD). Western blot analysis showed absence of PRKCD protein in both siblings (Figure 1). Comparison was made with other previously identified patients with homozygous mutations in PRKCD and in the new cases the protein loss was found to be more profound.

Conclusions:

These are the first children described with compound heterozygote mutations in PRKCD and from the protein expression results, the phenotype may be predicted to be more severe. Management of autoimmunity in the presence of immunodeficiency is complex.
A NEW TYPE OF PRIMARY IMMUNODEFICIENCY WITH PULMONARY ALVEOLAR PROTEINOSIS DUE TO OAS1 DYSFUNCTION


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Background:
Pulmonary alveolar proteinosis (PAP) is characterized by accumulation of surfactant-like substance in alveolar spaces and hypoxemic respiratory failure. Congenital forms of PAP (CPAP) are caused by mutations in genes (SFTPB, SFTPC and ABCA3) responsible for surfactant production, processing, or transport in alveolar type II epithelial cells. CPAP also develops in patients with mutations in genes (CSF2RA, CSF2RB and GATA2) responsible for surfactant catabolism in alveolar macrophages (AMs).

Methods:
We performed whole exome sequence (WES) analysis in a family affected by infantile-onset PAP who had no mutations in the above genes. We also performed Sanger sequencing of the candidate gene for two sporadic patients from different families manifesting similar clinical features; infantile-onset PAP, hypogammaglobulinemia, immature and small AMs, leukocytosis without abnormal distribution, splenomegaly, hyper reactivity and favorable response to immunoglobulin administration.

Results:
The same heterozygous missense variation in OAS1 was identified by WES in three affected siblings but not in unaffected family members. Deep sequence analysis with the next generation sequencer demonstrated 4%-mosaicism of this variation in DNA from their mother, indicating PAP observed in this family could be inherited as an autosomal dominant trait from the mother. Furthermore, we identified other de novo heterozygous missense variations of OAS1 in two additional cases. Pulmonary lesions in the two sporadic patients have been resolved after hematopoietic stem cell transplantation.

Conclusions:
Infantile-onset PAP with hypogammaglobulinemia observed in five patients from three families with mutations in \textit{OAS1} was considered as a new type of primary immunodeficiency.
AUTOIMMUNE DISEASES IN PRIMARY ANTIBODY DEFICIENCIES

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Background:

Primary antibody deficiencies are the most common inherited primary immunodeficiencies in humans, characterized by inability to produce antibodies. However, both systemic and organ-specific autoimmune diseases could be attributed to B-cell defects in these patients. The aim of study was to analyze the frequency and spectrum of autoimmune disorders in primary antibody deficiencies in Ukrainian patients.

Methods:

We performed retrospective study of autoimmune complications in 120 patients with primary antibody deficiency: X-linked agammaglobulinemia (n = 34), common variable immunodeficiency (CVID) (n = 22), hyper IgM syndrome (n = 8), IgA deficiency (n = 40), IgG subclass deficiency (n = 16).

Results:

Autoimmune disorders were found in 32 (26,6%) of 120 patients with primary antibody deficiencies. Autoimmune diseases were the most frequent in patients with hyper IgM syndrome (75,0%) than in those with X-linked agammaglobulinemia (35,2%) or CVID (22,7%). Autoimmune rheumatic diseases were found in 14 patients (11,7%), mostly as olygoarthritis, followed by celiac disease in 7 patients (5,8%), uveitis in 5 patients (4,2%), autoimmune cytopenia in 3 patients (2,5%). Most patients with antibody deficiencies have had autoimmune disorders by the time of diagnosis. The beginning of the replacement therapy with immunoglobulins has reduced the number and severity of autoimmune diseases in these patients.

Conclusions:

Primary antibody deficiencies have variable autoimmune manifestations. The patients with IgA deficiency have the biggest variety of autoimmune diseases. Rheumatic diseases were the most frequent autoimmune complications in patients with severe hypogammaglobulinemia. Autoimmune diseases should be suspected in patients with primary antibody deficiencies for early diagnosis and treatment.
E-POSTER DISCUSSION 8: AUTOIMMUNITY/AUTOINFLAMMATION

ESID7-0261

GENETIC VARIANTS FOUND BY WES IN PATIENTS WITH IMMUNE CYTOPENIA
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Background:
We aimed to evaluate the benefits and drawbacks of using whole exome sequencing (WES) as a diagnostic method in patients with chronic immune cytopenias (e.g. AIHA, ITP, AIN). Most patients presented with additional symptoms of immune dysregulation, e.g. CVID, lymphoproliferation and autoimmunity (DMT1, thyroiditis).

Methods:
30 patients (age 0-39) were sequenced on Illumina NextSeq 500 with mean coverage over 30x.

Results:
In ten patients (33%) we were able to find likely causative mutations. Four of them (including 3 siblings) carried a variant leading to CTLA4 deficiency. In one patient we identified a GOF variant in STAT3 recently described in immune dysregulation. In three patients we observed variants in genes associated with antibody deficiency (TACI, CD40L and IKBKG). One patient carried a novel variant in TERT. Another patient had a pathogenic variant in KMTD2 causing Kabuki syndrome. Of the remaining patients, four carried variants in genes associated with immune dysregulation, that we observed at lower frequencies also in healthy people (CASP10, PIK3CD). Twelve patients (36%) had either only heterozygous variant in genes causal for AR diseases (e.g. ITK, LRBA) or we have not yet found any relevant aberration. In the remaining four patients we were able to identify novel variants in genes related to immune dysregulation. However, these variants require extensive validation studies to prove their causality.

Conclusions:
WES helped to identify the genetic cause in one third of our patients. Because of the heterogeneity of genetic causes of immune cytopenias, we recommend to use WES over targeted gene panel sequencing.

A MUTATION IN THE OSM GENE IS ASSOCIATED WITH PERSISTING ANEMIA AND THROMBOCYTOPENIA

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Background:

Recurrent anemia and thrombocytopenia were reported for three sisters (P1, P2, P3) in a consanguinous marrocous family of 5 children (1 male, 4 females). Despite frequent erythrocyte transfusion, they have a normal life without any growth curve retardation or developmental defects.

Methods:

Whole exome sequencing was achieved on DNA from all the family member. Beside exhaustive flow cytometry analyses on blood and bone-marrow cells, the intrinsic hematopoietic potential of patients’ CD34+ cells was evaluated by in vitro differentiation assays.

Results:

In the three patients’ cells, we identified a homozygous mutation (c.507_508 insG) in the oncostatin (OSM) gene that leads to an open reading frameshift at the protein level (p.R170Pfs*124). The two parents and one sister were heterozygous for the mutation while the brother was homozgyous for the wild type OSM allele. In the bone marrow aspirate, no morphological anomalies were found. The lymphoid myeloid and erythroid lineage were normal but the megakaryocytes were rare or absent. The erythroid and megakaryocyte differentiation capacity were identical in the patients and control cells. In colony-forming unit assay, the CFU-GM and BFU-E counts were within the normal range. In the PB, the hematopoietic lineages were normally represented in the patients and their relatives.

Conclusions:

Our results suggest that OSM mutation is responsible for the anemia and thrombocytopenia observed in P1, P2 and P3. The absence of any intrinsic differentiation defect in the CD34+ progenitors suggests that OSM mutation could affect the medullar niche environment.
E-POSTER DISCUSSION 8: AUTOIMMUNITY/AUTOINFLAMMATION

ESID7-0270

NEXT-GENERATION SEQUENCING CONTRIBUTION FOR MOLECULAR DIAGNOSIS OF AUTOINFLAMMATORY DISEASES.

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Background:

Nowadays mutations in about 25 genes have been associated with autoinflammatory diseases (AIDs). In the last years next Generation Sequencing (NGS) has emerged as new diagnostic tool in this field.

Methods:

250 patients evaluated at our center from 2010 to march 2016 were enrolled. In the first 4 years targeted resequencing on 179 patients using a panel including 11 AIDs related genes, (MVK, MEFV, NLRP12, NLRP3, NOD2, TNFRSF1A, PSTPIP1, IL1RN, LPIN2, IL36RN, PSMB8) was performed. Subsequently 14 genes were added, obtaining a final design of 25 genes. Sequencing analysis were performed on MiSeq\textsuperscript{\textregistered} sequencing platform (Illumina, San Diego, CA). Validated variants were studied by \textit{in silico} analysis.

Results:

80 patients present variants in the AIDs-associated genes with a detection rate of 31%. Only variants with a frequency lower than 1% were considered. 25% of patients showed monogenic variants, 5% variants in two or more genes. 33% of total variants were found in NOD2 gene, 19% in MEFV, 16% in NLRP12, 12% in NLRP3, 9% in PSTPIP1 and 5% in TNFRSF1A and MVK.

Conclusions:

NGS leads to the identification of many genetic variants in different genes. The major challenge resides in the interpretation of the clinical relevance of identified variants, especially of those found in multiple genes. These could cooperate to determine a pathological phenotype. Moreover, variants with an allele frequency close to 1% may act as susceptibility polymorphisms to inflammation rather than disease-associated mutations. Therefore large-scale population studies, in vitro functional assays and careful correlation of genetic with phenotypic data are needed.
GENERATION OF FUNCTIONAL MYELOID CELLS FROM MEFV-MUTANT INDUCED PLURIPOTENT STEM-CELLS DEMONSTRATING A GENE-DOsing EFFECT

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Background:

Familial Mediterranean Fever (FMF) is a prototypic autoinflammatory disorder associated with MEFV pyrin-encoding gene mutations, characterized by unprovoked episodes of inflammation. In former studies we have demonstrated a specific phenotype of myeloid FMF-cells with hypersecretion of phagocyte-specific S100-proteins and IL-18, and a gene-dosing effect of mutated pyrin, on a protein level. In the present study we address characterisation of this phenotype in induced pluripotent stem cells (hiPSCs) from FMF-patients.

Methods:

Myeloid cells from MEFV-mutant patients with FMF and from healthy controls were generated by an embryoid body (EB)-based differentiation protocol for hiPSCs after selection from peripheral blood-derived (PB) CD34+ HSCs and re-genotyped. Terminally differentiated myeloid cells (monocytes and granulocytes) were phenotyped regarding extra- and intracellular marker expression as well as cell morphology and were subjected to functional assays such as phagocytosis and generation of reactive oxygen species. Cells were stimulated with LPS, ATP and S100A12 and cytokine secretion into culture supernatants was quantified by multiplexed bead array assay.

Results:

Terminally differentiated myeloid cells displayed phagocytic functions. Homozygous p.M694V patients, heterozygous patients and healthy controls differed by surface markers, by intracellular S100A8/S100A9 content, and by release of proinflammatory cytokines. Gene-dosing effects were seen for IL-1, IL-1RA, IL-10, and S100A8/S100A9.

Conclusions:

Here, we show efficient generation of mature monocytes and neutrophils from hiPSCs free from murine stromal cells in a patient population with MEFV-mutations. Previously described gene-dosing effects for cytokine and S100-protein secretion in patient serum and peripheral myeloid cells could be reproduced at hiPSCs derived myeloid cell level.
ESID7-0331

INTERLEUKIN-1 INHIBITOR (ANAKINRA) IN TREATMENT OF GRANULOMATOUS INFLAMMATION IN PATIENTS WITH CHRONIC GRANULOMATOUS DISEASE (CGD)

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Background:

CGD is a rare primary immunodeficiency with NADPH complex defects, manifesting by bacterial and fungal infections and granulomatous complications, that are often not directly related to infection and frequently treated by steroids.

Methods:

We report results of granuloma treatment in eleven CGD patients (10 boys and 1 girl). Diagnosis was confirmed by rhodamine test and by detection of relevant mutations. Granulomas were present in lungs (10 patients) and in liver (4 patients). Previous prolonged antibacterial and antifungal therapy failed to resolve the lesions.

Results:

IL1 inhibitor (Anakinra) was used at a dose 8-10 mcg/kg daily for 1-6 months. We demonstrate a significant (90%) reduction of granulomatous foci in all patients treated. In terms of side effects one patient had mild neutropenia, and one patient developed allergy to the drug and the treatment had to be discontinued after 2 weeks. IL1 synthesis by in vitro activated macrophages in the group of CGD patients was found to be increased compared to healthy controls.

Conclusions:

We report a novel approach to granuloma treatment in CGD patients that is potentially a better alternative to steroids in terms of efficacy and side effects.
COMBINED IMMUNODEFICIENCY AND PREVALENCE OF ATOPY AND AUTOIMMUNITY IN ROIFMAN SYNDROME

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Background:

Roifman syndrome (RS) is a complex syndrome encompassing immunodeficiency, epiphyseal dysplasia, dysmorphic features, retinal dystrophy and developmental delay, caused by unique mutations in RNU4ATAC. Most studies focused on the humoral defect associated with the syndrome. We describe here cellular immune aberration as well as the prevalence of atopy and autoimmune disorders in RS patients.

Methods:

Patient information was derived from the Canadian Centre for Primary Immunodeficiency Registry (n=8), and a review of the literature (n=7). Immunophenotyping and TCR repertoire were assessed by flow cytometry, and T cell proliferation was analyzed after stimulation with mitogens or antigens.

Results:

All patients suffered severe or repeated sino-pulmonary infections, but some had repeated HSV (n=6), PJP and other fungal infections (n=5). Nine patients had eczema and five patients suffered from repeated episodes of asthma. A wide spectrum of autoimmune manifestations were also recorded, including: immune cytopenia, autoimmune hepatitis, arthritis as well as endocrinopathy encompassing delayed puberty, gonadotropin deficiency, growth hormone deficiency, Diabetes Mellitus, hypothyroidism and hypoparathyroidism. B cell lymphopenia and antibody deficiency were identified in all patients. T cell lymphopenia, reduced memory T cells, lack of in vitro proliferative response to antigens and a skewed T cell repertoire were found in six patients.

Conclusions:

We describe here T cell dysfunction in addition to humoral deficiency, as well as the common occurrence of atopy and autoimmunity in RS.
LATE-ONSET CRYOPYRIN-ASSOCIATED PERIODIC SYNDROMES DUE TO SOMATIC NLRP3 MOSAICISM: EXPERIENCE FROM A TERTIARY CENTER IN SPAIN


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Background:

Cryopyrin-associated periodic syndromes (CAPS) encompass a spectrum of dominant IL-1β-mediated autoinflammatory diseases (AID). Early-onset urticarial skin-rash associated with fever, high acute-phase reactants and musculoskeletal and sensorineural inflammation are their hallmarks. Recently, somatic NLRP3 mosaicism has been shown as the underlying genetic mechanism in atypical AID presentations including adult-onset CAPS.

The aim was to identify the genetic cause and to describe the clinical features and response to treatments in three sporadic Spanish patients with late-onset AID.

Methods:

DNA was extracted from whole blood and isolated cell types. AID-associated genes were analyzed by amplicon-based deep sequencing (ADS) in an Illumina platform.

Results:

ADS detected variable degree of somatic NLRP3 mosaicism in all patients, with two of them carrying novel variants (p.Y563C and p.Q636E, with 6.4% and 18.4% allele frequency, respectively) and the other carrying an already known mutation (p.A352T in 18.7%). Functional studies performed in patient carrying the p.Q636E variant showed constitutively increased activation of NLRP3-inflammasome.

All patients were asymptomatic until their 50’s, when uricarial-like skin-rash, fever, arthralgia and increased inflammatory parameters first appeared. Additional symptoms occasionally detected included sensorineural deafness, hydrocephalus and low-level IgG-k paraprotein. Treatment with anti-IL-1 drugs resulted in quick control of clinical symptoms and normalised inflammatory markers.

Conclusions:
We herein describe three unrelated patients with late-onset CAPS carrying somatic NLRP3 mosaicism. Our findings expand the clinical spectrum of CAPS, demonstrate that mosaicism plays an important role in their pathogenesis and should be taking into account in the diagnosis of monogenic AID.
IMMUNOLOGICAL PHENOTYPE OF THE MURINE LRBA KNOCKOUT

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Background:

Biallelic mutations in the human Lipopolysaccharide Responsive Beige-like Anchor (LRBA) gene lead to a primary immunodeficiency known as LRBA deficiency, characterized by a broad range of clinical manifestations including autoimmunity, organomegaly, hypogammaglobulinemia and recurrent infections.

Methods:

Considering the phenotypic heterogeneity in patients and the severity of the disease, our aim was to assess the role of LRBA in immune cells and to understand the underlying pathomechanisms through the study of a Lrba knockout (Lrba⁻⁻⁻) mouse model.

Results:

LRBA-deficient mice did not show severe clinical or immunological signs of disease, either at steady state under specific-pathogen- free conditions, after vaccination with T-dependent and T-independent antigens, or in the context of acute infections with lymphocytic choriomeningitisvirus (LCMV) or Salmonella Typhimurium. Although Lrba⁻⁻⁻ mice were able to produce normal serum IgM and IgG and to mount a specific immune response after immunization, they showed elevated serum and secretory basal IgA levels. LRBA was dispensable for B- and T-cell development, as well as for in vitro B-cell proliferation, survival, isotype-switching and plasmablast differentiation. Interestingly, Lrba⁻⁻⁻ mice displayed decreased CTLA-4 expression by regulatory T cells and activated conventional CD4⁺ and CD8⁺ T-lymphocytes, reduced frequency of peritoneal B-1a cells along with diminished IL-10 production, and increased percentages of T follicular helper cells in Peyer’s patches, but without developing overt signs of autoimmunity.

Conclusions:
Our findings expand the role of LRBA in immune regulatory mechanisms previously reported in patients, and suggest a novel role in IgA production that is crucial for the protection of mucosal surfaces and gut-associated immune tolerance.
E-POSTER DISCUSSION 8: AUTOIMMUNITY/AUTOINFLAMMATION

ESID7-0490

VARIABLE PENETRANCE IN CTLA-4 DEFICIENCY
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Background:
Humans with heterozygous germ line mutations in CTLA-4 have reduced CTLA-4 expression, diminished Treg suppressor function and immune dysregulation with organ specific inflammation, autoimmunity and variable penetrance. Even though the role of CTLA-4 in immune dysregulation is widely studied, variable penetrance in the context of CTLA-4 is not fully understood.

Methods:
We aimed at studying variable penetrance in CTLA-4, its pattern, frequency and associated in vitro correlates. Among 90 patients from 37 families, there were 34 (38%) subjects with a CTLA-4 mutation and classified as “asymptomatic or limited autoimmunity” as opposed to “affected” (n:56, ~62%) who had major organ lymphocyte infiltration, cytopenia, early onset type I Diabetes Mellitus (T1DM), or lymphoma. The F:M ratio was 1.17 in the entire cohort; females dominated the asymptomatic/mild subset by 71%. Mean age for the asymptomatic/mild cohort was 37 ± 3.8 years (25 ± 2.0 yrs for the affected).

Results:
Affected cohort had significantly lower percentages of CD25+CD4+FOXP3+ T_reg cells than asymptomatic subjects. Ex-vivo T_reg function was decreased in patients, which was more striking upon co-culture with HD T_eff cells. In contrast, asymptomatic patient T_reg had normal suppressor activity. The majority of affected patients developed progressive CD4, B and NK cell lymphopenia CD4 T lymphocyte numbers were lower in affected patients.

Conclusions:
Our study reveals in vitro quantitative, functional and phenotypic differences among affected and asymptomatic CTLA-4+/−-cohorts. Thus, Treg dysfunction seem to be directly related to their clinical outcome.
ONSET OF THE DISEASE IN AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS) AS A CONSEQUENCE OF DIGENIC INHERITANCE


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Background:

Autoimmune lymphoproliferative syndrome (ALPS) is characterized by splenomegaly, lymphadenopathy, hypergammaglobulinemia and accumulation of TCRαβ+ CD4-CD8- T cells (called Double Negative, or DNT cells) along with autoimmune cytopenias in most patients. We described the first genetic basis of ALPS as dominant negative mutations in FAS more than 25 years ago. Our laboratory also showed that ALPS outcome in sporadic cases could be linked to somatic heterozygous mutations of FAS. However, one striking observation in the ALPS-FAS families was the partial clinical penetrance of some heterozygous germline mutations. In this context, we identified few years ago a secondary mutational event as a somatic mutation impairing the second FAS allele. Now, we hypothesized that a mutation in another gene could be the “second hit” in those ALPS families.

Methods:

We performed whole exome sequencing on ALPS families exhibiting a FAS mutation with partial clinical penetrance but without second event on FAS.

Results:

We identified one candidate gene, which is involved in the regulation of FASL expression. Two patients (P1 and P2) in one family carried a homozygous missense mutation in the FASLRegulator gene. By screening this candidate gene in the cohort, we identified a third patient (P3) in a second family exhibiting a homozygous mutation in the exon 14. In patient 1, we observed a FASL expression defect after re-activation of the blast by western blot that correlate with a functional apoptosis defect.

Conclusions:

This discovery provides a new mode of ALPS transmission and is the first digenic inheritance observed in autoimmune diseases.
Background:

The aim of our research is to improve understanding of molecular aetiology in children born with a faulty immune system by identifying causative genetic changes.

We investigated two patients from a consanguineous background with autoimmune lymphoproliferative syndrome (ALPS), manifesting as lymphadenopathy, hepatosplenomegaly, susceptibility to herpes viral disease, impaired Fas-dependent apoptosis, autoimmune thrombocytopenia and hemolytic anaemia. Both brothers developed lymphoma. Following hematopoietic stem cell transplantation, one patient rejected and died, while the other developed mixed chimerism, transient relapse of ALPS and disordered hematopoiesis.

Methods:

Whole exome sequencing revealed a homozygous, predicted damaging mutation within a novel disease gene. To investigate molecular pathogenesis, we reprogrammed patients’ fibroblasts using Yamanaka factors to induced pluripotent stem cells (iPSC). We proved their pluripotency by immunofluorescence and flow cytometry by expression of pluripotency markers, and their potential to differentiate into 3-germ layers.

We carried out feeder-free, serum-free differentiation of patients’ and non-affected iPSC to hematopoietic precursors in vitro. We characterised differentiating hematopoietic stem cells (HSC) by flow cytometry using panel of antibodies. In parallel, we performed colony-forming unit assay in semi-solid methylcellulose medium to evaluate the clonogenic potential of derived HSC.

Results:

We observed changes in total number and types of colony-forming units in patients’ iPSC in early and later stages of differentiation compared to controls. Our findings simulate clinical features of the disease.

Conclusions:

The study demonstrates an ideal in vitro model for studying this novel gene associated with autoimmune lymphoproliferative disease with potential for further studies.
NGS REVEALED UNEXPECTED GENETIC DEFECTS IN PATIENTS WITH LYMPHOPROLIFERATION AND POLY-AUTOIMMUNITY

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Background:

Autoimmunity can be a presenting feature of Primary immunodeficiencies (PIDs). Moreover, autoimmune manifestations are present in 30% of PIDs. We described the first monogenic cause of autoimmune lymphoproliferative syndrome (ALPS) as heterozygous mutations of the FAS gene. New genetic causes of ALPS with tissue infiltration, organ-specific autoimmunity and progressive loss of humoral immunity have been recently described with recessive LRBA mutations, CTLA-4 haploinsufficiency and STAT3 gain of function (gof).

Methods:

Using targeted Next Generation Sequencing (TNGS) or whole exome sequencing (WES) we identified such mutations in 51 patients presenting with lymphoproliferation and autoimmunity (25 CTLA4, 20 LRBA and 6 STAT3gof). However, in some instances, these NGS approaches revealed unexpected genetic defects.

Results:

A large heterozygous deletion (> 5kb) of the CTLA-4 gene was identified by TNGS whereas it remained undetectable by Sanger sequencing. We also found a homozygous CTLA-4 hypomorphic mutation in a patient with severe lymphoproliferation and autoimmunity. TNGS also led to the identification of a homozygous STAT3gof in a consanguineous pedigree with healthy heterozygous carriers. Molecular analysis evidenced a hypomorphic mutation with regards to the gain of function. Finally, we identified deleterious FOXP3 mutations in two unrelated patients presenting with unusual autoimmune features. One patient was presenting with poly-autoimmunity but neither with bowel disease nor with diabetes. The other patient presented with lupus features.

Conclusions:

NGS allowed the identification of unexpected genetic defects in autoimmune lymphoproliferations, extending their clinical spectrum or their mode of inheritance and suggesting the involvement of additional genetic modifiers.
E-POSTER DISCUSSION 9: MECHANISMS OF IMMUNE DYSREGULATION II

ESID7-0344

THE TRANSCRIPTION FACTOR Ets1 COOPERATES WITH IL17 SIGNALING TO PROMOTE AUTOIMMUNITY AND IMMUNODEFICIENCY

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Background:

Ets1 is a lineage-specific transcription factor that regulates B and T cell functions in development and disease. Mice that lack Ets1 (Ets1 KO) develop spontaneous autoimmune disease with high levels of autoantibodies. Naïve CD4+ T cells isolated from Ets1 KO mice differentiate more readily to Th17 cells and secrete IL-17, a cytokine extensively implicated in autoimmune disease pathogenesis.

Methods:

To determine if increased IL-17 production contributes to the development of autoimmunity in Ets1 KO mice, we generated Ets1/IL-17 Receptor double knock out (DKO) mice.

Results:

We found that the absence of IL-17 signaling did not prevent or ameliorate the phenotype of Ets1 KO mice, but rather that DKO animals exhibited worse symptoms with striking increases in the numbers of Th2 cells and Tfh cells as well as germinal center B cells, isotype-switched B cells, memory B cells and plasma cells. Furthermore, DKO mice develop spontaneous skin lesions colonized by Staphylococcus aureus (S. aureus). When DKO mice were experimentally infected with S. aureus they were unable to clear the bacteria. Curiously, the increased bacterial susceptibility was age-dependent as 2 month old DKO mice were not susceptible, whereas 4 month old mice were.

Conclusions:

Our current model is that excessive immune activation over time leads to production of autoantibodies that target immune effector mechanisms important for anti-bacterial immunity, such as anti-microbial peptides, thereby resulting in susceptibility to S. aureus. Our studies may have important implications in understanding the mechanisms that drive pathogenesis in human diseases characterized by both autoimmunity and immunodeficiency.
TEMPORAL GENE CO-EXPRESSION ANALYSIS IN THE INFANT HUMAN THYMUS

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Background:
Thymus reaches its final size at the first year of life. Moreover, thymic tissue suffers gender-related histogenomic changes during minipuberty. This work investigates temporal genomic changes in the human thymus throughout the first 30 months of life aiming to identify molecular pathways involved in early thymic functioning.

Methods:
Whole transcriptional profiles of infant thymus surgical explants grouped by age intervals – 0-6, 7-12, 13-18, and 19-30 months (25 males and 11 females) - were analyzed using the Weighted Gene Co-expression Networks Analysis (WGCNA), based on GO annotated genes.

Results:
WGCNA revealed 18 different modules represented by their module eigengene. Two transcriptional modules, cyan and pink – here named variable modules - were correlated (p-value <0.05) with patients' age intervals of 0-6 (downregulated genes) and 19-31 (upregulated genes) months. Conversely, four modules, blue, brown, green, and turquoise – constant modules - showed no significant gene expression variation along all time intervals but included relevant network hubs. Subnetworks obtained for cyan and pink modules encompassed many hubs associated to thymocyte development molecular pathways: e.g. STA3, TGF-beta, JNK, in cyan; NF-kappaB, Notch, integrin recycling, in pink. The subnetworks for constant modules displayed hubs associated to autophagic and apoptotic pathways (green), Hippo pathway (turquoise), and to transcriptional control and chromatin modification (blue and brown).

Conclusions:
The variable modules depict a progressive gene upregulation towards TEC survival and medullary functions (cyan), and T-cell development (pink). The constant modules are mostly related to thymocyte selection and egress. The overall picture shows an age-related transcriptional signature of the thymus functioning.
CONTRIBUTION OF DEFECTIVE NON-APOPTOTIC FAS SIGNALING TO IMMUNE DYSREGULATION IN AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS)

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Background:

ALPS patients show impaired generation of humoral memory for T independent antigens whereas they generate memory for self-antigens due to impaired FAS-dependent removal of autoreactive germinal center B cells. It is known that FAS signaling via caspase activation results in cell apoptosis. However, FAS ligation may also initiate or modulate non-apoptotic signaling as shown for example by its ability to activate NFkB. Recent data implicate a regulatory role of FAS in the modulation of mTOR signaling in DNT cells. Moreover, a recently described C194V mutation in FAS affects its translational modification (palmitoylation) leading to development autoimmunity in the mouse, without impairment of FasL mediated apoptosis. This supports the view that FAS may prevent autoimmunity with other mechanisms than inducing apoptosis.

Methods:

We hypothesize that FAS mutations impair this modulatory signaling, leading to hyper-activation of B cells.
We studied resting and activated B cells in ALPS patients in presence or absence of FAS ligand, by flow cytometry analysing relevant molecules to the BCR and CD40 signaling pathway.

Results:

We found that FAS ligation fails to induce downmodulation of BCR and CD40 signaling in ALPS patients B cells, as detected by PLCgamma 2, Akt and S6 phosphorylation. We are currently investigating the consequences at the B cell level of this observation.

Conclusions:

Defect in non-apoptotic FAS signaling may contribute to the immune dysregulation observed in ALPS.
Autoinflammatory Disease in children in Scotland-the spectrum of disease seen in the Scottish Paediatric and Adolescent Rheumatology Network clinics

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Autoinflammatory conditions are a spectrum of disorders increasingly presenting to and managed within Paediatric Rheumatology. We review cases presenting to clinics working within the Scottish Paediatric and Adolescent Rheumatology managed clinical network (SPARN); demonstrating the spectrum of conditions seen and the ability of the network to deliver local expert management to children with rare conditions.

Methods:
A total of 39 cases (definite and probable) were identified from clinics in 7 Health Board areas around Scotland and information gathered from paper and electronic records.

Results:
Cases seen are shown by diagnostic categories in the table.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
<th>Confirmed Genetic Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAPS</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>TRAPS</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Interferonopathy/AGS</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>MVKD</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>FMF</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PFAPA</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Periodic fever unspecified</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Lyn/tyrosine kinase mutation</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>miscellaneous</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>39</strong></td>
<td><strong>21</strong></td>
</tr>
</tbody>
</table>

Management:
17 children (10 CAPS, 3 TRAPS, 1 MVKD and 3 others) received anakinra during the course of their treatment; 8 remain on it. 5 children with CAPS moved on to canakinumab with good benefit. 1 child in the miscellaneous group unsuccessfully tried canakinunumab. 5 children received tocilizumab, 2 with MVKD and 1 with CAPS responded well. 2 children responded well to anti-TNF therapy. 8 children received colchicine alone or in combination. 8 children (all miscellaneous or interferonopathy categories) received various conventional immunosuppressive agents as part of their treatment. One child with an Interferonopathy responded well to ruxolitinib.
Conclusion:
The development of SPARN has resulted in increased recognition, diagnosis and treatment for these complex conditions. All therapeutic agents used have been facilitated by locally based teams, improving compliance and quality of life for children and families. Clear diagnostic and therapeutic pathways are now needed for use within the network.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0011

ANTI CD25 TREATMENT OF HUMAN DENDRITIC CELLS MODULATES THEIR CYTOKINE SYNTHESIS PROFILES AND THEIR CAPACITY TO ACTIVATE ALLOGENEIC CD4 T CELLS: A POTENTIAL TOLEROGENIC EFFECT

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Background:

Recently attention has been focused on role for dendritic cells DCs, in promotion of peripheral tolerance. It is currently believed that maturation/activation state of DCs might be a control point for induction of graft tolerance through modifications of activation state of T cells. We demonstrated in this study CD25 expression on human DCs upon LPS activation. DCs differentiated from monocytes were exposed to anti CD25 during maturation, this treatment affected abilities of human DCs to induce CD4T cell proliferation in response to alloantigens, maintained endocytic capacity, Anti CD25 treated DCs produce low levels of IL-12 and IFN and high levels of IL-10. All these characteristics suggest that DCs may be used in cellular therapy either to induce allograft tolerance (anti CD25 treated DCs) or to restore immunity against tumors (IL-2 treated DCs).

Methods:

1. DCs generation from monocytes
2. Flow cytometry analysis
3. FITC-dextran uptake Endocytic tracers
4. Isolation of CD4T lymphocytes
5. Allogeneic mixed lymphocyte reaction MLR
6. Cytokine and ELISA

Results:

-Mature human DCs express CD25
-Anti CD25 treated DCs display:
  reduced intracytoplasmic IL-2 production
  an impaired IL-12 and IFN synthesis and an increased IL-10 production
  conserved endocytosis capacities
  impaired allostimulatory properties on CD4T cells

Conclusions:

Clinical studies have indicated that subtle differences in maturation status of DCs might substantially impact on induced responses and turn T cell immunity into T cell tolerance. Thus, better understanding
of factors determining development and function of DCs will be a prerequisite to design strategies for prevention and/or treatment of solid organ allograft rejection. Furthermore, standardized criteria must be developed to investigate DC preparations, including cell purity, cell viability, cell phenotype and periodic evaluation of T-cell stimulatory ability of Ag-preloaded DCs.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0025

LOFGREN SYNDROME – A SUBFORM OF SARCOIDOSIS

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Background:

Löfgren syndrome consist of acute development of erythema nodosum often localized on tibial region, lymphadenopathy in hilar of lungs, fever and ankle joints involvement. Inducing factor(s) and pathogenesis are unknown. Therapy is based on non-steroid anti-inflammatory drugs (NSAID). In majority of patients this syndrome occurs only once. Löfgren syndrome is included into sarcoidosis, but there are important differences in clinical course. Sarcoidosis is chronic disease with relapses (often after steroid therapy), and/or progress leading to insufficiency of lungs. Immunological reaction in sarcoidosis is associated with reaction of T lymphocytes, mainly Th1, high proportion of T subpopulations CD4:CD8 (e.g. 5:1 or more). Macrophages involved in inflammation showed symptoms of hyperactivity.

Methods:

Typical histology for sarcoidosis (infiltration with lymphocytes and macrophages) was noted in EBUS biopsy of lymph nodes.

Results:

Patients

Löfgren syndrome occurrence, symptoms and course suggest different disease process with different therapy. We showed 42 patients (18 men and 24 women) with Löfgren syndrome presented as erythema nodosum (90.4% of patients), lymphadenopathy in hilar lymph nodes (95.2%) and ankle joints inflammation (83.3%). Therapy included NSAID and pain control. Symptoms resolved without continuation and/or relapse.

Conclusions:

Discussion

The typical course of sarcoidosis is chronic with exacerbations (relapses) treated with steroids commonly. Chronic inflammation and progress of disease are associated with prolonged activation of lymphocytes, overproduction of proinflammatory cytokines. Löfgren syndrome is an acute episode without chronic course, what indicate different pathomechanism despite of similar inflammatory process. Based on symptoms and clinical course the distinct entity for Löfgren syndrome is suggested.
Background:

*Trichilia monadelpha* Thonn J.J. de Wilde (TM) is a plant commonly used for the treatment of gastrointestinal disorders, inflammatory diseases and pain. This study evaluated the beneficial effect of the aqueous-methanol extract of stem bark of TM in rat colitis induced by trinitrobenzenesulfonic acid (TNBS).

Methods:

Thirty male Wistar rats weighing 180-200 g were randomly distributed to six groups of 5 rats; non-colitic, untreated colitic, colitic treated with prednisolone (2 mg/kg) or colitic rats treated TM (100 - 400 mg/kg). Rats were treated with TM orally for two days prior to colitis induction and thereafter for 7 days. Response to treatment was assessed both macroscopically and biochemically.

Results:

Treatment with prednisolone and TM significantly reduced the colonic damage score, weight/length ratio and leucocytes infiltration indicated by decreased colonic Myeloperoxidase activity (MPO) activity when compared to the untreated colitic animals (p≤ 0.05). Furthermore, TM at 200 and 400 mg/Kg doses and prednisolone (2 mg/Kg) significantly prevented the depletion of colonic total glutathione content (GSH) content levels in colitic rats (p < 0.0001). However, the extract was unable to prevent a similar depletion of superoxide dismutase (SOD) level, unlike the prednisolone.

Conclusions:

The methanol extract of the stem bark of *Trichilia monadelpha* ameliorates oxidative stress and inflammation in colitic rats. The beneficial effect can be further explored.
A NOVEL PYRIN-ASSOCIATED AUTOINFLAMMATION WITH NEUTROPHILIC DERMATOSIS MUTATION FURTHER DEFINES 14-3-3 BINDING OF PYRIN AND DISTINCTION TO FAMILIAL MEDITERRANEAN FEVER

Background:

Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis (PAAND) is a recently described monogenic autoinflammatory disease. The causal p.S242R MEFV mutation disrupts a binding motif of the regulatory 14-3-3 proteins within pyrin. Here we investigate a family with clinical features consistent with PAAND in whom the novel p.E244K MEFV mutation, located in the +2 site of the 14-3-3 binding motif in pyrin, has been found.

Methods:

Multiplex cytokine analyses were performed on p.E244K patient and control serum. Peripheral blood mononuclear cells were stimulated ex vivo with LPS. In vitro, inflammasome complex formation was evaluated by flow cytometry of Apoptosis-associated Speck-like protein containing a Caspase recruitment domain (ASC) specks. IL-1β and IL-18 production was quantified by enzyme-linked immunosorbent assay (ELISA). The ability of the p.E244K pyrin mutation to interact with 14-3-3 was assessed by immunoprecipitation.

Results:

PAAND p.E244K patient serum displays a different cytokine profile compared to patients with Familial Mediterranean Fever (FMF). In overexpression models, p.E244K pyrin was associated with decreased 14-3-3 binding and increased ASC speck formation. THP-1 monocytes expressing PAAND pyrin mutations demonstrated spontaneous caspase-1-dependent IL-1β and IL-18 secretion, as well as cell death, which were significantly greater than those of wild type and the FMF-associated mutation p.M694V.

Conclusions:
In PAAND, disruption of the +2 position of a 14-3-3 binding motif in pyrin results in its constitutive activation, with spontaneous production of IL-1β and IL-18, associated with inflammatory cell death. The altered serum cytokine profile may explain the different clinical features exhibited by PAAND patients compared to those with FMF.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0039

DYSREGULATED CYTOKINE PRODUCTION IN MONOCYTES FROM PATIENTS WITH CHRONIC GRANULOMATOUS DISEASE
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Background:

The secretion of tumor necrosis factor alpha (TNF-α) activates the cytokine cascade required for appropriate cellular responses. One important cellular target of TNF-α is the circulating monocyte, which produces interleukin-8 (IL-8) to activate and initiate neutrophil chemotaxis. TNF-α also stimulates IL-10 secretion, which ultimately terminates TNF-α synthesis. Patients with chronic granulomatous disease (CGD) display signs of persistent inflammation. The aim of this study was to elucidate the cytokine response in CGD patients following TNF-α stimulation compared with healthy controls.

Methods:

Heparinised blood samples were collected from 16 CGD patients without a definite infection and healthy volunteers after obtaining informed consent. Monocytes were incubated with TNF-α for 6 h. Incubated cells were harvested at 30-min intervals for mRNA analysis and supernatants were collected to determine IL-8 and IL-10 levels using enzyme-linked immunosorbent assays (ELISAs).

Results:

The average level of IL-8 and IL-10 production did not differ between CGD patients and healthy controls within the first 180 min; however, the average level of both IL-8 and IL-10 production decreased in healthy controls after 180 min but remained significantly elevated in CGD patients.

Conclusions:

A high and prolonged release of both IL-8 and IL-10 was observed in CGD patients relative to healthy controls following TNF-α stimulation. We hypothesise that the absence of phagocyte-derived reactive oxidants may be associated with the dysregulated production of pro- and anti-inflammatory cytokines in patients with CGD.
Background:

Autoinflammation and PLCγ2-associated Antibody deficiency and Immune Dysregulation (APLAID) is a complex disease due to dominant mutations in the PLCγ2 gene characterized by early-onset skin disease, recurrent bacterial infections and autoinflammatory symptoms involving joints, eye and gastrointestinal tract.

Objective: to describe the clinical, immunological and genetic features of two unrelated patients with APLAID syndrome.

Methods:

Clinical data were collected from patients’ medical charts. Genetic studies were performed by Sanger sequencing and next-generation sequencing (NGS) methods. Flow cytometry and cytokine profiling were performed to characterize the immunological features.

Results:
A 14 year-old girl (P1) and a 6 year-old boy (P2) presented with an early-onset (<1 year) inflammatory disease characterized by severe, generalized papulo-vesicular skin rash with granuloma formation and cutis laxa, recurrent sinopulmonary infections, bronchiectasis and bilateral keratitis (P1). Immunological investigations revealed antibody deficiency characterized by B lymphopenia with hypogammaglobulinemia (P1) and low IgM with poor antibody response to vaccines (P2). No abnormalities in T and NK cells were observed. NGS studies revealed novel, germline heterozygous PLCG2 variants (p.Ala708Pro and p.Leu845_Leu848del, respectively), which were predicted to be deleterious. Genetic investigations in patient’s parents confirmed their de novo nature. Further functional studies are ongoing.

Conclusions:

The coexistence of severe autoinflammatory features, recurrent infections and marked B cell defects should lead to the suspicion of PLCy2 mutations. This entity is a clear example of the new paradigm for PID involving both innate and adaptive immune systems, leading to the need of screening for PID to a wider spectrum of patients.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0044

A VERY LATE DIAGNOSIS OF FHL TYPE 5: RECURRENT EPISODES OF IMMUNE DYSREGULATION WITHOUT ESCALATION TO FULL-BLOWN HEMOPHAGOCYTIC LYMFHOHISTIOCYTOSIS

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Background:

Familial hemophagocytic lymphohistiocytosis (FHL) is characterised by immune dysregulation by impaired natural killer cells and cytotoxic T lymphocytes. Patients typically present at young age with fever, hepatosplenomegaly and cytopenias with hemophagocytosis, necessitating hematopoietic stem cell transplantation (HSCT). Type 5 FHL (FHL-5) is caused by syntaxin-binding protein-2 (STXBP2) gene mutations. Distinct mutations are associated with milder phenotypes, possibly delaying diagnosis.

Methods:

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Results:

Case report

A 42-year old male presented with a 40-year history of recurrent fever, cytopenias, neurological manifestations, colitis, eczema, lymphadenopathy and hemophagocytosis. No infectious pathogens were detected, yet antibiotic treatment and low dose steroids led to full recovery during these episodes. A disease-free period of 10 years was reported. In his thirties, he developed panhypogammaglobulinaemia with bronchiectasis. A prolonged bleeding time was detected. Lymphocyte subset analysis showed absence of peripheral B cells. NK degranulation and NK cell targeted lysis were absent. Illumina sequencing of a gene panel with 226 immunodeficiency associated genes revealed heterozygosity for the pathogenic c.1280-1G>C p.(?) and c.1654G>A p.(Gly552Ser) variants in the STXBP2 gene. An atypical course of FHL-5 was assumed.

Conclusions:
We present a unique FHL-5 phenotype in, to our knowledge, the oldest patient reported with this disorder. Concomitant symptoms as colitis, neurological manifestations, bleeding disorders and hypogammaglobulinemia were reported in other cases of atypical FHL. These cases were associated with later disease-onset, disease-free periods and prolonged survival without HSCT. The very atypical course of disease challenges the choice of optimal treatment. Increased insights into disease pathogenesis might guide future treatment decisions in atypical cases.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0050

DIFFERENT PRESENTATIONS OF LRBA DEFECT
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Background:

Common variable immunodeficiency (CVID) represents a heterogeneous group of disorders. In 2012, autosomal recessive mutations in LRBA (LPS-responsive beige-like anchor protein encoding), were described as a cause of CVID-like disease.

In Egypt, high rates of consanguinity and inbreeding, result in high rates of autosomal recessive disorders

Methods:

We present three cases from three different families with different presentation of LRBA defect.

Results:

Case 1: 7 year old male patient from a consanguineous family with history of male sibling death with the similar condition. At the age of 9 months he was diagnosed as type1 Diabetes Mellitus with autoimmune thyroiditis. At the age of 3 years he developed generalized lymphadenopathy, organomegaly, thrombocytopenia, chronic diarrhea and recurrent attacks of pneumonia. At the age of 5 years he developed severe Auto immune hemolytic anemia then pancytopenias. He had homozygous frameshift mutation in LRBA.

Case 2: 7 year old male patient from a consanguineous family. At one year old he developed generalized lymphadenopathy, recurrent attacks of pneumonia and arthralgia. At 5 years old he developed Classical Hodgkin's lymphoma. He had homozygous mutation in LRBA.

Case 3: 4.5 year old female patient from a consanguineous family. At one year old she had chronic diarrhea, failure to thrive, sinusitis, draining ears, eczema and autoimmune hemolytic anemia. She had homozygous mutation in LRBA and biallelic mutations in DOCK8.

Conclusions:

LRBA defect patients present early in infancy and can have different presentations.
SEASONAL VARIABILITY OF INFECTIONS IN PRIMARY IMMUNE DEFICIENCY

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Background:

Seasonal variability of bacterial and viral infections in the general population and select groups of patients is well-established. This topic is especially important in primary immune deficiency (PID), where the goal of immunoglobulin G (IgG) replacement therapy is protection against infections. However, both the extent of infection seasonality and its association with serum IgG levels in PID are unknown.

Methods:

We analyzed demographics, infection data, and serum IgG (trough) levels from seven CSL Behring studies of 20% subcutaneous IgG (SCIG) Hizentra in PID patients. Infections were diagnosed per local standard of medical care; serious bacterial infections (SBIs) were defined per FDA guidance.

Results:

Among 92/125 study patients with infections at any time, infection rate was highest in winter (peak in January, 77/789 cases, 9.8%) and lowest in summer (July, 48/789 cases, 6.1%). The most dramatic difference pertained to SBIs: 6/7 SBIs started in autumn and winter. However, IgG trough levels were stable throughout the year, with mean (SD) concentrations between 11.58 (2.68) and 12.35 (2.01) g/L.

Conclusions:

In patients on steady-state IgG replacement therapy, infections peak in winter, and have the lowest rate in summer. This substantiates the regulatory requirement of observing efficacy outcomes for at least 12 months in IgG studies of PID. Infection seasonality in PID does not correlate with the dynamics of serum IgG levels: on adequate replacement therapy, IgG trough levels remain stable throughout the year. IgG dose adjustment and/or prophylactic use of immune stimulants and antimicrobials in the high-risk months should be considered.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0053

HEMOLYTIC ANEMIA WITH INTRAVENOUS IMMUNOGLOBULIN IN IMMUNODEFICIENCY AND IMMUNE THROMBOCYTOPENIA: EFFECT OF ANTI-A DONOR SCREENING

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Background:

Hemolytic anemia (HA) is a rare complication of intravenous immunoglobulin (IVIG) therapy, considered to result from passive transfer of anti-blood group antibodies (isoagglutinins) with IVIG products. To reduce the quantity of isoagglutinins, anti-A donor screening was implemented for the IVIG Privigen® (CSL Behring) and donors with high titers were excluded. The cumulative incidence of HA with first-time administration of Privigen® for low-dose substitution therapy in immunodeficiency and for high-dose immune-modulatory therapy in immune thrombocytopenia was studied before (1/2008 to 12/2012) and after (10/2013 to 12/2015) implementation of donor screening.

Methods:

Patients were identified from a hospital-based administrative database of 862 US hospitals. HA within 30 days of first-time Privigen® use was assessed from manual records review. Crude cumulative incidence ratios of HA in first-time Privigen® administration with and without donor screening were estimated for each indication.

Results:

<table>
<thead>
<tr>
<th>Privigen indication</th>
<th>No. of patients</th>
<th>HA cases</th>
<th>Cumulative incidence / 1000 patients (95%CI)</th>
<th>No. of patients</th>
<th>HA cases</th>
<th>Cumulative incidence / 1000 patients (95%CI)</th>
<th>Crude CIR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune thrombocytopenia</td>
<td>2616</td>
<td>26</td>
<td>9.94 (6.50–14.5)</td>
<td>1911</td>
<td>8</td>
<td>4.19 (1.81–8.23)</td>
<td>0.42 (0.19–0.93)</td>
<td>0.027</td>
</tr>
<tr>
<td>Immunodeficiency</td>
<td>2530</td>
<td>9</td>
<td>3.56 (1.63–6.74)</td>
<td>1395</td>
<td>4</td>
<td>2.87 (0.78–7.33)</td>
<td>0.81 (0.25–2.61)</td>
<td>0.72</td>
</tr>
</tbody>
</table>
CI, confidence interval; CIR, cumulative incidence ratio; HA, hemolytic anemia

Conclusions:

The incidence of HA was higher in Immune thrombocytopenia than immunodeficiency, likely reflecting the higher IVIG dose for ITP. Donor screening was associated with a reduction of the risk of HA in immune thrombocytopenia.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0062

INFLAMMATORY BOWEL DISEASE ASSOCIATED WITH G6PC3 DEFICIENCY
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Background:
Glucose-6-phosphatase catalytic subunit 3 deficiency (G6PC3) deficiency causes severe congenital neutropenia and diverse extra-haematological manifestations, the full phenotypic spectrum of which has not yet been realised. Inflammatory bowel disease has been reported anecdotally in patients with G6PC3 deficiency but has not been studied systematically.

Methods:
Retrospective review of all children with G6PC3 mutations at the regional paediatric immunology service at Royal Manchester Children’s Hospital, UK.

Results:
Six children with G6PC3 deficiency had a median (range) age of 13 (3 - 18) years. Four patients had chronic debilitating diarrhoea. Endoscopy showed evidence of IBD with mucosal ulceration, crypt abscesses and inflammatory infiltrates but no granuloma. G-CSF therapy was commenced at a median (range) age of 8 (2 - 14) years, and in three patients coincided with the onset of bowel symptoms. Bowel symptoms resolved following matched-sibling donor HSCT in one patient. Notably, the two patients without gastrointestinal symptoms had a milder phenotype, no extra-haematological features and genotypes that are predicted to be less deleterious.

Conclusions:
We describe IBD as a major clinical feature in a series of patients with confirmed G6PC3 mutations. There may be a genotype-phenotype correlation. IBD can emerge later in the course of the disease, and may be driven by G-CSF conditioned neutrophils, thus amenable to HSCT. Further studies are underway to elucidate the functional role of G6PC3-deficient neutrophils in driving inflammatory colitis.
**Background:**

Lipopolysaccharide-sensitive beige-like protein (LRBA) is a broadly expressed cytosolic protein that is involved in lymphocyte receptor internalization, T and B cell activation and immunoglobulin synthesis. Hypogammaglobulinemia, inflammatory bowel disease-like enteropathy, autoimmunity, hepatosplenomegaly may be seen in LRBA deficient patients.

**Methods:**

We describe the heterogeneous clinical course of LRBA deficiency in two siblings, born to second degree consanguineous healthy parents, mimicking IPEX-like syndrome.

**Results:**

Patient 1 (3.5 year old boy) had chronic diarrhea beginning at the age of 1, growth retardation and dysmorphic features (low-set ears and hypertelorism), lymphoproliferation, hepatosplenomegaly, hypogammaglobulinemia, direct coombs test positivity, chronic glomerulonephritis and hypothyroidism. Colon biopsy revealed cryptitis and crypt abscess. He was followed-up with a preliminary diagnosis of immunodeficiency-polyendocrinopathy-enteropathy (IPEX) syndrome and regular IVIG replacement, antibiotic, antifungal prophylaxis and corticosteroid therapy were administered. No mutation was detected in the FOXP3 gene.

Patient 2, his elder sibling (8 year-old, boy) was admitted to our hospital about 4 years after the first case. He had recurrent respiratory tract infections, lymphoproliferation, hepatosplenomegaly, autoimmune hemolytic anemia and immune thrombocytopenic purpura, proteinuria due to focal segmental glomerulosclerosis, duodenitis and intermittent diarrhea.

Homozygous c.2496C>A, p.Cys382* mutation, leading to a the premature stop codon in the LRBA gene, was detected by whole exome sequencing in both male brothers, who had clinical findings of IPEX syndrome. The parents were shown to be heterozygous.

**Conclusions:**

The LRBA defect is characterized by recurrent infections, chronic diarrhea, autoimmunity, hepatosplenomegaly and should be kept in mind in cases with common variable immunodeficiency, IPEX syndrome and autoimmune lymphoproliferative syndrome.
VEOIBD PATIENT WITH NOVEL XIAP MUTATION SUCCESSFULLY TREATED WITH HLA-HAPLOIDENTICAL HSCT AFTER REMOVAL OF αβ+ T AND B CELLS
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Background:

XIAP mutations have been described in few IBD patients, in particular, with VEOIBD features. We describe a VEOIBD child who presented at 5 years of life. He had a complicated disease course and remained unresponsive to several lines of medical treatment options. Immunologic evaluation revealed mild decrease of IgG serum levels with poor antibody responses to tetanus toxoid, Haemophilus influenzae type b, Hepatitis B and reduction of transitional circulating B-cells. Of note, the patient showed persistent EBV viremia without EBV seroconversion.

Methods:

We performed Next Generation Sequencing (NGS) by Ion Torrent and functional studies (including flow-cytometry and western blotting) to investigate XIAP gene, protein expression and apoptosis before and after HLA-haploidentical HSCT.

Results:

The patient showed a novel and de novo hemyzigous mutation in XIAP gene. Functional studies revealed a reduction of protein expression in EBV-cell line and in the lymphocytes, granulocytes and monocytes subsets. The suspicious of a defective NOD2 pathway was demonstrated by the lack of IκBα degradation and an increased activation-induced cell death in PBMC. The child received haplo-HSCT after negative depletion of Tαβ+/CD19+ lymphocytes, using the mother as donor. Actually 1-year after HSCT, several functional studies were performed that revealed a complete immune-gastrointestinal recovery with XIAP expression in leukocytes and EBV-B-cell line. Six months and 1-year after HSCT we demonstrated the restored anti-apoptotic capacity and IκBα degradation suggesting a restored NF-kB activation.

Conclusions:

Our patient with a haplo-HSCT positive outcome underling how genetic analysis can allow a prompt diagnosis and lead to optimal treatment.
AN IMPAIRMENT OF ROS PRODUCTION IN MONOCYTES/MACROPHAGES MAY BE ASSOCIATED WITH INDUCTION OF GRANULOMATOUS ENTERITIS

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Background:

Reactive oxygen species (ROS) generated by the nicotinamide adenine dinucleotide phosphate (NADPH) are essential for intracellular killing of pathogens by phagocytes. In addition, ROS functions as pleiotropic signals in various inflammatory contexts. Chronic granulomatous disease (CGD) is a rare inherited disorder characterized by an inability of phagocytes to produce ROS, resulting in recurrent life-threatening infections and aseptic inflammatory bowel disease (IBD). Although dysregulated inflammatory cytokine production by monocytes and macrophages is thought to contribute to IBD in CGD, the underlying mechanisms are not fully understood.

Methods:

Although CYBB encoding gp91phox is one of the five causative genes for CGD, some types of the mutations also cause the syndrome of Mendelian susceptibility to mycobacterial disease (MSMD) by affecting monocyte-derived macrophages selectively. However, no IBD has been reported in patients with MSMD caused by CYBB mutations. Here is the first report of a patient who had a mutated CYBB resulting in impaired generation of ROS only in monocytes and developed IBD.

Results:

Pathological findings of his colons showed a marked infiltration of neutrophils and foamy macrophages, and the granuloma formation in the intestinal mucosa, which was similar to those of IBD associated with CGD. K562 cells transduced with his CYBB mutation demonstrated reduction of ROS production in response to lipopolysaccharide. In this patient, the inflammatory cytokine production by monocytes was increased compared with that of healthy subjects.

Conclusions:

Our results suggest that the inability of monocytes/macrophages to produce ROS would impact on the development of bowel inflammation.
Deficiency of adenosine deaminase 2 (DADA2), caused by bi-allelic loss-of-function mutations in CECR1 (cat eye syndrome chromosome region, candidate 1) followed by a decrease in ADA2 activity, is characterized by early-onset cerebral lacunar infarctions, cutaneous lesions (e.g., livedo racemosa), and chronic or recurrent systemic inflammation. Most DADA2 patients suffer systemic vasculopathy consistent with polyarteritis nodosa (PAN), although reports suggest wide phenotypic variability. The aim of our study was to describe the genetic and clinical diversity of the Japanese patients with DADA2.

Methods:

Five Japanese patients with confirmed DADA2 at the time 2017 were enrolled.

Results:

4 of 5 patients presented with early-onset cerebral infarction, as reported by Elkan (2014) or Zhou (2014). However, one patient revealed just fever and rashes with no evidence of vasculopathy. 4 of 5 patients were treated with corticosteroids, but all resistant. Now all of five patients started treatment with anti-TNF-α agent (infliximab, adalimumab, or etanercept) finally, then they have been free from symptoms. No ADA2 enzyme activity was detected in all patient’s plasma. Genetic analysis revealed two novel compound heterozygous mutations in one of five patients (c.984G>C p.Glu328Asp, c.706_708CTAdel p.Tyr236del), and the other are being tested.

Conclusions:

Accumulating evidence suggests that anti-TNF-α therapy is an effective treatment for DADA2. Early diagnosis of DADA2 would allow early intervention, which would prevent development of disabling complications.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0137

LRBA DEFICIENCY: CASE REPORT
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Background:
Lipopolysaccharide-responsive-beige-like-anchor (LRBA) protein deficiency causes an autosomal recessive combined immunodeficiency with severe autoimmune and inflammatory manifestations, including IBD and autoimmune cytopenias. Chronic diarrhea is a frequent and often severe symptom; in the majority of the cases, no pathogen is identified.

Methods:
10 years old girl was admitted with chronic diarrhea. She was diagnosed as type 1 diabetes when she was 9 months old. She had chronic diarrhea starting when she was 5 years old and she was diagnosed as Celiac disease, but she didn’t have any benefit from gluten free diet. She had a history of recurrent arthritis in her knees. Her arthritis and diarrhea was responsive to steroid therapy. There was no neutropenia, lymphopenia, anemia. Immunglobulin levels, lymphocyte subsets were normal but isohemaglutinin titer, hepatitis B vaccine response and memory B lymphocytes were low.

Results:
In the genetic analyze, it was detected a homozygous frameshift mutation in exon 23 of LRBA (A1184Sfs*34), which was confirmed by Sanger sequencing.

Conclusions:
In a patient with autoimmune manifestations even if not recurrent infections, it must be evaluated in terms of primary immune deficiencies/immune disregulation syndromes.
OLD GENE NEW PHENOTYPE: ADA2 DEFICIENCY


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Background:

With the advances in gene identification techniques genetic diagnosis for PIDs has become inevitable; however it also led to changes in the definition of disease characteristics. ADA2 deficiency (DADA2) was first described as a vasculopathy with early-onset strokes accompanied by mild immunodeficiency mainly affecting B cells. Recently, DADA2 was also identified in patients presenting with hypogammaglobulinemia without vasculopathy. Here we present a patient with DADA2, who has hypogammaglobulinemia and EBV induced lymphoma without vasculopathy.

Methods:

Case:

Results:

32 year-old female patient born out of consanguineous marriage presented to clinic with multiple lymphadenopathies and hepatosplenomegaly. Her past medical history was insignificant other than chronic hepatitis B infection. Lymphopenia and hypogammaglobulinemia were detected. Peripheral blood lymphocyte and B-cell subgroups were normal; however lymphocyte activation responses to phytohemaglutinin were low. Lymph node biopsy revealed EBV induced nodal peripheral T-cell lymphoma. EBV PCR copy numbers were 15000 copy/mL. Chemotherapy was initiated together with ivig replacement. During the disease course hemophagocytosis occurred in the bone marrow and remission was never achieved. She died following respiratory failure due to lung infiltration. Next generation sequencing based gene panel screening revealed a homozygous mutation on CECR1 gene, which was further confirmed by Sanger-sequencing.

Conclusions:

This is the first case of DADA2 reporting an immunodeficiency with EBV induced lymphoma without any rheumatologic symptoms. DADA2 should be considered as a PID with diverse spectrum.
NOVEL MUTATION IN X-LINKED INHIBITOR OF APOPTOSIS (XIAP) IN TWO CHINESE SINGAPORE BROTHERS WITH NUCLEOTIDE-BINDING AND OLIGOMERISATION DOMAIN (NOD)-2 SIGNALLING DEFECT

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Background:

X-linked inhibitory of apoptosis (XIAP) deficiency is an immune dysregulation syndrome characterised by recurrent splenomegaly and haemophagocytic lymphohistiocytosis (HLH), especially when triggered by Epstein-Barr virus. The genetic mutation is in the BIRC4 gene, but there is no consensus on an immunological diagnostic test. Based on the newly discovered role of XIAP in innate immunity, the stimulation of nucleotide-binding and oligomerisation domain (NOD)-2 by muramyl dipeptides (MDP) has recently been proposed as a diagnostic test for XIAP deficiency.

Methods:

We present two Chinese Singaporean brothers with a novel mutation in the BIRC4 gene, resulting in the deletion of one of seven exons. Their mother was found to be an asymptomatic carrier. The elder brother presented with Epstein-Barr virus (EBV)-associated chronic hepatitis at 7 years of age, requiring 2 years of valacyclovir and prednisolone. The younger brother presented at 4 months with incomplete HLH associated with EBV seroconversion, and was treated with a course of dexamethasone.

Results:

In both brothers, flow cytometry showed normal XIAP protein expression on T lymphocytes. Based on their mutations, this was not unexpected as most of the protein would be expressed. Activation induced cell death (AICD) assay showed excessive lymphocyte apoptosis upon stimulation compared to healthy control. The brothers also had markedly decreased IL6 and IL1b production on stimulation of NOD-2 by MDP after priming with EBV compared to controls, suggestive of defective NOD-2 signalling.

Conclusions:

Our results support the usage of AICD in combination with NOD-2 signalling assay for the immunological diagnosis of XIAP deficiency.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0155

AUTOSOMAL DOMINANT IMMUNE DYSREGULATION SYNDROME IN A PATIENT WITH CTLA4 DEFICIENCY

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Background:

The protein cytotoxic T lymphocyte antigen-4 (CTLA4), is an essential negative regulator of immune responses, and its loss causes autoimmunity and immune dysregulation.

Methods:

Here we present an 18 year old boy with CTLA4 deficiency.

Results:

The patient attempted to a local hospital with weakness and fatty stool when he was 10 years old. He was diagnosed as autoimmune hemolytic anemia (AIHA) with splenomegaly and treated with corticosteroids. He was hospitalized 2 more times for bronchopneumonia and AIHA. Laboratory tests showed panhypogammaglobulinemia. He was referred to pediatric immunology clinic for further evaluation. He had splenomegaly, failure to thrive, multiple lymphadenopathies, pancytopenia, panhypogammaglobulinemia and Cryptosporidium diarrhea. Lymphocyte subsets were normal with poor responses to vaccines, bone marrow aspiration and viral serology were also normal. Lymphadenopathies regressed after antibiotic therapy. Regular intravenous immunoglobulin replacement and prophylactic antibiyotherapy were started with the diagnosis of common variable immunodeficiency (CVID). Due to medical therapy refractory AIHA, he underwent splenectomy. During follow-up, he was treated for short stature and osteoporosis. He was hospitalized several times for fungal or bacterial bronchopneumonia and diarrhea due to Giardia lamblia or Cryptosporidium. He had lymphoproliferation during infectious episodes. Genetic analysis for X-linked lymphoproliferative syndrome (SH2D1A gene) was normal. Thorax computerized tomography showed chronic fibrotic lung changes and bronchectasis. He also had EBV and CMV viremia. We identified a heterozygous mutation in CTLA4 gene (c.518G>A, p.Gly173Glu) by whole exome sequencing.

Conclusions:

Many CTLA-4-deficient patients were clinically diagnosed with CVID. The phenotype of CTLA-4 deficiency includes autoimmunity, recurrent infections and lymphoproliferation.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0157

CASE REPORT OF EBV-POSITIVE B CELL LYMPHOMA IN A BRAZILIAN GIRL WITH HETEROZYGOUS GAIN-OF-FUNCTION MUTATION IN THE PIK3CD GENE.

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Background:

Mutation in PIK3CD gene causes ICV.

Methods:

The mutation PIK3CD: EXON 24: c. G3061A: p. E1021K was identified by whole-exome sequencing and targeted Sanger sequencing but was not found in the parents and in the two healthy brothers.

Results:

LSCO, born on 10/17/02, had onset of symptoms at 7 months manifested by recurrent sinopulmonary infections and progressive airway damage, otitis, lactose intolerance, lymphadenopathy and hepatosplenomegaly and several hospital admissions. From June 2010, she began her clinical follow-up at Hospital das Clínicas - UNICAMP, when was introduced antimicrobial prophylaxis. She showed persistent decreased numbers of CD4⁺ T cells (295 cells /mm³), increased numbers of CD8⁺ T cells (1314 cells/mm³), inversion of the CD4 / CD8 ratio (0.22), low number of CD19⁺ (252 cells/mm³), NKT (28 cells/mm³) and NK cells (160/mm³), hypergammaglobulinemia (IgG 1600 mg/dL), increased IgM (190 mg/dL), IgA <6.5 mg/d and IgE <4.38 UI/mL. Her 12-year-old brother with the same phenotype died of H1N1 infection. In August, 2013 lymph node immunohistochemistry showed atypical lymphoid hyperplasia, germinal center disintegration with CD23⁺, CD10⁺ on disaggregated lymphoid follicles, BCL2⁺ on perinodular lymphocytes, BCL6⁺ on disintegrated lymphoid follicles, Kappa⁺ and lambda⁺ on several plasmocytes, CD15⁺ in granulocytes, CD30⁺ on larger lymphoid cells, LMP-1 negative and Ki67⁺ in 80% of cells. Since 2012 she was relatively well but in May 2016 she manifested daily fever, lymphadenomegaly, hepatosplenomegaly and weight loss. The immunohistochemistry in an lymph node showed an EBV⁺ B cell Lymphoma.

Conclusions:

This aggressive form of her previous disease was associated with B cell lymphoma.
EXPLORING THE WARNING SIGNS OF PRIMARY IMMUNODEFICIENCIES AMONG PAKISTANI POPULATION
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Background:
Various warning signs to predict primary immunodeficiency diseases (PID) have been promoted by many international organizations. However, the ability of these warning signs to identify children with PID has not been rigorously tested. The main goal of this study was to determine the effectiveness of these warning signs in predicting defined PID among children who presented to Sir Ganga Ram Hospital, Lahore, Pakistan.

Methods:
A retrospective survey of 400 children was undertaken. The clinical records of 270 patients with a defined PID and 130 patients for whom detailed investigations failed to establish a specific PID were reviewed.

Results:
Overall, 96% of the children with PID were referred by hospital clinicians. The strongest identifiers of PID were a family history of immunodeficiency disease in addition to use of intravenous antibiotics for sepsis in children with neutrophil PID and failure to thrive in children with T-lymphocyte PID. With these 3 signs, 96% of patients with neutrophil and complement deficiencies and 89% of children with T-lymphocyte immunodeficiencies could be identified correctly. Family history was the only warning sign that identified children with B-lymphocyte PID.

Conclusions:
PID awareness initiatives should be targeted at hospital pediatricians and families with a history of PID rather than the general public. Our results provide the general pediatrician with a simple refinement of 10 warning signs for identifying children with underlying immunodeficiency diseases.
A "SYNDROME OF ASEPTIC ABSCESSES" CAUSED BY COMPOUND HETEROZYGOUS MUTATIONS IN FAM105B

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Background:

Symptoms of autoinflammation often resemble those of systemic infection.

Methods:

Case report.
A 7.5 y/o boy of non-consanguineous parents from Greece was admitted for abdominal pain. As an infant he had presented with pneumonia and a gluteal abscess which had been "difficult to treat". The child developed fever, neutrophilia of 40.000/ul, CRP of 400 mg/l and S100A8/A9 of 66.000 ng/ml, ESR however remained normal.
The patient was treated with antibiotics for suspected pneumonia. One week after fever onset, the boy developed a bubo-like swelling on his forearm. Pus was surgically drained, and a skin biopsy sent for histological evaluation. Within one week additional pyoderma-like skin lesions appeared. Imaging was suggestive of lung abscess in the left lower lobe. Surgical excision of a lung segment was performed with discharge of necrotic purulent material upon thoracotomy. No pathogens were grown or identified by eubacterial and panfungal PCRs including mycobacteria. With a working diagnosis of autoinflammatory disease, treatment with corticosteroids was started on day 16 following fever onset with resolution of fever and CRP. On day 19 a complex surgical intervention including splenectomy, resection of pancreatic tail and axillary dissection was performed as MR-imaging suggested multi-organ abscess formation.

Results:

Histologies of skin, lung and spleen showed non-characteristic inflammation with necrosis. Whole exome sequencing identified compound heterozygosity for the following mutations in the FAM105B (otulin) gene: c.[258G>A];[500G>C] p.[Met86Ile];[Trp167Ser].

Conclusions:

Identifying the pathophysiology of aseptic abscess formation has a direct implication for treatment. Anti-TNFα inhibition should prevent future inflammatory episodes and potentially life-threatening multi-organ abscess formations.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0170

RELATIONSHIP BETWEEN BIOMARKERS AND CARDIOVASCULAR RISK IN RHEUMATOID ARTHRITIS

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Background:

Patients with RA have an increased cardiovascular morbidity and mortality (CV) being the main cause of death. “Systematic Coronary Risk Evaluation” (SCORE) allows us to estimate the risk of death from cardiovascular disease as 10 years.

Objectives: To evaluate whether the presence of anti-peptide citrullinated (ACPA) antibodies is associated with increased frequency of cardiovascular disease (CVD) in patients with rheumatoid arthritis (RA) or with greater SCORE.

Methods:

Observational, analytical case-control study nested in a cohort of patients diagnosed with AR. Cases were considered the patients who have developed a myocardial infarction or cerebrovascular or ischemic heart disease and controls RA patients without CVD. They all determined ACPA levels.

Results:

Observational, analytical case-control study nested in a cohort of patients diagnosed with AR follow-up in the Valme hospital area. Cases were considered the patients who have developed a myocardial infarction or cerebrovascular or ischemic heart disease and controls RA patients without CVD. They all determined ACPA levels, the classic cardiovascular risk factors.

<table>
<thead>
<tr>
<th></th>
<th>ACPA+</th>
<th>ACPA-</th>
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</thead>
<tbody>
<tr>
<td>Cardiovascular events</td>
<td>12,50%</td>
<td>16,90%</td>
</tr>
<tr>
<td>SCORE Risk (&lt;5)</td>
<td>74,70%</td>
<td>75,70%</td>
</tr>
<tr>
<td>High risk score (5,10)</td>
<td>10,70%</td>
<td>6,90%</td>
</tr>
<tr>
<td>Very high score (&gt;10)</td>
<td>13,60%</td>
<td>18,40%</td>
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</tbody>
</table>

When analyzing separately the association between the presence of ACPA+ and classic cardiovascular risk factors, we found no statistically significant differences between ACPA (+) v ACPA-.
Conclusions:

We found no differences in the occurrence of cardiovascular events, or the SCORE in patients ACPA (+) v ACPA (-). While subgroups of patients with ACPA + have a greater tendency to be treated with biological therapy.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0171

ASSESSMENT OF BIOLOGICAL THERAPY IN PATIENTS WITH PSORIASIS ARTHRITIS

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Background:

Objective: To evaluate the effectiveness and long-term safety of TB in a series of patients with PSA.

Methods:

Observational descriptive study of a series of 25 patients with diagnosis of PSA with peripheral affection according CASPAR criteria, in treatment with TB.

Results:

25 patients diagnosed with PSA with TB treatment were reviewed. Etanercept was the drug most used in first line (36%), followed by Infliximab (32%), Adalimumab (16%), Golimunab (12%) and Certolizumab (4%). In 2nd line of treatment, also Etanercept was the most used followed of Adalimumab. A single patient with highly refractory APS was treated with Abatacept in the fourth line and is currently being treated with Ustekinumab in the 5th line.

At the cut-off point after the first biology, we found: All the patients who were in remission and with low activity.

The rest of the patients with TB, we found after treatment with the first drug 60% were in remission, 24% low activity and 16% remained with moderate activity.

In the following treatment lines, the decrease in DAS 28 does not show statistically significant differences. Moreover, in the evaluation of successive TB, the sample size does not allow us to establish efficacy data with statistical significance.

Survival time with the first drug was: the median 14 months (minimum 3 months and max 156 months)

Conclusions:

TB is an effective and safe option of treatment that should be considered in patients with moderate to severe PAs, who are resistant to classical therapies or who have contraindications to conventional drugs.
PROFILE OF A SERIES OF PATIENTS WITH PSORIATIC ARTHRITIS TREATMENT WITH BIOLOGICAL THERAPY.
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², Seville, Spain

Background:

The main aim was to describe the characteristics of a series of patients from the southern hospital area of Seville (Hospital Virgen de Valme) with PSA diagnosis in treatment with biological therapies (TB)

Methods:

Retrospective observational study of 25 patients diagnosed with APS with peripheral affection according to CASPAR criteria, in treatment with TB registered in the database of the UGC of Rheumatology of the hospital Virgen de Valme. Description of baseline sociodemographic characteristics, age, sex, APs form, cutaneous involvement, and indicated biological therapies

Results:

25 patients have been reviewed.

Of these, 68% were men, compared to 32% women. The mean age at present is 48 years, and the mean age at diagnosis is 37 years. Or this 92% has cutaneous involvement and 12% of nail involvement.

The clinical profiles were: 40% has polyarticular affection, 36% oligoarticular, 12% axial, 8% axial and peripheral, and 4% involvement of the distal interphalangeal.

The TB most used as first line of treatment was Etanercept in 36%, followed by Infliximab in 32%, and Adalimumab (16%). In the 2nd line of treatment, we again find Etanercept as the most used drug followed by Adalimumab, which was the most used also in 3rd line.

Finally we found the 84% of the patients were responders to the 1st biological therapy measured by a DAS28 <2.6 (almost remission) or, failing that, a DAS28 <3.2 (low activity) and / or an MAE.

Conclusions:

TB in general is effective in achieved high response rates after the first biological in real clinical practice conditions.
CARDIOVASCULAR RISK IN RHEUMATOID ARTHRITIS.

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Background:
Although there are conflicting data on the subject, it seems that there is a higher incidence of cardiovascular events (CVE) in Rheumatoid Arthritis (RA) compared to population control. We must consider the severity of the inflammatory process plays an essential role in accelerated atherosclerosis in this pathology. On the long term security of biological therapies, we find heterogeneous data in different studies.

The main aim was to evaluate the CV risk and prevalence of CVD in RA treated with biological therapies versus RA treated with classics disease modifying drugs.

Methods:
Retrospective cohort study. Cohort of 260 patients diagnosed with RA (ACR 1987). The CV risk was measured by the estimated SCORE of atherosclerotic cardiovascular mortality risk in 10 years, calibrated to Spain.

Results:
72% were women, age of 58.5 years. 48% were receiving biological therapy with or without concomitant DMARDs. 38% overweight and obesity in 32% of patients, 14% had diabetes mellitus and 39% hypertension; 51% had dyslipidemia and 18% were smokers. 61% ACPA + and 65% FR +. 13% of patients who did not realize biological therapy treatment showed a high Score (5-10) versus 3.7% of patients with biological therapy treatment, this difference was statistically significant.

Conclusions:
Patients treated with biological therapy have lower cardiovascular risk than patients treated with DMARs. We don’t found differences in the prevalence of CVE between groups. Probably the reduction of inflammation with TB has direct relation with cardiovascular risk reduction in patients with RA.
ARE THERE ANY RELATION BETWEEN DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS AND BIOMARKERS

Background:

Anti citrullinated peptide antibodies (ACPA) are the most specific serological markers of Rheumatoid arthritis (RA) with a specificity of 98-99% in some studies. The presence of autoantibodies such as rheumatoid factor (RF) and ACPA is related to more severe structural damage and joint destruction during disease progression.

To assess whether the presence of ACPA is associated with higher levels of disease activity score (DAS 28) in a sample of patients with RA.

Methods:

We performed a descriptive study of a cohort of patients diagnosed with RA. Demographic data, immunological profile (presence of RF and / or ACPA), treatment (DMARDs or biological therapy) and disease activity measured by DAS 28 were collected transversely.

Results:

260 patients, age of 58.5 years, 72% women, 48% were receiving biological therapy. 61% were ACPA positive, 65% were RF positive and 54.3% were ACPA and RF positive. Mean DAS28 was 2.6 for RF and ACPA negative patients, compared to 2.8 for patients that showed positivity for one or both autoantibodies, without statistically significant difference. With respect to the subgroup of patients receiving biological treatment (n = 122), no significant difference of the mean value of DAS28 was seen (regarding to the presence / absence of ACPA): DAS28 2.53 for ACPA positive versus 2.52 for ACPA negative.

Conclusions:

New studies suggest that, although ACPA positivity does maintain relationship with structural damage, there is no association with the severity of clinical activity measured by DAS28. We found no relationship between the presence of ACPA and / or RF and disease activity.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0178

A REGISTRY FOR HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS BY THE HISTIOCYTE SOCIETY AND THE EUROPEAN SOCIETY FOR IMMUNODEFICIENCIES

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Background:

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory syndrome. The full picture usually requires prompt aggressive treatment, particularly in primary HLH. Current treatment schemes in primary HLH utilize immunoglobulins, glucocorticosteroids, etoposide, ciclosporin A, and anti-CD52 antibodies, followed by hematopoietic stem cell transplantation.

Methods:

A joint registry for HLH has been set up by the European Society for Immunodeficiencies (ESID) and the Histioyte Society (HS). It includes patients with primary forms of HLH and patients who can initially not be reliably distinguished from primary HLH, i.e. predominantly infection-associated HLH, excluding patients with known autoimmune, autoinflammatory, malignant or metabolic disease, or visceral leishmaniasis. Patient data focus on information required to judge if a patient is a candidate for an interventional trial. They are documented electronically via Marvin (running since Mai 2016) or the ESID registry online tool (final testing of level 3 module under way), depending on center preference.

Results:

From May 2016 until March 2017, 32 centers have opened the registry. Seventeen patients have been registered, median age 1.2 years. The preliminary disease classification was primary (n=8), secondary (n=5), or undetermined (n=4) HLH. CNS involvement was found in 2 patients, a viral trigger in 7 patients. Three patients had received pre-treatment prior to transferal to the documenting center. One year follow-ups will deliver information on treatment, HSCT, and final disease classification.
Conclusions:

This is the first registry that combines the HLH expertise of HS and ESID and may serve as basis for future international interventional trials. Other centers are invited to join.
HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS SECONDARY TO PRIMARY IMMUNODEFICIENCY

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Background:

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening syndrome with high mortality rates. HLH develops due to underlying genetic defects, primary immunodeficiencies (PID), secondary to infections, malignancies, metabolic, autoimmune diseases.

Methods:

We presented 11 patients with HLH and PID.

Results:

Patients: 11 patients (M/F:8/3) with the diagnosis of Chédiak-Higashi (1), Gricelli (5), SCID (2), CD27 defect (1), EBV-associated lymphoproliferative disease (EBV-associated LPD) (1), CVID (1) were enrolled in study. Age at the beginning of symptoms, age of HLH was 3 months (1-24 months), and 11.5 months (2.5-85 months) old respectively. All had fever, splenomegaly, hemophagocytosis in bone marrow. Ferritin was high (>500 ng/ml) in nine, triglyceride was high (>265 mg/dl) in seven, fibrinogen was low (<150 mg/dl) in seven patients. All were fulfilled 5 out of 8 HLH criteria. All were given HLH-2004 protocol (IVIG/dexamethasone/cyclosporine A/etoposide), intrathecal therapy were added to four. Three patients were transplanted. Four died due to complications of HLH. HLH was the initial manifestation of PID in nine. There was a negative correlation between fibrinogen and intrathecal therapy need (r=−0.657, p=0.028).

Conclusions:

HLH may be the initial presentation in patients with PID. High suspicion in diagnosis and prompt initiation of treatment are important on prognosis.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0191

GASTROINTESTINAL MANIFESTATIONS IN A MONOCENTRIC CVID COHORT
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Background:
Gastrointestinal involvement is reported in 9-20% of Common Variable Immunodeficiency Diseases (CVID) patients. Acute or chronic infectious diarrhoea is the most common gastrointestinal symptom in CVID. Non-infectious enteropathy occurs in 10-12% of CVID patients and may resemble coeliac disease or inflammatory bowel disease.

Methods:
Clinical, laboratory and histological data of CVID children and adults referred to the PID Centre of the Fondazione-IRCCS Cà Granda in Milan have been reviewed.

Results:
Thirty-four CVID patients, 4 children and 30 adults, have been enrolled. Median age is 33.5 years, ranged 11-70. Prospective follow-up mean time is 9 years (range 2-37). All patients are under immunoglobulins replacement therapy.

Thirteen adult patients (38.2%) reported gastrointestinal symptoms: 3 epigastric pain/dyspepsia, 7 chronic/relapsing diarrhoea and 3 both symptoms. Infectious diarrhoea was demonstrated in 7 patients. H. Pylori, Salmonella, Campylobacter, Clostridium, Norovirus and Rotavirus were isolated.

Four patients were diagnosed for gastritis, four for celiac disease, five for lymphocytic colitis and one for ulcerative colitis.

Granulomatous liver disease was found in 3 patients. Biopsy confirmed non-caseating granulomas. Two patients were found to have chronic viral infection: Cytomegalovirus and Epstein-Barr virus, respectively.

Diarrhoea improved when treated with antibiotics according to isolated pathogen, but relapsing culture-negative events are frequent. Gluten-free diet ameliorates symptoms in celiac disease, but not solved. Two patients with lymphocytic colitis need chronic budesonide treatment to control diarrhoea. Two patients temporary solved symptoms with local mesalazine.

Conclusions:
Management of gastrointestinal involvement in CVID is challenging because intestinal biopsy specimens often have distinct histologic features, and these patients often fail to respond to conventional therapies.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0192

A CASE OF ADULT ONSET STILL'S DISEASE (AOSD) CASED BY A NOVEL SPlicing MUTATION IN TNFAIP3 SUCCESSFULLY TREATED WITH TOCILIZUMAB

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Background:

TNFAIP3 encodes the NF-κB regulatory protein A20. High-penetrance heterozygous mutations in TNFAIP3 cause a haploinsufficiency of A20, inadequate inhibition of NF-κB pathway and an autoinflammatory disorder resembling Behcet’s disease. Here we describe a patient who has a novel splicing mutation in TNFAIP3 but presenting with AOSD.

Methods:

The TNFAIP3 variant was identified using a targeted gene panel. The mutation was confirmed using Sanger sequencing.

Results:

32 years-old female patient presented at the age of 16 with high fever, abdominal pain, urticarial-like rash and polyarthritis. She continued to have ongoing problems with the rash, polyarthritis and systemic inflammatory response despite regular corticosteroids and DMARD’s. Eventually she responded to tocilizumab. She has two children who both have episodic sterile fevers associated with pharyngitis and cervical lymphadenopathy. Her father has a history of early onset rheumatoid arthritis.

The patient was found to have a novel heterozygous variant in TNFAIP3 c.1906C>T. This variant was not found in ExAC database. Further analysis shows that this single nucleotide variant at the terminal residue of TNFAIP3 exon 6 produces an alternatively spliced mRNA resulting in p.His636fsTer1. Further genetic analysis of family members shows that this variant does segregate with the inflammatory clinical phenotypes.
Conclusions:

Recently 2 additional cases of A20 haploinsufficiency have been published one presenting with ALPS-like disorder, and another with complex autoimmunity. Our case adds to the expanding spectrum of clinical phenotypes associated with A20 haploinsufficiency.
NEWBORN WITH FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: A DIAGNOSTIC AND THERAPEUTIC CHALLENGE

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Background:

Hemophagocytic lymphohistiocytosis (HLH) is an aggressive and life-threatening syndrome of excessive immune activation.

Methods:

Case Report

Results:

The authors report the case of a newborn male, first child of a healthy, non-consanguineous couple. The pregnancy was uneventful until 35th week when splenomegaly was found on the obstetric ultrasound. He was born at 39 weeks of gestation without complications. Moderate to severe splenomegaly was confirmed and a petechial rash was observed in the first hours of life. Thrombocytopenia was found and required platelet transfusions. Infectious, alloimmune and metabolic causes were excluded. On D12 neutropenia was detected (921/µL) and on D20 he had fever and pancytopenia. After exclusion of sepsis, elevated levels of ferritin (11341 ng/mL) and soluble CD25 (13365 U/mL) and hypofibrinogenemia (110 mg/dL) confirmed the diagnosis of HLH. A complete remission was achieved with dexamethasone, cyclosporine and alemtuzumab. He acquired CMV infection from maternal milk and was treated with ganciclovir without complications. A homozygous nonsense mutation was identified in UNC13D gene (familial HLH type 3). At the age of 4 months, he was submitted to haploidentical hematopoietic stem cell transplantation (HSCT). After seven months of follow-up, he is clinically well.

Conclusions:

Familial HLH is a rare disease, and its presentation and diagnosis in the neonatal period are infrequent. The diagnostic approach is complex and requires a high level of clinical suspicion. This disease is rapidly fatal in the absence of treatment. Therefore, early initiation of specific therapy and urgent HSCT determines the survival of these children.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0196

AN ATYPICAL CASE OF FAMILIAL MEDITERRANEAN FEVER: ARE WE MISSING ANOTHER PID?

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Background:

Familial Mediterranean Fever (FMF) is an autoinflammatory disorder that causes recurrent fever and painful inflammation of abdomen, lungs and joints.

Methods:

We present an atypical case of FMF: 7 year-old girl referred to Paediatric Immunoallergology Unit to assess hypergammaglobulinemia and high IgE.

Personal history:

- Pre-term birth (36 weeks), non-consanguineous parents from Morocco.
- Admitted to the neonatal unit due to sepsis suspicion. Negative cultures.
- Admitted to the paediatric ward at 60 days old due to prolonged fever, diarrhoea and anaemia.
- Admitted at 4, 14 and 36 months due to diarrhoea, anaemia and splenomegaly.
- Recurrent abdominal pain and microcytic anaemia since age 3.
- Sensorineural hearing loss.

Complementary explorations:

- High IgG (1823 mg/dl) and IgE (1314 UI/ml), microcytic anaemia.
- Genetic analysis: 3 MEFV mutations in heterozygosity.

Results:

Evolution:

The patient started taking colchicine (1mg/day) at age 8, showing improvement of general condition, anaemia, abdominal pain and hearing loss, but progressive increase of amyloid A, requiring dosage increase up to 3 mg/day.
In October 2016 (age 15) she started episodes of abdominal pain and vomiting every four weeks. In January 2017 she was admitted to Hospital due to clinical worsening, presenting an abrupt increase of inflammatory parameters, intestinal subocclusion, pericardial effusion and microcytic anaemia (Hb 8,1g/dl). Treatment was changed to Anakinra 100mg/24h with subsequent improvement. Study extension is carried out with other immunodeficiencies, inflammatory bowel disease, infections and other.

**Conclusions:**

- FMF can debut at early age with atypical manifestations that force an extensive differential diagnosis.

- Complications and presence of amyloid A must be carefully monitored.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0200

CLINICAL AND IMMUNOLOGICAL CHARACTERISTICS OF FIVE CHILDREN WITH LRBA DEFICIENCY FROM AN ANDALUSIA PATIENT COHORT

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Background:

LRBA (lipopolysaccharide responsive beige-like anchor protein) is involved in vesicular traffic and deficiency affects re-cycling of cytotoxic lymphocyte antigen 4 (CTLA4) to the plasma membrane; the later being a checkpoint receptor turning down immune responses. LRBA deficiency causes autoimmunity, lymphoproliferation, infections and enteropathy, commonly seen in ALPS, CID and CVID disorders. We present immunological and clinical characteristics of children with LRBA deficiency from Andalusia, Spain.

Methods:

NGS was performed using an AmpliSeq strategy on an Ion Torrent PGM platform. A 115 genes panel related to combined and humoral deficiency and lymphoproliferative disorders was designed. T and B lymphocyte immunophenotyping including CTLA4 expression of CD45RA⁻Foxp3+ T cells were analyzed.

Results:

In a cohort of 47 patients with suspected immune dysregulation syndromes, five children, four girls and one boy, aged 4-12 years, with previously no described LRBA mutations (3 homozygous, 1 double heterozygous and 1 heterozygous only) were identified. All children suffered from lymphadenopathy and splenomegaly; four from autoimmunity; two from recurrent infections, and one each from lung and inflammatory bowel disease (IBD). T and B cell immunophenotype showed low memory B and Treg (CD4/CD25/FOXP3) cells, elevated transitional B and α/β CD4-/CD8- double negatives T cells. Whilst unstimulated CTLA-4 expression was markedly reduced in LRBA patients, stimulation of T cells including the lysosomal degradation inhibitor bafilomycin A (BafA) resulted in a nearly normal CTLA-4 expression.

Conclusions:
Our patients with LRBA deficiency showed B cell (CVID Euroclass smB-TrhiCD21low) and T cell alterations (elevated DNT's and reduced CTLA-4 expression). An ALPS-like phenotype may justify screening for LRBA deficiency.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0201

A NOVEL HOMOZYGOUS MUTATION IN IL10RA CAUSING SEVERE INFANTILE ONSET IBD.
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Background:

Very early onset IBD (VEO-IBD) presents in patients younger than 6 years of age. Within these patients is a subset with infantile IBD, which develops in children less than 2 years of age. VEO-IBD can be caused by mutations in IL10 or IL10R and is associated with a more severe phenotype resistant to conventional therapy. Due to the prevalence of colitis as a presenting symptom of primary immunodeficiencies (PIDs), patients with very early onset and neonatal IBD may be initially referred to a clinical immunologist.

Methods:

The patient’s lymphocyte subsets and immunoglobulin levels were assessed in a clinical laboratory. Next generation sequencing using Ion Torrent S5 System using the PIDv2 panel with a coverage of 1232x.

Results:

The patient is an eight-month-old male who presented with severe bloody diarrhea, failure to thrive, and fever since two months of age. No evidence of infection was found. Immunoglobulin levels and absolute numbers of T, B, and NK cells were normal. Colonoscopy and biopsy showed severe colonic ulceration and diffuse inflammation. He had poor response to hydrolyzed formula and steroids. Ion Torrent sequencing revealed a novel homozygous mutation in IL10RA (c.499T>C, p.Tyr167His). He died due to intravenous catheter-related sepsis during the preparation for hematopoietic stem cell transplantation (HSCT).

Conclusions:

We present a novel mutation in IL10RA causing severe infantile IBD in a patient referred for evaluation of a primary immunodeficiency. We highlight the importance of including monogenic causes of colitis in targeted gene sequencing panels.
Background:

Defects in proteins of the inflammasome complex or in the NF-kB pathway are responsible for monogenic autoinflammatory syndromes. Since clinical features are usually non-specific and overlapping, molecular diagnosis is helpful in dissecting disease pathogenesis and may provide insights for therapeutic options.

Our aim is to characterize an inflammatory disease in a 12-year-old boy with early-onset episodes of recurrent fever, psychomotor and growth delay, multiple oral and genital ulcers and persistently increased acute phase reactants. We suspected that his inflammation is caused by the haploinsufficiency of A20 due to a chromosomal deletion that includes the TNFAIP3 gene.

Methods:

A CGH-array was performed due to patient’s syndromic features. Activity of the NF-Kb canonical pathway was assessed by Western Blot following stimulation of patient’s fibroblasts with TNF. Immunoprecipitation experiments with antibodies against K63b and immunoblotting against IKK (NEMO) and RIP1 were carried out to evaluate the ubiquitination function of A20.

Results:

CGH-array identified a 13 Mb genomic deletion in chromosome 6 that encompasses 35 genes. Patient’s fibroblasts showed a clearly decreased A20 expression, and consequently, an increase in the phosphorylated levels of NEMO IKBa, P65, JNK and P38 upon TNF stimulation. Patient’s cells displayed increased levels of total K63-Ubiquitin proteins and increased K-63 ubiquitination of RIP1 and NEMO levels.
Conclusions:

Karyotype and CGH-array allow a comprehensive diagnosis of syndromic patients with immune dysregulations and should be the first diagnostic step in these cases. Further analysis confirmed that the autoinflammatory phenotype in our patient is caused by the haploinsufficiency of A20.
SUCCESSFUL MISMATCHED BONE MARROW TRANSPLANTATION IN A PATIENT WITH IPEX SYNDROME USING POST-TRANSPLANTATION CYCLOPHOSPHAMIDE FOR GVHD-PROPHYLAXIS

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³Friedrich-Alexander-University Erlangen-Nürnberg, Nephropathology- Institute of Pathology, Erlangen, Germany

Background:

Immunedysregulation, polyendocrinopathy, X-linked (IPEX) syndrome is caused by defects in the FOXP3 gene leading to dysfunction of regulatory T cells and loss of self-tolerance. Patients present early in life with various phenotypes of immundysregulation including polyendocrinopathies, enteropathy and eczema. Hematopoietic stem cell transplantation is the only curative treatment and often associated with poor outcome.

Methods:

We report on a patient presenting with neonatal diabetes, who was diagnosed with a disease causing variation in FOXP3 (c.1010G>A, R337Q). At the age of 11 weeks he developed severe nephrotic syndrome with proteinuria (>11g/d), edema and low serum protein. Around the same time he manifested with protracted diarrhea, failure to thrive, hypothyroidism and mild eczema. Although no serum auto-antibodies could be detected, a kidney biopsy revealed anti-PLA2R antibodies bound to the glomerular basal membrane suggesting primary membranous nephropathy.

Results:

Pre-conditioning administration of alemtuzumab resulted in rapid drug excretion due to proteinuria, as shown by serum levels measured 3h and 65h after end of treatment. Since no effective serotherapy could be expected, post-cyclophosphamide was applied as GvHD-prophylaxis after myeloablative conditioning with fludarabine/busulfan and bone marrow transplantation from an unrelated donor with one antigen mismatch. On d+51 the patient has stable hematologic engraftment with full donor chimerism and >700/µl T cells reactive to CMV. He is in clinical remission with resolved proteinuria (<0.3g/d), and he has no signs of GvHD.

Conclusions:

In conclusion, we demonstrate successful bone marrow transplantation from a mismatched donor in a patient with IPEX syndrome receiving post-transplantation cyclophosphamide as GvHD-prophylaxis.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0231

TREATMENT WITH ANAKINRA. FIRST UKRAINIAN EXPERIENCE
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Background:
Since 2010 the number of patients with autoinflammatory diseases (AIDs) in Ukraine is growing up. The spectrum of AIDs in Ukraine is: 3 patients with confirmed CINCA/NOMID, 3 patients with confirmed FMF, 10 patients with periodic fever syndromes (no genetic testing) including cold induced-, TRAPS-, SAVI-like diseases and 42 patients with PFAPA. But only two patients receive anakinra on the regular basis. Here we present two patients with genetically confirmed CINCA/NOMID and HIDS.

Methods:
Diagnosis were varifcated by clinical picture, laboratory inflammatory markers, level mevalonic acid in urine and genetic tests.

Results:

HIDS. Glib, 3,5 years old boy. Manifestation of illness started just after birth with severe impact on boy’s development and frequent febrile episodes. Treatment with different medications was ineffective. The diagnosis was made at 18 month of age. Anakinra was initiated at 2,5 years.

CINCA/NOMID. Katia, 8 years old girl. Manifestation of disease started just after birth with severe symptoms: joints, skin, CNS, ear and eye involvement. Right diagnosis was delayed up to 3 years. Anakinra was initiated at 3,5 years.

After initiation of anakinra we observed dramatic improvement of disease course. Such treatment allowed to control aggressive inflammatory response and rapid regression of all symptoms.

Treatment with anakinra is carried out by parent resources and sponsors.

Conclusions:
Awareness about autoinflammatory disorders is still poor in Ukraine. Absence of easy access to treatment with anakinra in Ukraine is a real challenge for patients and pediatric immunologists. Searching way to facilitate access to anakinra is always a question to be resolved.
A DIAGNOSTIC CHALLENGE OF CVID WITH GRANULOMATOUS INFLAMMATION

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Background:

This is a case of palatal perforation due to granulomatous inflammation in the context of Common Variable Immune Deficiency (CVID).

Methods:

N/A

Results:

A 31-year old female with a background of Diamond-Blackfan anaemia, CVID, and bronchiectasis presented in December 2014 with palatal perforation.

The patient was initially treated as presumed ANCA- negative GPA based on the presence of necrotising granulomatous inflammation on histopathological specimens from nasal mucosa. Other differential diagnoses were excluded.

She showed a good response to high dose steroids and cyclophosphamide; however, she developed new lesions involving external aspects of the nose and cheeks. Following a multidisciplinary discussions and further skin biopsies it was thought that CVID with granulomatous inflammation is the most likely working diagnosis.

The patient has been trialed on different immunosuppressant therapies including cyclosporine A and methotrexate without significant clinical response. Currently she is receiving rituximab and methotrexate.
Conclusions:

The purpose of this report is to illustrate a case that continued to present a significant diagnostic and therapeutic challenge.

<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
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<tbody>
<tr>
<td><strong>Inflammatory markers:</strong></td>
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<tr>
<td>CRP</td>
<td>15</td>
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<tr>
<td>ESR</td>
<td>80</td>
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<tr>
<td><strong>U&amp;Es</strong></td>
<td>Within reference ranges</td>
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<tr>
<td>Hb</td>
<td>105</td>
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<tr>
<td>WBC</td>
<td>Within reference ranges</td>
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<tr>
<td>Platelet</td>
<td>449</td>
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<td><strong>LFTs:</strong></td>
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<tr>
<td>ALT</td>
<td>16</td>
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<tr>
<td>ALP</td>
<td>128</td>
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<tr>
<td><strong>Autoimmune and vascular screen</strong></td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Microbiology cultures from nasal biopsies</strong></td>
<td>Jan 2015: positive for E. Col which was sensitive to co-amoxiclav and ciprofloxacin. March 2015: negative for viral and atypical organisms</td>
</tr>
<tr>
<td><strong>Microbiology/virology/Atypicals</strong></td>
<td>Negative including HIV, hepatitis screen, syphilis, toxoplasma, HSV/7</td>
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<tr>
<td><strong>Radiology:</strong></td>
<td></td>
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<tr>
<td>CT sinuses (Mar 2014)</td>
<td>A small nasal septal defect</td>
</tr>
<tr>
<td>CT sinuses (Feb 2015)</td>
<td>Increased destruction of nasal septum, almost complete destruction of the hard palate (Figure 2)</td>
</tr>
<tr>
<td>MRI sinuses (Nov 2015)</td>
<td>Near complete absence of the nasal septum, absence of turbinates</td>
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<tr>
<td>MRI sinuses (May 2016)</td>
<td>Extensive erosive nasal septal process. No gross internal progression</td>
</tr>
<tr>
<td>PET scan (Aug 2016)</td>
<td>No evidence of asymptomatic disease elsewhere</td>
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</tbody>
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KAWASAKI DISEASE IN UKRAINIAN CHILDREN
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Background:

Kawasaki disease (KD) is acute inflammatory condition of childhood affecting medium size arteria. If untreated, it leads to heart insufficiency, carditis, coronary aneurysms formation including myocardial infarction. The number of patients with Kawasaki disease in Ukraine grows rapidly during last 5 years. Actually, the statistical data concerning KD in Ukraine is absent. Objective of this study was to describe clinical picture of KD in Ukrainian children, underline importance of this disease for Ukraine and to try establish its incidence for Ukrainian population.

Methods:

The study was retrospective. We collected data from 21 children with KD and we analyzed their clinical manifestations, cardiac involvement and coronary aneurysms formations.

Results:

All children 7 month to 10 years were citizens of Kyiv. 11 had complete KD on the day of IVIG administration and 10 incomplete disease. One 7-month year old girl died on 14 day of illness because of myocardial infarction. Coronary arteria involvement was seen in 60% of patients who received IVIG after 10 days if illness. Coronary aneurysms formation was in 83% children under 12 month of age, compare to older children (F-test 6,11; p=0,024; φ 0,57).

Conclusions:

We describe the largest group of KD patients presented so far in Ukraine. Estimated incidence of KD in Kyiv in 2016 was 5,3 / 100 000 of children under 5 years. The same indicator could be used for Ukraine because of population similarities.
CORRELATION OF VERY HIGH FERRITIN LEVELS WITH DIAGNOSIS OF HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS AND ASSOCIATED MORTALITY

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Background:

Hemophagocytic Lymphohistiocytosis can be fatal when uncontrolled. The symptoms mimic other illnesses, and delay treatment leading to mortality. Measurement of ferritin levels is simpler, cheaper and reported on the same day, as compared to IL-2Ra measurements which are complex. Here we analyzed the utility of quantitative ferritin measurements as an effective tool for the diagnosis of HLH.

Methods:

All patients with ferritin values above 5000ug/dl were included (retrospective analysis). Medical records were evaluated for supporting evidence of HLH (fever, splenomegaly, liver function tests, triglyceride and fibrinogen levels) as recorded by treating physicians. Mortality in patients with ferritin levels below 5000ug/dl was also assessed.

Results:

The mean age was 38 years (range 4-82). Bone marrow examination revealed 6 patients had HLH (had been done in 9 patients only). Fibrinogen level was done in 9, one had Hypofibrinogenemia. Triglyceride level was done in 16 patients, 6 patients had hyper triglyceridemia (37%). LFTs were done in all the patients of which 12 had high enzyme levels. Nine patients required ventilator support and ICU care of which 6 survived. \textbf{Nine out of 28 (32\%) patients with ferritin levels more than 5000ug/dl had died due to complications.} Nine patients were treated for HLH (steroids, etoposide and cyclosporine). \textbf{Whereas amongst patients with ferritin levels below 5000ug/dl 4\% had died (7 out of 148 patients).}

Conclusions:

High ferritin levels can correlate directly with increased mortality and can be used as a strong marker to start early treatment for HLH and reduce mortality.
Background:
The use of rituximab (RTX) is increasing, even in developing countries. It has become the first-line therapy or adjuvant to chemotherapy (CHOP) for various diseases, including B cell lymphoma and autoimmune diseases. Aim: We describe the immunological markers associated with RTX treatment of patients with non-Hodgkin lymphoma (NHL).

Methods:
Serial quantitative serum immunoglobulin were determined. Response to tetanus, diphtheria, and hepatitis B virus vaccine was tested in patients after vaccination. Pneumo-23IgG-specific anti-pneumococcal antibodies were evaluated. Immunophenotyping and lymphocyte proliferation against specific mitogens as well as different antigens were determined.

Results:
Seven patients were followed in two tertiary public hospitals in São Paulo state, Brazil. Median age was 56.0±5.0 years (range, 41.9–71.6 years). At baseline, the mean level of IgG was 333.7±40.8 mg/dL, IgM 40.9±11.3 mg/dL; IgA and IgE were under the limit of detection. Two patients had reduced or absent B cells and T cell subsets were at normal levels in five patients. All patients failed to mount an efficient post-vaccination immune response against hepatitis B virus, tetanus, diphtheria, and against the 23-valent pneumococcal polysaccharide vaccine. During treatment, human-IgG-immunoglobulin (IVIg) therapy was introduced in six patients after recurrent infections.

Conclusions:
 Poor response against pneumococcal vaccines increases the susceptibility of respiratory diseases in these patients. The benefits achieved with IVIg replacement for the control of recurrent infectious diseases is of paramount importance. Clinicians dealing with monoclonal antibodies against cancer therapy, especially RTX, should be aware of the increasing risks for symptomatic induced hypogammaglobulinemia and respiratory infections.
AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS) DUE TO CASPASE 10 DEFECTS: MANY FACES FOR A MONOGENIC DEFECT

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Background:

Autoimmune lymphoproliferative syndrome (ALPS) is a disorder of abnormal immunological homeostasis, due to an inability to regulate the process of lymphocyte apoptosis. The clinical symptoms include lymphoproliferative disease, manifested by lymphadenopathy, hepatomegaly, splenomegaly and an increased risk of lymphoma, as well as autoimmune disease, typically autoimmune cytopenias. Mutations in the Fas-dependent apoptotic pathway are the most common genetic defects in ALPS. Mutations in the gene encoding for caspase 10 (CASP10) have also been described, but their clinical implications are less clear.

Methods:

Here we report three ALPS patients carrying the heterozygous Ile406Leu mutation in CASP10. Analysis of the variant segregation in the family and apoptosis assays in CASP10 deficient patients will be performed.

Results:

P1) a 35-year-old female was referred to an immunologist for severe lymphadenopathy, joints pain, and recurrent episodes of fever lasting several weeks. PBL immunophenotype did not display increased frequency of double negative (DN) T cells. P2) A 8 years-old male patient had severe thrombocytopenia, massive lymphadenopathy, severe hypogammaglobulinemia and sinopulmonary infections. PBL immunophenotype did not display increased frequency of DN T cells. P3) A 11 years-old male patient presented with periodic fever, aphthas, joints pain and recurrent episodes of tonsillitis. PFAPA was initially suspected. PBL immunophenotype revealed a slightly increased frequency of DN T cells (2.7%), then the genetic test for ALPS was performed.

Conclusions:

Our data provides further insight into the clinical impact of CASP10 mutations. The Ile406Leu genetic variant, classified as a Variant of Unknown Significance, may be considered as a pathogenic mutation with reduced penetrance.
Background:

Common Variable Immunodeficiency (CVID) can present with a wide array of gastrointestinal involvements. The most common infections are caused by Giardia lamblia, followed by Salmonella and Campylobacter species. CVID patients can also present with non-infectious complications like inflammatory bowel disease-like (IBD), celiac-like enteropathy and nodular lymphoid hyperplasia.

Methods:

We report two cases of CVID and chronic Campylobacter infection mimicking IBD.

Results:

A 38 years old woman and a 39 years old man with CVID presented with malabsorption and chronic diarrhea. They had severely reduced IgG and absent IgA and IgM, both showing a SmB- phenotype. Endoscopic and histologic analysis were compatible with Crohn’s diseases, but clinical response to IBD targeted therapies was scarce. Multidrug resistant Campylobacter jejuni was isolated from stool sample from both patients and long term specific antibiotic therapies achieved clinical improvements.

Conclusions:

Chronic and recurrent Campylobacter infections in CVID patients can mimic IBD. Especially in deeply hypogammaglobulinemic patients long term therapies might be needed to eradicate the pathogen and multidrug resistance maybe a possible complication.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0260

INFLAMMATORY COMPLICATIONS IN CHRONIC GRANULOMATOUS DISEASE: A SINGLE-CENTER EXPERIENCE FROM NORTH INDIA

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Background:

Chronic granulomatous disease (CGD) is an inherited phagocytic disorder characterized not only by recurrent infections, but also various inflammatory complications related to granuloma formation and autoimmunity.

Methods:

Case records of 46 patients diagnosed with CGD at our Institute from August 1993-December 2016 were analyzed. Diagnosis of CGD was based on the nitroblue tetrazolium test (NBT) alone in eight, dihydrorhodamine (DHR) testing alone in two, and by both in 36 cases.

Results:

The ratio of cases with X-linked (XL) and autosomal recessive (AR) CGD was 17:27. Non-infective complications were noted in 20 patients (43.5%)- lung granulomas (10), colitis (8), hemophagocytic lymphohistiocytosis (HLH) (2), liver granuloma (1), intestinal obstruction (1), unexplained renal failure (1), atopiform dermatitis (1), and chilblains (1). Diagnosis of colitis was confirmed by colonoscopy and histopathology in 5 patients. Inflammatory colitis was the presenting manifestation of CGD in 5 patients (2 gp91phox, 2 p67phox, 1 p47phox, 1 unclassified AR-CGD). All 4 patients with p67phox defect had colitis. Mean delay between the onset of colitis and diagnosis of CGD was 2.4 years. HLH is noted in 2 patients with XL-CGD and it was a presenting feature of CGD in one of the cases. A carrier of XL-CGD developed features of lupus in form of polyarthralgia, malar rash, positive anti-nuclear antibody, and elevated anti-double stranded DNA titers. The presence of inflammatory complications did not influence the mortality or the survival functions.

Conclusions:

Inflammatory complications in CGD are under-recognized and a majority of them would require cautious use of immunosuppressive therapy.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0262

VARIABLE CLINICAL PHENOTYPES AND RELATION OF INTERFERON SIGNATURE WITH DISEASE ACTIVITY IN ADA 2 DEFICIENCY

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Background:

The deficiency of adenosindeaminase 2 (DADA2) is an autosomal recessive autoinflammatory disease, caused by CECR1 mutations. An upregulation of type I interferon-stimulated gene transcripts, so called interferon signature (IS), was described also in DADA2 patients. This abstract describe the clinical course of 5 DADA2 patients and assess IS as marker of disease activity.

Methods:

Blood was collected into PAXgene tubes and expression levels of IFI-27 IFI-44L IFIT-1, ISG-15, RSAD-2, SIGLEC, determined. IFN scores higher than 1.4 were considered positive

Results:

Four Caucasian patients were identified carrying CECR1 mutations. Mean age was 5.3 ± 2.7 years. Three of the patients presented with recurrent fever, livedo reticularis, persistent elevation of inflammatory markers, arthralgia/ arthritis. One of these patients showed early onset gastrointestinal involvement, posterior reversible encephalopathy with seizures, deafness and nephrogenic hypertension secondary to kidney infarction. His younger brother, carrying the same mutations, showed a very mild phenotype characterized by a single episode of prolonged fever with abdominal pain and arthralgia. One patient presented at 8 years with persistent livedo reticularis without signs of systemic inflammation. All patients showed a complete remission after treatment with etanercept. The interferon score before treatment was elevated in 4 out of 5 patients (except for the younger brother), and normalized after treatment

Conclusions:

Our data confirm the highly variability of DADA2 regarding age of onset, and severity even within families and, among patients with the same mutations. Furthermore, these data suggest that type I interferon score could be used in DADA2 patients as a biomarker of disease activity
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0304

SAVI SYNDROME INITIALLY PRESENTING AS A COMBINED IMMUNODEFICIENCY

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Background:

STING-associated-vasculopathy-with-onset-in-infancy (SAVI) is a type-I interferonopathy clinically characterized by systemic inflammation, severe autoinflammatory cutaneous and pulmonary vasculitis.

Methods:

We report a case of 15-months Italian girl referred to our Hospital due to persistent dermatitis and decreased T cells suggesting a combined immunodeficiency. At 3 days of life she developed a vesicular rash on cheeks and nose, later spreading on hands and feet with evolution in pustules and scars. The child had low-grade fever, recurrent cough and diarrhoea unresponsive to diet, but variably responsive to glucocorticoids and antibiotics. A chest CT was performed revealing a marked pulmonary involvement.

Results:

Blood investigations revealed moderate anaemia, thrombocytosis, increased acute phase reactants and hypergammaglobulinemia. Immunological investigations showed low CD3+, low CD8+, increased CD19+ cells and low T cell proliferation to OKT-3. We detected an impaired memory distribution, but presence of normal naïve T cells, excluding a typical T cell deficiency. The presence of low titer autoantibodies, the atypical vascular infiltrate in skin biopsy and the pulmonary involvement led us to investigate autoinflammatory disorders. Targeted-genome-sequencing revealed a de novo heterozygous mutation in TMEM173 (p.Asn154Ser) leading to the diagnosis of SAVI. Therapy with Ruxolitinib was started with a partial remission in the first month.

Conclusions:

Early onset atypical inflammatory symptoms should suggest genetic disorders with immune-dysregulation and alert different specialists (immunologist, dermatologist, rheumatologist). We describe a very young patient with SAVI presenting with severe dermatitis and features of immunodeficiency. Longer follow-up is required to monitor side effects and effectiveness of Ruxolitinib both on clinical and immunological phenotype.
DICARBONYL STRESS AND INFLAMMATION: THE AXIS UNITING TYPE 2 DIABETES AND PERIODONTITIS

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Background:

Methylglyoxal (MGO), a highly reactive α-oxoaldehyde, mainly derived from triosephosphates, is known to cause protein modifications and AGE formation more effectively than the parent hexose sugars. Diabetes mellitus (DM) is a systemic disease associated with serious complications like retinopathy, neuropathy, cardiovascular diseases, periodontitis etc. DM produces a hyper-inflammatory phenotype in certain cells due to the action of AGEs which in turn worsens the periodontitic conditions. In DM and PD; blood low-density lipoproteins (LDL) are subjected to numerous enzymatic and non-enzymatic modifications that intensify the atherogenicity and induce immunogenicity. LDL is glycated readily to form advanced lipoxidation end-products (ALEs) resulting in oxidative and dicarbonyl stress. Glycated LDL is capable of inducing inflammation, thus contributing to the progression of periodontitis. Compared to freely circulating modified LDL; modified LDL associated with the immune complexes have a more robust pro-inflammatory potential.

Methods:

Glycation of LDL using different concentrations of MGO for varied incubation time intervals was studied. The structural perturbations induced in LDL were analyzed by UV–Vis, fluorescence, circular dichroism and FTIR spectroscopy, molecular docking studies, thermal denaturation studies, Thioflavin T assay, isothermal titration calorimetry, comet assay, SEM and TEM. MALDI-TOF, ketoamine moieties, carbonyl content, HMF content were also quantitated in native and glycated LDL.

Results:

We report structural perturbations, increased carbonyl content, ketoamine moieties and HMF content in glycated LDL as compared to native analogue (nLDL).

Conclusions:

The results substantiate that in hyperglycemic state glycated LDL could obstruct normal physiological functions and might contribute in the development of secondary complications in diabetic patients like periodontitis etc.
DYSREGULATION OF INFLAMMATION: A FAMILIAR CASE WITH OVERLAP OF FEATURES.
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Background:
Diseases of immune dysregulation are a Primary immunodeficiency characterized by mutation in the genes which have critical roles in the regulation of immune responses and immunological tolerance. This group of PID patients presents phenotypic overlap including cutaneous features. Pyoderma Gangrenosum has been described in the pediatric group in association with inflammatory bowel disease, leukemia, rheumatoid arthritis, Takayasu disease, and several Immunodeficiencies among chronic granulomatous disorder, leukocyte adherence glycoprotein deficiency, Hyper IgE syndrome and selective deficiency of IgA.

Methods:
Here we present the immunological follow-up of male and female brothers in pediatric age of six and three years respectively, children are sons of unrelated parents. They presented with clinical manifestations such as periodic fever, inflammatory articular and digestive symptoms and different skin lesions. Both actually have been diagnosed of Pyoderma Gangrenosum, but the immune dysregulation subjacent it’s unclear as well as clinical response to multiple treatments.

Results:
Immunological studies included determination of plasmatic immunoglobulin G, A, M levels, leukocyte and lymphoid cell counts as well as their phenotype and function, the results were between normal ranges of values. Genetic analysis for most common autoinflammatory disorders was negative. Functional cellular studies including Burst test, migratory disorders and leukocyte adhesion was achieved and results wasn't pathological. The study of the skin biopsies on two occasions has not been conclusive.

Conclusions:
Combined treatment with corticoids and cyclosporine isn’t enough to scar skin lesions, and both required additional management with Anakinra, actually immune modulation with intravenous unspecific immunoglobulin’s Throws surprising results with faster remission in skin lesions.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0315

MUCOSAL IMMUNE PROFILE OF INFLAMMATORY BOWEL DISEASE IN CHRONIC GRANULOMATOUS DISEASE

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Background:

Inflammatory Bowel Disease (IBD) is a known manifestation of Chronic Granulomatous Disease (CGD). Mucosal immune profile of CGD-IBD as not been yet investigated. We report an immune analysis of the intestinal mucosa in two 23-year-old twins with X-linked CGD and IBD.

Methods:

Upper and lower endoscopy were performed for IBD assessment and treatment guidance. Biopsies from ileum, colon and rectum were taken from patients and from one healthy donor (HD). Along with routine histology, we analyzed the lamina propria mononuclear cells (LPMC) by flow cytometry to detect immunophenotype and intracellular cytokine concentration.

Results:

Patient II.2 had normal macroscopic findings with histologic signs of chronic inflammation. Patient II.3 showed macroscopic appearance of active disease in the left colon and histologic picture of active pancolitis. The percentage of NK-, NKT- and T-cells (mainly CD103-), as well as Tregs (CD4+CD25+CD127lowFoxP3+), were increased in both patients compared to HD, independently from the gut segment and the endoscopic appearance. Apoptosis study showed augmented cell death in both patients compared to HD. Cytokines profile revealed an upregulation of TNFα expression in the colon of both patients compared to the HD, without any differences in the production of IL17. Patient II.3 showed a higher frequency of INFγ secreting cells among colonic CD3+ LMPC compared to patient II.2 and HD.

Conclusions:

Two IBD-CGD patients showed a distinct mucosal immune profile of LPMC compared to HD, independently from endoscopic and histologic disease activity. INFγ was overproduced by LPMC of the patient with active mucosal lesions of CGD-IBD.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0318

A NON-MALIGNANT TUMORAL PRESENTATION OF A RARE PRIMARY-IMMUNODEFICIENCY
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Background:

Many PIDs typically associate non-malignant or malignant lymphoproliferations with recurrent infections.

Methods:

We report a 7 year-old patient presenting with chronic abdominal symptoms since early infancy along with recurrent ear nose and throat (ENT) infections. He was multi-investigated for his abdominal symptoms by echography, CTscan and PETscan.

Results:

Abdominal imaging revealed multiple masses highly metabolically active on PETscan. Colonoscopy confirmed nodules disseminating along the colon with masses in the caecum. Lymphoma was first suspected. Histology demonstrated a dense pleomorphic non-clonal T and B cell lymphoproliferation, together with eosinophilis ; germinal center architecture was blurred. These features were not consistent with a diagnosis of lymphoma. Immunological investigations showed decreased IgG2 subclass and increased IgM. Post-vaccine antibody responses were good for protein antigens but insufficient for pneumococcal antigens. Lymphocyte sub-populations showed decreased CD4+T and B cells with normal distribution. T lymphocytes proliferated well after stimulation with mitogens. After lymphoma and PIDs classically prone to lymphoproliferation were ruled out we undertook to explore further our patient with a whole exome sequencing screen. Data analysis based on a panel of genes enriched in « immunodeficiency related genes », revealed that our patient harbored an heterozygous c.3061G>A, p.Glu1021Lys, PI3K delta chain mutation. This mutation causes activated PI3K delta syndrome (APDS), a rare autologous dominant PID recently described.

Conclusions:

This PID displays variable phenotype but typically combined non-malignant lymphoproliferation to ENT and broncho-pulmonary recurrent bacterial infections and patients are prone to lymphoma. At time of diagnosis the patient had no bronchiectasis. He has just started on polyvalent immunoglobulin substitution.
A FAMILY WITH DEFICIENCY OF ADENOSINE DEAMINASE 2 (DADA2) PRESENTING WITH NEUTROPAENIA AND LYMPHOPAENIA IN ADULTHOOD

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Background:

Deficiency of adenosine deaminase 2 (DADA2) is an autosomal recessive disorder that causes systemic vasculopathy and early-onset recurrent stroke due to homozygous mutations in the Cat Eye Syndrome Chromosome Region, Candidate 1 (CECR1) gene. A recent study identified DADA2 as a cause of hypogammaglobulinaemia. We observed a syndrome of intermittent fevers and recurrent infections with concurrent panlymphopenia and neutropenia in a non-consanguineous family with healthy parents, two affected sisters and one unaffected brother.

Methods:

We used whole exome sequencing (WES), Sanger sequencing and ADA2 enzymatic assays to compare affected to non-affected members of the family.

Results:

Subtractive analysis of genetic variants between affected and unaffected members of the family revealed that the affected sisters carried compound heterozygous mutations at positions 506 and 1057 (c.506G>A, c.1057T>C) of the coding sequence of the CECR1 gene predicted to cause the amino acid changes p.Arg169Gln and p.Tyr353His in the encoded enzyme ADA2, respectively. The unaffected family members were heterozygous carriers for either of these two mutations. These results were corroborated using Sanger sequencing. One of the mutations (p.Tyr353His) is previously unreported. Both mutations were predicted to cause loss of function in ADA2. Enzyme activity analysis demonstrated complete lack of ADA2 function in plasma samples obtained from the affected sisters, compared to unaffected members of the family and healthy controls, indicating DADA2.

Conclusions:

Neutropenia and lymphopaenia, with or without hypogammaglobulinaemia and irrespective of age, could be a late presentation of DADA2, in the complete absence of the typical cardinal manifestations of the syndrome.
THE WIDENING SPECTRUM OF NLRC4-RELATED AUTOINFLAMMATION: CASE REPORT.

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Background:

NOD-like receptor (NLR) family members are cytoplasmic molecules that play a key role in innate sensing and immune response. Mutations in NLR family members: NLRP3 and NLRP12 are associated with sterile inflammatory syndromes referred to as cyropyrin-associated periodic syndromes (CAPS). Reports from the last 3 years have documented that mutations in another member of the family: NLRC4 gene can also be responsible for causing autoimmune symptoms.

Methods:

Herein we describe a case of a de novo missense mutation in NLRC4 gene as a probable cause of early-onset autoinflammatory syndrome in 1-year-old Polish girl.

Results:

To our knowledge this is the fourth report of individual with autoinflammatory presentation caused by NLRC4 variant. The early-neonatal onset of autoinflammatory episodes is similar, but clinical phenotype including chronic, sterile meningitis and retinitis has not been observed in previously described cases.

Conclusions:

This report gives further evidence for the role of NLRC4 variants in autoinflammation and expands the phenotypic spectrum of NLRC4-related disease.
COULD PFAPA SYNDROME AND PSORIATIC ARTHRITIS BE SPECTRUMS OF THE SAME PSTPIP1 GENE DEFECT?

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Background:

PAPA syndrome is an autosomal dominant disease characterized by sterile, deforming arthritis, skin ulcers (pyoderma gangrenosum), and severe cystic acne, is caused by mutations in the gene that codes proline-serine-threonine phosphatase interacting protein 1 (PSTPIP1).

Methods:

We describe a case of peridic fever in an patient with a PSTPIP1 defect son of a father with psoriatic arthritis.

Results:

A 3 year-old boy, born of non-consanguinous parents addmited with recurrent fever, aphtous stomatites, sore throat and maculopapular rash. His father was diagnosed with difficult control psoriatic arthritis in use of infliximab. The child had been treated for acute tonsilits several times, however fast test for strep throat used to be negative. Thereafter, he presented recurrent febrile peaks associated with tonsilits and canker sores, each 15 days, even during the use of antibiotics. The C-reactive protein was always elevated during the episodes of fever as neutrophilia. He received the diagnostic of PFAPA and treatment with corticosteroids during the crises was initiated with significant response. The daily use of colchicine was indicated with good response. After 6 months of cochicine use he developed intolerance with vomiting, abdominal pain and diarrhea that stopped with suspension of the drug. Currently, he is stable, using corticosteroids only at the crises. The genetic panel for autoinflammatory diseases showed a probable patogenic defect in PSTPIP1(NM-003978)-c1213C>T (p.R405C).

Conclusions:

The PSTPIP1 gene defect seems to be related with the autoinflammatory symptoms in these patients. We are expanding the investigation to better stabilish the relationship between them.
CGD: FEMALE PATIENT WITH INFECTIONS, VASCULITIS AND OSTEOPLASTIC TRACHEOBRONCHOPATHY
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Background:

We aim to report a case of a 17 y.o. female patient with a recent diagnosis of Chronic Granulomatous Disease (CGD), who presented vasculitis and underwent bronchoscopy suggestive of osteoplastic tracheobronchopathy during the investigation of chronic cough. This is a benign disease characterized by bone and cartilaginous proliferation in the posterior wall of the trachea with consequent narrowing of the large airways. Its etiology is related to a chronic inflammatory process, of infectious origin or not.

Methods:

Descriptive study based on medical records review.

Results:

Adolescent female patient with a previous history of low stature, cutaneous abscesses which started at 6 months of age, numerous pneumonias, lung abscess episode with the diagnosis of DGC initially discarded due to normal NBT at 4 y.o. She was followed by pneumology clinic with chronic cough and a diagnosis of osteoplastic tracheobronchopathy. She was again screened after chronic osteomyelitis by Staphylococcus aureus and leukocytoclastic vasculitis at 15 y.o., and the diagnosis of CGD was made by DHR. After initiation of prophylaxis with itraconazole and Sulfamethoxazole + Trimethoprim, she presented favorable evolution without new infectious intercurrences to date, but keeping chronic cough.

Conclusions:

We present the case of a patient with a clinical history strongly suggestive of CGD with normal NBT in initial investigation, but with altered DHR later. The patient presented a picture compatible with osteoplastic tracheobronchopathy. Besides that, she presented a vasculitis, not very usual in CGD patients. To date, there are no reports of the association between osteoplastic tracheobronchopathy and CGD.
Background:

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening syndrome due to a severe uncontrolled hyperinflammation triggered in most cases by an infectious agent. In the last years there were described more and more HLH cases in primary immunodeficiencies other than familial forms.

Methods:

A girl, from Romanian consanguineous parents, without suggestive family history, was diagnosed at 12 months of age with PNP deficiency (based on enzyme and genetic testing). From 13 months she developed persistent cough and diarrhea that didn’t responded to antibiotics. After 2 weeks she developed fever, hepatomegaly, splenomegaly, seizures and progressive jaundice. Laboratory tests showed: hemoglobin=6.8g/dl, PLT=73.000/mmc, WBC=3750/mmc (6000-17.000), neutrophils=2800/mmc, lymphocyte=849/mmc(2900-5100), B lymphocyte=20/mmc(700-1300), T lymphocyte=428/mmc(1800-3000), CD4+=4/mmc(1000-1800), CD8+=424/mmc(800-1500), NK=401/mmc(200-600), ferritin>25.000 ng/l, sCD25=20.124, TGP=136UI/ml, TG0=1586UI/ml, direct bilirubin=312µmol/l(<5), CRP= 52 mg/l (<5), d-dimer=2190ng/ml(<270), PCR for CMV and EBV were positive.

Results:

The diagnosis of HLH was established (6 from 8 criteria). We initiate treatment with Gancyclovir, Dexamethasone 10mg/m², and 1 dose of Etoposide. The hepatomegaly and splenomegaly didn’t accentuated, fever became more rarely and ferritin was 6000ng/l after 3 days but the WBC decreased < 1000/mmc. Taking in account the risk of sepsis the decision was to stop the Etoposide administration but reappeared high fever, seizures, progressive pancytopenia and hyperferritinemia, she developed sepsis and died.

Conclusions:

We can’t say the evolution was better if we continued with Etoposid but the treatment of HLH remains a challenge and must be individualized in each case of SCID taking in account the immunological parameter and the inflammation’s severity.
HYPERIGE SYNDROME AND DOCK8 DEFICIENCY: THE CASE OF A 2-YEAR-OLD ALBANIAN FEMALE

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¹Bambino Gesù Children's Hospital, University Hospital Pediatric Department, Rome, Italy
²Policlinico Agostino Gemelli, Dirigente Medico Terapia Intensiva Pediatria e Trauma Center Pediatrico, Rome, Italy

Background:

DOCK8 deficiency is an autosomal recessive combined immunodeficiency characterized by hyperIgE syndrome and early-onset severe clinical phenotype.

Methods:

We present the case of a 2-year-old Albanian female with a diagnosis of DOCK8 deficiency.

Results:

Patient presented to our hospital with a previous history of atopic dermatitis since birth unresponsive to topical steroids, and a sepsis caused by Staphylococcus aureus during the first year of life. The patient did not have facial/skeletal abnormalities. At the age of 2 years, the child was newly admitted to the hospital for a tricuspid endocarditis by S. aureus, associated with Parvovirus and Cytomegalovirus co-infection. The clinical course was complicated by a vasculitis-like purpura with necrosis of four toes, requiring cardiorespiratory support, plasmapheresis sessions, intravenous pentoxifylline, immunoglobulins and steroids. A bloodstream infection due to Candida parapsilosis, and an extensive pulmonary embolism requiring urgent surgery also occurred. The immunological investigations showed normal lymphocytes count with high EMRA CD8+ and gamma/delta T cells and absent Th17 cells, IgE 3366 kIU/L, eosinophilia, high ferritin and triglycerides. The genetic panel for HyperIgE syndrome identified a deletion in the DOCK8 gene causing loss of Dock8 protein expression. Patient is now in good general conditions; she receives monthly intravenous immunoglobulins and antimicrobial prophylaxis. The search for a compatible bone marrow donor is currently underway.

Conclusions:

Severe/recurrent atopic dermatitis, elevated IgE and recurrent S. aureus/Candida infections are red flags of a primary immunodeficiency. Early onset severe infections, increased susceptibility to virus and absence of craniofacial/skeletal abnormalities should suggest a DOCK8 deficiency, more than other genetic defects causing HyperIgE syndrome.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0350

WOULD BE PFAPA SYNDROME A SPORADIC CONDITION?
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¹Hospital da Polícia Militar de Minas Gerais, Pediatric, Belo Horizonte, Brazil
²Santa Casa de Misericódia de Belo Horizonte, Pediatric Rheumatology, Belo Horizonte, Brazil
³Universidade Federal de Minas Gerais, Allergy and Immunology Pediatrics, Belo Horizonte, Brazil

Background:

Periodic fever syndromes are characterized by short and recurrent attacks of fever and severe localized inflammation that occur periodically or irregularly and that are not explained by usual childhood infections.

Methods:

We present a case report of a family with periodic fever, aphthosis, pharyngitis, and cervical adenitis.

Results:

A ten years old girl who presents a periodic fever that onset before the age of one; during approximately three days, associated with abdominal and cervical pain and vomiting. The events do not prejudice her growth and development. In an outpatient investigation, it was questioned the hypothesis of periodic fever, aphthosis, pharyngitis, and cervical adenitis (PFAPA) syndrome. However, her younger brother presented the same symptoms and her mother either, when she was young. Therefore, genetic testing was required to exclude Monogenic Periodic Fever, (Tumor necrosis factor receptor - associated periodic syndrome (TRAPS), FMF, HyperIgD syndrome; Cryopyring-associated periodic syndrome), which are caused by mutations of genes involved in regulation of the inflammatory response. Laboratory Data: Increased acute phase reactant C Reactive Protein on the fever attacks periods; genetic information had negative results for MVK, MEFV, NOD2, PSTPIP1, NLRP3 and TNFRSF1A. Treatment: Present a good answer to Glucocorticoids and Colchicine.

Conclusions:

Although PFAPA syndrome is known as a sporadic disease, some authors defend that is not. The symptoms of PFAPA syndrome and others periodic fevers are largely overlapping. This case strongly suggests a familial pattern. Maybe we have a gene causing disease that is not covered by the panel or another non mendelian mechanism of inheritance.
Background:

Hyper-IgE syndrome (HIES) is a rare primary immunodeficiency classically characterized by high levels of IgE, recurring staphylococcal skin diseases, eczema, chronic mucocutaneous candidiasis and severe pulmonary infections. The majority of HIES is due to autosomal dominant mutation in the Signal Transducer and Activator of Transcription-3 (STAT3). In these patients, the inhibitory effect of STAT3 on TLR-signaling, IFN-γ pathway as well as the anti-inflammatory molecules production is defective. This in turn leads to abnormal and uncontrolled immune responses upon infections and Ag-stimulation.

Methods:

Description of clinical, immunological and genetic characteristics of STAT3-HIES patients diagnosed in our Centre.

Results:

4 caucasian patients (2M;2F) were diagnosed for STAT3 deficiency at mean age of 9.7yo (range 1-29yo). First severe systemic infection was reported within the first year of life in 1 out of 4 patients (mean age: 4.8 yo: age-range, 3month-8 yo). All patients presented recurrent respiratory infections and 2 out of 4 presented lung sequelae(Table 1). One patient with severe infective complications and no facial dimorphisms was diagnosed at 30 yo. Overall immunological findings (Table 2), showed lower Th17 responses and suboptimal CpG related B-cell responses.
<table>
<thead>
<tr>
<th>Table 1. Clinical characteristics</th>
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<tr>
<td></td>
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<tr>
<td>Gender</td>
</tr>
<tr>
<td>IgE SERUM</td>
</tr>
<tr>
<td>SKIN ABDUSES</td>
</tr>
<tr>
<td>PNEUMONIA</td>
</tr>
<tr>
<td>LUNG ABNORMALITIES</td>
</tr>
<tr>
<td>RETAINED PRIMARY TEETH</td>
</tr>
<tr>
<td>SCOLIOSIS</td>
</tr>
<tr>
<td>FRACTURES</td>
</tr>
<tr>
<td>HYPEREOSINOPHILIA</td>
</tr>
<tr>
<td>FACIAL DYSMORPHISM</td>
</tr>
<tr>
<td>MIDLINE</td>
</tr>
<tr>
<td>NEONATAL RASH</td>
</tr>
<tr>
<td>ECZEMA</td>
</tr>
<tr>
<td>RESPIRATORY INFECTIONS/ YEAR</td>
</tr>
<tr>
<td>CANDIDIASIS</td>
</tr>
<tr>
<td>OTHER INFECTIONS</td>
</tr>
<tr>
<td>LYMPHOMA</td>
</tr>
<tr>
<td>HYPEREXTENSIBILITY</td>
</tr>
<tr>
<td>Current AGE</td>
</tr>
</tbody>
</table>
Conclusions:

The variability of the clinical presentation and the absence of classical clinical signs, often determine a delay in the diagnosis. STAT3-HIES should always be considered in pediatric patients presenting with early onset atopic dermatitis, recurrent respiratory and skin infections, and high levels of IgE. Systematic reports on larger cohorts of children are needed to shed light on such disease or on other milder forms of HIES.

---

### Table 2. Immune and genetic characteristics

<table>
<thead>
<tr>
<th></th>
<th>PATIENT A</th>
<th>PATIENT B</th>
<th>PATIENT C</th>
<th>PATIENT D</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>11590/ul</td>
<td>8710/ul</td>
<td>8920/ul</td>
<td>8770/ul</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2100/ul</td>
<td>1520/ul</td>
<td>156/ul</td>
<td>710/ul</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>2540/ul</td>
<td>246/ul</td>
<td>2490/ul</td>
<td>1820/ul</td>
</tr>
<tr>
<td>CD3+</td>
<td>80.2%</td>
<td>71.6%</td>
<td>80.0%</td>
<td>71.9%</td>
</tr>
<tr>
<td>CD19+</td>
<td>6.0%</td>
<td>74.0%</td>
<td>13.7%</td>
<td>7.7%</td>
</tr>
<tr>
<td>NK (CD3-CD16+CD56+)</td>
<td>3.9%</td>
<td>12.3%</td>
<td>5.1%</td>
<td>15%</td>
</tr>
<tr>
<td>CD3+CD4+</td>
<td>43.3%</td>
<td>43.7%</td>
<td>46.5%</td>
<td>34.4%</td>
</tr>
<tr>
<td>CD3+CD4+CD27+CD45RA+</td>
<td>62.6%</td>
<td>70.0%</td>
<td>73.6%</td>
<td>59.3%</td>
</tr>
<tr>
<td>CD3+CD4+CD27+CD45RA-</td>
<td>23.4%</td>
<td>23.4%</td>
<td>25.5%</td>
<td>30.5%</td>
</tr>
<tr>
<td>CD3+CD4+CD27CD45RA+</td>
<td>10.8%</td>
<td>1.5%</td>
<td>0.9%</td>
<td>7.8%</td>
</tr>
<tr>
<td>CD3+CD4+CD27CD45RA-</td>
<td>3.1%</td>
<td>0.1%</td>
<td>0.1%</td>
<td>2.3%</td>
</tr>
<tr>
<td>CD3+CD4+CD31+CD45RA+</td>
<td>48.5%</td>
<td>70.8%</td>
<td>70.1%</td>
<td>49.4%</td>
</tr>
<tr>
<td>CD3+CD4+CD45RA&lt;0.0198</td>
<td>nd</td>
<td>2.2%</td>
<td>4.0%</td>
<td>nd</td>
</tr>
<tr>
<td>CD2+</td>
<td>38.3%</td>
<td>21.3%</td>
<td>14.6%</td>
<td>25.2%</td>
</tr>
<tr>
<td>CD3+CD4+CD31+CD45RA+</td>
<td>51.6%</td>
<td>81.9%</td>
<td>89.7%</td>
<td>34%</td>
</tr>
<tr>
<td>CD3+CD4+CD45RA-</td>
<td>0.26%</td>
<td>3%</td>
<td>0.8%</td>
<td>1.7%</td>
</tr>
<tr>
<td>CD3+CD4+CD45RA-</td>
<td>16.0%</td>
<td>11.9%</td>
<td>8.6%</td>
<td>10.6%</td>
</tr>
<tr>
<td>CD3+CD4+CD45RA-</td>
<td>32.6%</td>
<td>2.5%</td>
<td>2.1%</td>
<td>13.4%</td>
</tr>
<tr>
<td>CD3+CD4+IL7+ (ICP*)</td>
<td>0.2%</td>
<td>0.04%</td>
<td>0.2%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Treg CD25+IL7+FOX3+ on CD4+</td>
<td>nd</td>
<td>12.2%</td>
<td>nd</td>
<td>5.5%</td>
</tr>
</tbody>
</table>

|          | 15000 mg/dl | 9510 mg/dl | 9520 mg/dl | 7100 mg/dl |
| IgG, IgA, IgM (mg/dl) | 150 mg/dl | 197 mg/dl | 115 mg/dl | 115 mg/dl |
| Response to vaccinations | High | High | Low | High |
| CpG response | Reduced proliferation and differentiation | Normal proliferaion and reduced differentiation |
| T cell proliferation (PHA/OKT3) | Normal | Normal | Normal/Reduced |
| STAT3 mutation | N466S | R615S | Y637A | K882Q |
| STAT3 domain | DNA Binding | SH2 | SH2 | DNA Binding |

*ICS: Intra Cellular Staining on PBMC activated with PMA and ionomycin*
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0353

POST TRANSPLANT IMMUNE RECONSTITUTION IN A PATIENT WITH AUTOINFLAMMATORY SYNDROME DUE TO SAMD9L MUTATION

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2Immunology Clinical Center Dra Bezrodnik, Immunology, CABA, Argentina
3Gutierrez children’s Hospital, Immunology, CABA, Argentina
4Immunology Clinical Center Dra Bezrodnik, Immunology, CABA, Argentina

Background:

Autoinflammatory diseases are inborn errors of the innate immune system. They present with uncontrolled systemic and organ-specific inflammation that can resemble infectious conditions, that generally associate inflammation of the skin and others organs since the neonatal period raised inflammatory markers in blood. Fever is present most of the disorders, but not all of them.

Methods:

Present the post transplant immune reconstitution of a female patient with CANDLE like syndrome.

Results:

1y.o. baby with chronic and generalized pustulosis since birth. In her follow up she persisted with severe skin ulcers, splenomegaly, chronic anemia and thrombocytopenia with hypogammaglobulinemia and almost absent B cell counts. At 4 months suffered from respiratory distress needing mechanical ventilation. Refractory to standard immunosuppressant. No mutation in ADA2, TNFRSF1A, CECR1 and NLRP3. Given the severe compromise, new studies were performed: Altered score for interferonopathies and mutation in SAMD9L protein was found by WES. In 2016 she received unrelated HSCT(10/10). Nowadays normal IgA and IgM levels, with B cell engraftment (7.5% B memory cells and 13% B transitional cells). CD4 naive T cells:243/mm3. Normalized serum pro inflammatory cytokines with good evolution up to date. GvHD treatment stopped.
Conclusions:

CONCLUSION: Severe Autoinflammatory syndromes are still under study and represent a challenge for clinical immunologists. Further studies involving SAMD9L protein pathways will allow our understanding related with immune system. HSCT has been proposed as a curative treatment in this condition, presenting with auto inflammation and immunodeficiency.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0361

TREM-1 OVEREXPRESSION AND EARLY ONSET AUTOINFLAMMATORY SYNDROME: WOULD IT BE A NEW MECHANISM OF AUTOINFLAMMATORY DISEASE?
L. Cunha1, R. Souza1, F. Santos1, A.C. Silva2, N. Linhares3, S. Pena1, E. Vieira2, C. Souza3, Y. Kashiwabara1, J. Pinto1

1Universidade Federal de Minas Gerais, Imunologia, Belo Horizonte, Brazil
2Universidade Federal de Minas Gerais, Medicina Translacional, Belo Horizonte, Brazil
3Universidade Federal de Minas Gerais, Genética, Belo Horizonte, Brazil

Background:

Inflammatory pathways of autoinflammatory syndrome need to be elucidated.

Methods:

We reported the case of a 3-year-old boy, born from non-consanguineous parents, exhibiting recurrent fever since 2 months of age associated with rhinorrhea, sialorrhea and painful ulcers in the oral cavity.

Results:

At 23 months, he presented keratitis with opacity and corneal neovascularization in the left eye that evolved to low visual acuity. Laboratory tests showed increased makers of inflammation, anemia, neutrophilia and thrombocytosis. At 2 years of age, he was referred to our service with the hypothesis of autoinflammatory syndrome. The patient did not respond to colchicine and cyclosporine and exhibited partial response to corticosteroid. Several hospitalizations accompanied by fever, serosanguinolent rhinorrhea, oral cavity ulcers and necrotic skin lesions occurred. Biopsies of the lesions were inconclusive. He had severe esophagitis with hematemesis at the time of reduction of corticosteroid. At 32 months, he was admitted to pediatric intensive care unit with respiratory distress. We obtained good response to high dose of immunoglobulin. The patient had considerable improvement with anti-TNFα therapy (etanercept 25 mg/week) associated with corticosteroid. Plasma measurements of cytokines showed very high expression of TREM-1 and IL33 in comparison with normal healthy controls at the same age. The levels remained increased even out of exacerbations. It was also detected high levels of IL8 during the respiratory distress crisis.

Conclusions:

Anti-TNF therapy associated with corticosteroid probably exerted direct effect in the inflammatory pathway associated with this case. This is the first description of this pathway in autoinflammatory disease.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

PFAPA SYNDROME IN PAEDIATRIC PRACTICE – ONE CENTRE EXPERIENCE

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²FD Roosevelt Faculty Hospital, Department of Otorhinolaryngology, Banska Bystrica, Slovak Republic
³Comenius University in Bratislava- Jessenius Faculty of Medicine in Martin- University Teaching Hospital, Department of Clinical Biochemistry, Martin, Slovak Republic

Background:

PFAPA syndrome represent the most common form of autoinflammatory diseases in paediatric practice with self-limiting course and good prognosis. It is considered to be an idiopathic disease with unknown genetic background, however, recently several genes’ polymorphisms (MEFV, NLRP3, CARD8) were linked to its complex aetiology.

Methods:

The patients were selected from the Centre for Diagnosis and Treatment of Primary Immunodeficiencies in University Teaching Hospital in Martin (Slovakia). The diagnosis of PFAPA syndrome was established according to the classification criteria of Thomas et al. (1999).

Results:

Altogether, 109 children with PFAPA syndrome were identified: 46% boys (aged 2.78±1.1 years) and 64% girls (aged 4.25±1.9 years. Positive family history for PFAPA syndrome was found in 17% of the children. 100% children had recurrent fever with the interval of 2 – 6 weeks, 100% cervical lymphadenopathy, 94% tonsillopharyngitis and in half of the children, aphthous stomatitis was seen in clinical presentation. All the children showed positive clinical response to episodic application of prednisone (in 95% one dose, in 5% repeated dose). Prophylactic application of anti-inflammatory ketotifen improved the clinical course in 2/3 of the children, however, in 34% children, tonsillectomy with or without adenoidectomy completely resolved the recurrent flares of fever.

Conclusions:

PFAPA syndrome is very important part of differential diagnosis of recurrent fever in children. In general, it has good prognosis and self-limiting course, however, in some of the children tonsillectomy should be performed. In persistence of the symptoms and other typical clinical features, the diagnosis of PFAPA syndrome should be revised.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0364

VARIED PRESENTATIONS OF X-LINKED LYMPHOPROLIFERATIVE DISEASE WITHIN THE SAME FAMILY INCLUDING ISOLATED CNS HLH

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¹Faculty of Medicine- Ain Shams University, Paediatric Immunology, Cairo, Egypt
²Great Ormond Street Hospital, Department of Paediatric Immunology, London, United Kingdom

Background:

X-linked lymphoproliferative syndrome 1 (XLP-1) is a rare primary immunodeficiency disease caused by mutations in the SH2D1A gene. Patients develop severe immune-dysregulation often following Epstein-Barr virus (EBV) with manifestations including haemophagocytic lymphohistiocytosis (HLH), dysgammaglobulinaemia, and lymphoma.

Methods:

We present a family with 3 boys with a genetically confirmed diagnosis of XLP-1. The older siblings presented around 2 years of age but the younger sibling presented earlier. P1 had recurrent chest infections, panhypogammaglobulinemia, and EBV viraemia, requiring several courses of Rituximab and replacement immunoglobulin therapy. P2 developed EBV negative ileo-caecal lymphoma treated with chemotherapy followed by a successful haematopoietic stem cell transplant (HSCT) from an 8/10 mismatched unrelated cord blood donor. P3 was diagnosed shortly after birth and was monitored regularly. He was thriving and developing normally with normal immunoglobulin levels, lymphocyte subsets, and negative viral screening. However, at the age of 11 months, he developed refractory seizures with regression of developmental milestones. Infection screening was negative including CSF evaluation which revealed high protein levels. MRI brain showed multiple lesions within the white matter. A diagnosis of isolated CNS HLH was made and there was no evidence of systemic HLH. He commenced HLH 94 protocol with worsening of neurological symptoms and was treated with 3 doses of Alemtuzumab which led to modest improvement of MRI lesions and improvement of neurological status, but sequelae are evident.

Results:

Progression to HSCT is planned.

Conclusions:

The lack of genotype-phenotype correlation and unpredictable course of XLP-1, despite early diagnosis and close monitoring suggests that early transplantation may improve outcome, provided a suitable donor is available.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0370

IMMUNE DEFICIENCIES/DYSREGULATIONS UNDERPINNING LIMBIC ENCEPHALITIS IN CHILDHOOD: A CASE SERIES AND LITERATURE REVIEW.
A. Alawadhi¹, G. Clemence¹, G. Sébire¹
¹McGill University, Pediatrics, Montreal, Canada

Background:

Limbic encephalitis (LE) is a rare autoimmune syndrome affecting limbic structures and causing variety of neurobehavioral manifestations, e.g. memory change, temporal epilepsy, and psychiatric symptoms. This rare disease is featured by a well-recognizable combination of clinical, neuroimaging and/or pathological manifestations. Beyond the occasional association with anti-neuronal antibodies, no clear immune dysregulation(s) has been looked for - or associated with - LE in childhood. Our objective is to characterize the clinical and paraclinical features of non-paraneoplastic LE and to correlate them with underlying immune deficiencies.

Methods:

Retrospective series of seven patients with LE recruited at the Montreal Children’s Hospital with a focus on the immune- and neuro-phenotypes, including neuronal antibodies (Ab), lymphocyte sub-typing, key markers of immunoglobulin and complement systems, and genotyping.

Results:

Three out of seven patients were females. The age range at first symptom was between 1 to 16 years. Symptoms included temporal epilepsy (n=5), psychiatric symptoms such as ADHD or autistic symptoms (n=2), and memory changes (n=3). According to our preliminary results: (i) one patient was positive for both voltage gated potassium channel (VGKC Ab) and anti-thyroid peroxidase (TPO Ab), (ii) two patients were positive only for anti TPO antibodies without any thyroid dysfunction, (iii) two patient had low CD45CD19 cell counts, low immunoglobulins’ titres, (iv) two patients had low CD45CD16 cell count. Additional immunological, neuropsychological, and pedo-psychiatric assessments are pending. Most patients were treated with corticosteroids, IV immunoglobulins, or Rituximab with few improvement.

Conclusions:

Our preliminary results show immune dysregulations in three patients with LE, which will be further characterized.
Background:

Autoinflammatory syndromes are a group of conditions characterized by recurrent episodes of fever, rash and serositis. In many of the inflammatory syndromes genetic abnormalities and consequent disorder regulation of the innate immune system lead to hyperactivity of pro-inflammatory cytokines and subsequent inflammatory symptoms.

Methods:

We report the case of a child, the offspring of parents, who presented in the immediate neonatal period pustular eruption, joint edema, osteolytic lesions, respiratory difficulties and generalized coagulopathy.

Results:

Laboratory data showed persistent leukocytosis and increased C-reactive protein. The patient evolved requiring intermittent and non-invasive ventilatory support and the use of corticosteroids to improve the rash. A patient's whole exome sequencing was performed showing homozygous mutation (nonsense) of the IL1RN gene. He started treatment with canaquinumab (inhibitor IL-1β), the only drug available in Brazil at 9 months of age, given at a dose of 8mg / kg every 4 weeks. There was a progressive improvement in inflammation of the skin, joints, respiratory distress, nasal obstruction, aspect of upper airway secretions (being able to suspend invasive ventilation) and reduction of interleukin-1β levels. At 1 years and 2 months of age, the patient presented acute viral infection, evolved with severe respiratory distress and severe systemic inflammation, without response to the adopted measures, and died less than 24 hours after decompensation.

Conclusions:

Early-onset autoinflammatory syndromes should be considered in the differential diagnosis of recurrent sepsis. Canaquinumab seems to be effective to control the symptoms in this DIRA's patient.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0406

TWO CASES OF DEFICIENCY OF INTERLEUKIN 1 RECEPTOR ANTAGONIST (DIRA) PRESENTING WITH CHRONIC INFLAMMATORY ARTHRITIS AND REFRACTORY NAIL INFECTIONS

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¹Ege University Faculty of Medicine, Pediatric Immunology, Izmir, Turkey
²The University of Jordan School of Medicine, Pediatrics, Amman, Jordan
³French Institute of Health and Medical Research, Laboratory of Human Genetics of Infectious Diseases, Paris, France
⁴The Rockefeller University, St. Giles Laboratory of Human Genetics of Infectious Diseases, New York, USA

Background:

Deficiency of interleukin-1 receptor antagonist is a rare autoinflammatory disease involving neonatal onset pustulosis, periostitis and sterile osteomyelitis with a recessive mutation in IL1RN gene.

Methods:

Case 1: A 14-year-old girl developed pustular cutaneous lesions at one year of age. At the age of 12, she was hospitalized for arthralgia of her knees, elbows, ankles and arthritis of the left knee, with pustular cutaneous lesions. After discharge, she was referred to our clinic. She had bilateral inguinal, paraumbilical hyperpigmented scar lesions and paronychia of the thumbs. She also had contracture of the left knee, episcleritis, failure to thrive with an increase of acute phase reactants and hypergammaglobulinemia. A skin biopsy of hyperpigmented lesions demonstrated subepidermal pustular dermatosis. Genetic analysis revealed a homozygous mutation(p.R26X) in IL1RN.

Results:

Case 2: A 21-year-old girl was diagnosed as juvenile idiopathic arthritis due to the involvement of left elbow, bilateral ankles, and knees when she was six years old. She's had dystrophic nails since she was five years old and at the age of 11, Candida Albicans was detected in nail specimens. She had high levels of acute phase reactants, hypergammaglobulinemia, and uveitis. Her symptoms were under control with etanercept for the last ten months until she was readmitted with left hip pain with limited motion. Magnetic resonance imaging showed stage 3-4 sacroiliitis. A homozygous mutation(p.Arg29X) in IL1RN was detected.

Conclusions:

Interleukin-1 receptor antagonist deficiency is a rare disease which can be successfully treated with canakinumab and should be considered in patients with refractory arthritis and nail problems.
COMMON VARIABLE IMMUNODEFICIENCY WITH AUTOIMMUNITY AND BOWEL INFLAMMATORY DISEASE

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Background:

Common variable immunodeficiency (CVID) is a heterogeneous group of primary immunodeficiencies characterized by hypogammaglobulinemia and absent specific antibody responses to vaccination.

Methods:

Descriptive

Results:

39 yo male patient without familiar background. He suffered from several pneumonias during childhood and vitiligo. At 12 yo developed pneumonia after measles infection. At 20 yo he was hospitalized for pneumonia; hypogammaglobulinemia and chronic lung disease with bilateral bronchiectasis. Diagnosed with CVID started IVIG. That year, he began with chronic aqueous diarrhea diagnosed as ulcerative colitis.

At 38 yo he was diagnosed with hipotiroidism and insulin requiring type 1 diabetes. And developed at 39 yo. aqueous diarrhea with malnutrition and dehydration that required hospitalization. He had both ankles swollen and joint pain in feet, knees and hips. Mycosis in hands and feet

Videendoscopy: candida esophagitis and surface gastropathy. Duodenum biopsy: laying widened villi with atrophic crypt and isolated intraepithelial lymphocytes, lamina propia fibrosis with mild inflammatory infiltration. Jejunum: lymphangiectasis and xanthomas. Videocolonoscopy: chronic colitis with mild activity. Colonic mucosa showed crypt distortion with inflammatory infiltration in lamina propia. Under this clinical picture and taking into account his treatment with budesonide, gammaglobulin, ciprofloxacin, we treat with rapamycin, hydroxychloroquine and fluconazole. He improved diarrhea, ameliorate his general condition, gain weight and joint pain remitted. He is under SCIG and stable with 2 depositions per day

Conclusions:

CVID patients present with infectious manifestations, but non infectious manifestations are also comon. Complications related to autoimmunity and immune dysregulation are not present in all patients.

IgG replacement in patient with severe phenotype, does not ameliorate gastrointestinal involvement
IDENTIFICATION OF STAT1 GOF MUTATIONS IN 4 PATIENTS WITH 2 NOVEL MUTATIONS USING A NGS SEQUENCING PANEL

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Background:

The phenotype of STAT1 GOF mutations is heterogeneous with autoimmune/infectious disease manifestations (mostly) chronic mucocutaneous candidiasis (CMC). More than 50 mutations (mainly in the coiled-coil domain (CCD) and DNA-binding domain (DBD)) have been reported.

Methods:

Patients with autoimmune/infectious disease manifestations were analysed using a customised in-house NGS panel covering 115 PID-related genes.

Results:

4 patients (3 families) were identified. P1 (mother) and P2 (daughter) presented with CMC in early childhood. P1 has suffered from a malar rash diagnosed as rosacea and severe oesophageal candidiasis requiring repeated endoscopic treatment. Her 5-years old daughter has a suffer from CMC, recurrent sinusopulmonary infections, interstitial lung disease with bronchiectasis. A novel STAT1 mutation (Pro326Ser) in the DBD was identified in both patients. P1/P2 showed low Th17 cells and memory B cells were markedly reduced in P2.

P3 is a 36-years old woman with recurrent, treatment refractory and now azole resistant CMC and recurrent visceral leishmaniasis (VL). NGS revealed a previously described (Ala267Val) mutation. Switched memory B cells, regulatory T cells and PHA T cells stimulation were reduced.

P4, a 10 years old girl with recurrent EBV/CMV infections, had a recently described mutation (Leu351Phe) presenting with low Th17 cells.

Conclusions:

NGS panels are feasible first-tier diagnostic tools in patients with autoimmunity/infectious manifestations. STAT1 GOF immunophenotypic alterations are heterogenic, decreased Th17 levels being most consistent.

We report a not previously reported association of STAT1 GOF and recurrent VL. The impact of STAT1 GOF mutations remains to be elucidated, however Th17 immunity has shown to be highly relevant in the clearance of VL.
NOVEL MUTATION OF IL10RB GENE ASSOCIATED WITH REFRACTORY DIARRHEA

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\textsuperscript{4}Division of Immunology Boston Children's Hospital- and Department of Pediatrics-, Harvard Medical School, Boston, Iran

Background:

Interleukin-10 (IL10) is an immunomodulator cytokine that deactivates macrophages and decreases T cells proinflammatory cytokines production. Biallelic mutations in IL10 and IL10 receptor genes cause early onset Inflammatory Bowel Disease in infants. We present here a case of a male with IL10 receptor subunit beta (IL10RB) deficient patient with initial impression of food allergy associated with poor response to aminoacid based formula.

Methods:

Comprehensive clinical and immunological phenotyping was combined with a targeted sequencing for identifying molecular diagnoses.

Results:

A 5 month old infant who referred with chief complaint of chronic diarrhea, anal ulcer and severe failure to thrive (FTT). In past medical history he was born from a consanguineous parents and he presented with chronic and bloody diarrhea since 20 days old, irritability due to painful defecation and mild to moderate eczematous lesion on his face. Amino acid based formula commenced but the response was poor to this regimen. At 4 month old the patient admitted due to refractory diarrhea and basic evaluation revealed the presence of chronic inflammatory disorder. Endoscopy showed fragile mucosa and multiple aphthous lesion. Targeted gene panel sequencing revealed a novel homozygous null mutation within \textit{IL10RB} gene (c.92C>T, p.S31P, affecting extracellular domain of the protein) and the patient referred for bone marrow transplantation.

Conclusions:

IL10 and IL 10 receptors' deficiencies are rare disorders. These conditions present with FTT, chronic diarrhea and can be in differential diagnosis of food allergy if the patient do not response to hypoallergenic formula.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0444

TNFRSF13B (TACI) MUTATIONS IN TWO NON-RELATED BRAZILIAN BOYS WITH THE COMMON VARIABLE IMMUNODEFICIENCY DISORDER (CVID)

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Background:

Approximately 7%-10% of CVID patients carry mutations in the gene encoding the transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), a member of the TNF-receptor family.

Methods:

Whole-Exome-Sequencing (WES) was performed to search for a possible causative gene mutation.

Results:

Patient 1#: 21 years old, begins symptoms at 9 years of age. He had the antecedent of chickenpox at 3 years of age; mononucleosis at 4 years old; chronic sinusitis; 2 pneumonias (1 and 6 years old) and recurrent tonsillitis becoming tonsillectomy; gastroesophageal reflux and Herpes zoster at 11 years of age, lymphadenopathy, splenomegaly, hypogammaglobulinemia, leucopenia and thrombocytopenia.

Patient 2##: 16 years old, begins symptoms at 8 years of age. He had the antecedent of ecchymosis associated to thrombocytopenia, lymphadenopathy, adenotonsillectomy at 4 years of age, Herpes zoster at 13 years of age. He showed elevated vitamin B12, reduced number of CD19+ B cells, elevated αß TCD3+CD4-/CD8- (DNT); Hypogammaglobulinemia; EBV IgG+.

<table>
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<td>TCRαβ+CD4+CD8</td>
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<td>CD3+CD16+CD56+</td>
<td>109/mm³</td>
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<td>CD3+CD16+CD56+</td>
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Both patients are using mycophenolate and intravenous Human Immunoglobulin every 28 days. The WES showed about 65,000 SNPs and after filtering criteria remained about 11,000. Using the Phenolyzer tool the gene TNFRSF13B (score of 69.7) was identified.

Conclusions:

Both patients have a heterozygous TNFRSF13B mutation causing changes in the protein.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0460

A CASE OF AN INFANT WITH GRANULOMAS AND VIRAL, FUNGAL AND BACTERIAL SUSCEPTIBILITY WITH AUTOINFLAMMATION

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Background:

The autoinflammatory diseases have been classified by some as primary immunodeficiencies (PID), where diagnosis and treatment is challenging.

Methods:

We present a 2 year 9 months old girl, with family history of one brother who died at 1 yo with recurrent infections, BCG lymphadenitis, abscessed lymph node granulomas, high white blood cells count (WBC) (70,000 cell/mm³), elevated acute phase reactants, hypergammaglobulinemia, and fatal hemophagocytic lymphohistiocytosis.

Results:

She started with cytomegalovirus infection at 1.5 months of age treated with ganciclovir. Then she presented recurrent low grade fever, gastrointestinal (GI) symptoms and recurrent oral candidiasis. Treatment with prednisone and colchicine was started because the persistence of fever. She developed lymph node enlargement, hepatomegaly and splenic cysts. The lymph node biopsy reported caseous granulomas without isolated pathogen. The laboratories showed elevated WBC, acute phase reactants, IgG (1,100 mg/dL), ferritin (2820 mg/dL) and normal IgD levels. She had normal lymphocyte subsets, lymphoproliferative response to PMA (25 ng/mL), PHA (10 mcg/mL) anti CD3-CD28, dihydrorhodamine, functional status of IFN-γ/IL-12 axis and perforin expression. In 2015 a targeted next generation sequencing was performed (250 genes related to PID). The physical exam shows hepatomegaly and livedo reticularis.

She had presented central nervous system, lung and GI invasive infections that needed admission to the Intensive Care Unit in six occasions treated with antiviral, antifungal, antibacterial and antimycobacterial drugs for long periods of time and intravenous immunoglobulin at immunomodulator and substitutive doses.

Conclusions:

Increasing number of new defects in autoinflammation and immunodeficiencies have been reported, leading us to amplify the research in this field.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0467

A NOVEL CASE OF COPA SYNDROME HIGHLIGHTS THE POTENTIAL SEVERITY OF ARTICULAR INVOLVEMENT

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Background:

Copa syndrome is a recently discovered immune dysregulatory disease characterised by polyarticular arthritis and progressive interstitial lung disease with pulmonary haemorrhages as a possible complication. The disease is caused by heterozygous mutations of \textit{COPA} gene, which encodes for the coatomer subunit α, a regulator of protein transport between Golgi and the endoplasmic reticulum.

Methods:

Molecular analysis of \textit{COPA} gene was performed by Sanger sequencing.

Results:

We followed a case of a young girl that presented at 3 year of age with polyarticular arthritis involving metacarpophalangeal joints, hip and cervical spine. Lab results showed elevation of inflammatory markers, high titer rheumatoid factor and antinuclear antibodies. Lung CT scan performed because of persistent cough without evidences of infectious origin, showed interstitial lung disease with tree-in-a-bud appearance and air-filled cysts. The patient was treated with oral and intra-articular steroids with good response, however methotrexate and abatacept failed to control disease progression. Anti-TNF drugs were avoided because of lung involvement. Targeted genetic analysis of \textit{COPA} gene showed the reported c.698G>A mutation. The patient was lost to follow up for 3 years during which therapy was discontinued. At a subsequent control she showed a very severe osteoarthritis with joint damage and deformities of the hands, feet, limitation of elbow, shoulders and cervical spine movement with fusion of the cervical vertebrae. Hydroxychloroquine and mycophenolate were started with a partial response.

Conclusions:

We report a novel case of COPA syndrome with a severe articular involvement, underlining the importance of early treatment to avoid loss of joint function and deformities.
HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR IL10R DEFICIENCY LEADING TO VERY EARLY-ONSET INFLAMMATORY BOWEL DISEASE: 4 YEARS FOLLOW-UP
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Background:

Interleukin 10 is a key anti-inflammatory cytokine and a master regulator of intestinal mucosa homeostasis that can inhibit proinflammatory responses of both innate and adaptive immune cells.

Methods:

Case report

Results:

A 6-year-old boy, son of healthy non-related parents, presented in the neonatal period with profuse diarrhea, perineal inflammation and eczematous skin lesions with partial response to amino acid formula. Laboratory workup revealed microcytic anemia and elevated serum inflammatory markers (microbiology studies were negative). During his first year of life, recurrent episodes of bloody diarrhea with mucus were noticed associated with multiple and persisting perianal fissures and subsequently failure to thrive. An increased fecal calprotectin and ulcerations of the recto-sigmoid mucosa with inflammatory infiltrate and crypt abscesses on histological examination led to the diagnosis of indeterminate colitis. Serum immunoglobulins, lymphocyte immunophenotyping, oxidative burst and FOXP3 expression in regulatory T cells were normal. Functional tests of the IL10 axis revealed a defect of the IL10-Rβ (confirmed by genetic studies). Bowel rest with total parenteral nutrition and immunosuppressive therapy (prednisolone and azathioprine) led to partial and temporary clinical improvement. Ileostoma was made at 18 months and 11 months later he was submitted to hematopoietic stem cell transplantation (HSCT). After 4 years of follow-up, he is clinically stable without gastrointestinal symptoms and normal endoscopic findings.

Conclusions:

IL10R deficiency is a rare cause of IBD which should be excluded in all cases of very early, severe and refractory colitis associated with perianal disease. Treatment options are limited and HSCT seems the most attractive (and potentially curative) therapy.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0474

CLINICAL CASE: AGAMMAGLOBULINEMIA AND INTRACTABLE DIARRHEA

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Background:

In recent years, auto-inflammatory disorders have shown a variety of forms of presentation, including B-cells involvement. We present a case of a patient with agammaglobulinemia and inflammatory intestinal disease.

Methods:

Review of Clinical History.

Results:

Boy with non-consanguineous parents, healthy twin brother (bichorionic, bivitelin pregnancy).

Meconium ileus with intestinal perforation at birth.

Multiple episodes of severe abdominal cramping, vomiting and secretory diarrhea that compromise his general state and requires fasting and parenteral nutrition to improve.

Absence of B-cells and agammaglobulinemia with no mutation in BTK. Normal CD4 but diminished CD8 with a pattern of activation (CD8+DR+24%)

Absence of plasma cells and T-cells infiltrate (CD4+ and CD8+) in lamina propria with subtotal vellocitary atrophy in two intestinal biopsies. Cystic Pancreatic Fibrosis was discarded.

He received IVIG every 21 days, oral budesonide and azathioprine with no infections but no improvement of the intestinal manifestation. Then he started with parenteral nutrition, weekly IVIG and sirolimus.

He also has thin, sparse hair but normal skin. A progressive pigmentary retinitis was found recently.

At 1 yo he presented cardiorespiratory arrest secondary to metabolic compromise and severe dehydration due to profuse diarrhea, he died of septic shock a few month later.

No significant mutation appears in BTK; TRNT1, TTC7A nor SKIV2L.

Conclusions:
Agammaglobulinemia could be the expression of a more complex inflammatory disease in which intestinal involvement may be the main clinical manifestation. Finding a molecular diagnosis could help us to understand the association between the inflammatory compromise and the B-cell ontogeny.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0475

CLINICAL CASE: AGAMMAGLOBULINEMIA, SKIN LESIONS AND CHRONIC DIARRHEA

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Background:

Diseases of immune dysregulation are very complex diseases, usually with specific organ damage (lungs, skin, etc), with or without infections, and as part of the immune compromise the B cells could be involved. We present a patient with immune dysregulation and agammaglobulinemia.

Methods:

Review of Clinical History

Results:

Girl 1 yo. No relevant family history. Since birth widespread xerosis that progressed to severe erythematous and squamous rash with intense itching and severe eosinophilia. Profuse chronic diarrhea and failure to thrive. Several effusive otitis.

Agammaglobulinemia with absent of B cells. Normal T and NK cells and Lymphocyte proliferation.

At 2 months she started IVIG, high-dose corticosteroids and cow’s milk protein was contraindicated, improving both eosinophilia and eczema.

Skin biopsy: Psoriasiform dermatitis. Hair and lashes biopsy: Uneven distribution of the pigment and morphological abnormalities of the bark, incomplete fractures (trichoclasis), lacerations, and detachments of cortical fibers. All compatible with Trichorrhexis.

Colon biopsy: Non-Specific chronic colitis.

Several febrile episodes with persistently positive blood cultures to different bacteria. In the context of febrile syndromes she presented hyperglycemia and euthyroid disease.

Thinking about a disregulatory immunodeficiency and because she continued with frequently reactivations of the skin lesions she started treatment with sirolimus and acitretin.

She died of septic shock at 1 year old. Cariotype and spink5 gene study are still pending.

Conclusions:
We present a case and immune dysregulation and agammaglobulinemia, still without molecular diagnosis. Finding a molecular diagnosis could help us to understand the association between the immune dysregulation and the B-cell ontogeny.
SYNONYMOUS MUTATIONS DO MATTER FOR NFKB1 AS WELL: EXPANDING PHENOTYPE IN THREE PATIENTS WITHIN THE SAME FAMILY

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Background:

The nuclear factor k light-chain enhancer of activated B cells (NF-kB) signaling pathway is a cornerstone for cellular processes involving T,B,NK cells and myeloid cells. Accordingly, disease-causing mutations in NF-kB pathway genes have been reported to cause hypogammaglobulinemia and autoinflammatory symptoms.

Methods:

We studied in deep a patient suffering from Langerhans’ cell histiocytosis (difficult to treat, requiring clofarabine), disseminated non-tuberculous mycobacteria -NTM- (Mycobacterium genavense), sclerosing mesenteritis, failure thriving, HLH-like episodes and gut failure. RAG1/2, IFNγR1, GATA2, RMRP genes were sequenced. Functional assessments: STAT5 phosphorylation, IL12/IFNg axis. Western blot for p105/p50 in unstimulated/stimulated PBMCs, RT-PCR from gDNA. cDNA sequencing in unsorted and sorted T,B,NK cells.

Results:

Targeted NGS revealed a synonymous mutation (c.705 G>A / wt) leading to exon 8 skipping in the three related subjects. Surprisingly, the older sibling and the father were healthy with normal immunoglobulin levels and basic lymphocyte subsets. The proband partially recovered the T-cell defect but lately developed panhypogammaglobulinemia with impaired polysaccharide responsiveness. mRNA NFKB1 transcripts were decreased to 30% compared with a healthy donor. In the three affected subjects WB showed p105 phosphorylation, suggesting haploinsufficiency was the disease causing mechanism. perforin staining, CD107a degranulation and citotoxicity were normal.
Follow up

0.8 year

LCH

M. Genavense

Hypogamma

Clofarabine

HLH-like episodes

Failure thriving

Gl intolerance

Steroids

TPN
cDNA amplification from mRNA exons 7-9

WB NFKB1 p105/50 (P-105)

NFKB1 mRNA T-cell
Conclusions:

Synonymous mutations do matter. In patients with NFKB1 deficiency disseminated NTM infections can occur. This family represents the incomplete penetrance in NFKB1 and expands the phenotype of this defect with a novel NTM infection.

**NFKB1 : c.705 G>A / wt ⇒ exon 8 skipping**

*Synonymous variant not found in Exac/1000G/dbSNP*
SUCCESSFUL ONE-LOBE VENTILATION IN A CHILD WITH SEVERE PLEURO-PNEUMONIA AND HYPER-IgE SYNDROME

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2Children’s Hospital Bambino Gesù, Pediatric Intensive Care Unit, ROMA, Italy
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Background:

Autosomal dominant hyper-IgE syndrome (AD-HIES), is a complex primary immunodeficiency, with high susceptibility to to pyogenic infections. Here we reported a case of severe pneumococcal pneumonia in a child with AD-HIES complicated with tension pneumothorax successfully treated by one-lung ventilation.

Methods:

An four years old female affected by AD-HIES (heterozygous mutation in STAT3) was hospitalized for streptococcal right pneumonia with pleural effusion treated by chest drainage. Chest CT (computer tomography) evidenced right lower lobe consolidation associated with cystic lesion and bronchopleural fistula (Figure 1). She initially received multiple antibiotic therapy and monthly Igev, with a progressive improvement.

One month later she was readmitted due to the development of massive right hydropneumothorax, confirmed by chest CT (Figure 2). Streptococcus pneumoniae persisted positive in pleural effusion.
Antibiotic therapy, pleural drainage and later surgical toilette was done. Right pneumonectomy was considered without improvement.

**Results:**

A conservative approach with one-lobe ventilation was chosen instead of surgical one. One-lobe ventilation was done in order to allow pleural healing. Pre-extubation chest CT showed the re-expansion of right lung (Figure 3).

**Conclusions:**

AD-HIES presents a susceptibility to pyogenic pneumonia. Mutation in STAT3 generates low level of Th17 cells, which compromises clearance of extracellular bacterial infections, specially in bronchial epithelium, implying the development of abscesses, bronchiectasis and pneumatocele. STAT3 mutation leads likely to abnormalities in tissue remodeling after surgical intervention⁴, so patients a conservative approach is recommended. In our case prolonged antibiotic therapy, chest drainage and one lung ventilation allowed to avoid lobectomy pneumonectomy in order to preserve respiratory function.
T FOLLICULAR HELPER CELLS IN PATIENTS WITH CTLA-4 HAPLOINSUFFICIENCY

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Background:

A critical subset of effector CD4+ T cells, T follicular helper (Tfh) cells, mediate the differentiation of B cells into memory cells and plasma cells. Importance of Tfh cells in humoral immunity has been proven in primary immunodeficiencies.

We have analyzed circulating Tfh cells (cTfh) in patients with germline mutations in CTLA-4, an immunedysregulatory disorder with variable penetrance, B cell lymphopenia and/or hypogammaglobulinemia.

Methods:

Frequency of circulating Tfh cells (cTfh) was analyzed by flow cytometry in the peripheral blood samples of both clinically affected and asymptomatic patients. cTfh cells were identified as CD3+CD4+CD45RA⁻CXCR5+ population, expressed as % of CD4T cells, and compared using non-parametric T test.

Results:

We found a statistically significant increase (P< 0.0001) in cTfh subset in both affected patients (Median +/- SD: 28.4 +/- 10.9) and their asymptomatic yet mutation positive relatives (Median +/- SD: 20.6 +/- 8.4%) as compared to healthy donors (Median +/- SD: 11.5 +/-3.9%).

Although most affected patients had reduced B cell numbers and hypogammaglobulinemia, none of the asymptomatic relatives had low Ig levels and maintained B cells. The change in the frequency of cTfh did not correlate with the patient’s age, or prior treatment.

Conclusions:

Tfh and B cell interaction is pivotal in B cell differentiation and humoral response. The role of Tfh has been studied in other monogenetic immunodeficiencies with hypogammaglobulinemia and a decrease in Tfh cells was shown. In contrast, our CTLA-4 mutation positive cohort with or without hypogammaglobulinemia shows increased cTfh, suggesting quantitative deficiency in CTLA-4 expression may lead to their dysregulation.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0491

EARLY ONSET AUTOINFLAMMATORY SYNDROME SECONDARY TO MUTATION IN THE TYROSINE-PROTEIN KINASE LYN: RISKS AND BENEFITS OF COLCHICINE THERAPY

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Background:

In mouse models, p.Y508F mutation in tyrosine-protein kinase Lyn is associated with severe anaemia, autoimmune glomerulonephritis and positive ANA. We describe the clinical course of a 16-year-old patient with a de novo mutation in the regulatory tyrosine residue 508 in LYN, noting benefit and risk of colchicine therapy.

Methods:

Clinical evaluation and case-note review were performed. Next generation sequencing (NGS) gene panel detection of the de novo mutation was confirmed by Sanger sequencing.

Results:

A child with history of rash since birth and recurrent painful skin lesions associated with fever, fatigue, testicular pain and swelling, poor growth and elevated serum amyloid (SAA) showed poor response to anakinra and developed an episode of glomerulonephritis requiring renal replacement therapy. A trial of colchicine led to dramatic improvement in SAA and growth but intermittent rash and fatigue persisted. Subsequent clinical deterioration led to addition of tocilizumab, which was later discontinued due to worsened symptoms. After 3 years on colchicine, the patient experienced abrupt deterioration with diarrhoea and 9kg weight loss. Gastrointestinal histology was suggestive of colchicine toxicity requiring discontinuation with improvement. Targeted NGS revealed p.508F mutation in LYN. Subsequent introduction of etanercept therapy resulted in normal SAA, improved growth and weight gain, self-reported improved cognition and sustained clinical remission on treatment.

Conclusions:

An American patient with a similar but more severe phenotype has previously been found to have a de novo mutation in LYN (p.Y508*). Our case highlights the potential role of colchicine, and anti-TNF therapy in controlling inflammation, but caution is indicated regarding colchicine toxicity.
Adaptive and innate immune response in the murine leaky SCID model RAG1S732C

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Background:

Augmented susceptibility to infectious agents, including chronic virus infections, as well as immune dysregulation, inflammation, and autoimmunity characterise primary immunodeficiencies (PIDs). One prototypic PID develops as a consequence of recombinase-activating genes 1 and 2 (RAG1/2) mutations, proteins that are central for lymphocyte development. Hypomorphic RAG mutations, with a minimal expression and function of the mutant protein - still enabling variable lymphocyte development - are found in patients suffering from leaky severe combined immunodeficiency (leaky SCID).

A murine model carrying a hypomorphic RAG1 mutation (RAG1S732C) has been previously described to display lymphopenia, spontaneous antibody secreting cell expansion and autoantibodies. So far, innate and adaptive immune responses to replicating persistence-prone viruses have not been described.

Methods:

We have studied lymphocytic choriomeningitis virus (LCMV) - a prototypic persistence prone virus - infection of RAG1S732C mice.

Results:

LCMV infection of heterozygous and wildtype mice, led to lymphoproliferation with expansion and later contraction of virus specific cytotoxic T cells. Those CD8+ T cells were functional regarding cytokine production and virus elimination. Homozygous RAG1S732C animals were unable to expand virus specific CD8+ T cells and did not mount virus-specific antibody responses and showed continuous viraemia up to one month post-infection. Rather surprisingly, even the innate immune response was impaired in homozygous RAG1S732C after LCMV infection, assessed by serum type I interferons measurement.

Conclusions:

In conclusion, although homozygous RAG1S732C mice have low amounts of B and T cells, neither innate nor adaptive immune responses against persistence prone virus are detected in vivo. Investigation regarding virus-driven autoimmunity is currently on-going.
Background:

We report an 18-year-old male with CTLA-4 haploinsufficiency with multiple autoimmune and lymphoproliferative features, who developed severe, adenovirus-infection-induced Macrophage Activation Syndrome (MAS). Between age 2-7 years he developed type 1 diabetes mellitus and recurring Evan's Syndrome (autoimmune cytopenias with splenomegaly) and lymphadenopathy, requiring prednisolone, anti-D, danazol, rituximab, IVIG treatment. He remained panhypogammaglobulinemic post-rituximab.

Methods:

Immune workup showed low NK cells with normal cytotoxicity, low class switched B cells, normal Treg number, increased DNT cells (6-8%) but normal IL-10, sFasL, vit. B12 levels; no ALPS gene mutations were found.

Results:

Between age 7-8 he had spontaneous temporal lobe haemorrhage, and pulmonary nodules, hilar lymphadenopathy, psoriasiform skin rash and cerebellar lesions appeared, showing angiocentric T cell vasculitis and lymphocytic infiltration. Microbiologic and oncologic workup were negative and mycophenelate (MMF) was added to IVIG. Autoimmune enteropathy developed later. Due to persistent lymphadenopathy, splenomegaly and ALPS phenotype, MMF was switched to sirolimus. At 13 years, adenovirus URTI progressed, despite discontinuation of sirolimus and additional IVIG, to alveolar/interstitial infiltration and pulmonary edema with biopsy confirming bronchiolitis obliterans. No other pathogen was identified. In addition, persistent fever, pancytopenia, very high levels of CRP, triglycerides, ferritin, D-dimer, sol-CD25, sol-CD163, LDH and creatinine developed. Bone marrow showed hemophagocytosis. Stabilization and recovery occurred with pulse methylprednisolone and high dose IVIG. In the past 5 years, combined MMF/SCIG therapy has controlled the autoimmunity.

Conclusions:

This is the first report that immune dysregulation in CTLA-4 haploinsufficiency (heterozygous mutation c.436G>A, p.G146R identified in 2015) may predispose to virus-induced hyperinflammation with MAS as well as to autoimmunity.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0542

Adenosine Deaminase 2 (ADA2) is a new member of nucleic acid sensors
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Background:

Biallelic mutations in ADA2 cause an immune dysregulation syndrome termed “Deficiency of ADA2” or “DADA2”. Even if it is clear that lack of ADA2 expression leads to uncontrolled inflammation, the function of the protein and the nature of the factors that sustain the inflammatory process is still not known. Pattern recognition receptors (PRRs) are a group receptors evolved to recognize conserved microbial structures named pathogens associated molecular patterns (PAMPs). Nucleic acid sensors belong to this family. To date there is a large consensus that most of the nucleic acid receptors have been identified; nevertheless, our results indicate that ADA2 belongs to this family.

Methods:

Immunohistochemistry, Proteomics, Molecular Biology, Cellular Biology, EMSA

Results:

ADA2 is localized in phagolysosomal vesicles and can bind and degrade DNA and RNA with an acid pH-optimum between 4.5 and 5. These results suggest that ADA2 is an endonuclease acting on non-self nucleic acids. In striking agreement the proteomes of monocytes from DADA2 patients show a significant skewing towards interferon type I (IFN-α and IFN-β) and type II (IFN-γ) induced proteins, hallmarks of the immune sensing of nucleic acids.

Conclusions:

Our results indicate that ADA2 is a novel member of the family of nucleic acid sensor and they offer the key for deciphering the immuno-pathogenesis of DADA2. Importantly our observations, together with an immediate impact on the management of DADA2 patients, will open new important research fronts in the study of mechanisms linking nucleic acid-induced inflammation and vascular or bone marrow diseases.
Hepatosplenomegaly, cholestasis and inflammatory syndrome: from a suspicion of inherited metabolic disorder to a primary immunodeficiency diagnosis


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Background:

A 11-days-old newborn was referred to our hospital due to hepatosplenomegaly, cholestasis and cytopenia with high suspicion of an inherited disorder. He was the 3th child of non-consanguineous parents. One brother deceased during newbornhood because of fulminant sepsis due to Burkholderia cepacia and macrophage activation syndrome. Because of the persistence of cholestatic liver disease and progressive increase of inflammatory parameters, several biochemical and genetic investigations were performed in order to exclude a metabolic disorder or a primary immunodeficiency with immune dysregulation. The inflammatory phenotype of the disease and deterioration of clinical condition led to the employment of steroid therapy in addition to broad spectrum antibiotics with a good clinical and biological response and temporary remission of symptoms. Investigations revealed no significant finding supporting any metabolic diseases, including the early suspicion of Gaucher's disease.

Methods:

Targeted gene sequencing panels exclude familial HLH, autoinflammatory disorders and primary immunodeficiencies associated with immune dysregulation.

Results:

After discharge, whole exome sequencing revealed a hemizygous c.45+1G>T change in CYBB leading to the diagnosis of Chronic Granulomatous Disease (CGD). In accordance with genetic results, neutrophil oxidative burst was abnormal. At present, the patient is under prophylactic antifungal and antibacterial medications.

Conclusions:

We report a remarkable case of CGD with an atypical and very early onset. This case highlight the importance of considering rapidly CGD in differential diagnosis in case of early inflammatory complications even in absence of severe infectious diseases or classical hallmarks of the disease in order to avoid delay in diagnosis and allow an early and focused management.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0545

Sporadic hemophagocytosis lymphohistiocytosis (HLHs) are associated with a gamma interferon signature independently of the associated diseases: Report of a National, prospective cohort of 124 patients.

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Background:

The pivotal role of gamma interferon (IFN-γ) in genetic HLH (HLHg) is well established. Cytotoxic deficient CD8 cells produced abnormal levels of IFN-γ, that activates macrophages resulting in pro inflammatory cytokines production and hemophagocytosis. Currently, the role of IFN-γ in sporadic HLHs is not established.

IFN-γ signature is investigated in HLHs by analyzing serum secreted cytokines by macrophages, in 124 patients classified depending on their associated disease: hematologic neoplasia (n= 40), autoimmune/inflammatory diseases (n=21), and idiopathic (n= 63).

Methods:

Serum levels of II2R-a, IP-10 (CXCL10), TNF-α, IL1-β, II-6, II-10, IL-18 were analyzed by ELISA.

Results:

A significant positive correlation between serums cytokines levels with IFN-γ, IP-10 was found in the whole or associated disease’s groups of HLHs. (Spearman’s test, p<0,05), and also between IFN-γ and CD8DR+ cells.

Cluster analyzes classified patients in three cytokines profiles.
1 (n=34): High level IP-10, IL-2RS, IFN-γ and low levels of TNF-α, IL1-β, IL-6
2 (n=76): Low level of all cytokines.
3 (n=15): High level of all cytokines

Spleen size, ASAT, and triglycerides levels were significantly higher in group 1. Hematologic neoplasia and autoimmune diseases were associated with group 1 and 2 respectively. Severity of
HLHs, defined by hospitalization in ICU, etoposide requirement and death, was more frequent in the group 1.

**Conclusions:**

Proinflammatory cytokines secreted by macrophages are highly correlated to IFN-γ levels in HLHs, regardless the associated diseases. Levels of serum cytokines is dependent on the associated disease, providing a strong rationale for treatment targeting IFN-γ in HLHs.
INFLAMMATORY DISORDERS & DYSREGULATION OF INFLAMMATION

ESID7-0547

NOVEL LPS-RESPONSIVE BEIGE-LIKE ANCHOR (LRBA) MUTATION IN TWO UNRELATED PATIENTS OF GEORGIAN JEWISH DESCENT

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Background:

Biallelic mutations in LRBA are associated with early onset immunodeficiency and immune dysregulation. The most frequently described features include antibody deficiency, enteropathy and immune cytopenias.

Methods:

We describe two unrelated patients of Georgian Jewish descent with LRBA deficiency and heterogeneous clinical course.

Results:

The first patient presented at the age of 3 months with chronic diarrhea and FTT. Small bowel biopsies revealed severe villous atrophy and diffuse lymphocytic infiltrates. At the age of 3 years he developed Type I diabetes, exocrine pancreatic insufficiency and continued to suffer severe FTT. At age 13 he was diagnosed with poorly differentiated, metastatic, gastric adenocarcinoma and died a year later of his disease. A diagnosis was reached more than 10 years after he died.

The second patient was healthy until the age of 25 when presented with ITP. Thrombocytopenia responded to steroid and IVIg treatment but 5 years later a significantly enlarged spleen was removed. In the next several years he was followed closely for persistent lymphadenopathy and multiple biopsies showed reactive LNs. At 36 years he developed B symptoms and was diagnosed with Hodgkin's lymphoma. 2 years after initial remission the patient relapsed and underwent autologous stem cell transplant. He suffered a second relapse 1.5 years later.

Both patients were found to have a homozygous nonsense mutation (c.6640C>T; p.Arg2214X) resulting in an early stop codon upstream of the LRBA BEACH domain.

Conclusions:

These two cases emphasize the diverse clinical phenotype of LRBA deficiency and could suggest an increased risk for malignancy.
A novel heterozygous mutation in NLRP3 associated with Schnitzler Syndrome in Brazil: case report

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Background:

Schnitzler Syndrome (SS) is a complex autoinflammatory disease (AID) without clear genetic basis sometimes associated with mutations in NLRP3. This paper aims to report the case of an adult patient with SS associated with heterozygous mutation in the NLRP3.

Methods:

Review of clinical and laboratory data was obtained after consent was granted. Genomic DNA was obtained from peripheral blood and Sanger Sequencing was performed to confirm the mutations.

Results:

I.N.P., 48, male, complained of 10 years of recurrent 3-4 days episodes of fever, arthralgia, myalgia, bone pain with urticarial rash. He had elevated neutrophils, CRP and ESR on flares. A monoclonal gammopathy, IgM kappa was found and myeloma was ruled out. The frequency of episodes was 4-5 in a year and as amyloidosis was ruled out, colchicine was started. A variant of unknown significance (NLRP3; c.A2176G - p.S726G) in heterozygosity in the was found.

Conclusions:

This patient fulfills Strasbourg clinical criteria (2 obligatory: chronic urticaria and monoclonal IgM plus fever, leukocytosis, bone pain and elevated CrP). The risk of developing amyloidosis and multiple myeloma is unknown. Genetic analysis is not obligatory but when done, mutations in NLRP3 are associated with the disease. Infevers database, the mutation found (p.S726G) was described associated with NOMID. Anti-IL1 seems effective to control fever, skin rash and also amyloidosis. However, these drugs don’t seem to control the progression to myeloma or lymphoma. About 25% control with colchicine.
Flare induced by canakinumab shot in a Deficiency of Interleukin One Receptor Antagonist (DIRA) Brazilian Patient: case report

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Background:

Deficiency of interleukin one receptor antagonist (DIRA) is a rare autoinflammatory disease. Clinically, it present with osteomyelitis in specific bones without fever and pustular skin rash. So far, just one report by Ulusoy et al 2016 reported canakinumab in DIRA. Canakinumab is a very safe human anti-IL1beta soluble monoclonal antibody. Until now, no antibody against anti-IL1 has been reported or flare induced by this drug has been reported.

Methods:

Patient data and laboratory analysis were revised after the consent term was assigned.

Results:

J.G.S.A. is a 7 year old boy with the diagnosis of DIRA since 5 years. Canakinumab 150 mg every 4 weeks (4mg/kg) was promptly started after the diagnosis with excellent improvement. However, in the 5th and 6th application of canakinumab, the child developed vomiting and diarrhea without etiology and resolved spontaneously. Immediately after the 7th application, he had vomiting and pain in thorax. An X-ray was performed, as well as laboratory analysis (figure 1) that confirmed a disease flare. This was treated with prednisone 1mg/kg for 5days with resolution.

Conclusions:

Mortality in DIRA has clearly decreased after anti-IL1. Up until now, just one report of a DIRA treated with canakinumab has been published without long outcomes. The drug is very safe and a large number of autoinflammatory diseases could be treated, since only mild side effects are observed. So far, no reports of flares induced by canakinumab have been reported.
Background:

Introduction: The γ-globulin replacement therapy is essential for the management of Predominantly Antibody deficiencies, although it is important for other Primary Immunodeficiencies and/or certain cases. The subcutaneous infusion (SCIG) has been introduced in Greece in 2006 and fSCIG is the only available since 2016.

Methods:

Methodology: Patients and families were trained in the Hospital and home therapy follows. Data from 30 patients under fSCIG, are reviewed. Serum Immunoglobulins levels were measured by nephelometry (Siemens Dade Behring BN II).

Results:

The ratio of male/female patients is 21/9. The majority of them (16/30) suffer from Common Variable Immunodeficiency, 11/30 from Btk deficiency, 1/30 from Hyper IgM Syndrome, 1/30 patient has Di-George Syndrome and 1/30 patient has Ataxia-Telangiectasia. All patients were managed with SCIG before starting fSCIG, which was initiated between 61/2 and 59 years of age. IgG levels prior to fSCIG were 363-1180 mg/dl, while IgG ranged from 489 to 1170 mg/dl after 1-15 months. Headache and pyrexia are noticed in one patient in every infusion and migraine was reported in another patient during the first 2 infusions. At the site of infusion all patients feel discomfort and have swelling which resolves 24-48 h later.

Conclusions:

The study demonstrates the safety of self-administered fSCIG at home, which improves treatment satisfaction, flexibility and independence of both patients and their families. Due to the recent initiation of treatment in some of our patients, further clinical assessment and laboratory investigation are required for safe conclusions regarding the treatment choice.
INGID
ESID7-0335

ENABLING SELF-TREATMENT THROUGH A NOVEL PATIENT-DRIVEN FACILITATED SUBCUTANEOUS IMMUNE GLOBULIN (fSCIG) ADMINISTRATION

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Background:

To meet patient lifestyles or due to fear of needle-sticks, complications at infusion sites and adherence problems, fSCIG provides an opportunity to empower patients with an alternative. We present results from a novel self-treatment fSCIG using a consistent, accurate and constant pressure infusion system (CPIS) together with an adjustable flow rate controller (AFRC) calibrated to the viscosity of the drug. The CPIS has an intuitive and easy operation to provide accurate infusion flow rates. A high flow infusion set (24G) was used to allow up to 300 ml/hr flow rates to infusion sites.

Methods:

fSCIG infusion data was recorded in a structured questionnaire at two departments specialized in treating primary immunodeficiency patients. Patients received education and training by an experienced nurse at the out-patient clinic.

Results:

69 fSCIG infusions were recorded. Patients’ age was between 25 to 81 years (mean 50 years). Males administrated 54% and females 46% of the infusions. The nurse assesses when a comfort level of performing self-treatment is reached which resulted in 93% of all infusions recorded. Average infusion time was 83 min. The AFRC used in the CPIS shows high quality performance, usability and safety with a total mean value score of 97 out of a maximum of 100 points.

Conclusions:

A CPIS with a calibrated AFRC demonstrates to be convenient and empower patients to successful self-treatment and home-therapy. It is easy to operate, safe and delivers ultimate therapeutic outcome for patients’ continual improvement of their well-being without triggering intolerable site reactions that patients sometimes have described.
Background:

Gene therapy protocols are becoming increasingly more available for patients with primary immune deficiency (PID). The sites that offer this novel therapy are still few, which necessitates travel and relocation of study subjects who wish to participate. As this experimental therapy is highly regulated and monitored for subject safety a model was needed to monitor the study subjects once they returned to their home countries.

Methods:

Using the advantages of technology such as Skype™ and email, a model for collaborating with the study subject’s referring care team to monitor for adverse events, protocol compliance and coordinate for collection of research sample was created.

Prior to the return of a subject to their home facility, a protocol training session is conducted by the gene therapy team with the subject’s care team at their home location. Using videoconferencing the protocol care is reviewed in detail with identification and discussion of monitoring and follow up care. To assist with collecting study specific data, a document was created that provides the team with the data to be collected at each visit. These documents are easily translated to subject/care team’s native language if necessary. Once collected, the data sheets are emailed, scanned or faxed back to the gene therapy team.

Results:

This model provides the oversight and monitoring of the study subjects, while allowing the subjects and families to return home adding to their quality of life.

Conclusions:

The home care team gets to maintain their primary relationship while gaining the benefit of participating in the research process.
Background:

Humoral immunodeficiency is a common group of primary immunodeficiency. IVIg is a choice for treatment them along with antibiotic prophylaxis. These patients could be have a better quality of life with early diagnosis and early substitution of IVIg. Here we want the report of the patients that received IVIg infusion in Mofid Children hospital between 2013 until 2017.

Methods:

The patients file extracted and data about age ,sex , type of diseases and time on IVIg treatment were extracted for them.

Results:

Total number of patients that received IVIg during these years are 33 patients. 23 of them are male and 10 of them are female . The youngest patient is 4months old age and oldest one is 20 yrs. Mean of patients ‘age is 7 yrs ± 4yrs SD.

IVIg infusion doses are 5 to 20grs for the patients with 2 to 4 weeks intervals.

4 patients have had side effect of IVIg that included: Fever, chills, headache, hypertension, dyspnea. Nausea.

Type of PID are ; 2 patients have bruton diseases, 7 have CVID. 5 patients have HyperIgM.,7 cases with ataxia telanjectasia, 1cases with ICF, 4 case of SCID before BMT and 2 cases after BMT 2 cases with CID . one cases of HLH after BMT ,2 cases with hyper IgE syndrome,

Conclusions:

PID patients received IVIg treatment by IV infusion on regular bases with early diagnosis have better quality of life.
INNATE IMMUNITY

MICROBICIDAL ACTIVITY OF NEUTROPHILS OF PATIENTS WITH X-LINKED AGAMMAGLOBULINEMIA
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Background:
Neutrophils as well as antibodies take the most active positions in the response system to extracellular pathogens. So the study of mechanisms of phagocytic link of the patients with primary agammaglobulinemia is of a great significance.

Methods:
10 patients aged 10-25 years old with XLA, were under observation. Indicators of Immune System were assessed before and after 2 years of substitution therapy. ELISA and NBT-test was used to detect the production of α-defensines and active forms of oxygen by neutrophils. Control group consists of 10 blood donors.

Results:
Before the beginning of substitute therapy oxygen-dependent inhibition of neutrophil phagocytic metabolism was registered, which is proved by the reducing NBT to 84,00±2,00 (in control 99,1±3,0) and stimulating coefficient 1,4±0,2 (in control ,1±0,1). In the same time synthesis of α-defensines (572,5±20 ng/ml) is several times more than reference value (75±25 ng/ml). The results of assessment of the parametres mentioned before and after 2 years of regular IVIG therapy showed increase the level of spontaneous production of active forms of oxygen (95,1±2,2ng/ml). However stimulating coefficient of NBT-test remaining below the control (1,4±0). The content of α-defensines in blood serum stabilized at the level of control (109±35,0 ng/ml).

Conclusions:
Patients with XLA have a functioning defect such as inhibition of production of active forms of oxygen and strengthening the extracellular antimicrobial activity. Immunoglobulin substitution leads to normalization of extracellular production of antimicrobial peptides, increases spontaneous oxygen dependent metabolic activity of neutrophils, but it doesn’t restore their adaptation potential up to normative criterions.
INNATE IMMUNITY

ESID7-0015

NEUTROPHIL-SPECIFIC GRANULE DEFICIENCY WITH NOVEL NONSENSE MUTATION IN CEBPE GENE GOT ATYPICAL MYCOBACTERIAL INFECTION

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Background:

Neutrophil-specific granule deficiency (SGD) is a rare autosomal recessive primary immunodeficiency disorder caused by CCAAT/enhancer binding protein-ε (C/EBPε) gene characterized by defective neutrophil chemotaxis, disaggregation, bilobed neutrophil nuclei, lack of neutrophil-specific granules, and exhibit increased susceptibility to pyogenic infections.

The encoded C/EBPε protein is a member of the leucine zipper family of transcription factors, which regulate proliferation, differentiation, and apoptosis in a variety of cell types primarily in myeloid cells.

Methods:

objectives: -
We were presenting a patient with a novel nonsense mutation in C/EBPε gene getting an atypical mycobacterial infection.

Results:

case reports: -
The patient is a ten years old Saudi girl for consanguineous parents, presented at the age of 9 months with pyogenic cervical lymphadenitis and recurrent pulmonary infection. Later, she got persistent fever, weight loss and persistent pulmonary atelectasis found to have a pulmonary atypical mycobacterial infection.

Conclusions:

Up to our knowledge, we were presenting a novel nonsense mutation for a rare SGD disease and novel clinical presentation with atypical mycobacterial pulmonary infection. Finally, we suggest testing for specific abnormal neutrophil morphology in case of invasive atypical mycobacterial infection especially if tests for other immunodeficiency disorders were negative.
INNATE IMMUNITY

ESID7-0017

SPECTRUM OF PRIMARY IMMUNE DEFICIENCY IN CHILDREN AT A TERTIARY CARE HOSPITAL IN PAKISTAN

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Background:

Primary Immunodeficiencies (PID) has been recognized in the West for years but its recognition and diagnosis has been an issue in developing countries. This study aims to present the spectrum of PID from Pakistan, based on clinical and molecular methods.

Methods:

Thirty five patients with features suggestive of a primary immunodeficiency disorder, from Pakistan were followed over a period of 6 years at Aga Khan University Hospital (AKUH). Immunoglobulin levels, dihydrorhodamine (DHR) flow cytometric tests were performed at AKU. Molecular testing of the suspected PID patients was performed in collaboration with Boston Children hospital.

Results:

Out of 35 suspected cases, 15 (42.8%) children were diagnosed to have primary immunodeficiency. The age of patient ranges between 3 months to 16 years. Chronic granulomatous disease (CGD) was the most common diagnosis (33.3%), followed by severe combined immunodeficiency (20%), hermansky pudlak syndrome (13.3%). 1 each (6.7%) was diagnosed with X-linked Bruton agammaglobulinemia, Wiscott Aldrich syndrome(WAS) , C3 complement deficiency, leukocyte adhesion defect type 3 (LAD3) and immunodeficiency centromeric instability and facial anomalies syndrome( ICF 2). Recurrent diarrhea was the most common clinical presentation (60%, n=9) followed by oral thrush (n=7, 46.6%). 5 children (33.3 %) presented with recurrent pneumonia, skin rash and abscesses. 93.3% (n=14) had family history of consanguinity and sibling deaths were seen in 53.3 % (n=8) of diagnosed cases. (Table)

Conclusions:

A high index of suspicion is key to the diagnosis of PID. Consanguinity, recurrent infections, sibling death supported by laboratory investigations are main features for diagnosing PID.
INNATE IMMUNITY

ESID7-0028

IMMUNOGLOBULIN ADMINISTERED AT REPLACEMENT DOSAGES MODULATES INNATE AND ADAPTIVE IMMUNE CELLS OF PATIENTS WITH PRIMARY ANTIBODY DEFICIENCIES

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Background:

Most of the studies analyzed the immunomodulatory effects of IVIg explored by in vitro experimental conditions or analyzed the in vivo action of immunoglobulins administered at high dosages in inflammatory-autoimmune diseases.

Methods:

Innate and immune cells from HD and from CVID and XLA patients before and after IVIg administration were analyzed for receptors expression, stimulation by E. coli, oxidative burst activity, ERK signaling.

Results:

The effects seem to be beneficial on innate cells in that dendritic cells maturate, pro-inflammatory monocytes decrease and neutrophil function is preserved. IVIg infusion have a normalizing effect only when cell phenotype and function were altered. When receptor expression was high as on monocytes. IVIg administration reduced CD11b and Siglec 9 expression, while when the receptors expression was not increased as on PMN, IVIg had no effect. IVIg did not affect the PMNs and monocytes functional ability to respond to an E. coli stimulation. The effects are less clear on adaptive immune cells. More complex and less understood is the interplay of IVIg with defective B cells of PAD patients.

Conclusions:

Intravenous immunoglobulin administered at replacement dosages modulates innate and adaptive immune cells in PAD in a different manner to what observed when high dosages are used or when their effect is analyzed by in vitro experimental conditions. The paucity of data underlies the need of more studies on patients with PAD before drawing conclusions on the in vivo mechanisms of action of IVIg based on in vitro investigations.
INNATE IMMUNITY

ESID7-0069

HOST GENETIC DETERMINANTS UNDERLYING SEVERE INFLUENZA INFECTION

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\textsuperscript{2}Aarhus University, Department of Clinical Medicine, Aarhus, Denmark
\textsuperscript{3}Aarhus University, Department of Biomedicine, Aarhus, Denmark
\textsuperscript{4}Aarhus University Hospital, Department of Clinical Immunology, Aarhus, Denmark
\textsuperscript{5}Aarhus University, Department of Molecular Biology and Genetics, Aarhus, Denmark
\textsuperscript{6}Aarhus University Hospital, Department of Anaesthesiology and Intensive Care East Section, Aarhus, Denmark

Background:

Influenza virus infection is normally self-limiting, but in rare cases patients experience severe disseminated disease, necessitating hospitalization and ventilator support. Little is known of why these patients seem to have a particularly severe course of disease. A previous study identified IRF7 deficiency in a child with severe influenza (Ciancanelli, et al. Science, 2015). In our study we investigate whether increased susceptibility to severe influenza infection might be caused by host genetics.

Methods:

We included 12 adult patients, who were hospitalized with severe Influenza A virus H1N1 infection, admitted to the intensive care unit and receiving ventilatory support during the Swine flu epidemic 2009/2010. Whole exome sequencing was performed, and genetic variants were identified by Ingenuity analysis (and other software). Patient cells together with reporter assays were used to functionally characterize the impact of the variants on patient immune responses.

Results:

We identified several variants located in genes important for host responses against influenza virus. Specifically, in a 48 year-old male two variants were identified in the cytosolic RNA sensor RIG-I. Both variants exhibited significantly decreased signaling activity (IFNb promoter reporter assay) in response to influenza virus. Patient PBMCs did not show increased susceptibility to influenza infection, but studies in patient fibroblasts are currently ongoing.

Conclusions:

We identified mutations in RIG-I, which we suggest may have conferred increased susceptibility to severe influenza in one patient. Moreover, heterozygous genetic variants in the transcription factor IRF7 and the (Interferon-inducible) enzyme RNase L were identified in two other patients, the impact of which is presently being examined.
INNATE IMMUNITY

ESID7-0101

CHRONIC GRANULOMATOUS DISEASE IN HONG KONG: REGION-SPECIFIC CHARACTERISTICS AND OUTCOMES

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²Queen Mary Hospital, Department of Paediatrics and Adolescent Medicine, Hong Kong, Hong Kong S.A.R.

Background:

Primary immunodeficiencies, such as chronic granulomatous disease (CGD) remain under-recognised in many parts of the world. CGD patients are classically susceptible to a wide variety of catalase-positive organisms, but autoimmune complications are becoming increasingly recognized. The epidemiology of implicated pathogens and complications also varies per geographical location.

Methods:

We retrospectively reviewed CGD patients in Hong Kong and report on region-specific characteristics and outcomes. We identified 19 patients with genetically-confirmed chronic granulomatous disease diagnosed at our centre (Queen Mary Hospital, Hong Kong) between 1999 and 2016.

Results:

There were 15 males (14 X-linked, 1 autosomal recessive) and 4 females (all autosomal recessive). The median age of diagnosis and follow-up were 1.5 (0-41) years and 14 (1-50) years, respectively. Eleven patients (58%) had confirmed carriers in their relatives after screening. Their mutations, complications and outcomes are summarized in Table 1. "Major infections" were defined as microbiologically-confirmed infections which required hospitalization. In contrast to western cohorts, Mycobacterium, Klebsiella, Salmonella, Aeromonas spp. were the most commonly isolated pathogens (Figure 1). Half (9 patients) had a history of microbiologically confirmed mycobacterial infection. Over a quarter (26%) of patients experienced a variety of autoimmune complications. Their clinical features and outcomes are summarized in Table 2. These patients had significantly lower rates of major infections (mean 0.2±0.4 vs. 2.2±1.9 episodes/patient-year, p=0.036).
Table 1: Mutations, complications and outcomes of 19 CGD patients from 1999-2016

<table>
<thead>
<tr>
<th>Mutations</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XL-CGD</td>
<td></td>
</tr>
<tr>
<td>- CYBB</td>
<td>14 (74%)</td>
</tr>
<tr>
<td>AR-CGD</td>
<td></td>
</tr>
<tr>
<td>- NCF1</td>
<td>3 (21%)</td>
</tr>
<tr>
<td>- CYBA</td>
<td>2 (11%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complications</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Major infections/ patient-year (mean ± SD)</td>
<td>1.5 ± 1.8</td>
</tr>
<tr>
<td>Autoimmune complications</td>
<td>5 (26%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Transplantation</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>Deceased</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>2 (10%)</td>
</tr>
</tbody>
</table>
Figure 1: Frequency of pathogens from microbiologically confirmed samples

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYCOBACTERIUM</td>
<td>15</td>
</tr>
<tr>
<td>KLEBSIELLA</td>
<td>13</td>
</tr>
<tr>
<td>SALMONELLA</td>
<td>10</td>
</tr>
<tr>
<td>AEROMONAS</td>
<td>6</td>
</tr>
<tr>
<td>BURKHOLDERIA</td>
<td>4</td>
</tr>
<tr>
<td>STREPTOCOCCUS</td>
<td>4</td>
</tr>
<tr>
<td>MORAXELLA</td>
<td>3</td>
</tr>
<tr>
<td>ESCHERICHIA</td>
<td>3</td>
</tr>
<tr>
<td>ASPERGILLUS</td>
<td>3</td>
</tr>
<tr>
<td>PSEUDOMONAS</td>
<td>3</td>
</tr>
<tr>
<td>STAPHYLOCOCCUS</td>
<td>3</td>
</tr>
<tr>
<td>ENTEROBACTER</td>
<td>3</td>
</tr>
<tr>
<td>OTHERS *</td>
<td>15</td>
</tr>
</tbody>
</table>

* Acinetobacter, Actinomyces, Bacillus, Candida, Chromobacterium, Citrobacter, Enterococcus, Neisseria and Serratia spp.
Conclusions:

We report on region-specific patterns of infections and identified significantly lower rates of major infections in patients with autoimmune complications. It is imperative for physicians to recognise these important geographic differences when tailoring specific diagnostic investigations and treatment.

<table>
<thead>
<tr>
<th>#</th>
<th>Diagnosis</th>
<th>Mutation</th>
<th>Gender</th>
<th>Age of diagnosis (years)</th>
<th>Duration of follow-up (years)</th>
<th>Major infections per year</th>
<th>Autoimmune complications</th>
<th>Treatment; Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AR-CGD</td>
<td>CYBA homozygous mutation, c.371G&gt;T, p.A124V</td>
<td>F</td>
<td>1.5</td>
<td>2.5</td>
<td>0.0</td>
<td>- Autoimmune perimyocarditis</td>
<td>Systemic steroids and sulfasalazine; in remission</td>
</tr>
<tr>
<td>2</td>
<td>AR-CGD</td>
<td>NCF1 homozygous mutation, c.75_76delGT, p.V25fsX31</td>
<td>F</td>
<td>0.0</td>
<td>6.0</td>
<td>0.0</td>
<td>- Granulomatous lung disease</td>
<td>Systemic steroids; methotrexate; in remission</td>
</tr>
<tr>
<td>3</td>
<td>XL-CGD</td>
<td>CYBB X-linked c.546_548delITTC, p.F216del</td>
<td>M</td>
<td>1.5</td>
<td>21.5</td>
<td>0.0</td>
<td>- Bronchiectasis</td>
<td>Systemic steroids and IFN-γ withheld; in remission</td>
</tr>
<tr>
<td>4</td>
<td>XL-CGD</td>
<td>CYBB X-linked c.163T&gt;A, p.F54S</td>
<td>M</td>
<td>0.5</td>
<td>27.5</td>
<td>0.1</td>
<td>- HLA-B27 positive juvenile idiopathic arthritis</td>
<td>Sulfasalazine; active disease (Failed methotrexate, leflunomide, thalidomide, intravenous immunoglobulin and steroids)</td>
</tr>
<tr>
<td>5</td>
<td>XL-CGD</td>
<td>CYBB X-linked c.7517&gt;C, p.W52R</td>
<td>M</td>
<td>38.0</td>
<td>1.0</td>
<td>0.1</td>
<td>- Granulomatous hepatitis</td>
<td>Systemic steroids; died from fulminant hepatitis</td>
</tr>
</tbody>
</table>

Table 2: Clinical features and outcome of 5 patients with autoimmune complications
INNATE IMMUNITY

ESID7-0105

NATURAL KILLER CELL SUBSETS IN PRIMARY IMMUNODEFICIENCY PATIENTS
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²Marmara University Medical Faculty, Division of Pediatric Allergy and Immunology, Istanbul, Turkey

Background:
Natural killer (NK) cells are component of innate immune system and play major role in the host-
rejection of both tumors and virally infected cells. On the other hand, it is known to have increased risk
of malignancy for the patients with ataxia telangiectasia (AT), hyper-IgM and common variable
immunodeficiency (CVID). In fact, studies on NK cells and their functions are very limited for these
diseases.

Methods:
In this study, peripheral-blood activating receptors of NK cells and cytolytic enzymes were investigated
in CVID, Hyper-IgM, AT and agammaglobulinemia patients in comparison to healthy subjects. NK cells
were evaluated using anti-CD3, -CD4, -CD16, -CD56, -NKp30, -NKp44, -NKp46, -NKG2D and HLA-
DR. For intracytoplasmic staining, anti-perforin and anti-granzyme monoclonal antibodies were used.
Analyses were performed in lymphocyte, CD3 CD16⁺ and CD3 CD56⁺ gates. mRNA expressions
of NKp30, NKp46, NKG2D. granzyme and perforin by RT-PCR were also obtained.

Results:
Our findings indicate that CD3 CD56⁺ and CD3 CD16⁺ NK cells and granzyme⁺ cells were higher in AT
patients compare to CVID patient’s. CD3 CD56⁺HLA-DR⁺ NK cells and granzyme positivity in AT
patients were also higher than healthy subjects. CD3 CD16⁺ and CD3 CD16⁺CD56dim NK cell subsets
were increased in AT patients compared to CVID patients. Moreover, AT patients express higher
mRNA of NKp46, perforin and granzyme than CVID patients.

Conclusions:
Even though activator receptors and cytolytic enzymes of NK cell are highly expressed in AT group
compared to the other primary immunodeficiency, the functions of NK cells may be improper which
should be further investigated in relation to cancer susceptibility.
INNATE IMMUNITY

ESID7-0119

FREQUENCY OF MANNOSE-BINDING LECTIN PROTEIN DEFICIENCY AMONG PRIMARY IMMUNODEFICIENCY PATIENTS

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2Research Center for Immunodeficiencies-Children's Medical Center-, Tehran University of Medical Sciences, Tehran, Iran

Background:

Primary immunodeficiencies (PIDs) are inherited disorders in which one or several components of the immune system are defective

Methods:

A total of 51 PID patients participated in this cross-sectional study. A detailed questionnaire was completed by patient’s interview for all patients to record demographic data, clinical and laboratory data. The levels of MBL were determined in the serum of patients by a sandwich enzyme-linked immunosorbent assay (ELISA) technique.

Results:

MBL deficiency was found in 29.4% of cases. In patients with MBL deficiency, the rate of meningitis, sepsis, pneumonia, and otitis media were higher than patients with normal MBL levels. Immunoglobulin replacement therapy reduced the rate of infectious complications in PID patients; however, these reductions were more apparent in patients with normal MBL levels than patients with MBL deficiency.

Conclusions:

Antibody deficient patients with a concomitant immune defect in MBL production have higher rates of recurrent infections despite receiving Immunoglobulin replacement therapy.
DOCK8 DEFICIENT PATIENTS HAVE IMPAIRED RESPONSES TO NUCLEIC ACID LIGANDS LEADING TO DECREASED TYPE I IFN PRODUCTION

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²Çukurova University, School of Medicine, Adana, Turkey

Background:

Patients with combined immunodeficiencies (CID) associated with STAT1 gain-of-function (GOF) mutations or STAT3 or DOCK8 deficiencies suffer from a distinct set of persistent infections. Since pattern recognition receptor (PRR)-mediated recognition of pathogen associated molecular patterns is paramount in establishment of protective immunity against pathogens, we sought to assess the integrity of the PRR dependent immune activation in such patients.

Methods:

For this, peripheral blood mononuclear cells (PBMCs) isolated from patients or healthy subjects were stimulated with various PRR agonists, followed by cytokine measurements and/or assessment of signaling intermediates.

Results:

Patients with STAT1 GOF mutation, STAT3 and DOCK8 deficiencies showed normal levels of pro-inflammatory cytokine (TNF-α, IL-6 and IL-1β) production in response to TLR ligands and inflammasome activators. DOCK8 deficient patients had significantly diminished type I IFN and chemokine response (IFN-α and IP-10) to multiple nucleic acid based ligands (D-type CpG, HSV-DNA, cGAMP and polyI:C), whereas patients with STAT1 GOF mutation and STAT3 deficiency showed normal levels of type I IFN production.

Conclusions:

Results indicate that DOCK8 deficiency might specifically impair the nucleic acid sensing pathways which are essential for prevention of viral infections.
INNATE IMMUNITY

ESID7-0182

DYSREGULATED INNATE LYMPHOCYTES IN PATIENTS WITH PRIMARY ANTIBODY DEFICIENCY TREATED WITH IVIG
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¹Center for Experimental Therapeutics, Department of Oncology- Faculty of Biology and Medicine- University of Lausanne, Lausanne, Switzerland
²Ludwig Cancer Research Center, Department of Fundamental Oncology- Faculty of Biology and Medicine- University of Lausanne, Lausanne, Switzerland
³Allergy Unit, Department of Rheumatology- Immunology and Allergology- University Hospital/Inselspital Bern, Bern, Switzerland
⁴Division of Immunology and Allergy- University Hospital and Medical Faculty Geneva, Department of Medical Specialties, Geneva, Switzerland

Background:

Primary antibody deficient (PAD) patients present reduced IgG and IgG subclass levels and suffer from recurrent bacterial and viral infections. Standard treatment in these patients is low-dose immunoglobulins. A number of mechanisms for the immune modulation and anti-inflammatory actions of high-dose IVIG have been described. Here, we explored the impact of low-dose IVIG on the innate and adaptive immune system in PAD with recurrent respiratory tract infections.

Methods:

The frequency and phenotype of Innate lymphoid cells (ILCs), natural killer cells (NK) and CD4 T cells were evaluated in peripheral blood mononuclear cells obtained from 9 PAD patients, longitudinally during IVIG treatment (pre-treatment, month 3, month 6, month 12).

Results:

Frequency of total ILCs was increased in pre-treatment PBMC in PAD patients, with an expansion of ILC1, mainly at the expenses of ILC2. The frequency of total NK was instead reduced. However while the proportion of CD56dimCD16+ NK population contracted in the patients, immature CD56negCD16+ NK cells were expanded. IVIG therapy partially restored frequencies and proportion of the different ILC subsets, but not of NK cells.

Conclusions:

Our data indicate differences in the frequency and phenotype of several cell subsets of the innate and adaptive immune system in treatment-naïve PAD patients compared to healthy donors. Treatment partially restores proportions of cell subsets to the level observed in healthy individuals, reflecting an immune modulation effect of low-dose IVIG therapy.
Clinical and Laboratory Features of Three Patients with the Defect of Glucose-6 Phosphatase Catalytic Subunit 3 (G6PC3)

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¹Hacettepe University, Pediatric Immunology, Ankara, Turkey

Background:

G6PC3 deficiency is a systemic disease, causes congenital neutropenia.

Methods:

We reported clinical features of three G6PC3 deficient patients.

Results:

Patient 1: A three-years-old male was admitted for recurrent oral ulcers, infections, neutropenia. He had fish-like mouth, depressed nasal bridge, hepatosplenomegaly, prominent abdominal wall veins, umbilical hernia, hypotonia. An homozygous missense mutation (p.P44L(c.131C>t) was detected.

Patient 2: A 14.5- year-old male, was referred for neutropenia, recurrent pneumonia, oral/genital aphthous lesions lasting for 6 years. He had periodontitis, gingivitis, clubbing, pectus carinatum, mild pulmonary hypertension, secundum ASD, bronchiectasia. An homozygous non-sense mutation (c. 565C>T, p. Arg189*) was detected.

Patient 3: A 6.5-year-old female, referred at 22 months with recurrent soft-tissue infections, skin abscesses, otitis media, refractory diarrhea, neutropenia. She had mid-face hypoplasia, depressed nasal bridge, marked abdominal wall veins, developmental delay, secundum ASD, mild pulmonary hypertension. An homozygous missense mutation p.Trp59Arg in exon 1 was detected.

All patients had lymphopenia and reversed CD4/8 ratioMaturation arrest in BM was present in Patient 1, but not present in patient 2 and 3.

Conclusions:

Clinical presentation in G6PC3 deficiency varies over a wide range. G6PC3 gene defect should be considered if other systemic findings associates with lymphopenia in addition to neutropenia.
INNATE IMMUNITY

ESID7-0187

NEW MUTATION OF SEVERE CONGENITAL NEUTROPENIA:
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¹children hospital -Immunology research center of Tabriz -TB and lung research center of Tabriz-
Tbariz university of medical science, pediatrics, Tabriz, Iran
²Ahvaz university of medical science, Genetic, Ahvaz, Iran
³children hospital-Tabriz university of medical science, pediatrics, Tabriz, Iran

Background:
Severe congenital neutropenia (SCN), is a rare disorder characterized by abnormally low levels of
neutrophils (less than 200/mm³) that play an essential role in fighting bacterial infections. So,
neutropenic patients susceptible to many infections, that, in some cases, may result in life-threatening
complications. Cutaneous and visceral abscesses are common findings in these cases. From the
perspective of genetic inheritance, most common variants include SCN3 which has an autosomal
recessive inheritance pattern, and SCN1 which is autosomal dominant.

Methods:
Hereby, a new case of SCN syndrome with multiple cutaneous abscesses will be reported.

Results:
Case presentation:
A 16 month old boy with multiple admissions due to cervical abscesses and recurrent fevers,
pneumonia, and gastroenteritis was again admitted to the immunology ward. Although his problems
began after 2 months of age, but diagnosis of severe congenital neutropenia was suspected in his 10
months of age by laboratory analysis with absolute neutrophils less than 200/mm³. Treatment with
GCSF and for Bone marrow transplantation, genetic analysis was done.

Conclusions:
Conclusion:
Whole exome sequencing revealed a de novo heterozygous mutation in ELANE gene of the patient
(NM_001972: exon3:c.G253A) that leads to amino acid change p.G85R which could be deleterious
based on most predictors and has not previously reported.
INNATE IMMUNITY

ESID7-0199

IMMUNE DEFICIENCY HIDING IN THE BOWELS: A PAEDIATRIC CASE

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¹Ninewells Hospital, Paediatrics, Dundee, United Kingdom
²Ninewells Hospital, Immunology, Dundee, United Kingdom
³Great Ormond Street Hospital, Immunology, London, United Kingdom

Background:

A ten-year-old girl presented via the paediatric immunology service with recurrent sinusitis, persistent respiratory infections and lethargy. Initial investigation highlighted elevated IgG and undetectable IgA levels.

Within four weeks of presentation she developed intermittent abdominal pain and vomiting. Her IgG anti-endomysial antibody test was positive and she was referred to paediatric gastroenterology for investigation of possible Coeliac disease. Upper and lower gastrointestinal endoscopy revealed mucosal inflammation with epithelioid grauloma within both oesophagus and colon.

Methods:

Incomplete clinical correlation of endoscopy findings with intermittent, self-resolving gastrointestinal symptoms and concern regarding immune status prompted further investigation. A neutrophil oxidative burst (DHR) test was requested and found to be abnormal with a pattern suggestive of deficiency in p47phox. This was confirmed by protein analysis and a diagnosis of chronic granulomatous disease (CGD) was made.

Results:

CGD is a rare immunodeficiency (prevalence 1:250,000) caused by defects in protein components within the NADPH oxidase complex. 70% of CGD cases are the result of X linked inheritance conferring deficiency in gp91phox protein and result in serious bacterial and fungal infections. P47phox deficiency is of autosomal recessive inheritance and represents 25% of CGD patients with a milder phenotype.

Conclusions:

Whilst GI symptoms are common in CGD, literature suggests only a small number of patients initially present with these symptoms. As this case highlights, a high index of suspicion and clinical vigilance are required in cases of possible IBD where even mild infectious symptoms are present, as use of immunosuppressant medication in these patients can result in severe infectious consequences.
INNATE IMMUNITY

ESID7-0243

ISOLATED ASPERGILLUS NIDULANS CEREBELLAR ABSCESS IN A CHILD WITH ABNORMAL CARD9/DECTIN 1 SIGNALLING

G. Giardino1, A. Irwin2, F. Ladomenou3, K. Gilmour3, A. Bamford2, A. Worth3

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2Great Ormond Street Hospital for Children- NHS Trust, Department of Infectious Disease, London, United Kingdom
3Great Ormond Street Hospital for Children- NHS Trust, Department of Immunology, London, United Kingdom

Background:

Aspergillus nidulans rarely causes infections in human, almost exclusively in chronic granulomatous disease (CGD).

Methods:

We report the case of a previously fit 15-month old male presenting with diarrhea, weight loss and subsequent progressive deterioration with torticollis, right facial weakness and truncal hypotonia with limb hypertonia.

Results:

Brain scans revealed secondary obstructive hydrocephalus and a region of signal abnormality in the left cerebellar hemisphere, suggestive of inflammatory/infective process. Cerebellar biopsy showed patchy inflammatory infiltrate predominantly of giant cells and fungal hyphae, suggesting fungal infection associated with granulomatous inflammation. PCR on the cerebrospinal fluid revealed A. nidulans. Clinical and radiological parameters deteriorated despite treatment with amphotericin, voriconazole and micafungin. WBC counts and CRP remained low despite invasive infection. Immunological work-up showed mild lymphopenia, normal DHR, neutrophil phagocytosis, immunoglobulins, IgE levels, specific antibody response to vaccination, standard lymphocyte subpopulations, T-memory compartment and proliferative response to PHA and CD3. Impaired proliferative response to candida was detected. Defective production of proinflammatory cytokines (IL1beta and TNFalpha), compared to the control, was detected after beta-glucan stimulation, suggesting impaired Dectin1/CARD-9 pathway. Cytokine production was normal after stimulation with TLR ligands and Zymosan, suggesting a normal function of Dectin1/CARD9 independent innate immune response. CARD-9 sequencing was normal and targeted next generation sequencing panel, containing genes relevant for immunodeficiencies with susceptibility to fungal infections, is still ongoing.

Conclusions:

In conclusion, we report a case of A. nidulans infection in absence of neutropenia, immunosuppression and CGD. Considering the recent evidences, CARD-9/Dectin1 defect should be considered in the differential diagnosis in invasive fungal infections.
INNATE IMMUNITY

ESID7-0244

RECURRENT HSV1 ORAL INFECTIONS AND PULMONARY BACTERIAL INFECTIONS DUE TO NF-kappa-B-ESSENTIAL MODULATOR (NEMO)-DEFICIENCY IKBKG EXON 3 VARIANT c.337G>A; p.Asp113Asn

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²Charité University Medicine, Department of Pediatric Pneumology- Immunology and Intensive Care Medicine, Berlin, Germany
³University Hospital Ulm, Institute for Transfusion Medicine, Ulm, Germany

Background:

NEMO-deficiency can present without ectodermal dysplasia. Furthermore, in distinct IKBKG mutations, Toll-like receptor (TLR) signalling is unimpaired, thus making diagnosis difficult.

Methods:

We describe a patient with recurrent bacterial airway infections and oral HSV1 infections. The boy showed no response to pneumococcal polysaccharide vaccination as the only significant immunologic abnormality. IgG, IgA, IgM and IgG subclasses were normal as well as antibodies to Tetanus-Toxoid. Canonical TLR2/6, TLR4, TLR7/8 and TNFR-dependent signalling seemed unimpaired.

Results:

An IKBKG Exon 3 mutation (c.337G>A; p.Asp113Asn) was identified. This had been reported previously in patients with NEMO-deficiency without ectodermal dysplasia (Orange et al, J Allergy Clin Immunol:2004). No further bacterial infections occurred after initiation of immunoglobulin replacement. Frequent oral HSV1-lesions, however, persisted. A 20- year old maternal cousin had the same mutation, but no history of bacterial infections or HSV1-lesions. Summing up for this IKBKG mutation there seems to be in 3 different individuals differing from severe sick to healthy. Furthermore in this case, no specific antibody deficiency was detected, confirming a strict lack of phenotype-genotype correlation in IKBKG mutations, that has not been reported previously.

Conclusions:

IKBKG sequencing should be performed in boys with polysaccharide specific antibody-deficiency and herpes virus infections independent of signs of ectodermal dysplasia or unimpaired TLR-signalling.
INNATE IMMUNITY

ESID7-0254

UNUSUAL PRESENTATION OF DEFICIENCY OF THE 8th COMPONENT OF COMPLEMENT: INVASIVE MENINGOCOCCAL DISEASE AND FITZ-HUGH-CURTIS SYNDROME

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Background:

Terminal complement deficiencies are rare primary immunodeficiencies that markedly increase susceptibility to invasive meningococcal disease. The European prevalence of congenital complement deficiencies (excluding MBL deficiency) has been calculated at 0.03% of the population. C8 deficiency is the reported cause of 8% of complement deficiencies in Europe.

Methods:

The functional activity of the classical and alternative complement pathways was measured by haemolytic assay in a 25 year old female patient. Mannan-binding lectin was measured by ELISA. Serum complement factors were measured by radial immunodiffusion. DNA sequencing of a panel of genes known to cause immunodeficiency was performed.

Results:

The patient was admitted with a history of fever, lethargy and right sided abdominal pain. Laboratory testing revealed elevated CRP and leukocytosis. Peripheral blood cultures were positive for Neisseria meningitidis that was subsequently identified as serotype E. CT imaging demonstrated free pelvic fluid and peri-hepatitis suggestive of Fitz-Hugh-Curtis syndrome.

Laboratory testing showed absent alternative pathway and reduced classical pathway haemolytic assays. C8 was within reference range in a serum sample (table 1). Genetic analysis identified a homozygous p.Arg428Ter mutation in the C8B gene on chromosome 1p32.2 (OMIM 120960, figure 1). The patient received immunisation against meningococcus serogroups A, B, C, W and Y. Family testing is underway (family tree shown in figure 2).
Table 1. Results of complement haemolytic assays and individual complement components

<table>
<thead>
<tr>
<th></th>
<th>Result</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical pathway</td>
<td>11 U/ml</td>
<td>23-46U/ml</td>
</tr>
<tr>
<td>Alternative pathway</td>
<td>&lt;31%</td>
<td>66-125%</td>
</tr>
<tr>
<td>C3</td>
<td>1.11g/L</td>
<td>0.75-1.65g/L</td>
</tr>
<tr>
<td>C4</td>
<td>0.22g/L</td>
<td>0.14-0.54g/L</td>
</tr>
<tr>
<td>C5</td>
<td>175mg/L</td>
<td>70-120mg/L</td>
</tr>
<tr>
<td>C6</td>
<td>&gt;120mg/L</td>
<td>40-80mg/L</td>
</tr>
<tr>
<td>C7</td>
<td>72mg/L</td>
<td>50-80mg/L</td>
</tr>
<tr>
<td>C8</td>
<td>55mg/L</td>
<td>40-280mg/L</td>
</tr>
<tr>
<td>C9</td>
<td>367mg/L</td>
<td>50-250mg/L</td>
</tr>
</tbody>
</table>
Figure 1. Sequencing chromatogram showing homozygous p.Arg428Ter mutation in C8B gene
Conclusions:

We speculate that pelvic inflammatory disease due to an unusual serogroup of *Neisseria meningitidis* was the presenting feature of a congenital C8B deficiency.
INNATE IMMUNITY

ESID7-0290

A CASE OF NON-PULMONARY SARCOIDOSIS DUE TO NOVEL HYMORPHIC MISSENSE MUTATION OF NCF2
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Background:
Sarcoidosis is a systemic inflammatory disease characterized by non-caseating epithelioid cell granulomas. The etiology of the disease remains unknown.

Methods:
A 15-year-old boy was referred to our hospital because of sustained cough and fever with hepatosplenomegaly. Laboratory tests showed pancytopenia, renal dysfunction and elevated levels of serum lysozyme, sIL-2R and ACE. PET-CT image disclosed increased FDG uptakes of liver, spleen and kidneys. The renal biopsy revealed interstitial nephritis with non-caseating epithelioid cell granulomas. Therefore, the patient was diagnosed as having non-pulmonary sarcoidosis. He was treated with corticosteroids and mycophenolate mofetil (MMF), and his pancytopenia and renal dysfunction improved. After 4 months of immunosuppressive therapy, he suffered from recurrent invasive pulmonary aspergillosis. MMF was switched to methotrexate (MTX), but 5 months later, he developed perianal and inguinal duct abscess and osteomyelitis of the right upper arm. After 4 months, invasive pulmonary aspergillosis was relapsed.

Results:
Whole exome sequencing (WES) revealed novel homozygous missense mutation (p.Tyr394Asp) of NCF2 in the patient and his brother who had a history of perianal abscess. Reactive oxygen species (ROS) production of patient's neutrophils was partially impaired by DHR-123 and luminol assay. Residual ROS activity might be associated with their clinical course.

Conclusions:
We identified novel homozygous hypomorphic missense mutation of NCF2 in a case of non-pulmonary sarcoidosis by WES analysis. Hypomorphic mutation of chronic granulomatous disease-related genes could cause sarcoidosis. Molecular diagnosis might be important for patients with sarcoidosis because immunosuppressive therapy would be harmful for such patients.
INNATE IMMUNITY

ESID7-0293

DISSEMINATED BCG INFECTION ACCOMPANIED BY REDUCED T CELL EXPRESSION OF CD5
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Background:

Disseminated BCG infection is a rare and potentially fatal condition associated with different PIDs including a deficient IL-12/INF-γ axis. T cell expressed CD5 acts inhibitory on TCR mediated signalling. In T cells, the CD5 locus is under expressional control by GATA3.

Methods:

We performed flow-cytometric analysis of the IL-12/IFN-γ axis in an elderly man with severe (liver and renal granulomas and therapy induced DRESS syndrome) disseminated BCG infection secondary to BCG instillation in the bladder. As a child, our patient had received several BCG vaccinations without ever turning Mantoux positive.

Results:

A lymphocyte marker study revealed general T cell activation (34% HLA-DR positivity among CD3+ T lymphocytes). The patient had markedly fewer CD5 positive B and non-B lymphocytes (the latter: 45.7% vs. control: 81.7%) and was singled out by a pronounced population of non-B lymphocytes displaying CD5 dim expression. His monocytes showed normal expression of IFN-γR1 and normal pSTAT1 phosphorylation in response to INF-γ (also in the presence of his own serum). Compared with a control, the patient's monocytes responded with clearly reduced pSTAT4 phosphorylation in response to IL-12.

Conclusions:

We report a case of severe disseminated BCG infection likely due to deficient IL-12 signalling. The case patient also displayed reduced frequencies of CD5 positive B and T lymphocytes and habored a population of CD5 dim T lymphocytes not found in the controls. As reduced IL-12 signalling favors increased GATA3 expression, the latter being able to suppress CD5 expression, his aberrant CD5 T lymphocyte expression profile might be secondary to deficient IL-12 signalling.
INNATE IMMUNITY

ESID7-0345

A PATIENT WITH A GATA2 MUTATION: CASE REPORT
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IZMIR, Turkey
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Background:

The GATA2 gene is a member of the GATA transcription factor family that plays critical roles in haematopoiesis. The most common clinical manifestations are; MDS, severe viral and nontuberculous mycobacteria infections, pulmonary alveolar proteinosis, solid organ tumors, lymphoedema, venous thrombosis, endocarditis, hearingloss, hypothyroidism.

Methods:

Patient with pustular psoriasis was referred to our clinic due to frequent and serious infections. In 2011 fever, malasia, loss of 30 kg/y, hepatosplenomegaly, LAPs and pancytopenia had been reported. He had been splenectomized due to hypersplenism and the biopsy revealed granulomas with caseification necrosis. Mycobacterium tuberculosis grew in the bone marrow (BM) specimen. He was diagnosed as miliary tuberculosis. Multidrug therapy had started. The patient had been hospitalized by reason of pneumonia between 2013 and 2016. In 2015 he had been diagnosed as sarcoidosis based on typical HRCT findings. The necrotizing granulomatous lymphadenitis was detected on lymph node specimen. The skin Bx revealed molluscum contagiosum. He developed herpes virüs infection on his lip.

Results:

On admission a persistent lymphopenia accompanied by monocytopenia was noticed (Table). Immunoglobulins were normal although the B cells were diminished by flow-cytometry. A BM biopsy showed hypocellularity and dysmegakaryopoiesis. A heterozygous mutation in GATA2 (c.1186C> T missense) was established.

Conclusions:

Here we report a case of GATA2 mutation previously diagnosed as sarcoidosis/tuberculosis. Increased nontuberculous mycobacterial infection has been reported in the literature, but differently, our case presented with Mycobacterium tuberculosis. Bone marrow transplantation is planned for the patient and the preparations are ongoing.
INNATE IMMUNITY

ESID7-0357

ELEVATED EXPRESSION OF MIR-378, MIR-518F, MIR-520D, AND MIR-1205 IN CHILDREN WITH SEVERE AND RECURRENT INFECTION WITH HERPES SIMPLEX VIRUS

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²Jagiellonian University Medical College, Department of Internal Medicine, Krakow, Poland

Background:

Severe and/or recurrent infections with Herpes simplex virus type I (HSV-1) are observed in a large group of patients treated in immunology facilities, yet the pathomechanism of these infections remain largely unknown. Recent findings indicate an important role of regulation of gene expression by microRNA (miRNA) in development and function of NK cells. That is why, the aim of the study was to determine miRNA expression profile in NK cells in children suffering from severe and/or recurrent infections with HSV-1.

Methods:

NK cells were isolated from peripheral blood mononuclear cells using magnetic sorting, while miRNA expression was determined using TaqMan real-time PCR method. The study included 40 patients and 40 age-matched control subjects.

Results:

As a result, we detected four miRNA: miR-378, miR-518F, miR-520D, and miR-1205, which expression was significantly elevated in a group of patients, in comparison to healthy control subjects. Among these miRNA, only miR-378 was previously described to influence NK cell function, namely to regulate perforin and granzyme B expression. MiR-518F might be related to regulation of TRAIL expression in NK cells, while the role of miR-520D, and miR-1205 in NK cells is unknown. The role of all four miRNA in regulation of antiviral response of NK cells is currently being evaluated and should be updated in the poster.

Conclusions:

At this stage, it is not clear whether the observed alteration of the expression of miRNA is a cause of observed disturbance of antiviral response or a result of prolonged viral infection.
A NOVEL POINT MUTATION IN COMPLEMENT FACTOR D RESULTING IN LOSS OF ALTERNATIVE COMPLEMENT PATHWAY ACTIVITY

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⁴Royal Free London NHS Foundation Trust, University College London Institute of Immunity and Transplantation, London, United Kingdom

Background:

Complement factor D (CFD) is a serine protease involved in amplifying the alternative pathway (AP) of complement. Previously described mutations in CFD all lead to absence of serum CFD and severe immunodeficiency. We identified a novel single-nucleotide polymorphism of CFD in a 19-year old presenting with meningococcal septicaemia lacking other immune defects. The resulting transcript, R201P, produces undetectable AP activity despite normal serum titres of CFD.

Methods:

Patient serum was obtained and tested for AP haemolytic activity (AP50). Serum CFD titres were measured by ELISA. Reconstitution assays were performed using purified CFD, Factor B and properdin (CompTech). Recombinant CFD was generated via mammalian cell culture. Recombinant CFD activity was assayed by incubation with C3b and Factor B, followed by gel electrophoresis of cleavage products. Surface plasmon resonance (SPR) was used to assess binding of recombinant CFD to C3bB.

Results:

Sanger sequencing of the CFD gene revealed a G to C mutation, resulting in R201P CFD. Patient serum demonstrated negligible AP50 which could be reconstituted only by addition of wild-type CFD. At physiological concentrations (0.04μM), R201P CFD showed significant reduction (>80%) in cleavage of Factor B relative to wild-type. Furthermore, SPR indicated R201P CFD was unable to bind to C3bB.

Conclusions:

This is the first description of a mutation that abrogates CFD function by preventing binding to its natural substrate, C3bB. Residue 201 plays a critical role in enabling binding of CFD and ultimately, AP function. This finding has potential implications for the therapy of diseases of AP over-activation such as age-related macular degeneration.
INNATE IMMUNITY

ESID7-0393

GM-CSF DOES NOT IMPROVE NEUTROPHIL COUNT IN A YOUNG PREGNANT WOMAN WITH HOMOZYGOUS JAGN1 DEFICIENCY

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Background:

Biallelic mutations in JAGN1 have been identified as a cause of severe congenital neutropenia (SCN). In vivo experiments with the respective mouse model and in vitro experiments with human JAGN1 deficient bone marrow granulocytes suggest that GM-CSF restores a qualitative defect. The safety profile of G-CSF in pregnant SCN patients with other genetic defects is acceptable.

Methods:

GM-CSF treatment results in a patient with biallelic JAGN1 mutation.

Results:

A girl with SCN descending from consanguineous parents suffered from life-threatening infections in childhood and adolescence. G-CSF doses of up to 50µg/kg/d only resulted in a negligible increase of neutrophil count. Her bone marrow (under G-CSF administration) showed severely reduced granulopoiesis, however all stages of maturity were present; no malignancy or dysplasia detectable. The oxidative burst test was normal. When a pregnancy was detected (at neutrophil counts of 0.1–0.3/nL) at 17 years, GM-CSF (Sargramostim®) was administered at week 12 after a thorough risk-benefit analysis, up to a dose of 320 µg/m²/d for 7 days. No increase in neutrophil count was noted. Later, mutation analyses revealed a homozygous missense mutation in JAGN1 (c.63G>T, p.Glu21Asp), previously described as pathogenic. An acquired heterozygous stop-mutation in the intracytoplasmatic region of the G-CSF-receptor gene (p.Q741X) was identified. After a pregnancy with manageable infections, a healthy boy with normal neutrophil count was born. The patient is currently considering stem cell transplantation from an HLA-identical sibling donor (heterozygous carrier).

Conclusions:

The role of GM-CSF in patients with SCN based on JAGN1 mutations remains to be determined.
INNATE IMMUNITY

ESID7-0400

NOVEL STAT-1 GAIN-OF-FUNCTION MUTATION ASSOCIATED WITH CHRONIC MUCOCUTANEOUS CANDIDIASIS

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²Gloucestershire Royal Hospital, General Paediatrics, Gloucester, United Kingdom
³Royal Free Hospital, Department of Immunology, London, United Kingdom
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Background:

A thriving 4 month old baby boy was admitted with low saturations and respiratory distress; he required intubation and ventilation. His chest radiograph showed diffuse interstitial pneumonitis and BAL grew *Pneumocystis jirovecii*. He had a history of persistent oral candidiasis.

Methods:

Extensive virology tests including HIV were negative. Initial investigations showed marginally raised IgM and low IgG with normal lymphocyte subsets and proliferation. His CD40 ligand and CD40 expression were normal. He made a good recovery; over the following 12 months he continued to thrive, his immunoglobulin results normalised (other than absent IgA) and he had normal vaccine responses. However, he has persistent issues with oral and nappy candidiasis when prophylactic fluconazole is discontinued.

Results:

The most common cause of congenital chronic mucocutaneous candidiasis disease (CMCD) are signal transducer and activator of transcription 1 (STAT1) gain-of-function mutations. These result in impaired STAT1 dephosphorylation and diminished IL-17-producing T-cells. The majority of mutations affect the coiled-coil domain of signal transducer and activators of transcription 1. Gene sequencing for this boy revealed a novel heterozygous variant in DNA-binding domain of STAT1 p.Q330K, the adjacent residue to a described pathogenic mutation. Mutations in the DNA-binding domain (DBD) are less frequent but have been described.

Conclusions:

Functional assays were performed to test the likely significance of this mutation which has shown a significant increase in phosphoSTAT1 post stimulation with IFN-alpha as compared to the healthy control individual.
RARE COMPOUND HETEROZYGOTE MUTATIONS IN COMPLEMENT COMPONENT 4 (C4) LEADING TO PRIMARY C4 DEFICIENCY

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Background:

Homozygote deficiency of C4A is associated with systemic lupus erythematosus and with Type I diabetes mellitus; homozygote deficiency of C4B is associated with susceptibility to bacterial meningitis.

Methods:

Case report: A 30 years old African American female with recurrent pruritic acute inflammatory skin lesions of two months duration, resolving over a week time with itchy scars.

Associated morbidities: Type I diabetes, eczema, seborrheic dermatitis, chronic dry mouth, idiopathic chronic constipation, asthma, recurrent bronchitis, allergic rhinitis/conjunctivitis, and recurrent severe sinus infections with a history of sinus surgery due to “fungus in sinus”. Known adverse reactions: Tongue swelling/general pruritis to Pistachio nuts, and throat swelling to sulfa drugs.

Family History: Mother with Type I diabetes mellitus and Both parents with “bad allergies”.

Physical Examination was remarkable for few scar skin lesions over upper extremities and positive dermatographism.

Results:

Lab Results: Remarkable for positive IgE mediated skin testing to multiple pollen and perennial allergens. Lung functions: mild restrictive lung disease with reactive airway component, elevated total serum IgE level of 120 IU/mL, elevated sedimentation rate of 41 mm/1st Hour and deficient serum complement C4 (8) and CH50 (26), evaluated several times. Genomic Southern blot analyses and C4 protein allotyping revealed a rare compound heterozygote with deficiency of C4A on one haplotype and deficiency of C4B on another haplotype. The patient has only two copies of C4 genes.

Plaquenil 200mg bid was associated with a remarkable improvement of her skin lesions.

Conclusions:

Review of skin lesions was compatible with cutaneous lupus erythematosus (conformational biopsy with immuno-histochemical staining is pending).
INNATE IMMUNITY

ESID7-0426

ECTODERMAL DYSPLASIAS – NEED FOR EARLY RECOGNITION AND TARGETED MANAGEMENT
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Background:

Ectodermal Dysplasias (EDs) are genetic disorders affecting the development or function of tissues that originate from embryonic ectoderm such as hair, sweat glands, nails and teeth. One subtype is associated with Immunodeficiency (ED-ID). Individuals affected by ED face a lifetime of special needs and hence early diagnosis would help with adequately meeting such needs.

Methods:

Clinical and Investigative findings were obtained from two ED patients.

Results:

Case 1: A 23 year old male has had recurrent respiratory tract infections since childhood. He has partial anodontia with narrowed upper arch and class III skeletal malocclusion (Figure 1). There is extreme heat intolerance due to reduced sweating since childhood. There was dry skin with thin, sparse body and scalp hair and specific antibody deficiency. No other family members are affected. Results of molecular studies are awaited.

Case 2: A 13 year old male gets recurrent respiratory tract infections over several years and had a tonsillectomy at the age of three years. He has extreme heat intolerance and has markedly reduced sweating since infancy. He has conical upper incisors (Figure 2) and specific antibody deficiency. No other family members have similar symptoms. Molecular studies are in progress. Conclusions:

Some forms of Ectodermal dysplasia may be associated with immunodeficiency disorders. Early diagnosis and appropriate management should help reduce complications such as hyperthermia and irreversible brain damage especially in infancy or life-threatening respiratory and other invasive infections. Awareness of this group of disorders should help clinicians make a timely diagnosis.
INNATE IMMUNITY

ESID7-0450

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS PRESENTATION OF CHRONIC GRANULOMATOUS DISEASE
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Background:

Chronic Granulomatous Disease (CGD) is a primary immunodeficiency caused by a defect in the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Hemophagocytic lymphohistiocytosis (HLH) has been reported in patients with CGD. However, HLH as the presenting manifestation of CGD is rare.

Methods:

We report a 3-month-old male who presented with fevers and abdominal distension and a 16-month-old male who presented with fever and cough. Both met diagnostic criteria for HLH and CGD.

Results:

Cases: C.M. presented at 3 months of age with a 4-day history of fever and abdominal distension. His labs were significant for a prolonged PT, PTT, elevated INR, low fibrinogen, ferritinemia, and elevated triglycerides. He had a maternal cousin and great uncle with CGD. His bone marrow biopsy was unrevealing. He met criteria for HLH and was started on chemotherapy with clinical improvement. L.D. presented at 16 months of age with a 5-day history of fever, diarrhea, and cough. He developed a metabolic acidosis with elevated liver enzymes and INR. He underwent a bone marrow biopsy, which exhibited hemophagocytes. Despite the initiation of decadron, etoposide, and IVIG, he clinically deteriorated and expired.

Conclusions:

These two cases illustrate the importance of considering an underlying immunodeficiency in the differential diagnosis when evaluations patients presenting with HLH.
CLERECUZIO SYNDROME: CASE DESCRIPTION
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Background:
Clericuzio syndrome, poikiloderma with neutropenia (PN) is a rare congenital disease. Autosomal recessive mutations in C16ORF57 (USB1) gene have been recently identified as a genetic cause of PN.

Methods:
We describe a four years old boy who was diagnosed with PN based on poikiloderma since the first year of life, pachyonychia and severe neutropenia. He also had facial dysmorphism, bilateral deafness, recurrent infections, lymphadenopathy, anemia and intermittent thrombocytopenia.

Results:
Bone marrow was normal except for single-shot increased blasts (up to 11%). In opposite to majority of patients reported previously his serum immunoglobulins were normal. An unusual feature was molluscum contagiosum since two years of age. Treatment with G-CSF 5 mcg/kg/daily was successful in resolving neutropenia.

Conclusions:
Since molluscum contagiosum usually reflects T cell defects, based on our case immunological defects in PN require further investigation.
INNATE IMMUNITY

ESID7-0469

A NOVEL MUTATION IN A PATIENT WITH X-LINKED CHRONIC GRANULOMATOUS DISEASE: A CASE REPORT
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²Hospital da Criança José Alencar, Immunology, Brasília, Brazil

Background:

INTRODUCTION

Chronic granulomatous disease (CGD) is a primary immunodeficiency disease (PID) of phagocytes caused by mutations in components of nicotinamide dinucleotide phosphate (NADPH) oxidase resulting in absent or reduced oxidative burst.

Methods:

CASE REPORT

We report a case of a 20-years old male patient, born from non-consanguineous parents and no family history of recurrent infections. He had been vaccinated as scheduled. He had a history of several pneumonias, hyperplasia of lymph nodes and sporadic colitis during childhood. At 17 years old, he was hospitalized with a diagnosis of pneumonia and was treated with antibiotics. Additionally, he had progressive infiltration in the thoracic wall and fifth costal arch.

Results:

Immunological tests results, including lymphocytes subset and complement levels, were all normal. However, the dihydrorhodamine (DHR) assay showed an altered response (MFI: 6347) of stimulated granulocytes in comparison to a healthy control (MFI: 191745). The quimioluminescence assay was performed and the results confirmed the impaired production of reactive oxygen species. The expression of the cytochrome b558 and the p22-phox protein were evaluated with different fluorescent antibodies and the results support the characteristic X-CGD pattern. Genomic DNA was isolated from blood with EDTA. All the 13 exons of CYBB gene were amplified by polymerase chain reaction (PCR) and sequenced by Sanger method.

Conclusions:

The genetic analysis identified a novel missense mutation c.951T>A: p.V295E in the exon 8 of the CYBB gene. This mutation resulted in the absence of the NADPH oxidase activity and caused a low level of gp91-phox protein expression in the cytochrome b558 complex.
A NOVEL HOMOZYGOUS SPLICE-SITE MUTATION IN INTRON 1 OF THE HAX1 GENE CAUSES SEVERE CONGENITAL NEUTROPENIA

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Background:

Severe congenital neutropenia (SCN) represents a heterogeneous group of immunodeficiencies associated with low neutrophil count, which can result in life threatening bacterial infections. Current treatment options are administration of granulocyte colony stimulating factor (G-CSF) or allogeneic hematopoietic stem cell transplantation. Among others, mutations in HCLS1 associated protein X-1 (HAX1) are causative for SCN3 (Kostmann disease). Transcript variants resulting in different HAX1 isoforms have been described. Only mutations affecting both isoform A and isoform B are associated with a neurological phenotype.

Methods:

A 20-month old girl born to consanguineous parents of Turkish ancestry presented with a history of severe deep bacterial skin infections, several episodes of impetigo, and chronic purulent otitis. Neurological development and family history were inconspicuous. A bone marrow smear displayed maturation arrest at the promyelocyte stage. Given the consanguineous background in the girl’s family, autosomal recessive inheritance was considered most likely and HAX1 was sequenced.

Results:

A new homozygous HAX1 mutation (c.53+1G>A) in intron 1 altering the donor splice site signal was identified. As most of the reported mutations affect exon 2-7, further evaluation of how isoform A and isoform B are affected by the new mutation will be presented. The patient is responsive to G-CSF treatment.

Conclusions:

A novel homozygous splice-site mutation in HAX1 causes Kostmann disease in a consanguineous Turkish family
INNATE IMMUNITY

ESID7-0521

THE EFFICACY OF LONG-TERM VORICONAZOLE THERAPY ON SIX CHILDREN WITH CHRONIC GRANULOMATOUS DISEASE AND INVASIVE FUNGAL DISEASE; A RETROSPECTIVE OBSERVATION

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2Ondokuz Mayıs University Faculty of Medicine, Pediatric Infectious Diseases, Samsun, Turkey
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Background:

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency. It is characterized by life-threatening and/or recurrent infections caused by bacteria and fungi. Invasive fungal diseases are the most common causes of death among CGD patients. There is no information about long-term voriconazole therapy in children with CGD and invasive fungal disease (IFD). We aim to report the efficacy of long-term voriconazole therapy in these patients.

Methods:

This retrospective study includes six CGD patients. All data were recorded from their hospital registries. CGD was diagnosed based on the results of a dihydrorhodamine-1,2,3 assay and genetic analysis. Long-term voriconazole therapy was started after clinical and/or laboratory diagnosis of IFD in CGD patients. Patients who had used voriconazole for six months or more were included in this study.

Results:

All six patients were male. Two had an X-linked recessive subtype of CGD (X-CGD), and the others had autosomal recessive subtypes of CGD. The mean age at which CGD was diagnosed was 7.6 ± 4.1 years. The mean age at first IFD was 9.5 ± 5.3 years. The mean duration of voriconazole therapy was 21.6 ± 17.5 months. Aspergillus spp. was showed in three patients. Five patients did not suffer from IFD second time. The sixth patient, who has X-CGD, suffered from multiple fungal osteomyelitis and recurrent IFD. None of the six patients experienced any side effects of long-term voriconazole therapy.

Conclusions:

Long-term voriconazole therapy may be successful for most children with CGD and IFD.
BACKGROUND:

IPEX (Immunodysregulation Polyendocrinopathy Enteropathy X-linked) syndrome is caused by mutations in the gene encoding the transcription factor FOXP3, which leads to the loss of function of regulatory T (Treg) cells. The absence of functional Treg leads to the development of autoimmune manifestations in the first months or years of life.

METHODS:

We have launched a gene therapy approach designed to selectively induce a Treg program in CD4+ T cells by expressing FOXP3. We took advantage of the availability of IPEX patients samples and of Scurfy mice, which present a spontaneous mutation in the Foxp3 gene and develop a disease very close to human pathology.

RESULTS:

We demonstrated that FOXP3 gene transfer into murine CD4+ T cells enables the generation of potent regulatory T cells, as shown by their in vitro functional suppression ability. Likewise, CD4+ T cells from IPEX patients acquired in vitro suppressive function upon transduction with a lentivirus encoding the FOXP3 gene. Moreover comparison of the transcriptional profile of these regulatory CD4^{IPEX/FOXP3} cells to natural Treg by RNA-seq analysis demonstrated a similar repression of cytokine transcripts (IL4/5/13, CD40L) and IL7R, induction of IL1R2 and activation of Treg genes (IL2RA, IKZF2, CTLA4).

CONCLUSIONS:

Therefore, both murine and human studies show that the introduction of a functional copy of the FOXP3 gene into Foxp3-deficient CD4+ T cells may be sufficient to restore immune tolerance. In vivo analysis of the ability of CD4-FOXP3 to prevent Scurfy disease by adoptive transfer is currently under evaluation.
MORPHOLINO ANTISENSE OLIGONUCLEOTIDE-BASED CORRECTION OF HYPOMORPHIC ZETA-CHAIN-ASSOCIATED PROTEIN KINASE 70 (ZAP70) MUTATION IN AN ADULT WITH COMBINED IMMUNODEFICIENCY

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Background:

The ζ-associated protein of 70 kDa (ZAP-70) is required for T cell activation. ZAP-70 deficiency in humans has typically resulted from null mutations, leading to severe combined immunodeficiency in early childhood, requiring stem cell transplantation for survival. "Leaky" variants are less well characterized but may require distinct approaches to restore immune function. We aimed to dissect the genetic mechanism of "leaky" ZAP-70 deficiency in an adult and to pursue mutation-specific therapy to restore immune function.

Methods:

Whole-exome and Sanger sequencing, in silico analysis, and RT-PCR were used to identify the mutation. Evaluation of primary and transfected cells by immunoblotting, by induction of target genes by qPCR, and by cellular proliferation studies were used to determine the effect of the mutation on protein expression and function, as well as the effect of an antisense morpholino oligonucleotide targeting the specific mutation.

Results:

The c.1272C>T, while synonymous, created a de novo, intra-exonic donor splice site that predominated over wild-type donor splice site, resulting in a truncated null isoform of ZAP-70. In the homozygous state, little wild-type protein was expressed. Morphant silencing of this mutation enhanced wild-type ZAP-70 protein expression, molecular signaling from the T cell receptor, up-regulation of key cytokine genes, and ex vivo proliferation of patient's primary T cells.

Conclusions:

These findings provide proof-of-concept that morpholino-based silencing of immunodeficiency-causing mutations can improve molecular and cellular function. This approach potentially expands the therapeutic armamentarium against genetic diseases of immunity.
Background:

Previously, we have discovered homozygous nonsense mutations in the zinc finger transcription factor ZNF341 to lead to an autosomal-recessive disorder resembling the STAT3-associated autosomal-dominant Hyper-IgE syndrome (HIES): Patients of four consanguineous families with homozygous STOP codons presented with the typical clinical symptoms of HIES, consisting of chronic eczema, elevated IgE levels, recurrent skin abscesses and respiratory tract infections. Moreover, they had dental and facial abnormalities and reduced TH17 cells, but no viral infections and wild-type STAT3, Tyk2, PGM3 and Dock8 sequence.

Methods:

Consistent with the STAT3-like phenotype, a transcriptome study on patient-derived PBMCs revealed drastically reduced STAT3 mRNA expression, which was confirmed by real-time qPCR and Western Blot. To evaluate ZNF341 as a potential transcription factor for STAT3 we performed artificial overexpression systems and ChIP.
Results:

Overexpression of wildtype ZNF341 in HEK293T cells lead to an enhancement of endogenous STAT3 mRNA expression. Moreover, in the artificial overexpression system, we could detect the underlying mechanism: Wildtype ZNF341 enhances STAT3 expression by binding to its promoter, while the mutant variants showed impaired transcriptional activity, partly due to failure of nuclear translocation. In addition, ChIP and ChipSeq data further confirmed that ZNF341 binds to the STAT3 promoter (and other target genes to be revealed at this meeting) and revealed sequence motifs recognized by ZNF341.

Conclusions:

This widely uncharacterized zinc finger transcription factor targets STAT3 and thereby is an important regulator of immune-competence. The reduced STAT3 expression caused by the novel homozygous nonsense mutations in ZNF341 is the underlying mechanism explaining the STAT3-like phenotype in the investigated families.
A NATION-WIDE SURVEY OF HAPLOINSUFFICIENCY OF A20 REVEALS THE FREQUENT COINCIDENCE OF AUTOIMMUNITY IN JAPAN

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Background:

A20, encoded by TNFAIP3 gene, is a negative regulator in the tumor necrosis factor (TNF) - NF-kB signaling pathway. Recently, the haploinsufficiency of A20 (HA20) caused by heterozygous mutations of TNFAIP3 gene, has been revealed to present as early onset autoinflammatory disease resembling Behçet’s disease. Initially, six families of HA20 patients were described by Zhou Q and her colleagues. Subsequently, three additional families or sporadic patients had already been reported. In this study, we performed the multi-center survey about the HA20 patients found in our research community, primary immunodeficiency database in Japan (PIDJ).

Methods:

We summarized the detailed clinical manifestations, genetic analyses and cytokine profiles, and several immunological parameters of Japanese patients with HA20.

Results:

A total 30 patients in 9 independent families were included in this study. Among them, 22 patients were genetically identified as having HA20 and 8 patients showed Behçet’s disease-like phenotype with the autosomal dominant inheritance trait but their genetic analysis was not available. One nonsense mutation, one missense mutation, five frameshift mutations and two mutations located on the splice sites were found. All these mutations were evaluated to be functionally pathogenic by several in vitro assays. The production levels of proinflammatory cytokines were increased. The excess differentiation of Th17 cells, but not Th9 cells, were observed. Interestingly, not only autoinflammatory phenotypes but also several autoimmune disorders, including psoriatic arthritis, Hashimoto’s thyroiditis or autoimmune lymphoproliferative syndrome were identified.

Conclusions:

Our study suggests the possibility of the unexpected various phenotypes of HA20.
Background:

In recent years, biological networks emerged as powerful tools in modeling a multitude of signaling pathways, assigning putative roles to yet uncharacterized genes or proteins and visualizing complex relationships among biological entities. Harnessing their potential, we give an in-depth systems biology based characterisation of autoimmunity and autoinflammation.

Methods:

As the basis of our analysis, we have constructed a comprehensive and multi-layered AutoImmunOme, a biological subnetwork within the human Interactome. The AutoImmunOme includes disease genes responsible for an autoimmune and/or autoinflammatory phenotype, curated and annotated interactions and pathways that take part in processes related to autoimmunity/autoinflammation; transcriptomic signature of the diseases and drug-gene interaction data.

Results:

Using 707 autoimmunity/autoinflammation genes, we have confirmed that the AutoImmunOme forms a highly connected disease cluster, with 480 of the genes forming a large connected component (z score: 9.54). Furthermore, we find that genes associated with a particular disease cluster significantly in the same network neighbourhood within the AutoImmunOme. To allow for segregation of disease subtypes and to unravel potential novel functional clusters, we have performed state-of-the-art functional enrichment analysis, and described potential subgroups of the AutoImmunOme disease module. Finally, as our approach allows us to translate complex measures into quantifiable data, we have compared the network based distance of the AutoImmunOme to other immunological diseases.

Conclusions:

Our network based analysis revealed insights into the genetic basis of the molecular processes governing autoimmunity/autoinflammation. Given the broad adaptability of our analysis, we hypothesize that our approach may be applicable in other diseases as well.
ABSENCE OF Γ-CHAIN IN KERATINOCYTES ALTERS CHEMOKINE SECRETION RESULTING IN REDUCED IMMUNE CELL RECRUITMENT

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2University of Wisconsin-Madison, McArdle Laboratory for Cancer Research, Madison, USA
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Background:

Loss of function mutations in the common gamma (γc) chain cytokine receptor subunit give rise to severe combined immunodeficiency (SCID) characterised by lack of T and natural killer cells and infant death from infection. Haematopoietic stem cell transplantation or gene therapy offer cure but despite successful replacement of lymphoid immune lineages a long-term risk of severe cutaneous human papilloma virus (HPV) infections persists, possibly related to persistent γc-deficiency in other cell types.

Methods:

We generated a γc-deficient keratinocyte model, stimulated these cells with IL-15 and analysed chemokine secretion. In addition, migration experiments with dendritic cells and T cells towards supernatants collected from keratinocytes were carried out.

Results:

We demonstrated that keratinocytes, the only cell type directly infected by HPV, express functional γc and its co-receptors. Following stimulation with the γc-ligand IL-15, γc-deficient keratinocytes demonstrate significantly impaired secretion of specific chemokines including CXCL1, CXCL8 and CCL20 resulting in reduced chemotaxis of dendritic cells and CD4+ T-cells. Furthermore, γc-deficient keratinocytes also exhibit defective induction of T-cell chemotaxis in a model of stable HPV18 infection.

Conclusions:

Our findings suggest that persistent γc-deficiency in keratinocytes alters immune cell recruitment to the skin which may contribute to the development and persistence of warts in this condition and would require novel treatment approaches.
Background:

RAS-Associated Autoimmune Leukoproliferative Disorder is a nonmalignant condition that typically presents in childhood with monocytosis, lymphocytosis, autoimmunity, splenomegaly and lymphadenopathy. Despite sharing some overlapping clinical features and genetic defects with Juvenile Myelomonocytic Leukemia (JMML), RALD is indolent. Optimal therapeutic management of RALD and JMML differs, necessitating accurate diagnostic discrimination of RALD and JMML.

Methods:

Here we share the clinical spectrum of our cohort of 15 patients (Male:Female:NRAS 7:0:KRAS:1:7; median age 13 years; range 1.5-43yrs for KRAS and 3 to 58 yrs for NRAS cohort)(Table) including immune correlates and genomic landscape of RALD for the presence of non-RAS cooperating mutations found in JMML.

Results:

Peripheral blood and bone marrow morphology and immunophenotyping revealed activated monocytes (CD16+) and polyclonal B lymphocytosis with normal karyotypes in all the patients tested(N=12). Increased cytokine-IL10-2-6, splenomegaly with low HDL was noted in eight. Whole exome sequencing (WES) was performed on PBMCs from 7 patients (4 KRAS, 3 NRAS). One patient(‘85.1) died with no evidence of malignancy. One (58.1NRAS) has developed 2 lymphomas over his lifetime, another (104.1KRAS) developed TTP requiring plasma exchange and rituximab. None have undergone BMT.
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Conclusions:

EXOME sequencing revealed single somatic mutations in RAS genes with absence of cooperating leukemogenic mutations. This work suggests that in the spectrum of so-called RASopathies, RALD represents a non-malignant proliferative hematopoietic disease that is clinically distinct from JMML. In future; yearly immuophenotyping and whole EXOME or targeted sequencing of blood for leukemogenic genes may be incorporated into the long term follow up of RALD patients as a lymphoma-leukemia surveillance.
Background:

Immunodeficiencies secondary to RAG1/2 mutations has a widening clinical spectrum with emerging phenotype of combined immunodeficiency with autoimmunity and granulomas (CID-G/AI).

Methods:

Peripheral T and B cell compartments of a patient with CID-G/AI phenotype secondary to rag2 mutation with partially preserved recombinase activity (19.6%) have been assessed with flow cytometry. Single cell cloning of BCR heavy and light chain variable regions and as well as whole repertoire analysis has been performed to assess changes in B cell receptor repertoires to identify autoreactive B cell clones.

Results:

The patient's clinical phenotype was notable for autoimmune (ITP) and infectious complications including meningitis, pulmonary nodules and disseminated varicella infection with continued presence of anti-cytokine antibodies to IL-12, IFN alpha and αβδω omega. Reduced frequency of transitional and naïve B cells were detected while marginal zone and CD21-/low B cells were increased in peripheral blood. Single cell cloning of BCR variable regions and as well as whole repertoire analysis of marginal zone and CD21-/low B cell heavy chains revealed increased frequency of V4-34 V genes associated with autoreactive properties. In connection to altered B cell repertoire increased number of follicular helper T cells were detected.

Conclusions:

Our data indicate break in B cell tolerance in RAG CID-G/AI phenotype with a result of skewed B cell populations enriched in autoreactive B cell clones.
MTE - Meet the expert 5

ESID7-0264

NFKB1 HAPLOINSUFFICIENT PATIENTS SHOW IMPAIRED PROLIFERATION, PLASMABLAST FORMATION AND IMMUNOGLOBULIN PRODUCTION EX VIVO

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Background:

Common Variable Immunodeficiency (CVID) is a heterogeneous disorder characterized by recurrent infections, low immunoglobulins and poor vaccination response, admixed with variable autoimmune and inflammatory features. In a cohort of 846 primary immunodeficiency cases with genomes sequenced as part of the NIHR BioResource – Rare Diseases project, we identified NFKB1 as the gene most enriched for likely disease-causing variants.

Methods:

WGS data were analyzed to identify rare loss-of-function variants. PBMCs were isolated from both clinically affected and unaffected NFKB1 variant carriers (NFKB1+/−), including 4 sporadic cases and 5 pedigrees (n=17, age: 11–77 years). Apart from extensive immunophenotyping, PBMCs were CFSE-labeled and cultured with T-cell-dependent and independent stimuli to examine B-cell proliferation, plasmablast formation and immunoglobulin production ex vivo.

Results:

Of the 390 cases with a CVID diagnosis, 16 (4%) had a novel heterozygous frameshift, nonsense, splice-site, gene deletion or conserved missense NFKB1 variant. The analyzed sporadic and familial NFKB1+/− individuals showed symptoms ranging from none to recurrent respiratory tract infections, autoimmunity, and lymphomas. Partial penetrance is noted, disease correlating with older age. Serum IgG and IgA levels were low in all adult NFKB1+/− individuals. The low-to-absent switched memory B-cells in all NFKB1+/− individuals was accompanied by low surface IgG and IgA. All clinically affected NFKB1+/− patients had an increase in CD21low B-cells. Proliferation, plasmablast formation and immunoglobulin production (apart from IgM) was impaired upon stimulation with CpG/IL-2 and anti-IgM/anti-CD40/IL-21 in all NFKB1+/− individuals.
Conclusions:

*NFKB1* haploinsufficiency is the commonest monogenic cause of CVID and results in a defect in the formation of immunoglobulin-producing B-cells.
APPLICATION OF WHOLE EXOME SEQUENCING IN CONUNDRUM OF DYSGAMMAGLOBULINEMIA

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\textsuperscript{2}Division of Clinical Immunology- Department of Laboratory Medicine-, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden

Background:

A correct molecular diagnosis in patients with primary immunodeficiencies is crucial for the classification of disorders with a heterogeneous clinical/immunological phenotype and subsequent therapeutic management. Primary antibody deficiency is a group of primary immune diseases with highly diverse clinical features and, in most cases, an as yet unknown etiology.

Methods:

Clinical and immunological phenotyping of a cohort of consecutive dysgammaglobulinemic patients with unknown genetic defects after having been subjected to classical targeted sequencing underwent whole-exome sequencing analysis using a previously described filtering pipeline.

Results:

Exome sequencing analysis was performed on 110 probands (54.5\%male, 94.5\%childhood onset) born to predominantly consanguineous parents (82.2\%) and thus expected to carry homozygous mutations with an autosomal recessive pattern of inheritance. This method and subsequent immunological investigations identified the disease-causing variants in 88\% of the patients. Clinical and immunologic phenotypes of the remaining 13 patients were investigated for potential modifier genes. Medical implications of the definite genetic diagnosis were reported in 49\% of the patients, including hematopoietic stem cell transplantation, follow-up visits schedule and tertiary preventive screening tests (such as reducing radiation exposure for radiosensitive patients), targeted medication and prenatal diagnosis.

Conclusions:

Due to misclassification of the conventional clinical and immunological phenotyping for targeted sequencing, employing next generation sequencing as a preliminary step of molecular diagnosis approach to patients with dysgammaglobulinemia is essential and could help in many facets of management and treatment of the patients and their family members. This study also illustrates the power of exome sequencing in identification of novel and candidate genes underlying primary antibody deficiency.
Background:

Immunodeficiencies are associated with inflammatory bowel disease. The frequency and types of primary immunodeficiencies in an unselected cohort of patients with Very Early Onset Inflammatory Bowel Disease (VEO-IBD) is not known.

Methods:

A joint Gastroenterology-Immunology clinic was established. We enrolled >500 consecutive VEO IBD patients (with onset of IBD before 5 years of age). Whole exome sequencing (>250), flow cytometry (>150) and pathology review (>200) was performed.

Results:

Features that differed in the VEO-IBD clinic compared to later onset IBD were higher rates of ostomies and colectomies, as well as higher levels of apoptosis, eosinophils and villous blunting. Sixteen patients with recognized primary immune deficiencies were identified. Four new candidate genes were identified and several predicted damaging heterozygous variants in the IL-12/23 pathway were found. Overall, we have identified 10% of VEO-IBD with monogenic primary immunodeficiencies. Five bone marrow transplants have been performed which have been curative and gene-targeted therapies have also been effective. Pan-enteric disease, villous blunting and abnormal lymphocyte subsets were common in the group with monogenic defects.

Conclusions:

The identification of children with monogenic immunodeficiencies can define appropriate treatment and bone marrow transplantation can be curative. Screening flow cytometry, pathology and disease severity appear to have high sensitivity for identifying those with monogenic disease.
CONGENITAL DIARRHEA WITH PROTEIN LOSING ENTEROPATHY, RECURRENT INFECTIONS, AND ALTERED LIPID METABOLISM IN PATIENTS WITH NOVEL DGAT1 MUTATIONS.

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Background:

Congenital diarrheal disorders (CDD) can be a manifestation of a variety of underlying conditions, including early-onset inflammatory bowel disease (EO-IBD) or non-inflammatory diseases such as congenital chloride or sodium diarrhea. Compounding hypogammaglobulinemia can also result from the loss of protein through the gastrointestinal tract, leading to increased infections. These conditions can be attributed to monogenic defects as evidenced recently with the identification of human IL10-receptor deficiency.

Methods:

We used targeted next generation sequencing, or combined homozygosity mapping and exome sequencing to uncover molecular etiologies underlying CDD. Western blotting (WB) and immunohistochemistry (IHC) was used to detect protein expression. Lipid droplet staining was performed on patient-derived dermal fibroblasts.

Results:

We identified 4 novel mutations in the gene diacylglycerol-acyltransferase-1 (DGAT1) in 6 patients from 4 families suffering from early-onset chronic diarrhea and hypoproteinemia with sometimes lethal course of disease. The corresponding protein product is involved in the terminal step of triglyceride synthesis. Previous studies have illustrated two DGAT1 mutations underlying CDD, but the molecular pathomechanism is poorly understood. The mutations resulted in a lack of protein product as shown by WB and IHC. Furthermore, patient-derived dermal fibroblasts show a lack of lipid droplet formation upon treatment with oleic acid, pointing to lack of DGAT1 function. Reconstitution with wild-type DGAT1 protein reverted lipid droplet formation phenotype.

Conclusions:

Our findings show that biallelic mutations in DGAT1, an enzyme crucial for the metabolism of dietary fat, led to impaired formation of lipid storage in the form of lipid droplets and manifests as chronic diarrhea leading to hypogammaglobulinemia.
ADULTS WITH UNCLASSIFIED ANTIBODY DEFICIENCY: LOW QUALITY OF LIFE DESPITE ‘MILDER’ IMMUNOGLOBULIN ABNORMALITIES

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Background:

Patients with milder hypogammaglobulinemia who present with recurrent ‘normal’ - mainly respiratory - infections and fatigue as their main complaints form the largest group of patients with primary immunodeficiency. Also for them, early detection and adequate treatment is important.

Methods:

3-year observational cohort study. The TAAQOL-TNO-AZL Questionnaire for Adult Health-Related Quality of Life (HR-QoL) was part of the pre-first-visit intake procedure; 99/113 patients (≥18yrs) with abnormalities in serum immunoglobulin(s) and/or specific antibodies, gave informed consent to use these data for research purposes. TAAQOL-results were compared with age-related Dutch reference data (n=4120). Due to the negatively skewed distribution, the analysis was performed using the Mann-Whitney test, and median and inter-quartile range as summary measures.

Results:

43/99 were referred by a pulmonologist. 10 qualified the ESID-definition for CVID (IgG+IgA decreased - relevant symptoms - specific antibodies decreased), 89 were qualified as ‘unclassified antibody deficiency’ (unPAD) (=all antibody deficient minus monogenic agamma/hyperIgM minus CVID). In 60, HRCT-scan was performed; 5/7 (71%) CVID and 30/53 (57%) uAD showed bronchiectasis. HR-QoL was severely decreased in all domains (p=<0.001-0.01/domain).

Conclusions:

Although our care pathway mainly diagnosed so-called ‘milder’ unPAD-patients, more than half showed bronchiectasis. This is lower than in the CVID-group (71%) but still a significant proportion. HR-QoL was significantly decreased in all domains, an important problem for patients in their daily lives. Lately, more attention has been paid to this for CVID-patients; our data show that this is just as much a problem for unPAD-patients, who do not at all consider their health problem ‘mild’.
Background:

Chronic active Epstein-Barr virus infection (CAEBV) is a very rare disease with high morbidity and mortality, which was characterized by chronic or recurrent infectious mononucleosis-like symptoms, such as fever, hepatosplenomegaly, persistent hepatitis and extensive lymphadenopathy with high viral loads in their peripheral blood. Here we want to explore the genetic defect and immune defect under a case with CAEBV related acute-on-chronic liver failure.

Methods:

Whole exome sequence (WES) and flow cytometry were applied in the patient and his sister’s samples.

Results:

The patient from non-consanguineous family, who display recurrent EBV infection (EBV-DNA in serum $10^3$-$10^5$ IU/mL) from 5 years old with fever and abnormal ALT level (40-600 IU/mL). Slightly decreased CD4+ cells count in blood and very low IgM level in serum were found in this patient. Auto-antibody and HBV, HCV antibody were negative in serum. At 24 years old, the patient died of liver failure, which was induced by Chinese Herbal Medicine. WES was applied in the patient and his sister, no reported genetic disorder on CAEBV was found.

Conclusions:

CAEBV related acute-on-chronic liver failure is a very rare disease but is associated with a high case fatality rate. Specific immune defect may be associated with CAEBV infection.
MEASURING AND IMPROVING EXPERIENCED QUALITY OF CARE IN A PEDIATRIC IMMUNOLOGY OUTPATIENT CLINIC: A CLINICAL NEED SURVEY

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Background:

Different tools assess the quality of care on many levels. In recent years, patient satisfaction has become more important, with a special focus on patient centered care. There is however limited data on the experienced quality of care in pediatric immunology patients.

Methods:

We developed and validated a satisfaction questionnaire specifically for immunology patients in the outpatient clinic of the Wilhelmina Children’s Hospital (a tertiary pediatric immunology center). The questionnaire contained questions regarding communication, daily life, logistical factors and other items divided amongst 9 domains. The validation focused on both content- and construct validity using Cronbach alpha and intraclass correlation coefficient in SPSS. We next used this validated questionnaire to measure experienced quality of care in a larger patient population.

Results:

We noted a response rate of 67% (10/15 patients) during our validation process and 53% (28/53 patients) during the questionnaire distribution. With the exception of one domain (diagnostic phase) the mean satisfaction scores of experienced quality of care were above 4.0 on a 5-point scale. Open-end questions provided specific advise for improvements.

Conclusions:

In general, patients were very satisfied with the provided medical care. We found, however, three areas eligible for quality improvement. We are thus focussing on three topics for quality improvement: education, communication and counselling. More specifically, we made changes to the hospital’s website to better facilitate education. We will increase the use of digital communication tools (such as a patient portal) and doctors use extra feedback rounds during patient consultations.
A NOVEL MUTATION IN PIK3D CAUSING LATE ONSET COMBINED IMMUNODEFICIENCY

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Background:

Genetic mutations cause primary immunodeficiencies, which predispose to infections. Here we describe activated PI3K-δ Syndrome (APDS), associated with a dominant heterozygous mutation (c.970C>G, p.Arg324Gly) in the p110δ protein, the catalytic subunit of phosphoinositide 3-kinase δ (PI3Kδ), encoded by the PIK3CD gene.

Methods:

Fourteen year-old boy, born to non-consanguineous healthy parents was admitted with complaints of recurrent respiratory tract infections for 2 years. He had been diagnosed as common variable immunodeficiency at another hospital and had been receiving regular IVIg replacement.

Results:

On admission, he had generalized peripheral lymphadenopathies, hepatosplenomegaly with low IgG (IgG: 567 mg/dl) and undetectable IgM and IgA levels. Lymphocyte subsets were as follows; CD3+ T cells:70%, CD3+CD4+ Thelper cells: 18% (low for age), CD3+CD8+ T cytotoxic cells:47%, CD19+ B cells:9%, and CD16/56+ NK cells:18%. *Moraxella catarrhalis* grew in the stupum. EBV and CMV DNA copies were found positive. Thorax CT revealed bronchiectasis on right upper lobe and left lingula with mosaic oligemic pattern on lower lung areas. He did not have any clinical or laboratory autoimmune findings. An autosomal dominant mutation was identified in the PIK3CD gene by whole exome sequencing. He has been treated with subcutaneous immunoglobulin replacement, antibiotics and antiviral prophylaxis.

Conclusions:

PI3Kδ is expressed predominantly in cells of hematopoietic lineage and is the major PI3K isoform signaling downstream of T and B cell antigen receptors, Toll-like receptors, co-stimulatory molecules and cytokine receptors in T, B and myeloid cells. The APDS patients are characterized by recurrent sinopulmonary infections, CD4 lymphopenia, lymphadenopathy, EBV and/or CMV viremia and sometimes lymphoma.
OTHERS

ESID7-0081

URINARY TRACT INFECTION CAUSED BY UREAPLASMA UREALYTICUM IN IMMUNOCOMPROMISED HOSTS
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Background:

Ureaplasma urealyticum is a human urogenital pathogen, and is known to cause infection or colonization in the urogenital tract.

Methods:

We describe two cases of U. urealyticum infection in a patient with common variable immunodeficiency (CVID) and a cancer patient.

Results:

A 14-year-old female with CVID manifested with recurrent episodes of urinary tract infection. The patient complained of frequency, dysuria and suprapubic pain. The symptoms persisted over a year in spite of treatment with 3rd generation cephalosporin or trimethoprim/sulfamethoxazole. Repeated urinalysis showed pyuria and bacterial cultures were negative. Subsequently U. urealyticum was isolated by special urine culture. The patient showed dramatic symptom improvement after 14 days of doxycycline treatment.

A 17-year-old male with acute lymphoblastic leukemia who underwent haploidentical stem cell transplant developed hemorrhagic cystitis and epididymitis. The patient complained of dysuria, gross hematuria and painful scrotal swelling. Repeated urinalysis showed hematuria, pyuria and bacterial cultures were negative. BK virus and adenovirus were negative. Subsequently, U. urealyticum was detected by urine PCR. The patient showed dramatic symptom improvement after 14 days of doxycycline treatment.

Conclusions:

A high index of suspicion for U. urealyticum is required especially in culture negative recurrent urinary tract infection or hemorrhagic cystitis of immunocompromised host.
CLINICAL AND DEMOGRAPHIC CHARACTERIZATION OF PRIMARY IMMUNODEFICIENCIES IN CHILDREN WITH RECURRENT INFECTIONS AT HOSPITAL UNIVERSITARIO HERNANDO MONCALEANO PERDOMO, NEIVA - COLOMBIA

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Background:

Primary Immunodeficiencies (PIDs) are conditions that predispose patients to acquire infectious, autoimmune, allergic, and malignant diseases. PIDs are more common in children than in adults. Children suffering recurrent infections (RIs) might have a PID too, thus, they require prompt study so that early diagnosis is made and treatment is established. Epidemiological data on PIDs is short in our region and timely diagnosis is deficient. Our objective was to characterize the clinical, immunologic and demographic characteristics of the pediatric patients newly diagnosed with PID that attended to the Hospital Universitario Hernando Moncaleano Perdomo in Neiva, Colombia from June 2013 to December 2016.

Methods:

Clinical records of the children seen for RIs during the study period were checked, records of children with diagnosis of PID supported with clinical findings and immunological tests were selected for analysis. Records of the patients that had PID ruled out, based on immunological tests, were used as control group.

Results:

299 children consulted for RIs during the chosen period. 24 (17 male) were newly diagnosed with a PID. Age of onset of symptoms had a median of 7 months. The most frequent infection was pneumonia. The most common PIDs were humoral deficiencies.

Conclusions:

The rate of new PID cases per year was 6.8. The most common PID group were the Predominantly Antibody Deficiencies (n=15). Molecular diagnosis is lacking in our patients. We expect this report improves awareness and timely diagnosis of PIDs in our region.
AN ATYPICAL CASE OF LATE ONSET TYPE I INTERFERONOPATHY
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Background:
Type I interferonopathies are newly considered group of inflammatory diseases. These rare genetic disorders most frequently affect the brain and the skin within the first year of life. Since the original description in 1984, a wider spectrum of this disease presentation has been recognized.

Methods:
We aim to present a clinical case, which could be described as a clinically distinct phenotype of type I interferonopathy.

Clinical case: 24 yrs-old homozygous twins were studied in the main Lithuanian hospitals due to many severe different syndromes. First symptoms appeared at the age 19 and during 5 years they had a wide variety of signs: intermittent thrombocytopenia, chilblain like lesions, recurrent bacterial skin infection, recurrent fever, skin and nail mycosis, warts, sensorineural polyneuropathy, livedo reticularis, hirsutism, striae, obesity, cholelithiasis, dyslipidemia, recurrent hypoglycemia, astigmatism, hypokalemia, preserved intellect, limited communication abilities.

Results:
Brain CT scan for one girl (A) showed calcification in the pineal gland, for another (B) - hypodense area between midbrain and pons. Girl A was diagnosed with autoimmune thyroiditis, lichenoid dermatitis (skin biopsy). Girl B also has a chronic pancreatitis, sick sinus syndrome, leukocytoklastic vasculitis (skin biopsy).

Lymphocyte phenotyping was within the norm. Genetic work up showed IFIH1 gain-of-function mutation.

Conclusions:
We suppose, that presented clinical case could be described as a different (late onset) phenotype of the disease. Review of a literature showed that the same mutations of type I interferonopathy are associated with clinically distinct phenotypes and there is no definite explanation for this variability yet.
THE STUDY OF SERUM LEVEL OF M1 MACROPHAGE IN ECHINOCOCCOSIS

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Background:

Objective Echinococcosis is a zoonotic parasitic disease of human and mammals caused mainly by the larval stages of Echinococcus. In the process of defense, macrophages polarize gradually into two different subtypes. It suggested that there is a close bond between macrophages polarization and the outcomes of different diseases. Characterized by expressing CD16/32⁺、CD68⁺、CD86⁺、MHC II⁺, M1 had a strong ability of inducing an anti-inflammatory effect on immune system to maintain homeostasis. Through high expression of IL-6, low expression of IL – 10 and TGF-β1 to eliminate pathogen.

Methods:

Method Therefore, in this research, we tracked the M1 relative cytokine of infected mice in different period through RT-PCR, immuhistochemistry and CBA.

Results:

Result The RT-PCR and CBA have found that M1 and the relative cytokine increased in the early stage and decreased in late. The immuhistochemistry show the same phenomenon.

Conclusions:

Conclusion Macrophages as a nonspecific immune response cells and the antigen presenting cell of specific immune response play an important role to preserve host integrity in the development of hydatid diseases. After the polarization,M1 appeared in the early period, which probably is a way to protect host from pathogen.
THE HEMATOLOGICAL MALIGNANCIES IN CHILDREN WITH ATAXIA-TELANGIECTASIA EXPERIENCE OF SINGLE CENTER

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Background:

Ataxia- telangiectasia (AT) is a rare disease with multi-organ presentation including neurodegeneration, primary immunodeficiency and increased predisposition to malignancy. The aim of this study is to assess the first symptoms, diagnostic and treatment results of hematological malignancies in children with AT, treated in the Department of Pediatric Hematology, Oncology and Transplantation in Lublin.

Methods:

Between 2000-2016 four boys and two girls with AT from the age at 4 to 16 years (median age 10, 5 years) were diagnosed with: two cases of non-Hodgkin lymphoma, one patient Hodgkin's lymphoma and T-ALL in three children.

Results:

The first signs of cancer in the study group were non-specific symptoms of the respiratory system, infections, and lymphadenopathy. All patients presented mediastinal tumor. Early diagnosis was limited by the inability to use X-ray images. In addition, our patients had aggressive histological type of tumors. For those reasons, the stage of disease in the group was advanced. Treatment of patients with AT were frequently delayed or reduced because of the impaired tolerance to cytotoxic drugs. Three children completed treatment and are in complete remission, one patient is on treatment, one boy died from disease progression and one girl died after treatment. This outcome is worse as compare to non-AT children with leukemia and lymphoma.

Conclusions:

In patients with AT early diagnosis of malignancy is difficult due to similarity of infections in immunocompromised patients and first signs of lymphoproliferation. Non-specific symptoms of the respiratory system were the first observed. Patients in AT need multidisciplinary management with regular oncological check-up.
SYNDROMIC PRESENTATION OF SEVERE CONGENITAL NEUTROPENIA: VPS45 DEFECT WITH P.E238K MUTATION.

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Background:

Severe congenital neutropenia (SCN) is heterogeneous group of disorders caused by the maturation, homeostasis and functional defects of neutrophils. Mutations leading to defective vacuolar protein sorting 45 (VPS45) protein have been reported in patients with SCN, having recurrent bacterial infections and infant onset myelofibrosis. Here we report a patient presenting with SCN and multisystemic symptoms having VPS45 pE238K mutation.

Methods:

Case:

Results:

2 months old female patient was referred to our clinic with severe leukopenia detected on the 15th day of life. She was born out of a first cousin consanguineous marriage. She had prominent forehead, almond shaped palpebral fissures, bulbous nasal tip and high palate. Mild axial hypotonia and horizontal nystagmus with poor pupillary light reflex were present on neurological exam. Mild anemia and lymphopenia with profound neutropenia was detected in blood count. Lymphocyte subset analysis revealed CD8 lymphopenia, Trec representing CD4+CD45RA+CD31+ T cell counts were normal. Activation responses to phytohemaglutinin and antiCD3 were low. The profound neutropenia was persistent. During the follow-up, hemoglobin levels decreased without reticulocyte response, and thrombocyte counts lowered gradually. Since she had recurrent abscesses in deep tissues (perianal, orbital and intraabdominal), we started G-CSF. Meanwhile, optic nerve hypoplasia was detected with orbital MRI, and sensoryneural hearing loss was present in right ear. Genetic analysis of the patient with NGS based gene panel screening revealed a homozygous c.712G>A p.E238K mutation in VPS45 gene.

Conclusions:

The p.E238K mutations of VPS45 gene have syndromic presentation of multisystemic involvement through mainly neuromotor retardation associated with progressive bone marrow failure.
AD-HIES: LONG-TIME OF PROSPECTICAL FOLLOW UP IN ADULT PATIENTS

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⁹, , , , , Italy

Background:

This study aims to describe a cohort of adult patients with autosomal dominant Hyper-IgE Syndrome (AD-HIES) with a long time follow-up.

Methods:

Adult AD-HIES patients recorded in the IPINet Registry with available follow-up have been considered.

Results:

Twenty-three of the 37 HIES patients recorded are adults (62%). AD-HIES patients with follow-up are 13 (8M/5F), all carriers of mutation on STAT3 gene. The average time of follow-up is 17 years (range 5-40). Mean age at last follow-up was 33.5 years (DS±9.96).

Age at disease onset ranged 1 months-4.5 years. Onset symptoms were infections for 84.6%, dermatologic involvement for 7.7%, both manifestations for 38.5% patients. Mean age at clinical diagnosis was 22.2 years (DS±12.84). Diagnosis delay ranged 9-45 years.

At diagnosis, all the patients started prophylaxis: 92.3% antibiotics, 69.2% antifungals and 7.7% antivirals.

Table shows manifestations at clinical diagnosis and at last follow-up.

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Present at diagnosis</th>
<th>At last follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>eczema</td>
<td>92.3%</td>
<td>69.2%</td>
</tr>
<tr>
<td>skin abscess</td>
<td>84.6%</td>
<td>69.2%</td>
</tr>
<tr>
<td>chronic mucocandidiasis</td>
<td>84.6%</td>
<td>38.5%</td>
</tr>
</tbody>
</table>
pneumonia 92.3% 92.3%
pneumatocele 69.2% 76.9%
lobectomy 53.8% No more
osteoporosis 23.1% 53.8%
abnormal bone fractures 30.8% No more

At clinical diagnosis, serum IgE mean levels was 14725 (DS±17514.4), at last follow-up 12101 (DS±12131.8). The 63.3% of patients had positive RAST to more than 70% of the aero- and food allergens tested, but they had skin prick tests positive only for 8%-40% of the corresponding allergens. Two atopic patients are reported.

Two patients died aged 28 and 39 years for massive pulmonary hemorrhagia and for cancer, respectively.

**Conclusions:**

Prophylactic therapy improves outcome and can impact AD-HIES patients' expectation of life
ICF SYNDROME: TWO SIBLING WITH TWO DIFFERENT CLINICAL PICTURES

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Background:

The ICF syndrome is an autosomal recessive condiditon comprised of Immunodeficiency, Centromeric instability, and Facial anomalies. Mutations in the gene DNMT3B, which encodes a DNA methyltransferase, are causative in approximately half of individuals with this syndrome (ICF-1). Mutations in the ZBTB24 gene, which encodes a transcriptional repressor involved in DNA methylation, were identified in some of these patients (ICF-2). Mutations in the cell division cycle associated 7 (CDCA7) gene was referred ICF-3.

Methods:

We presented two siblings who have same homozygous mutation.

Results:

Case 1: 10 years old boy was referred with recurrent infections when she was 14 months old. In the physical evaluation, he had hypertelorism, low ear, flat face, micrognati, high palate. In the laboratory, anemia, neutropenia, agammaglobulinemia, low isohemaglutinin, low CD19+ B cells ratio were detected. He was diagnosed as ICF syndrome with cytogenetic test. It was given antibiotic prophylaxis and IVIG replacement. DNMT3B and ZBTB24 mutations were negative. It was detected a homozygous mutation in the CDCA7 gene. He is under the subcutaneous immunglobulin and antibiotic prophylaxis, he doesn’t have any serious infection and hospitalization.

Case 2: 5 years old sister was admitted with pneumonia when she was 3 months. She had characteristic face, agammaglobulinemia and low CD19+ B cells. She had homozygous CDCA7 mutation. IVIG and antibiotic prophylaxis was started after treatment of pneumonia. She couldn’t gain weight because of recurrent infections and chronic diarrhea. She was 6 kg when she was 2. She went to bone marrow transplantation when she was 30 months. She is 5 and she is on 10 percentile and she doesn’t have any serious infection.

Conclusions:

We presented these two sibling cases for the different clinical course although having same mutation.
DIAMOND-BLACKFAN ANEMIA LIKE PRESENTATION IN PATIENTS WITH CECR1 MUTATION: MIGHT BE MORE COMMON THAN PREDICTED IN DBA PHENOTYPE
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Background:
CECR1 or ADA2 deficiency is an autosomal recessive disorder with phenotypic heterogeneity. Most of the patients present with childhood polyarteritis nodosa, whereas in rare occasions Diamond-Blackfan anemia (DBA) like phenotype or stroke have also been reported. The patients usually have mild or no propensity to infections, whereas the immunological evaluations may reveal abnormalities.

Methods:
Out of 30 patients who underwent molecular work-up with a diagnosis of DBA, 5(17%) revealed CECR1 mutation. Herein we present five patients (M:F=3/2) who were referred with a diagnosis of DBA for molecular testing and was confirmed to have CECR1 gene mutations in WES and further confirmed with Sanger sequencing.

Results:
Of the patients with CECR1 mutations, the median age at diagnosis of DBA and molecular testing were 4 months-old(1-6 mo) and 4 years of age(27 mo-23 yo), respectively. History revealed recurrent urinary tract infections in 1, and recurrent pneumonia and bronchitis in another patient. Three patients revealed recurrent aphtous stomatitis and one had a history of stroke. None had vasculitis. Three of the patients had low IgA and IgG levels compared to age appropriates. On the other hand CD19 levels were lower compared to age appropriates in 2 patients. Three of the patients had DBA resistant to steroid treatment and were on transfusion programme. One of the patients was initiated IVIG but transfusion dependency persisted. Two patients underwent HSCT after molecular work-up and all are currently alive.

Conclusions:
The causes phenotypic heterogeneity and the pathogenesis of DBA-like phenotype in some of the CECR1 deficient patients is unknown.
A DEEP INTRONIC MUTATION OF c.1166-285G>T IN SLC46A1 IS RESPONSIBLE FOR HEREDITARY FOLATE MALABSORPTION IN TWO UNRELATED JAPANESE PATIENTS

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Background:

Hereditary folate malabsorption (HFM) is an autosomal recessive disease caused by mutations in \textit{SLC46A1} encoding the proton coupled folate transporter (PCFT). Folate deficiency due to defects of folate uptake in the intestine and choroid plexus results in various clinical features including megaloblastic anemia, thrombocytopenia, combined immunodeficiency, and neurodevelopmental disorders. We previously reported compound heterozygous mutations of c.566G>T (p.G189V) and c.1166-285G>T (p.A389GfsX20) in Patient 1 with HFM.

Methods:

We performed sequence analysis of \textit{SLC46A1} in Patient 2, a 3-month-old girl with possible HFM. We further characterized and compared her clinical features with those of Patient 1 and previous reports.

Results:

Patient 2 was diagnosed with HFM caused by the same deep intronic mutation of c.1166-285G>T as Patient 1. It appears to be a homozygous mutation because the heterozygous c.1166-285G>T mutation identified in her parents. Since these two patients are unrelated, this could be a hot spot or a common mutation at least among the Japanese population. These two patients shared clinical feature of undetectable levels of serum folate concentration, megaloblastic anemia, thrombocytopenia, and hypogammaglobulinemia. Significantly reduced proliferation of their lymphocytic cell lines in media with low concentration of folate indicated PCFT could also function for folate transport at hematopoietic lineages in the settings of folate deficiency.

Conclusions:

Here we report two patients with HFM who shared the same deep intronic mutation of c.1166-285G>T (p.A389GfsX20).
IMMUNODEFICIENCY CAUSED BY ACTIVATING MUTATIONS IN THE SUBUNITS OF PI3K AND TREATMENT WITH SIROLIMUS

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Background:

Class-IA PI3Ks are composed of two subunits; a catalytic (p110α,p110β,p110δ) and a regulatory subunit (p85α/p55α/p50α, p85β, p55γ). Heterozygous mutations in PIK3CD encoding the p110delta cause senescent T-cells, lymphadenopathy, and immunodeficiency (PASLI) in which Akt/mTOR(mammalian-target-of-rapamycin) signaling was activated. More recently a heterozygous splice site mutation in PIK3R1 has been shown to cause a similar syndrome.

Methods:

Here, we present clinical and immunological features of a total of four unrelated patients harbouring activating mutations in the genes encoding PI3K subunits (two patients each with mutations in the PIK3CD and PIK3R1 genes). In addition, the response to sirolimus treatment is evaluated.

Results:

Laboratory investigation revealed normal lymphocyte counts, a decreased ratio of CD4+/CD8+ cells, reduced B-cell numbers, increased number of senescent CD8+ T cells, low serum IgA and elevated IgM. All patients exhibited hepatosplenomegaly and enlarged peripheral lymph nodes and one (P4) underwent splenectomy due to refractory thrombocytopenia. The mean duration of sirolimus therapy was 13.5±3.6 months. We observed a remarkable decrease in the size of lymph nodes, liver and spleen after a 4-6 week of sirolimus treatment. Additionally, there was a slight increase in the ratio of CD4+/CD8+ cells, with a modest decrease in the number of senescent CD8+ T cells in P2 and P3. The most common adverse effects attributed to sirolimus use were oral ulcers and mild hyperlipidemia.

Conclusions:

Sirolimus appeared effective in the treatment of PASLI disease, with a quick and sustained response. While lymphadenopathies and splenomegaly relieved under treatment a longer term follow up is warranted.
OTHERS

ESID7-0242

PREFERENCES AND MOTIVATIONS FOR TREATMENT OPTIONS. OVERVIEW OF DECISIONS TAKEN BY THE PARENTS OF UNDERAGE PATIENTS WITH PID - RESEARCH RESULTS

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Background:

The psychological factors of PID therapy such as motivations and preferences of patients are extremely important to explore. In the case of underage patients, the situation is even more complicated. Not only child’s emotions and behaviors affect the decision process; his/her parents’ feelings are even more important.

Methods:

Immunoprotect's studies were conducted using an online questionnaire and paper questionnaires delivered to hospitals in Poland. The research involved 36 parents of underage patients. Among their children 19 were treated with SCIG and 17 with IVIG.

Results:

The collected data presents: parents' preferences about types of treatment, their motivations, opinions about both SCIG and IVIG, including the greatest advantages and disadvantages of each type of therapy. 51% of parents chose once-a-month home SCIG therapy for their children, 9% preferred once-a-week home SCIG infusions, 40% of parents wanted their children to be treated with IVIG: 31% chose hospital-based IVIG and 9% a home-based therapy. Main reasons for choosing SCIG are: perspective of having higher, more stable IgG levels (65,2%), less side effects (47,8%) and the fact, that SCIG option gives more freedom in a personal life (43,5%). Main motivations for IVIG therapy are: medical/nursery care during infusion (77,8%), the possibility of having only one infusion monthly (66,7%) and the fact that the procedure is done by the medical personnel (38,9%).

Conclusions:

We believe that the collected data will let all of us better understand the motivations of PID patients' parents and thereby better respond to their needs while trying to eliminate their greatest concerns.
HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH HETEROZYGOUS STAT1 GAIN-OF-FUNCTION MUTATION

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Background:

Signal transducer and activator of transcription 1 (STAT1) is an important transcription factor for cellular response in many cell types. Human STAT1 GOF mutations lead to chronic mucocutaneous candidiasis and autoimmunity. The patients have commonly been treated with antifungal drugs, however, an established therapeutic approach is lacking, mainly due to the heterogeneity of the clinical symptoms and variable severity. A few reports described severely affected patients who underwent hematopoietic stem cell transplantation (HSCT), with variable outcomes. Here we describe two patients with STAT1 GOF mutations presenting in infancy with a combined immunodeficiency (CID) phenotype and received HSCT.

Methods:

Clinical data and laboratory findings including interferon-induced STAT1 phosphorylation were evaluated.

Results:

Patient 1 (P1) presented with oral candidiasis, CMV infection and cavitary lung lesions caused by Mycobacterium tuberculosis. Patient 2 (P2) suffered from oral candidiasis and CMV pneumonitis. Targeted panel sequencing has identified heterozygous missense mutations (T385M, C324F) in the STAT1 gene in both patients. The patients were transplanted with HLA-matched donors, using a reduced intensity conditioning. While P1 successfully recovered from her disease and currently doing well, P2 has developed systemic CMV reactivation and GvHD following a HSCT and died from severe lung disease. In vitro studies on peripheral blood cells isolated from P1 demonstrated a correction in the STAT1 hyper phosphorylation that was abnormally active prior to HSCT.

Conclusions:

STAT1 GOF mutations can present with a severe early onset CID phenotype and may be treated with HSCT. However, the variable outcome of HSCT in our two cases underscores the necessity of developing improved therapies.
OTHERS

ESID7-0250

REFERENCE VALUES FOR LYMPHOCYTE SUBSETS IN HEALTHY CHILDREN AND ADOLESCENTS; JMF MARMARA CENTER

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Background:

Immunophenotyping of lymphocytes plays an important role in diagnosis, classification, and prognosis of immunological and hematological diseases such as immune deficiencies, lymphoproliferative disorders, and autoimmune diseases. In order to diagnose and treat immune deficiencies, it is crucial to identify accurate and up-to-date reference percentage rates and absolute numbers of lymphocyte subset for each age group, which are known to be influenced by environmental factors, antigogenic stimuli, ethничal differences, present vaccination schedule and laboratory factors.

Methods:

Babies, children, adolescents, and hospital staff who were accepted to be healthy after examination were determined to be our population universe. Based on previous researches, life span was divided into 10 groups and each group consisted of minimum 30 participants according to law of great numbers. Total number of 297 children were admitted for our research. All the groups were homogenously scattered according to number, age, and gender. Complete blood count and immunophenotyping of T, B, NK lymphocytes, and subgroups of B, Thelper vs T cytotoxic cells were simultaneously analyzed. Double negative T cells, recent thymic immigrants, and HLA-DR expressions were also assessed for their reference values.

Results:

Based on our findings, percentage rates and absolute counts for lymphocyte subsets were expressed in tables as reference values and in graphs as percentiles.

Conclusions:

Our findings in general had similarities with previous reports. However, we hereby presented for the first time broader reference values for lymphocyte subgroups including different age groups which is specific to our population and applicable to daily medical practice.
Hypomorphic JAK3 and IL2RG Mutations Presenting with a Predominantly Antibody Deficiency Phenotype

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Background:

Hypomorphic mutations in the genes that are typically associated with severe combined immune deficiency (SCID) can lead to milder and atypical presentations. Here, we describe two cases presenting predominantly with an antibody deficiency phenotype, who were found to have mutations in the SCID-causing genes.

Methods:

Clinical and laboratory features of two patients were collected retrospectively from medical reports.

Results:

Patient-1 (P1), a ten-year-old female, born to consanguineous parents presented with recurrent lower respiratory tract infections (LRTI) starting from age of 4 years. Immunologic evaluation revealed normal levels of Immunoglobulin G, A, M and E, however, with a complete absence of IgG2 and IgG4. CD3+CD4+ T-cell numbers were reduced but B and NK-cells were normal. Whole exome sequencing in the family revealed a homozygous missense mutation in the Janus-kinase-3 (JAK3) gene (c.3196T>C, p.C1066R) in the patient, segregating with an autosomal recessive inheritance pattern. The patient is being evaluated for hematopoietic stem cell transplantation (HSCT).

Patient-2 (P2): A thirty-year-old male was referred for the evaluation of recurrent LRTI and bronchiectasis, which then required surgical resection of the lung segments. Laboratory evaluation revealed slightly reduced IgG and IgM, normal IgA but absent isohemagglutinins. There was a reduction in the numbers of CD3+CD4+ T-cells, B-cells and NK-cells. Panel targeted sequencing revealed a homozygous promoter gene mutation (X:70331494, c.-105C>T) in interleukin 2 receptor gamma (IL2RG) gene, which was previously linked to leaky SCID.

Conclusions:

The cases presented underscore the value of high throughput/panel sequencing methods in PID work-up. Timely molecular diagnosis may be of significant therapeutic importance as illustrated by our two cases.
OTHERS

ESID7-0258

FREQUENCIES OF PD-1- POSITIVE T CD3+CD4+, T CD3+CD8+ AND B CD19+ LYMPHOCYTES IN ADULT PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY

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Background:

PD-1 maintains tolerance and inhibits autoimmune responses. Common variable immunodeficiency (CVID) is one of the most frequent humoral immunodeficiency diseases of unclear etiology. The aim of this study was to evaluate the percentage and absolute counts of PD-1 positive T and B cells in adult patients with CVID.

Methods:

The study group included 30 patients and the control group comprised of 20 age- and sex-matched healthy individuals. The frequencies of PD-1 positive T and B cells were assessed with the use of flow cytometry method.

Results:

Results showed significantly higher frequencies and absolute counts of PD-1 positive CD3+CD4+ T cells, CD3+CD8+ T cells and CD19+B cells in patients with CVID in comparison to the healthy volunteers. Moreover, higher mean fluorescence intensity of PD-1 was found on CD3+CD4+ T cells, CD3+CD8+ T cells and CD19+B cells in the study group than in the control group.

Conclusions:

Obtained results suggest that PD-1 protein might involved in the pathogenesis of CVID.
MULTIPLE FACES OF CVID PRESENTED IN THE ONE PATIENT

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Background:

We present the case of 20y old man with CVID, who was long treated for autoimmune hemolytic anemia and bronchiectasis. First clinical manifestation of CVID were repeated respiratory infections and meningitis at the age of 11. Hodgkin's lymphoma was diagnosed at the age of 16, with remission after chemotherapy.

Methods:

In May of 2015 patient was hospitalized for bilateral bronchopneumonia, severe immune thrombocytopenia and anemia. Within the differentiation of conditions, it was necessary to exclude lymphoma relapse and search for an infectious cause, which could be the reason for the deteriorating immune cytopenias. The chest CT shows large necrotic aspergillosis. Antinfective agents in combination of meropenem and voriconazole were started. Concurrently continued treatment with corticosteroids, immunoglobulins and hemosubstitution, with partial improvement of anemia and thrombocytopenia. Given the risk of developing respiratory insufficiency, surgical treatment was not indicated. PET/CT excluded lymphoma relapse. Within three months the situation was getting worse with progression of bronchopneumonia and cytopenia with fatal outcome.

Results:

Infections are frequent and life-threatening complication in patients with CVID. It is necessary to think about the broad spectrum of bacteria, mycobacterial infections, viral and fungal pathogens. Invasive mycoses are more common in the group of immunodeficiency disorders such as defects of phagocytosis than in patients with humoral deficiency. In patients with CVID these infections can occur as a result of combined humoral and T-cell response defects. In our case conditions were worsened by long term corticotherapy and the deepening thrombocytopenia.

Conclusions:

The key is early antifungal therapy, surgical intervention and treatment with immunoglobulins.
PURINE NUCLEOSIDE PHOSPHORYLASE DEFICIENCY PRESENTING AS SEVERE COMBINED IMMUNE DEFICIENCY, NEURODEVELOPMENTAL DELAY AND AUTOIMMUNE HEMOLYTIC ANEMIA

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Background:

Purine nucleoside phosphorylase (PNP) deficiency is an autosomal recessive metabolic disorder characterized by severe combined immunodeficiency, autoimmunity (especially autoimmune hemolytic anemia) and by a complex of neurologic manifestations. PNP protein catalyzes the phosphorolysis of deoxyinosine and deoxyguanosine and expressed at the highest levels in lymphoid tissues. Consequently the lymphoid system is predominantly affected in PNP deficiency. We describe a four-year-old boy with neurodevelopmental delay, autoimmune hemolytic anemia, severe lymphopenia, neutropenia diagnosed as PNP deficiency.

Methods:

Case Report

Results:

The patient was admitted to our clinic with pallor and jaundice. He was born as first baby out of a third cousin consanguineous marriage, following an uneventful pregnancy with normal birthweight. He had been hospitalizated for pneumonia and encephalitis when he was 8 months and 1,5 years old. On his physical examination, there were pallor, jaundice, motor mental retardation, respiratory distress and hepatosplenomegaly. Laboratory results revealed severe anemia (hemoglobin 4,7 gr/dl) and leucopenia (1130/mm³), lymphopenia (300/mm³) and neutropenia (550/mm³), direct coombs: positive, LDH : 886 IU/l, haptoglobinine <14 mg/dl, uric acide < 1.5 mg/dl, IgG : 2050 mg/dl, IgM :433 mg/dl IgA <6,5 mg/dl. In lymphocyte subgroup analysis all of the lymphocyte subsets were low. Because of existence of autoimmune hemolytic anemia, motor mental retardation, recurrent invasive infections, decreased uric acid level, PNP gene analysis was studied. Homozygous p. R58X mutation was determined. Bone marrow transplantation was planned.

Conclusions:

As a result patients with developmental delay and hypouricemia should be screened for PNP deficiency, particularly in the presence of lymphopenia and autoimmune hemolytic anemia.
HUMAN BI-ALLELIC IRF8 MUTATIONS CAUSES DENDRITIC CELL DEFICIENCY AND MULTI-LINEAGE IMMUNE DYSREGULATION

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Background:

The transcription factor interferon regulatory factor 8 (IRF8) has multiple roles in immune cell development and function and the phenotype of bi-allelic human IRF8 mutation has been inferred from a single reported case with homozygous IRF8^K108E mutation.

Methods:

Here we describe a patient presenting with recurrent viral infection, granulo-proliferation and intracerebral calcification, found to have compound heterozygous IRF8 mutations on whole exome sequencing.

Results:

R83C is a conserved residue in the DNA binding domain and R291Q is an invariant residue in the interferon association domain. Neither mutant was able to regulate the Ets/IRF composite element or interferon-stimulated response element but R291Q retained BATF/JUN interactions. Consistent with the K108E mutation, dendritic cells and monocytes were absent, but tissue macrophages and Langerhans cells were preserved. Neutrophils were consistently elevated, dysplastic and hypofunctional. B cell development was affected with fewer memory cells, impaired class-switching and reduced frequency and complexity of somatic hypermutation. CD4⁺ T cell polarization was skewed towards Th2, effector memory CD8⁺ T cells were decreased and CXCR3 expression virtually absent. Gene expression profiling of purified lymphoid subsets revealed complex patterns of differentially regulated genes, more than half involved in interferon responses.

Conclusions:

Human bi-allelic IRF8 mutation results in dendritic cell and monocyte deficiency, granulo-proliferation and a complex, multi-lineage immunodeficiency.
GAIN-OF-FUNCTION (GOF) MUTATIONS IN SIGNAL TRANSCLUDER AND ACTIVATOR OF TRANSCRIPTION 1 (STAT1): A CASE WITH CHRONIC MUCOCUTANEOUS CANDIDIASIS MIMICKING COMBINED IMMUNODEFICIENCY

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Background:

Chronic mucocutaneous candidiasis (CMC) is a general name used for the chronic/recurrent, non-invasive Candida infections of skin, mucous membranes and nails. CMC may be secondary to factors affecting the immune system or a finding of primary immunodeficiencies (PID).

Methods:

A 26-month-old girl was admitted with the complaints of recurrent oral moniliasis, diarrhea and respiratory infections. She had a severe varicella infection and frequent hospitalizations with infections. She had failure to thrive and mild mental-motor retardation. Parental consanguinity and a sibling loss were warning signs for a PID.

Results:

*Candida albicans* grew in oral mucosa swab. Immunological work-up revealed hypergammaglobulinemia with normal IgE levels. Percentages of lymphocyte subgroups, oxidative burst activity, foxp3 expression and *in-vitro* T-cell proliferation response were normal.

Imaging studies revealed calcific parenchymal nodules (≤5mm) in lungs and a calcific nodule (8 mm) in liver. Bronchoscopy showed normal airway anatomy with mucopurulent secretions. No bacterial, mycologic, parasitic and mycobacterial agents were isolated in bronchoalveolar lavage fluid. Tuberculin test was negative and interferon-gamma release assay was positive leading to anti-tuberculosis treatment. Antiviral treatment for CMV and EBV positivity and antifungal and antibacterial prophylaxis were started.

Infections with variable pathogens suggested preliminary diagnosis as combined immunodeficiency, CMC and mendelian susceptibility to mycobacterial diaseases. Genetic analysis revealed a pre-defined heterozygous GOF mutation (c.1154 C>T, p.Thr385Met) in the gene coding STAT-1 molecule.

Conclusions:

STAT-1 GOF mutations are responsible for nearly half of CMC cases. This may cause variable infectious and noninfectious findings as autoimmune diseases, tumors and aneurysms.
ESID7-0292

CLINICAL AND IMMUNOLOGICAL ASPECTS IN A COHORT OF 13 RAG DEFICIENT PATIENTS. 
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Background:

RAG genes mutations in humans are associated with a variety of clinical phenotypes, ranging from the typical SCID and Omenn (OS), inevitably fatal early in life if not recognized and treated, to forms with a milder clinical course and delayed presentation characterized by variable association of infections and autoimmunity due to hypomorphic mutations.

Methods:

We enrolled 13 RAG patients analyzed by Sanger sequencing and NGS in a study focused on the possible correlation between clinical and genetic data.

Results:

In our cohort of PID patients, we found a high number of typical and atypical patients with RAG-deficiency. We determined RAG1 mutations in 5 patients (4 with classical OS phenotypes, among them one with nephropathy, and 1 presenting with Miller Fischer syndrome) and in other 2 CID (Combined Immunodeficiency) patients analyzed successfully by Sanger. The clinical variability of these patients represents an effective example how NGS has highlighted unpredictable RAG patients in which the phenotype did not suggest this diagnosis. Indeed, we identified a RAG1 deficiency in a patient followed for many years for CVID (Common Variable Immunodeficiency). Other 2 diagnosis were typical SCID and 3 patients were referred from other centers, among them one had a severe nephropathy.

Conclusions:

Several possible phenotypes are related to a RAGs protein deficiency, thus immunological markers more than clinical presentation are considered now suggestive for diagnosis. The unpredictable outcome of these recently discovered RAG deficiency pose a challenge for clinicians on the more suitable treatment and proper timing of definitive treatment, such as stem cell transplantation.
THROMBOTIC MICROANGIOPATHY IN RAS-ASSOCIATED AUTOIMMUNE LEUKOPROLIFERATIVE DISEASE: CASE REPORT

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Background:

RAS-associated autoimmune leukoproliferative disease (RALD) is an autoimmune lymphoproliferative syndrome (ALPS) related disorder caused by gain-of-function somatic mutations in NRAS and KRAS genes. Patients show lymphadenopathy, splenomegaly, hypergammaglobulinemia, autoimmunity, and partial overlap with juvenile myelomonocytic leukemia (JMML). Monocytosis is an essential feature. Few cases are reported at present. They have shown broader phenotypic features than initially described.

Methods:

We present a 6-year-old boy admitted to hospital at 1 year 8 months. Physical examination revealed severe hepatosplenomegaly and multiple enlarged lymph nodes. Infectious and metabolic causes were excluded.

Results:

Persistent monocytosis, increased IgG and IgM levels, and normal “double-negative” T cells (1.57%) were established. Activated T cells were found to be resistant to apoptosis upon withdrawal of IL-2. Heterozygous somatic mutation p.G13D in NRAS confirmed the diagnosis of RALD. The patient was being closely monitored when at 4 years 11 months, epistaxis, hematemesis and hematomas appeared. Hemolytic anemia with schistocytes and decreased platelet counts conducted to the diagnosis of thrombotic microangiopathy (TM) related to RALD, provided that all other well known causes of TM were excluded. Plasmapheresis was instituted in addition with corticosteroids and rituximab. After improving his condition for one year, he suffered a relapse that required plasma administration and sirolimus as a treatment.

Conclusions:

We present a patient with diagnosis of RALD who developed TM. TM has not been reported previously associated to NRAS mutation to our knowledge, and results a critical aspect in the prognosis of the patient. Although RALD is a benign condition, a close follow up must be kept.
CLINICAL AND IMMUNOLOGICAL EVALUATION OF FIVE PATIENTS WITH ATYPICAL 22q11.2 DELETION SYNDROME

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Background:

The peculiarity of the 22q11.2 deletion syndrome is the great phenotypic heterogeneity making it a classic example of a syndrome with variable expressivity and incomplete penetrance. The reasons for this variability have not been completely elucidated. Deletions in 22q11.2 region are a consequence of non-allelic homologous recombination (NAHR) due to misalignment of low copy repeats (LCRs) during meiosis. Eight LCRs (named LRC22-A to H) have been identified, but only the four centromeric ones (LCR22-A to D) are implicated in this syndrome. As known, 90% of patients share a 'classic' ~3Mb deletion between LCR22-A and LCR22-D.

Methods:

We analyzed a cohort of 23 patients, focusing on the genetic and immunological data of 5 cases with atypical deletions.

Results:

In one case the deletion was mediated by LCR-A and B, whereas in the other 4 cases the mechanism of deletion seems not to be mediated by a NAHR event. Analysis of the additional Copy Number Variations (CNVs) elsewhere in the genome was also performed. Two rare CNVs were detected in one patient, and their gene content could influence the phenotype. Physical examination revealed a wide heterogeneity; however, global developmental delay and/or mild mental retardation, more prominent in language, was found in all patients and autistic traits in two. None of them had cardiac malformations. An "extended" immunophenotype revealed a severe T cell immunodeficiency in all patients, particularly in CD8+ subset, and in both naïve and recent thymic emigrants subsets.

Conclusions:

The variable extension of the deleted region could be a cause underlying the phenotypic heterogeneity.
HPO CODING IN PRIMARY IMMUNODEFICIENCY DISORDERS


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Background:

The NIHR BioResource – Rare Diseases project has recruited over 2,000 individuals with a primary immunodeficiency including their affected and unaffected relatives. The cohort comprises of 1400 sporadic and familial cases, with 70% having CVID, CID or Antibody Deficiency as the main diagnosis. Patients’ information were collected to include both quantitative and qualitative laboratory measurements and free text clinical synopsis. To integrate this diverse clinical information into the analyses of the genomic data, we adopted the Human Phenotype Ontology (HPO) as the standardised vocabulary of phenotypic abnormalities.

Methods:

Patients’ information were collected on paper forms, which were manually entered into a local database, then automatically transcribed into HPO using a rule system we developed. Terms assigned from free text fields were manually checked, taking between 10-20 minutes per patient. The main difficulties encountered were spelling mistakes, negative statements, and lack of ontology terms for immunophenotypes.

Results:

The 751 patients coded to date have on average 7.42 terms per patient (range 1-23). Those HPO encoded phenotypes have been used in conjunction with genomic data to facilitate in the interpretation of genetic variants, and are included in research reports for patients in whom we identified a likely or clearly pathogenic variant.

Conclusions:

Despite the lack of specific terms to encode for measurements commonly used to diagnose patients with primary immunodeficiency, HPO is a powerful tool for standardising phenotypes across different individuals, projects, disease cohorts, enabling their incorporation into a range of statistical models and bioinformatics tools.
SPECTRUM OF MUTATIONS IN WISKOTT ALDRICH SYNDROME AT TERTIARY CARE CENTER, INDIA


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Background:

Wiskott Aldrich Syndrome is an X-linked disorder with a wide spectrum of clinical manifestations ranging from intermittent X-linked thrombocytopenia (iXLT) to more severe classic lethal WAS. It is usually presented with clinical triad of eczema, thrombocytopenia and immune-deficiencies. Underlying molecular mechanism is attributed loss-of-function mutations present in Wiskott-Aldrich syndrome (WAS) gene. WAS gene encodes for Wiskott-Aldrich syndrome protein (WASp), which is involved in actin polymerization and thus cytoskeletal remodeling.

Methods:

All male child with thrombocytopenia and micro-thrombocytopenia were investigated for WAS. Clinical WAS score was calculated and WASp protein quantification was done by flow-cytometry. Molecular confirmation were carried out by Sanger’s sequencing method.

Results:

Out of 42 clinically suspected patients of WAS, 27 were molecularly confirmed to have WAS gene mutation. Detected mutations were found to be spread over whole gene sequence, including introns. Total of 23 mutations were identified in these 27 patients out of which 7 mutations were novel. 17 mutations were single nucleic acid change (15 transition and 2 transversion), 5 Deletion and 1 insertion. 15 mutations were exonic (4 missense, 6 nonsense and 5 frameshift) and 6 were intronic. Max mutation were found in exon 1 and exon 2 (39.13%).

Conclusions:

Although, targeted WAS gene sequencing strategies are not commonly employed, initial screening of Exon 1 and 2 may be recommended in resource constrained settings.
THE IMPORTANCE OF A PSYCHOLOGICAL CARE FOR PID PATIENTS – THE EXAMPLE OF A HANDBOOK ON LIFE MANAGEMENT FOR PID PATIENTS AND THEIR FAMILIES

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Background:

It is estimated that the success of any therapy in up to 70% depends on non-medical factors. This means that even a correct medical treatment may not give you the expected results if the patient will not cope with the disease in his everyday life. That is why a holistic approach to the PID patients is so important - educating them not only on medical but also psychological issues.

Methods:

Based on patient interviews and the results of our surveys, we have developed a set of key areas for the education of patients with PID. Basing on them, we have written the world's first psychological handbook specifically for PID patients and their families.

Results:

We focused on 4 key areas:

a) communication with family, loved ones and caregivers

b) coping with stress and other burdens related to illness

c) planning the path of a life - education, career, starting a family - with taking into account factors relevant to the patient with PID

d) patient's self-esteem building

Poster presents a universal model describing the key areas of psychological education of PID patients based on the example of our handbook and points to the importance of a non-medical aspects in the success of a therapy. Conclusions:

We believe that drawing attention to the psychological aspects of care of the PID patients will have a positive impact on the effectiveness of therapeutically processes as well as on patients' quality of life.
Background:

Primary immunodeficiencies (PID) occur in 1:10,000 population. The incidence of PID is unknown in Mexico.

Methods:

We report the rates of new cases of PID (x 100,000 patients registered with a family physician) in the 3 care levels at IMSS, between 2007 and 2015, according to their age group, sex, state and IDC-10 codes. know the incidence of PID at the Instituto Mexicano del Seguro Social (IMSS).

Results:

There were 27,758 PID cases, and an increase by 89% between 2007 and 2015. Half of the cases occurred in women. The states with more cases were: Mexico City (5,408), Jalisco (3,345) and Nuevo Leon (2,670). The 20-59 years of age group had 9,655 cases (34.78%); pediatric groups (0-4 years and 5-19 years) had 14,034 (50.56%) cases, and >60 years had 4,069 (14.65%) cases. The number of cases by care level were: 9,864 (35.53%), 12,388 (44.62%) and 5,506 (19.83%), in the first, second and third level, respectively. The rate for PID, in the first level was 18.46, in the second 25.46, and 15.11 in the third level. The more frequent IDC-10 PID groups were: D72 (other disorders of white blood cells) 58.33%, D84 (other immunodeficiencies) 17.37%, D80 (predominantly antibody defects) 8.86%, D82 (associated with major defects) 6.89% and G11 (ataxia) 3.25%.

Conclusions:

There is an increasing trend in PID rates. Half of the cases occurred in pediatric population. Antibody deficiencies were a sixth part of the expected. There is a low and inaccurate PID registry.
OTHERS

ESID7-0347

HYPOGAMMAGLOBULINEMIA, B-CELL ABNORMALITIES AND MENTAL RETARDATION WITH HYPERACTIVITY IN TWO SISTERS

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Background:

We present two sisters from non-consanguineous family, age nine (patient A) and seven (patient B) years, with syndromic features accompanied by IgG, IgA, and IgM hypogammaglobulinemia, B-cell lymphocytopenia and B-cell subset abnormalities.

Both patients suffer from mild mental retardation, attention deficit disorder and hyperactivity. Patient A manifested with pubertas praecox, patient B is treated for coeliac disease. Serious, atypical or frequent infections were not observed.

Methods:

First immunological examinations were carried out at the age of five (patient A) and three years (patient B).

Results:

Both patients revealed panhypogammaglobulinemia: IgG patient A: 2.33g/l; patient B: 2.49g/l (ref. range: 6.00-13.00), IgA A: 0.05g/l; B: 0.13g/l (0.60-2.20), IgM A: 0.24g/l B: 0.17g/l (0.40-1.60), very similar results were obtained during the last four years of follow-up.

Both patients had low anti-tetanic toxoid antibodies that increased after revaccination but decreased below protective level during the follow-up in patient A. Low anti pneumococcal antibodies temporary increased after protein conjugated pneumococcal vaccine decreasing again below reference range in both patients.

No abnormalities in major T-lymphocyte s subsets (CD3+,CD4+,CD8+) were observed. NK cell counts stayed within normal range.

The patients displayed marginal and low B-cell percentages A: 6.00% B: 1.00%(4.00-26.00). Assessing B-cell differentiation stages the increase of CD24++CD38++ transitional cells was the most predominant feature: A: 29.10% B: 45.00%(ref. range 4.50-9.20).

Genetic analysis ruled out microdeletion syndromes. Fragile X syndrome and subtelomeric abnormalities were not detected.
Conclusions:

Because of absence of clinical immunodeficiency symptoms and technical difficulties with immunoglobulin administration due to hyperactivity, immunoglobulin treatment was not initiated yet. NGS is considered.
SEXUAL DIMORPHISM IN HUMAN INFANT THYMUS: AIRE NETWORKS

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Background:

In the thymus AIRE functions as a transcriptional regulator and controls the ectopic expression of hundreds of genes. Estrogen-mediated downregulation of AIRE starts at puberty onset and may well influence gender susceptibility to autoimmune diseases (Dragin et al. 2016. J Clin Invest. 126:1525). Recently, our group showed that AIRE has essentially the same expression levels in males and females along minipuberty.

Methods:

Here we studied the expression (DNA microarray) of AIRE and of 41 AIRE-regulated genes in human thymus surgical explants obtained from infants up to 6 months (7 males and 7 females). We also investigated Aire protein expression in all these cases by immunohistochemistry.

Results:

The male (M) and female (F) groups showed similar expression of Aire protein. Gene coexpression networks (GCNs) were inferred for M and F groups considering AIRE and 41 AIRE-centered genes. The networks for M and F groups were rather different considering gene-gene link strengths. The AIRE network for M group revealed that 20 genes (48%) had high link strength connections (1.00 – 0.70 covariation values). Conversely, for F group the majority of the genes (88%) had link strength connections below 0.49.

Conclusions:

These results indicate sexual dimorphism in AIRE networks. Moreover, network analysis showed that AIRE may regulate thymic expression of CAND1, POLR2A, MCM6, C1QBP and MCM2 genes (link strength ≥ 0.90) in the M group.
AP3δ DEFICIENCY – A COMPLEX DISEASE OF IMMUNODEFICIENCY AND NEUROLOGICAL IMPAIRMENT

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⁴University Hospital Freiburg, CCI - Centre for Chronic Immunodeficiency, Freiburg, Germany

Background:

AP3δ is part of the heterotetramer protein complex - AP3. This complex is important for lysosome biogenesis and vesicular budding from the early endosomal compartment. Thereby AP3 sorts integral membrane proteins from the endosome to the secretory lysosome in immune cells. AP3 deficiency leads to unstable expression of the complex and mis-sorting of lysosomal proteins to the cell membrane. We studied a patient with albinism, neutropenia, immunodeficiency, neurodevelopmental delay, generalized seizures, and impaired hearing but with no mutation in genes so far associated with albinism and immunodeficiency.

Methods:

We have carried out plasma membrane profiling of AP3δ deficient human T cells from a patient with Hermansky-Pudlak Syndrome Type 10 to identify proteins that use the AP3 sorting pathway.

Results:

Our results identify a number of lysosomal transporters and adhesion molecules.

Conclusions:

The lysosomal transporter may account not only for the immunodeficiency, but also for the neuronal phenotype seen in HPS10 patients.
MULTIPLE COMPLICATIONS IN A FAMILY WITH ATAXIA-TEANGIECTASIA

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4King’s College Hospital, Paediatric Liver Unit, London, United Kingdom
5Great Ormond Street Hospital, Department of Immunology, London, United Kingdom

Background:

Ataxia-telangiectasia (A-T) causes progressive cerebellar ataxia, immunodeficiency, telangiectasia, radiation sensitivity and increased incidence of cancer.

Methods:

We present a consanguineous Pakistani family with 3 siblings suffering from A-T with a homozygous mutation (c.6198+538_6347+131 del 1002). P1 a male, in addition to the A-T phenotype had skeletal deformities, ectopic kidney and global developmental delay. He suffered respiratory infections and chronic diarrhoea. He had abnormal liver function tests with irregularly dilated bile ducts suggestive of cholangiopathy on ultrasound. At aged 13 years, he has persistent ulcerative granulomatous skin lesions. P2 a female without abnormal morphological features, suffered from recurrent diarrhoea including cryptosporidial infection and had similar hepatic ultrasound, non-specific liver biopsy and MRCP suggestive of cholangiopathy. She has not developed skin granulomas. Recently she developed Mycobacterium avium infection in her respiratory tract, treated with combination antibiotics. She developed elevated transaminases and multiple echoic lesions on liver ultrasound. Biopsy showed multiple granulomatous lesions but no evidence on microscopy, culture or PCR of infection with mycobacteria but low level EBV positivity (CT 35) of doubtful significance. At aged 10 years she is on immunoglobulin replacement. P3 had only 2 attacks of infection (impetigo and chest infection) and has normal morphology and development. He required immunoglobulin replacement early because of low IgG levels.

Results:

All three siblings have low T cell numbers and very low naïve CD4 cell counts with partial or complete IgA deficiency and IgG deficiency in one.

Conclusions:

This family illustrates a variety of complications including Cholangiopathy which is unusual in A-T.
OTHERS

ESID7-0385

MANAGEMENT OF SECONDARY IMMUNODEFICIENCY AT A TERTIARY REFERRAL CENTRE- KINGS COLLEGE HOSPITAL LONDON
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Background:
Antibody deficiency can manifest as primary disorders arising from genetic causes, or secondary to a wide range of circumstances including malignancies, chronic inflammatory/ infective diseases, drugs and iatrogenic causes. The Department of Health (DOH 2011) identifies a selection criteria for commencing immunoglobulin therapy for patients with secondary immunodeficiencies including, recurrent bacterial infection despite continuous antibiotics, IgG levels of <5 g/L and poor vaccine responses. Yet the literature on management of these patients remain limited.

Methods:
A cohort of 79 patients with secondary immunodeficiency was identified at Kings College Hospital London, between March 2016 and March 2017. The electronic medical records were retrospectively analysed with regards to age, sex, primary medical condition, drug therapy, immunoglobulin therapy, vaccine responses and the department who managed their condition.

Results:
The cohort was female dominant, with mainly haematological and respiratory disease background. Over half these patients were on steroid therapy. Of the 79 patients identified, 60% were under the care of the Immunology department, whilst the remaining 40% were either shared care or managed by other departments alone. Over half these patients were receiving immunoglobulin therapy, yet 10% were not on recommended treatment regimes. Less than half these patients were checked for vaccine responses.

Conclusions:
With an increase in identification of patients with secondary immunodeficiencies across many disciplines, it is important to ensure good communication between the different specialities to help optimise good patient care. Other specialities need to familiarise themselves with national recommendations to ensure effective patient management.
BACKGROUND:

There is little information about nutritional status in patients with primary immunodeficiencies (PID). Our aim is to know the nutritional status of our population, in order to identify comorbidities that can increase the risk of malnutrition in the long term.

METHODS:

Cross-sectional study using non-invasive methods (Body Mass Index [BMI], bioimpedance [muscle mass measurement]) to identify sarcopenia considered as less than 19%, and finally the MUST tool. Inclusion criteria: diagnosis of PID, over 18 years old, ability to perform bioimpedance.

RESULTS:

Of the 27 patients, mean age: 36 years, age range: 18-70 years, 60% women and 40% men, of which: 87% had Common Immunodeficiency Variable, 7% X-linked agammaglobulinemia, and 3% selective IgG-subclass deficiency and Good syndrome.

Population with normal BMI 55%, overweight 30% and 15% obesity, none with low weight, according to the criteria adjusted to Mexican population.

Bioimpedance: resulted average muscle mass 28.09%, only 7.4% present muscle mass less than 20% with risk of developing sarcopenia.

According MUST: 74% low, 15% intermediate and 11% high risk, because our population does not have a significant amount of weight loss.

CONCLUSIONS:

According to IMC we have a significant percentage for overweight and obesity, which exposes them to risk of metabolic diseases that must be prevented. On the other hand, patients with sarcopenia should be adequately assessed as this is considered a marker of poor prognosis in the case of infections. According to MUST our patients are at risk of malnutrition, so they must be sent to nutrition for their proper approach.
OTHERS

ESID7-0392

ABSENCE OF B-CELLS AND DEEP NK-CELL LYMPHOPENIA MIMICKING PRIMARY IMMUNE DEFICIENCY IN NEONATAL PERIOD DUE TO ANTI-LYMPHOCYTE MATERNOFETAL ALLOIMMUNISATION

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Background:

Neonatal cytopenias due to allo-immunisation are common, and affects predominantly platelets, red blood cells and neutrophils. In case of neonatal alloimmune thrombocytopenia (NAIT), anti-HLA type I antibodies can be found with debatable role in disease.

Lymphopenia at birth is always suggestive of primary immune deficiency, and should prompt further evaluation.

We here describe a newborn boy, with total lymphopenia, no B cells, low NK cells, thrombocytopenia, due to maternofetal immunisation to HLA class II antigens.

Methods:

Laboratory work-up and follow-up of this case includes repeated complete blood counts; serial flow cytometry immunophenotyping (including CD3, CD4, CD8, CD19, CD20, CD22, CD31, CD45RA, CD45RO, CD16, CD56, HLA-DR staining); lymphocyte proliferation assay; KRECs/TRECs determination on dried blood spot; antiplatelets antibodies determination; anti HLA type I and II determination (ELISA)

Results:

<table>
<thead>
<tr>
<th>FACS</th>
<th>Day 5</th>
<th>Day 15</th>
<th>Day 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Lymphos</td>
<td>2470</td>
<td>4890</td>
<td>4950</td>
</tr>
<tr>
<td>CD19 (µl)</td>
<td>10</td>
<td>150</td>
<td>680</td>
</tr>
<tr>
<td>CD3 (µl)</td>
<td>2290</td>
<td>4410</td>
<td>3910</td>
</tr>
<tr>
<td>CD3/CD4 (µl)</td>
<td>1900</td>
<td>3510</td>
<td>3130</td>
</tr>
<tr>
<td>CD3/CD8 (µl)</td>
<td>390</td>
<td>840</td>
<td>680</td>
</tr>
<tr>
<td>NK cells (µl)</td>
<td>70</td>
<td>250</td>
<td>240</td>
</tr>
<tr>
<td>CD3/HLA DR (µl)</td>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Naive T cells subsets were normal for age. Proliferation assays were normal. KRECs/TRECs analysis are pending.

Both antiplatelets and anti HLA class II were present in mother's serum

Conclusions:
This is the first case of a transient lymphopenia due to anti-HLA type II antibodies. As expected in this setting, B cells and NK cells, that express constitutively HLA-DR antigen were the most impacted cell types. Resolution occurs within 3 months, as in other allo-immune neonatal cytopenias.
FATIGUE IN PEDIATRIC PATIENTS WITH PRIMARY IMMUNODEFICIENCY

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Background:

Fatigue is a commonly reported clinical symptom in children with primary immunodeficiencies (PIDs) and has a profound effect on patients’ quality of life. The exact prevalence and level of fatigue in pediatric patients with PID is currently unknown. Therefore, the aim of this study is to objectively assess the level of fatigue in pediatric patients with PID.

Methods:

In March of 2017 a cross-sectional questionnaire-based survey was performed in children visiting a tertiary university hospital in the Netherlands. Parents scored fatigue of their child with the age specific PedsQL Multidimensional Fatigue Scale. A higher score indicates less fatigue, with a maximum score of 100 indicating no fatigue. Univariate descriptive statistics were conducted on the data.

Results:

Preliminary data of 20 children (mean age (range): 10.7 years (range 2-17 years); 70% male) were studied. 35% of patients had IgA deficiency, 30% were single cases with other types of PIDs, 25% CVID and 10% ADA2 deficiency. The mean (SD) total fatigue score was 59.6 (SD 16.8). For IgA deficiency mean score was 54.2 (SD 15.6), for ADA2 deficiency 59.7 (SD 25.5), for CVID 62.2 (SD 21.4) and for other type of PIDs 63.8 (SD 14.5).

Conclusions:

Surprisingly, our preliminary data indicate that parents of patients with IgA deficiency score their child to be the most fatigued. Further research in a larger sample and with multivariate analysis are ongoing to determine well-powered prevalence and level of fatigue in patients with PID.
Background:

Intrauterine growth restriction (IUGR) induces a thymic atrophy due to massive apoptosis of thymocytes. In such context, the expression of specific proteins of medullary thymic epithelial cells (mTECs) is supposed to be altered. We aimed to characterize the neonatal thymic morpho-phenotype in rats born after IUGR.

Methods:

Sprague-Dawley rats were exposed to normal or to low-protein diet (LPD, 9% casein) during gestation. At postnatal day (PND) 2, the thymus was harvested and frozen, then imbedded in paraffin. Slices were either stained with hematoxylin/eosin or labeled with immunofluorescent (IF) antibodies directed against cytokeratins (CK) 5, 14 and 8+18 and MHC I and II and AIRE.

Results:

At PND2, the CMR was significantly lower (3.00 vs. 3.49; p=0.049) in thymic slices from newborn IUGR rats. The IF expression intensity (mean in arbitrary units ± SD) of CK5 (9.88±0.85 vs. 8.64±1.31; p=0.42), CK14 (15.55±1.24 vs. 16.95±1.11; p=0.42) and CK8+18 (4.15±0.48 vs. 4.43±0.40; p=0.67) was not significantly different between groups; neither was the expression of AIRE (14.26±1.36 vs. 14.76±1.88; p=0.86), MHC I (13.57±0.76 vs. 11.72±0.30; p=0.08) and MHC II (10.42±1.37 vs. 7.99±1.69; p=0.33).

Conclusions:

These findings confirm the abnormal morpho-phenotype of the thymus observed after exposition to IUGR, with a reduced CMR indicating the massive thymocytes apoptosis in such context, and show no effect of IUGR on the expression of specific proteins of mTECs. Further studies may address functional analysis of mTECs and thymic output to better understand the mechanisms of the short-term and long-term effects of IUGR on immune functions.
OTHERS

ESID7-0443

TRANSFUSION-ASSOCIATED GRAFT-VERSUS-HOST DISEASE IN A SEVERELY IMMUNOCOMPROMISED PATIENT

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Background:
A 40-years old HIV-seropositive male was diagnosed with Hodgkin lymphoma (Ann Harbor Stage IV) in 2016. Standard chemotherapy was started after interruption of HAART and he presented progressive headache and altered mental status due to cryptococcal meningitis. Despite treatment with amphotericin B the patient had clinical deterioration and was admitted to ICU with pneumonia, pyelonephritis and septic shock. Multiple antibiotic regimens and anticonvulsants were used as well as several transfusions of non-irradiated blood products. He developed a post-transfusion maculopapular rash which was treated with hydrocortisone and promethazine. He also presented diarrhea and new skin lesions that evolved to purpuric macules and plaques, some of them progressing to blisters.

Methods:
Skin biopsies hypothesizing drug eruption or transfusion-associated graft-versus-host disease (TA-GVHD) were done, considering multiple transfusions and concomitance of conspicuous skin lesions, fever and diarrhea in a severely immunocompromised patient.

Results:
Histopathological examination showed interface dermatitis with dermo-epidermal detachment and apoptotic basal layer keratinocytes. Edema and focal hemorrhages in papillary dermis were observed, with predominantly perivascular infiltrates, and some eosinophils. Necrosis of eccrine sweat glands was also detected. These findings were consistent with TA-GVHD.

Conclusions:
Although HIV infection is not considered a risk factor for TA-GVHD this condition may represent an additional hazard in patients with associated immunosuppressive disorders such as Hodgkin lymphoma (particularly under chemotherapy) and sepsis. TA-GVHD is a rare complication of blood transfusions - particularly in immunocompromised individuals - and must be ruled out even when a drug eruption is the most likely diagnosis.
THYROID CARCINOMA IN A CHILD WITH ACTIVATED PHOSPHOINOSITIDE 3-KINASE Δ SYNDROME (APDS): A SOMATIC EFFECT OF A GERMLINE MUTATION

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Background:

Introduction: Activated phosphoinositide 3-kinase δ (PI3Kδ) syndrome (APDS) is a primary immunodeficiency caused by heterozygous gain-of-function mutations in the PI3KCD or PI3KR1 genes. The hyperactivation of PI3Kδ causes downstream upregulation of AKT and mTOR, resulting in hypogammaglobulinemia, lymphoproliferation and T cell senescence. The clinical phenotype of APDS is characterized by recurrent upper and lower respiratory infections with encapsulated bacteria as well as chronic, recurrent or persistent infection with Herpesviruses. Lymphoproliferation, autoimmune and autoinflammatory manifestations and increased risk of lymphoma are other hallmarks of the disease.

Methods:

Case report: A 15-year-old girl presented with recurrent upper and lower respiratory infections from early infancy, with development of bronchiectasis at 7 years of age. Her immunological phenotype consisted of hypogammaglobulinemia, absence of switched memory B cells and T helper cell lymphopenia with low naïve T cells. At the age of 13 years old she developed a papillary thyroid carcinoma with local lymph node and lung metastases. She was treated with total thyroidectomy and radioactive iodide.

Results:

She was diagnosed with APDS due to a gain-of-function mutation in PIK3CD (p.Glu1021Lys).

Conclusions:

Discussion: The combination of both a rare primary immunodeficiency and a very rare childhood cancer together with the description of upregulated PIK3CD in some thyroid cancers urges us to consider that the germline activating mutation in PIK3CD indeed underlies the development of thyroid cancer in this patient. This is relevant both from the perspective of early diagnosis of the malignancy but also from the perspective of targeted treatment, which is available for PIK3CD gain-of-function mutations.
OTHERS

ESID7-0455

ESTIMATION OF DISEASE FREQUENCY AND OUTCOMES IN RARE DISEASES: A SYSTEMATIC LITERATURE EXAMPLE ON WISKOTT-ALDRICH SYNDROME (WAS)

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Background:

There are several studies describing the epidemiology of primary immunodeficiencies (PIDs) globally, however, disease specific data is rarely summarised. These data are vital to improve disease understanding to support commercial and clinical development strategies and for regulatory and policy purposes.

Rare disease epidemiology face major challenges with limited sample size, sparse literature, complex diagnoses and lack of diagnostic codes in Electronic Medical Records. The aim of this study was to estimate WAS incidence/prevalence and to describe outcomes following hematopoietic stem cell transplantation (HSCT) from the literature.

Methods:

PubMed, Embase and PID registries were searched and all identified articles were assessed in a multi-stage screening process. Methods on how best to select and summarise published data will be described.

Results:

Thirty articles were used and the incidence of WAS ranged from 0.01 in Spain to 24 per million live births in Australia. Most recent estimates came from Iran and Greece. WAS prevalence ranged from 0.60 (Morocco) to 2.0 (France) per million inhabitants.

Seventeen articles were selected with 5-year overall survival between 62.5-92% and the proportion of WAS patients alive following HSCT ranged from 60-100%, largely depending on donor type. Chronic graft versus host disease (GvHD) developed in 25% of the WAS patients 1 year post-HSCT, with mean frequency ranging from 0% to 42%. Acute GvHD was reported in 45-67% of patients following HSCT.

Conclusions:

Dated disease frequency estimates, increased newborn screening, improved diagnostic techniques, and more established networks and registries, highlight the need for more recent estimates.
Background:

Monoallelic STAT1 gain of function (GoF) mutations have been identified as the predominant cause of chronic mucocutaneous candidiasis (CMC) in which susceptibility to non-invasive candida species is mediated by a deficiency of Th17 T-cells. More recently, a different STAT1 GoF mutation (F172L) has been demonstrated in a child diagnosed with Common Variable Immune Deficiency (CVID) who also had candida infections.

We have identified several monoallelic STAT1 missense mutations adults with CVID who do not exhibit fungal susceptibility but instead present with antibody deficiency, bacterial infections and autoimmunity.

Methods:

Using baseline and stimulated (IFN-α) phospho-STAT1 flow cytometry assays in primary cells and EBV LCL cell lines, we have shown that L60F and V266I STAT1 mutation confer gain of function.

Results:

We have also demonstrated that elevated phospho-STAT1 is not a generalised feature in patients with CVID, in comparison to matched healthy individuals.

Conclusions:

These findings suggest that STAT1 GOF mutations identify a subset of CVID, possibly with an increased risk of autoimmunity.
Background:
A male child presented to us at 1 year of age with pneumonia. He was symptomatic since 3 months of age, had recurrent sino-pulmonary infections, otitis media and blood stained stools. His elder brother was also symptomatic and had similar complaints. No history suggestive of X-linked disease in the family was forth coming.

*part of study was presented in ICID 2017(Bangalore, India)

Methods:
Investigation: In view of clinical profile, micro-thrombocytopenia with low IgM, diagnosis of WAS was suspected. WASp protein quantification through flow-cytometry gave ratio of SI of patient: control = 0.622 and that of brother was 0.697. Leukocytes and buccal swab DNA samples were sequenced.

Results:
Sanger sequencing of WAS gene reveled an in vivo reversion of mutations in exon 10, (Type 1) "C" according to the Genbank report, 1189_1190delC leading to frameshift at 397 and creating stop codon (TGA) at 444, P397fsX444; and (Type 2) "ACCGCCACCACC" according to the Genbank report, 1188_1199del, leading to "PPPP" deletion at position 401-404, 401_404del. Identical mutation was observed in his brother while his mother was an heterozygous carrier of the mutation Type 1. Due to reversion (Type 2), the effect of WAS mutat ion (Type 1) is likely to become less damaging in the index patient and his brother. Buccal swab samples of both children revealed presence of only Type 1 mutation, suggestive of mosaicism, which needs further characterization.

Conclusions:
We report a novel mutation with reversion of stop codon formation in leukocytes of two brothers with Wiskott-Aldrich Syndrome.
IDENTIFICATION OF A NOVEL WAS MUTATION IN A SOUTH AFRICAN PATIENT PRESENTING WITH ATYPICAL WISKOTT-ALDRICH SYNDROME

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Background:

Wiskott-Aldrich syndrome (WAS) is a rare X-linked recessive primary immunodeficiency disease characterized by immune deficiency, eczema, thrombocytopenia and bloody diarrhea. Here, we describe a one-year old male South African patient who presented at 6 weeks with suspected WAS but platelets that were normal in size. The use of exome sequencing revealed a novel mutation in WAS, thereby allowing for an accurate clinical diagnosis.

Methods:

The index case, an HIV negative, severely malnourished infant of non-consanguineous parents was referred for chronic diarrhea and extensive cytomegalovirus (CMV) pneumonia requiring intubation. His course was complicated by culture negative septic arthritis, food allergy, episodes of leucoclastic vasculitis, eosinophilic gastritis, inflammatory bowel disease and recurrent wheezing. Blood was drawn from the index case and both parents and DNA extracted. Whole exome sequencing (WES) was performed and the data was processed using an in-house bioinformatics pipeline, TAPER™.

Results:

A hemizygous missense mutation c. 397 G>A in exon 4 of WAS was identified. Sanger sequencing subsequently revealed that the variant was heterozygous in the mother and absent from the father of the index case. Moreover, this variant was excluded from all healthy controls that were screened.

Conclusions:

The identification of novel variants associated with disease has the potentially to greatly improve our understanding of the pathobiology of a disease. The current study made use of WES and a custom designed bioinformatics tool to identify the disease-causing variant in the patient. Following definitive molecular diagnosis using WES, we could offer the family genetic counselling and prenatal diagnostic testing for future pregnancies.
COEXISTENCE OF 2 RARE AUTOSOMAL RECESSIVELY INHERITED DISORDERS MANIFESTING WITH IMMUNE DEFICIENCY; IL-12 RECEPTOR β1 AND BIOTINIDASE DEFICIENCIES

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Background:

In this report, we described an infant with both partial biotinidase and IL-12Rβ1 deficiencies as these two entities are rare and unrelated inherited disorders.

Methods:

Genetic testing to confirm the diagnosis of IL-12Rβ1 and Biotinidase deficiency was done as part of genetic consultation with Medical Genetics Department of Çukurova University Faculty of Medicine.

Results:

One-month-old girl was diagnosed as partial biotinidase deficiency with newborn screening programme. Mutation analysis revealed a compound heterozygous mutation BTD: c.1330G>C (p.Val444Leu) / c.196_197dupCATC (p.Leu69HisfsTer24). At the age of 6 months, a nodule on her left axilla with purulent discharge was noticed which was related BCG vaccination. Acting from this point a suspicion of IL-12Rβ1 deficiency was improved with a mutational analysis revealed a homozygous c.783+1G>A mutation on IL-12Rβ1 gene. Interferon-gamma and anti-tuberculosis treatment were initiated together and the nodule with purulent discharge regressed dramatically.

Conclusions:

Here, we want to emphasize to considering coexistence of two rare autosomal recessively inherited diseases in a patient due to the high rate of consanguinity in our country.
STRONGYLOIDES STERCORALIS INFECTION IN THE PRESENCE OF SELECTIVE IgA AND IgG2 SUBGROUP DEFICIENCY

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Background:

Introduction: Strongyloides stercoralis (S. Stercoralis) is an intestinal nematode that causes infection especially in tropical and subtropical regions. Most of the affected individuals are asymptomatic, but important clinical situations were seen in patients with immunodeficiency. This report presents an immunodeficient patient with S. stercoralis infection.

Methods:

Case report: A 22-year-old male patient presented with recurrent lower respiratory tract infections and dispeptic complaints. The patient had a history of recurrent diarrhea. Some tests were performed and after all selective IgA and IgG2 subgroup deficiency, hypochromic micrositeranemia and eosinophilia were detected. (IgA: 0.305 g / L (0.82-4.53), IgG2: 2.06 g / L (2.42-7.07), Hgb: 11.5 g /dl (13-17), Hct: 38% (39-51), MCV: 67.7 fL (80-100), eosinophil count: 2000 /mm3). Upper gastrointestinal system endoscopy was performed and parasitic microorganisms compatible with microfilaria were detected in the pathological examination of the duodenum, where the thrown salt sight and mucosa carding were observed. Gaita examination showed giardia intestinalis cysts and metranidazole, albendazole and Ivermectin treatments were administered respectively. Two weeks after Ivermectin treatment no parasites were found in repeated gaita examinations.

Results:

Discussion and conclusion: Immunodeficiency should be suspected in cases of parasitosis caused by recurrent, treatment-resistant, multiple or rarely observed agents.

Conclusions:

If immunodeficient patients have gastrointestinal complaints such as diarrhea and dyspepsia rare parasites such as S. Stercoralis should be thought as the causative agent and diagnostic procedures should include endoscopic procedures in addition to gaita examination.
AUTOSOMAL DOMINANT HYPER-IGE SYNDROME (AD-HIES) AND TUBERCULOSIS: HOW TO MANAGE THE INFECTION?

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Background:

AD-HIES is a multi-system disease which exhibits elevated IgE levels, connective tissue disorders and recurrent infections. An increased susceptibility to Mycobacterium infections has been proposed.

Methods:

We present a case of AD-HIES with pulmonary tuberculosis with the subsequent associated management difficulties

Results:

17 year-old boy, born in Mauritius from non-consanguineous parents, with a history of recurrent soft tissue, lymph node and lung abscesses from the age of 8 months. At 5 years, after having moved to the U.K., he presented with a liver abscess and was diagnosed with AD-HIES (STAT-3 heterozygous mutation). On antifungal and antibacterial prophylaxis he then had a prolonged period (7 years) of good health with only eczema and severe facial acne, until recently, presenting with liver abscess (S. aureus) and intraabdominal fungal lymphadenitis (C. albicans). Due to the recurrence of these infections, a bone-marrow transplant (BMT) was planned. While still on treatment, he developed a new productive cough, with constitutional symptoms and fever. CT chest showed nodular changes, consistent with tuberculosis, and sputum culture identified M.tuberculosis (proved non-resistant). Investigations showed no evidence of disseminated infection. Started on standard TB quadruple therapy with additional moxifloxacin. Steroids were added and weaned within a month. Symptomatically he recovered quickly although sputum clearance was slow.

Currently on treatment, there is still uncertainties regarding adequate duration and timing of BMT.

Conclusions:

This case highlights the lack of evidence for the management of atypical infections in patients with primary immune deficiencies – and moreover, the need to capture such patients in national and international registries.
NOVEL MUTATION IN FOXN1 GENE PREDISPOSES TO SEVERE EBV INFECTION AND BURKUT’S LYMPHOMA

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Background:

Nude severe combined immunodeficiency (SCID) is a rare immunodeficiency due to deficiency of FOXN1, a transcription factor essential for the development and function of thymic epithelial cells (TECs). The study of animal models with Foxn1 mutations showed critical importance in TEC differentiation, homeostatic maintenance and T-cell lymphopoiesis. Lack of regular T-cell development and selection and nail dystrophy.

Methods:

We describe 4-months old boy, who presented to us with generalized lymphadenopathy. He was found to have alopecia, nail dystrophy, massive generalized lymphadenopathy and hepatosplenomegaly.

Results:

He had pancytopenia with WBC of 2800, Neutrophils; 1770, lymphocytes; 480, thrompocytes; 61 (cells/µL) and Hb; 9 g/dL. Elevated Immunoglobulin M of 22 g/L, normal IgG 4.34 and low IgA < 0.05 (g/L). He had severe panlymphopenia with CD3⁺ of 45, CD4⁺ 4, CD8⁺ 9, CD19⁺ 14, CD16⁺ 349 (cells/µL), CD3⁺/CD4⁺/CD45⁺/RA=1%, CD3⁺/CD4⁺/CD45⁺/RO=96%. T-cell receptors (TCR) γδ were 90% and αβ =10% and CD3⁺/CD4⁺/CD8⁺ (double negative) = 83%. He had very high EBV viral load of 339.095 by PCR. Lymph node biopsy showed high grad Burkitt’s lymphoma. He was started on IVIG, IV Ganciclovir, chemotherapy, and work up for BMT was initiated. The genetic study showed homozygous novel mutation in FOXN1gene: c.1579_1580del p.(Thr527*); Chr17(GRCh37): c.1579_1580del. Both parents were heterozygous for the same mutation. However, he developed fulminante pseudomonas sepsis and died with 6-hours despite aggressive IV antibiotics.

Conclusions:

This is the first report of novel mutation in Foxn1 gene caused nude SCID, that could predisposes this rare disorder to fulminante EBV infection, Burkitt’s lymphoma and uncontrolled sepsis.
Genetic diagnosis of two patients with Hermansky Pudlak syndrome type 2 in Iran


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Background:

Hermansky Pudlak syndrome type 2 (Hps2) (OMIM#608233) is a rare autosomal recessive disorder with mutation in AP3B1 gene, which encodes B3A subunit of the Adaptive protein 3 (AP3) complex. AP3 mediates sorting of transmembrane proteins to lysosomes and related organelles. HPS2 belongs to the groups of immunodeficiency disease with hypopigmentation including Griscelli and Chediak Higashi. Genetic diagnosis would help to differentiate these defects.

Methods:

Five unrelated patients suspected to Hps2 with a clinical phenotype of partial albinism, neutropenia, thrombocytopenia, hypogammaglobulinemia, leucopenia, gastroenteritis, recurrent fever, and chronic diarrhea. By rule out of Griscelli and Chediak Higashi, they suspected to have Hps2 mutation. So, the AP3B1 gene was considered for the study. Blood samples was taken from the patients after taking informed consents and DNA extracted from PBMCs. Polymerase chain reaction (PCR) was accomplished for exon 1 to 27 of AP3B1 gene and PCR products were subjected to sequencing analysis.

Results:

A large deletion from exon 10 to 24 in AP3B1 gene which is confirmed with RT-PCR and a deletion in exon 14 of this gene was found by next generation analysis in two patients respectively.

Conclusions:

This is the first genetic report of Iranian patients with HPS2. This study can help HPS2 patients to consider for HSCT and also provide valuable information for genetic counseling especially for those who have a history of immunodeficiency in their families and prenatal diagnosis.
Cutaneous manifestations in patients with Primary Immunodeficiency Disease in the Balearic Islands

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Background:

Primary immunodeficiencies (PID) are rare diseases associated with serious or life-threatening medical complications. Documentation of patients with PIDs extends our knowledge about the characteristic features of these disorders and is essential for the development of diagnostic and therapeutic strategies. Cutaneous manifestations are common in PIDs and may be the presenting clinical manifestations.

Methods:

Our series of PIDs from Balearic Islands in Spain comprises 150 patients with an overall prevalence of 12 per 100,000 inhabitants and 33% (n=48) of these patients were diagnosed in paediatric age (age <16 years). IgA deficiency represented the most common entity in this group (n=29; 60%), followed by Di-George anomaly (n=7; 15%), CVI (n=5; 10%), and X-linked agammaglobulinemia (n=5; 10%).

Results:

Our retrospective study showed that 10% patients (n=5) presented with skin manifestations as an early feature of the disease, including five patients with dermatitis resistant to treatment: two males with Wiskott-Aldrich-syndrome due to a mutation in the WAS gene presenting with new-born eczema, a female with autosomal STAT3 negative hyper-IgE syndrome (HIES) and eczematous rash, a male with immune dysregulation polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome with atopic dermatitis, and a male with Omenn syndrome showed erythroderma from birth. Other cutaneous manifestations included mucocutaneous candidiasis in a patient diagnosed with STAT1-GOF mutation, as well as other skin infections. Bacterial skin infections were more prevalent in congenital defects related to the number and/or function of phagocytes, as we observed in our patients with HIES and X-linked chronic granulomatous disease.

Conclusions:

Skin manifestations are common findings among several PIDs and may aide in the early detection and treatment of immunologic defects.
COMPLEMENT FUNCTION TEST (CH50) FOR MONITORING THE USE OF ECULIZUMAB IN A CASE OF ATYPICAL HAEMOLYTIC URAEMIC SYNDROME

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Background:

Atypical haemolytic uraemic syndrome (aHUS) is a rare thrombotic microangiopathy that is characterized by haemolysis, platelet consumption and renal injury.

The latest knowledge on aHUS reveals ongoing complement activation. Literature review shows controversial views on CH50 in monitoring eculizumab use. Some used CH50 to decide spacing out eculizumab use, while others suggested that CH50 measured during treatment did not parallel disease activity. There was also no consensus for CH50 cut-off.

Methods:

A boy presented at 18 months of age with gastroenteritis and status epilepticus. Renal function was deranged. Blood film showed microangiopathic haemolytic anaemia and thrombocytopenia, while ADAMTS13 antigen and activity were normal. The only positive microbiological finding was influenza A. He was diagnosed to suffer from aHUS. Plasmapheresis, steroid and renal replacement therapy were given with gradual recovery of renal function and haematological parameters. Genetic workup revealed no known pathogenic mutations for complement factor H (CFH), I (CFI) or B (CFB), MCP (membrane cofactor protein, CD 46), C3, THBD (thrombomodulin) and DGKE (diacylglycerol kinase e) genes. No anti-factor H antibodies were present. CFH was normal while CFI was borderline low. Ten months after complete remission, aHUS recurred at 30 months old. 3 courses of Eculizumab, a monoclonal anti-C5 antibody, were given with gradual recovery. His progress was monitored by biochemical, haematological parameters and CH50, which was used as a guide for eculizumab use.

Results:

In our case, CH50 after first eculizumab and just before third eculizumab therapy both showed reduced CH50.

Conclusions:

We demonstrated that CH50 can only be used for complementing biochemical and haematological parameters in disease monitoring.
Background:

A coordinated data collection in immunologic disorders is updated by clinical pharmacists in real-time and aimed at the continuous systematic analysis and interpretation of health data is carried out by OBSERVIG. In 2013, a monocentric prospective observational study oPTIMuM (risk factors, patient profile and tolerance of intravenous immunoglobulins) is setting up to evaluate the relationship between risk factors for adverse events and the infusion procedures and tolerance of polyvalent or specific IVIG in immunomodulation.

Methods:

An oPTIMuM form includes patient demographics, treatments, indications, biomonitoring and risk factors. It is complete continuously by all junior hospital pharmacists and senior clinical pharmacists during hospitalisation in previously untreated or treated patients. A toolkit oPTIMuM is implemented to standardize data acquisition. A pilot study is added in order to establish a predictive score IVG’s intolerance: oPTIMuM score.

Results:

An analysis of database associate with oPTIMuM score is done to evaluate the profile type for intolerant patients (age, indications, treatment and target risk patients: diabetics, obesity, hypertension, pre-existing renal insufficiency, concomitant dose of nephrotoxic drugs).

This score describe tolerance profile of patients receiving IVIG in immunomodulation and compliance with recommendations issued by OBSERVIG working group infusion procedures. Clinical pharmacists improved the personalized protocol infusion for this group of patients (dose schedule, limitation of infusion rate, hydration, premedication).
Conclusions:

The oPTIMuM score remain to be validated on a wider population in immunomodulation. The OBSERVIG network improve quality of comprehensive care and secure the use of IVIG.
Studies on the presence of bacteria in the mucous membranes of the nose and throat in patients with primary immunodeficiency

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Background:

The aim of the study was to analyze the microflora of the mucous membranes of the nose and throat in the context of the presence of Lactobacillus and Bifidobacterium probiotic bacteria in children with primary immunodeficiency (PNO) and in children with recurrent respiratory tract infections (RRTI); And in which the PNO was excluded.

Methods:

The study included 74 patients (non-infected): 34 patients with PID, 20 with RRTI, 20 healthy people. All were marked: WBC, Gran., CRP, IgG, IgA, IgM, IgE, lymphocyte panel (CD3 +, CD4 +, CD8 +, CD19 +, CD56), Vit. D3, nasal and throat mucus was analyzed.

Patients included in the study included 78 species of microorganisms identified and identified using MALDI Biotyper Identification and identification

Results:

Streptococcus pneumoniae and Moraxella catharralis are the most common pathogens in the mucous membranes of the nose and throat.

Among the strains considered potentially probiotic, 6 species were isolated:

Lactococcus lactis in 1 patient in throat culture
Lactobacillus casei in 1 patient in throat culture
Lactobacillus fermentum in 5 patients in throat culture
Lactobacillus oris in 1 patient in throat culture
Lactobacillus paracasei in 3 patients in throat culture
Lactobacillus rhamnosus in 6 patients in throat culture.

Conclusions:
Among the strains considered potentially probiotic, 6 species were isolated: Lactococcus lactis, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus oris, Lactobacillus paracasei, Lactobacillus rhamnosus.
PARALLEL SESSION : ORAL ABSTRACT PRESENTATIONS

ESID7-0009

THE EXTENDED PHENOTYPE OF 15 PATIENTS WITH GAIN-OF-FUNCTION IN STING
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Background:

Gain-of-function mutations in TMEM173 encoding STING underlie a type I interferonopathy, poorly responsive to conventional immunosuppressive therapies and associated with high morbidity and mortality. This disease is variably characterized by early-onset systemic inflammation with fever, a severe skin vasculopathy, and interstitial lung disease (ILD).

Methods:
We provided clinical, radiological, histological and immunologic data of 15 patients from 11 families.

**Results:**

The patients carried the p.V155M (N=9), p.N154S (N=3), p.V147M (N=1), p.C206Y (N=1) or p.R281Q (N=1) substitution in *TMEM173* resulting in gain-of-function in STING. The median disease onset was 3.1 years [0.1 – 13]. Failure to thrive was almost constant and a severe growth failure below – 3 SD was observed in 7 patients. The core features, systemic inflammation, skin vasculopathy and ILD, were observed respectively in 14/14, 12/15 - with amputations in 3/12 - and 14/15 patients. Histological analysis of the lungs revealed macrophage alveolar infiltration, lymphoid infiltrate and follicular hyperplasia. Four patients had end-stage respiratory failure, among which 2 underwent lung transplantation. Severe destructive polyarthritis was seen in 2 patients, a phenotype so far not described in STING patients. Most patients presented with T-cell defect, in particular low counts of central memory and effector memory CD8+ cells. When tested, T-cell proliferation was normal in response to mitogens but impaired in response to antigens. Out of 13 alive patients, 8 are currently treated with a JAK1/2 inhibitor and promising efficacy was confirmed.

**Conclusions:**

We described the largest cohort of STING patients, extending the clinical and immunological phenotype and emphasising heterogeneity of this condition.
MUTATION IN EFL1, AN SBDS PARTNER, IS ASSOCIATED WITH INFANTILE PANCYTOPENIA, EXOCRINE PANCREATIC INSUFFICIENCY AND SKELETAL ANOMALIES

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Background:

During ribosome maturation, the 40S ribosomal subunit binds the transcribed mRNA; then joins the 60S ribosomal subunit to form a functional 80S ribosome. To prevent premature association of the ribosomal subunits, eIF6 binds the 60S subunit within the nucleus. Its release in the cytoplasm enables ribosomal maturation; this step is impaired in SDS.

Methods:

Exome analysis was used to identify the molecular basis of pancytopenia, exocrine pancreatic insufficiency and skeletal abnormalities in four patients from two unrelated families. Fluorescence microscopy was used for the localization of the yeast eIF6 homologue, Tif6-GFP in yeast WT and mutant cells. Human and yeast EFL1 proteins, WT and mutant, were expressed in S. cerevisiae BCY123 strain. Circular dichroism and Small-angle X-ray Scattering were used to assess the folding and flexibility of these proteins. Green malachite colorimetric assay was performed to determine EFL1 GTPase activity.

Results:

The four patients were homozygous for the R1095Q mutation in the EFL1 protein. Residue R1095 is conserved in all members of the EFL1 protein family. The mutation did not affect EFL1 expression level or fold but increased the protein flexibility. The mutation did not alter EFL1 GTPase activity or its activation by SBDS and by the 60S subunit. Nonetheless, Tif6-GFP was localized to the cytoplasm in mutant yeast cells in contrast to its nuclear localization in WT cells.

Conclusions:

The EFL1 R1095Q mutation is associated with SDS-like disease; similar to the pathomechanism of SDS, homozygosity for this mutation perturbs the release of Tif6 from the 60S subunit, uncoupling it from EFL1-GTP hydrolysis.
PROMIDISα: A TCRα SIGNATURE FOR THE MOLECULAR DIAGNOSIS OF IMMUNODIFICENIES CAUSED BY HYPMORPHIC V(D)J RECOMBINATION/DNA REPAIR DEFECTS

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**Background:**

Diversification of immune repertoires (TCR and Ig) is critical to face all foreign antigen situations and avoid autoimmunity. The V(D)J recombination, initiated by the RAG1/2 lymphoid specific factors, accounts for this diversification through a cut-and-past process of somatic DNA rearrangement. Impaired V(D)J recombination results, in its more severe form, in Severe Combined Immunedeficiency without T and B cells (T-B-NK+ SCIDs), revealed soon after birth from infectious complications. Hypomorphic mutations in RAG1/2 or the DNA repair genes Artemis, PRKDC, Cernunnos, and DNA-LigaseIV preserve residual VDJ recombination activity, allowing development of B and T cells to some extent but resulting in alteration of their immune function, causing Combined Immune Deficiency (CID), Common Variable Immune Deficiency (CVID), Late Onset Immune Deficiency (LOCID) or autoimmunity often revealed in adulthood. Identification of the molecular causes in these adult patient remains challenging.

**Methods:**

We developed a biomarker based on the analysis of the TCRα repertoire by NGS in peripheral lymphocytes.

**Results:**

14 patients from newborn to adults with hypomorphic mutations in various VDJ recombination factors presented with a typical TCRα signature distinct from that of 27 healthy controls. Several of these patients were indeed diagnosed with the help of this biomarker, pointing to candidate gene for sequencing. The same assay proved to be very robust also in the identification of ATM patients, which harbor a distinct TCRα signature.

**Conclusions:**
PROMIDISa is a very efficient tool to help diagnosis of hypomorphic VDJ recombination deficiency and ATM patients. It may prove useful in the future for other immunodeficiency conditions.
TARGETED EXOME SEQUENCING FOR PRIMARY IMMUNE DEFICIENCIES LEADS TO IDENTIFICATION OF ARPC1b AS THE CAUSE OF A NOVEL COMBINED IMMUNE DEFICIENCY

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³Tor Vergata University, Department of Systems Medicine, Rome, Italy
⁴San Raffaele Scientific Institute, Center for Translational Genomics and BioInformatics, Milan, Italy
⁵San Raffaele University, Vita Salute, Milan, Italy
⁶San Raffaele Scientific Institute, Division of Experimental Oncology- Unit of B-cell Neoplasia, Milan, Italy
⁷San Raffaele Scientific Institute, Division of Immunology- Transplantation- and Infectious Diseases- Biocrystallography Unit., Milan, Italy
⁸Children’s Hospital Bambino Gesù, University Department of Pediatrics- Unit of Immune and Infectious Diseases, Rome, Italy
⁹National Institute of Allergy and Infectious Diseases- NIH, Laboratory of Host Defenses, Bethesda, USA
¹⁰Istituto Giannina Gaslini, Unit of Pediatrics 2, Genova, Italy

Background:

Next generation sequencing (NGS) technology has opened great perspectives for the characterization of patients with unsolved Primary Immunodeficiencies (PID). We focused on patients with combined immunodeficiency (CID) and identified a novel immunodeficiency in a patient with an autoimmune/autoinflammatory phenotype resembling Wiskott-Aldrich syndrome (WAS), caused by ARPC1B gene.

Methods:

Haloplex target system panel; ex-vivo (proliferation, flow cytometry) and in-vitro assays (actin polymerization, migration, confocal and electron microscopy) to characterize defects in different cell subsets; gene editing for in-vitro model of the disease.

Results:

Haloplex panel with more than 600 genes among those related to PID and candidate genes was validated and applied to 32 patients. Mutations in SCID and CVID genes were found in 11%, possible candidate genes are under validation in 18% of patients and the gene(s) are under investigation in 58% of patients. In particular we studied a patient resembling WAS disease (microthrombocytopenia, eczema, recurrent pneumonias, vasculitis, enterocolitis) with normal WAS gene in whom we identified a frame-shift mutation in the ARPC1B gene encoding for a component of ARP2/3 complex. ARPC1B protein was reduced. F-actin polymerization and migration to SDF1α receptor showed alterations in lymphocytes morphology and actin expression. Platelets showed a
decreased expression of PAC1. We are currently studying the effect of the mutation by gene editing and lentiviral vector gene addition.

Conclusions:

These studies show the great potential of NGS in the identification of molecular basis of PID and warrant further development for a wider application of these platforms to identify the treatment of choice.
WHOLE GENOME SEQUENCING OF 1350 INDIVIDUALS UNCOVERS NOVEL GENES AND NON-CODING VARIANTS CONTRIBUTING TO PRIMARY IMMUNODEFICIENCY DISORDERS

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⁶Academic Medical Center, Department of Experimental Immunology, Amsterdam, United Kingdom

Background:

As part of the UK NIHR BioResource – Rare Diseases project, we recruited 1400 patients with primary immunodeficiency (PID) and additional 670 affected and unaffected relatives. This cohort includes sporadic and familial cases of both pediatric and adult disease onset, from broad recruitment criteria and limited pre-screening for known causes of PID.

Methods:

We performed whole genome sequencing (WGS) of 1350 individuals, including 912 unrelated index cases. To provide genetic diagnoses, we screened for pathogenic variants in the IUIS 2015 gene list. To search for novel causes of PID, we performed pedigree analyses of familial cases; trio analyses of sporadic cases; and cohort-wide analyses of high impact variants and CNVs in coding, promoter and enhancer regions.

Results:

We obtained diagnostic yield of 10%. Causal variants were identified in 31 different genes, including multi-exon and promoter deletions in DOCK8 and LRBA, respectively. We observed several novel genotype-phenotype correlations in genes such as DKK1, TTR37 and CD40LG. The availability of WGS data enabled us to identify undeclared relatives recruited through different centres, leading to appropriate genetic counselling.

We identified 15 novel candidate genes, which we are currently validating by co-segregation analyses, functional cell assays, CRISPR editing of patient cell lines, and mouse models. Notably, 16 of the ~500 predominantly CVID cases are explained by novel loss-of-function variants in NFKB1, establishing NF-kappa-B haploinsufficiency as the most common monogenic cause of CVID.

Conclusions:

WGS of a large, heterogeneous PID cohort is a powerful tool for identifying novel causal variants, including large deletions over coding and regulatory regions.
DEFECTIVE ACTIN ARP2/3 ACTIVATOR FUNCTION CAUSED BY A ARPC1B MUTATION RESULTS IN A NOVEL WISKOTT-ALDRICH SYNDROM LIKE IMMUNODEFICIENCY

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²University Medical Center, Unit for Special Laboratory Diagnostics- University Children's Hospital, Ljubljana, Slovenia
³University Clinic of Pulmonary and Allergic Diseases Golnik, Department of Allergology and Clinical Immunology, Golnik, Slovenia
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Background:

Polymerisation of actin filaments is initiated by actin nucleators. Among them Arp2/3 complex has ability to form branched actin networks and is regulated by members of the Wiscott-Aldrich syndrome protein (WASp) family. Mutation in WASp gene can cause immunodeficiency characterised by recurrent bacterial infections, eczema, thrombocytopenia and autoimmune diseases.

We present three related patients with WAS features and a mutation in a ARPC1B gene (Arp2/3 activator subunit).

Methods:

All patients (2 females, 1 male–relatives) presented in neonatal period with eczema, thrombocytopenia and bloody diarrhoea. Later they developed recurrent bacterial infection of the lung and skin, therapy resistant inflammatory bowel disease, various autoimmune features (AI vasculitis, AI thrombocytopenia, pernicious anaemia) and allergic reactions. They have mild cellular deficiency, slightly decreased phagocytic assays, decreased IgM and elevated IgA and IgE antibodies.

Whole exome sequencing was performed on an index patient.

A simple, rapid and very specific functional test to evaluate Arp2/3 complex function was developed, consisting of in vitro fMLP stimulation of mononuclear cells and flow cytometric detection of intracellular polymerised actin with fluorescinated phalloidin.

Results:

We identified a novel missense mutation in ARPC1B (Arp2/3 activator subunit) that was predicted disease causing with several in silico prediction tools.
Median fluorescence intensities (MFI) of FITC-phalloidin stained actin in monocytes and neutrophils as evaluated by flow cytometry are shown in table 1.

Table 1. Average median fluorescence intensity (MFI) with standard deviations of neutrophils and monocytes before and after 20 seconds of stimulation with fMLP in three different groups – homozygous (patients), carriers, and healthy subjects (without ARPC1B mutation). Relative increase in MFI was significantly lower in patients when compared to carriers and healthy subjects (p < 0.005).

<table>
<thead>
<tr>
<th></th>
<th>before stimulation</th>
<th>after stimulation</th>
<th>increase in MFI</th>
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<tbody>
<tr>
<td><strong>Neutrophils</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>homozygous (n=9)</td>
<td>10.3 ± 2.9</td>
<td>11.4 ± 2.3</td>
<td>11%</td>
</tr>
<tr>
<td>carriers (n=4)</td>
<td>16.5 ± 9.8</td>
<td>25.3 ± 5.9</td>
<td>60%</td>
</tr>
<tr>
<td>without mutation (n=3)</td>
<td>8.9 ± 5.0</td>
<td>17.7 ± 7.5</td>
<td>99%</td>
</tr>
<tr>
<td><strong>Monocytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>homozygous (n=9)</td>
<td>3.5 ± 0.7</td>
<td>3.7 ± 0.5</td>
<td>5%</td>
</tr>
<tr>
<td>carriers (n=4)</td>
<td>4.9 ± 1.0</td>
<td>8.8 ± 1.5</td>
<td>80%</td>
</tr>
<tr>
<td>without mutation (n=3)</td>
<td>3.8 ± 2.3</td>
<td>7.92 ± 4.5</td>
<td>107%</td>
</tr>
</tbody>
</table>

**Conclusions:**

We report three patients with novel WAS-like immunodeficiency caused by mutation in ARP2/3 activator subunit.

Functional fMLP/phalloidin test can efficiently discriminate homozygous symptomatic patients from asymptomatic heterozygous carriers and can be used as a screening test for actin-polymerisation defects.
PARALLEL SESSION 2: JAK STAT & PI3K INHIBITORS

ESID7-0077

THERAPEUTIC EFFICACY OF JANUS KINASE INHIBITION VERSUS INTERFERON GAMMA NEUTRALIZATION IN PRECLINICAL MODELS OF HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Background:

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening disorder of the immune system characterized by the proliferation of lymphocytes and histiocytes, which copiously secrete IFNγ and other proinflammatory cytokines. HLH is classified as primary, reflecting the presence of germline mutations in genes regulating lymphocyte cytotoxicity, or secondary, reflecting the presence of HLH symptoms without identifiable germline mutations. Primary HLH can be modeled through the infection of perforin knockout mice with Lymphocytic Choriomeningitis virus (LCMV), while secondary HLH can be modeled via repeated CpG-mediated stimulation of Toll-like Receptor 9. We recently reported that treatment with the Janus Kinase (JAK) inhibitor ruxolitinib significantly ameliorates disease in both HLH models.

Methods:

We sought to understand ruxolitinib’s mechanism(s) of action by comparing its effects to those obtained following antibody-mediated neutralization of IFNγ in both models of HLH.

Results:

In the model of primary HLH, ruxolitinib treatment resulted in greater reductions in splenomegaly, CD8 T-cell number and cytokine secretion, and serum TNFα levels compared to IFNγ neutralization. Effects of JAK inhibition were longer-lasting than those obtained with IFNγ neutralization, as all mice survived following withdrawal of ruxolitinib, compared to only 20% of animals in which IFNg neutralization was discontinued. In the model of secondary HLH, ruxolitinib was again superior to IFNγ neutralization in reducing splenomegaly, numbers of inflammatory macrophages, neutrophils, CD4 and CD8 T cells, and hypercytokinemia.

Conclusions:

These data reveal that ruxolitinib mediates its effects via mechanisms extending beyond the inhibition of IFNg signaling and provide evidence for JAK inhibition as a novel and potentially more effective HLH treatment.
HUMAN RELA HAPLOINSUFFICIENCY RESULTS IN AUTOSOMAL DOMINANT CHRONIC MUCOCUTANEOUS ULCERATION

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Background:

The treatment of chronic mucocutaneous ulceration is challenging and only some patients respond selectively to inhibitors of tumor necrosis factor-alpha (TNF-a). TNF-a activates opposing pathways leading to caspase-8-mediated apoptosis as well as NF-kB-dependent cell survival.

Methods:

Whole exome sequencing was performed on the proband, her two affected siblings, her affected mother, and her healthy father. NF-kB activation was assessed in the patients' fibroblasts using a luciferase assay. Gene expression was assessed by RNA-Seq. Cellular apoptosis was determined by flow cytometry and histological analysis of tissues.

Results:

We investigated the etiology of autosomal dominant mucocutaneous ulceration in a family whose proband was dependent on anti-TNF-a therapy for sustained remission. A heterozygous mutation in RELA (NM_021975: c.559+1G>A), encoding the NF-kB subunit RelA (p65), segregated with the disease phenotype and resulted in RelA haploinsufficiency. The patients' fibroblasts exhibited increased apoptosis in response to TNF-a, impaired NF-kB activation, and defective expression of NF-kB-dependent anti-apoptotic genes. We show that Rela⁻/⁺ mice have similarly impaired NF-kB activation, develop cutaneous ulceration from TNF-a exposure, and exhibit severe dextran sodium sulfate-induced colitis ameliorated by TNF-a inhibition. WT>Rela⁻/⁺, but not Rela⁻/⁻>WT, bone marrow chimeras develop TNF-a-driven ulceration, indicating a stromal-cell intrinsic defect.

Conclusions:

These findings demonstrate an essential contribution of biallelic RELA expression in protecting stromal cells from TNF-a-mediated cell death, thus delineating the mechanisms driving the effectiveness of TNF-a inhibition in this disease.
PARALLEL SESSION 4: MANAGEMENT OF PID IN LESS ADVANTAGED COUNTRIES AND REGIONS

ESID7-0166

ATYPICAL PRESENTATIONS OF PRIMARY IMMUNODEFICIENCY DISEASES: A CHALLENGE FOR DIAGNOSIS

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²Faculty of Medicine- Cairo University, Clinical and Chemical Pathology, Cairo, Egypt

Background:

Most of PIDs are identified by their classic clinical and immunologic features and confirmed at molecular level. However, phenotypic-genotypic correlation is not always consistent rendering a definitive diagnosis very challenging.

Methods:

We present four cases with non-classical presentations and an unexpected diagnosis by extended molecular workup including WES, NGS and Sanger sequencing.

Results:

Case 1: 3 years old female presented at age of 11 months with delayed milestones, recurrent pneumoniae, autoimmune hemolytic anemia and hepatic affection, reduced CD3, CD4 and CD8 counts with elevated IgM. WES detected homozygous mutation in PNP. Case 2: 18 months old male, presented with history of eczema, diarrhea and pneumoniae since the age of 5 months, CBC showed anemia, thrombocytopenia and eosinophilia; Immunological work up showed normal CD counts, IgE level 266 mg/dl, normal expression of WASP. NGS revealed PGM3 defect. Case 3: 3.5 years old male from consanguineous family, history of perianal cellulitis, thigh abscesses, oral candidiasis and attacks of hemolytic anemia. CBC showed lymphopenia, normal serum immunoglobulins apart from markedly elevated IgE, CD4 lymphopenia, normal CD19 and normal DHR test. NGS revealed homozygous mutation in RAG1. Case 4: 4 years old female from non-consanguineous family presented with exfoliative skin rash, history of recurrent diarrhea, at age of 2, developed severe anemia and became transfusion dependent. Patient had low CD4, borderline CD19 counts; elevated IgG and IgM levels. Compound heterozygous mutation was detected in RAG1 gene sequencing.

Conclusions:

Increasing physician’s awareness together with extended molecular work up are strongly needed for patients with such atypical presentations.
PARALLEL SESSION 4: MANAGEMENT OF PID IN LESS ADVANTAGED COUNTRIES AND REGIONS

ESID7-0399

CLINICAL FEATURES AND OUTCOME OF ATAXIA-TELANGIECTASIA PATIENTS
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Background:

Ataxia-telangiectasia (ATS) is the association of combined immunodeficiency with progressive cerebellar ataxia. The objective of this study is to analyze the clinical and evolutive characteristics of this disease.

Methods:

We retrospectively analyze medical records of patients with ATS in the pediatric immune-hematology unit, Tunis between 2000 and 2016

Results:

110 patients were enrolled. Mean age at onset was 3 years (2 months-18 years). Consanguinity was present in 85% of cases. A familial history of similar cases was noted in one-third of the patients. Infectious manifestations were: pneumonia (67%), bronchiectasis (35%), ENT infections (25%) and digestive infections (15%). Cerebellar syndrome had interested 100 patients. Two patients had allergic symptoms. Three patients had autoimmune manifestations. Four patients had cancer: pancreas head cancer (n = 2), Hodgkin's lymphoma (n = 1) and lung cancer (n = 1). B lymphopenia was observed in 49 cases, and T lymphopenia in 30 patients. Responses to PHA were low in 15 patients. Proliferation to specific antigens was low in 40 patients. The three classes IgG, IgM and IgA were normal in only 15 patients. 14 patients had hyper IgM syndrome. 71 patients died (63%) at an average age of 12 years +/- 5 years. For survivors, the quality of life was definitely compromised by neurological impairment.

Conclusions:

ATS is a relatively frequent immune deficiency in Tunisia. The prognosis of ATS is severe as it reflects the rapid neurodegeneration, occurrence of respiratory infections, and an increased risk of cancer. The management remains symptomatic.
INFLAMMATORY BOWEL DISEASE ASSOCIATED WITH XIAP DEFICIENCY CAN BE CURED BY HEMATOPOIETIC STEM CELL TRANSPLANTATION

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2Tokyo medical and dental university, Department of Community Pediatrics- Perinatal and Maternal Medicine, Tokyo, Japan

Background:

XIAP deficiency is a rare immunodeficiency characterized by recurrent hemophagocytic lymphohistiocytosis (HLH), and often associated with refractory inflammatory bowel disease (IBD). The natural course of XIAP deficiency is poor, and hematopoietic stem cell transplantation (HSCT) is the only curative therapy. Nevertheless, outcome of HSCT for XIAP deficiency remains unsatisfactory compared with those for SAP deficiency and familial HLH. We investigated the outcome of HSCT for Japanese patients with XIAP deficiency.

Methods:

A spreadsheet questionnaire was sent to physicians who had provided HSCT treatment for patients with XIAP deficiency in Japan.

Results:

Eleven patients with XIAP deficiency underwent HSCT until the end of 2016 in Japan, and 9 patients (82%) survived, and the outcome was better than that reported by a previous study. All survived patients received fludarabine-based reduced intensity conditioning (RIC) regimen. Seven patients (64%) suffered from IBD before HSCT, and all were refractory to conventional IBD treatment, which became the indication for HSCT. Intriguingly, IBD improved remarkably after HSCT and maintained remission without any treatments for IBD. IBD was relieved during the conditioning regimen, at least by the point of engraftment. Colonoscopic and pathological findings completely improved to normal colon appearance after HSCT in some cases, and Pediatric Ulcerative Colitis Activity Index was...
remarkably decreased.

Conclusions:

National survey for XIAP deficiency in Japan revealed that the outcome was prominent, and IBD associated with XIAP deficiency could be cured by HSCT with RIC regimen.
PARALLEL SESSION 5: THE 5 MOST DIFFICULT PROBLEMS IN PID HSCT

ESID7-0299

OUTCOME OF CHILDREN WITH PRIMARY IMMUNE DISORDERS INFUSED WITH BPX-501 DONOR T-CELLS GENETICALLY MODIFIED WITH NOVEL SUICIDE GENE (INDUCIBLE CASPASE-9) AFTER T-CELL DEPLETED HLA-HAPLOIDENTICAL HSCT

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\textsuperscript{2}Ospedale Pediatrico Bambino Gesù, Oncology, Rome, Italy
\textsuperscript{3}Children’s Hospital of Los Angeles, Oncology, Los Angeles, USA
\textsuperscript{4}Great Ormond Street Hospital, Oncology, London, United Kingdom
\textsuperscript{5}Texas Children’s Hospital, Oncology, Houston, USA
\textsuperscript{6}Children’s Healthcare of Atlanta, Oncology, Atlanta, USA
\textsuperscript{7}Bellicum Pharmaceuticals, Clinical, Houston, USA

Background:

Allogeneic haploidentical HSCT offers curative therapy for children with Primary Immune Disorders (PIDs) who lack an available HLA-identical donor. T-cell depletion mitigates the risk of GVHD after haplo-HSCT, but is associated with extended immunodeficiency leading to complications due to infections. We performed αβ TCR-depleted haplo-HSCT with post-transplant infusion of BPX-501 gene modified T-cells to allow for more rapid immune reconstitution. Upon occurrence of GVHD, administration of rimiducid (AP1903) dimerizes the Caspase 9 suicide switch and rapidly induces apoptosis of the transduced BPX-501 cells and mitigates the GVHD.

Methods:

We report on a large multicenter, prospective Phase I-II study. Patients were infused with BPX-501 T-cells ~ 18 days post-transplant. 28 children have >100 days follow-up, 23 >180 days and 16 >1 year. 12 SCID, 6 WAS, 4 CGD, 2 CID and 4 other PIDs were treated. All patients received myeloablative therapy and low dose ATG. No pharmacologic GvHD prophylaxis was given.

Results:

All patients engrafted with no secondary graft failure. Immune reconstitution was brisk. See figure for CD3, CD4, CD8 counts. 9 patients had aGvHD Grade I-II and 1 patient had mild cGvHD. All GvHD resolved with standard care or rimiducid. Cumulative incidence of TRM remains very low (3.6%).
Conclusions:

These results indicate that α/β depleted haplo-HSCT, followed by infusion of donor BPX-501 cells with a suicide gene mechanism, is an effective alternative therapy for those children with PIDs lacking a suitable HLA-matched donor.
INDUCED PLURIPOTENT STEM CELL MODELS OF BLAU SYNDROME REVEAL AN INFLAMMATORY RESPONSE IN PRIMED MACROPHAGES

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2Chiba University Graduate School of Medicine, Department of Dermatology, Chiba, Japan
3Kansai Medical University, Department of Dermatology, Osaka, Japan

Background:
Blau syndrome is a hereditary autoinflammatory disorder associated with heterozygous mutation in NOD2. This disease causes a juvenile-onset systemic granuloma, mainly affecting skin, joints, and eyes. The underlying mechanisms of Blau syndrome leading to inflammation are still unclear, and there is currently no effective specific treatment for this disease. NOD2 is expressed in monocytic lineage and encodes a pathogen-recognition receptor that recognizes muramyl dipeptide, found in bacterial cell wall, and then transduces signals leading to NF-κB activation. Although it is supposed that increasing of NF-κB signal by mutant NOD2 affects the pathogenesis of Blau syndrome, previous reports describing the function of mutant NOD2 showed controversial results in regard to the activation of NF-κB pathway.

Methods:
To clarify the relation between disease associated-mutant NOD2 and the inflammatory response, here we established induced pluripotent stem cells (iPSCs) from Blau syndrome patients. The iPSC model is free from the influence of the in vivo cellular environment, such as medication or endogenous cytokines. To precisely evaluate the in vitro phenotype of iPSC-derived cells, we also established isogenic iPSC pairs sharing the same genomic backgrounds but different NOD2 genotypes using the CRISPR-Cas9 system.

Results:
RNA-sequence data revealed that NOD2-mutated macrophages derived from disease-specific iPSCs were in a pre-activated state. Functional analyses using iPSC-derived macrophages suggested a priming signal through the up-regulation of NOD2 as a critical mediator of the inflammatory manifestations in this disease.

Conclusions:
Our data support the significance of autoinflammation in primed macrophages in the pathophysiology, providing an opportunity for probing therapeutic targets of Blau syndrome.
Background:

Pyogenic arthritis, Pyoderma gangrenous, and Acne (PAPA) syndrome is a rare auto-inflammatory disease caused by a gain of function mutation in PSTPIP1/CD2BP1.

More than 20 mutations have been described of which A230T, E250Q and E250K are known to be PAPA associated mutations. The E250K mutation has been identified in Hyperzincemia/Hypercalprotectinemia (Hz/Hc) syndrome, the severest form of PAPA syndrome. Other PSTPIP1 mutations can cause inflammatory disorders such as Pyoderma gangrenosum and Juvenile idiopathic arthritis.

Associated disease symptoms have been attributed to dysregulation in cells involved in innate immunity, especially in neutrophils. However, it remains unknown how cellular functions become compromised by these mutations due to the difficulty in isolating and handling neutrophils.

Methods:

Peripheral blood neutrophils were isolated from five patients with PSTPIP1 mutation and healthy donors. Production of reactive oxygen species (ROS) and cell death were assessed. Wild type and mutant PSTPIP1 recombinant proteins were generated and transduced into healthy neutrophils. Patient autologous iPS cells with the E250K mutation were generated and were differentiated into neutrophils.

Results:

Greater ROS production and increased susceptibility to cell death in patients’ neutrophils compared to healthy donors were observed.

Similar observations were made when recombinant mutant PSTPIP1 protein, but not wild type PSTPIP1 proteins, were transduced into healthy neutrophils. Differentiated iPS-derived neutrophils
displayed the same phenotype as peripheral neutrophils when subjected to the same functionality and apoptosis assay.

**Conclusions:**

Mutant PSTPIP1 per se exerts deleterious effect. Aberrant neutrophil ROS production and apoptosis may be implicated in pathogenesis of PAPA syndrome.
DYSREGULATED IL-12 RELEASE IS ASSOCIATED WITH INFLAMMATORY COMPLICATIONS IN INDUCIBLE T-CELL CO-STIMULATOR (ICOS) DEFICIENCY

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7University of Medical Sciences, Department of Pediatrics and Neonatology- School of Medicine, Kashan, Iran
8Tehran University of Medical Sciences, Research Center for Immunodeficiencies- Pediatrics Center of Excellence- Children’s Medical Center, Tehran, Iran
9Newcastle University, Primary Immunodeficiency Group- Institute of Cellular Medicine- Medical School, Newcastle upon Tyne, United Kingdom
10National Institute for Health Research NIHR, Cambridge Biomedical Research Centre, Cambridge, United Kingdom

Background:

ICOS deficiency has been categorized as a combined immunodeficiency often complicated by enteropathies, autoimmunity, lymphoproliferation and malignancy. We report 6 new patients and three novel ICOS mutations resulting in CVID-like phenotype and show that dysregulated IL-12 release is associated with inflammatory complications in this condition.

Methods:

Combination of whole exome and Sanger sequencing was used to identify novel mutations. Standard clinical and immunological evaluation was performed. Combination of FACS and ELISA-based assays was used to study cytokine responses and ICOS/ICOSL expression following stimulation of whole blood and PBMCs with multiple TLR ligands, anti-CD3 Ab and PHA

Results:

Novel ICOS mutations included: homozygous c.323_332del, homozygous c.451 C>G and compound heterozygous c.58+1G>A c.356T>C. Clinical features and immunological evaluations are detailed in Tables 1 and 2. Patients 1 and 2 had multiple inflammatory complications, including granulomatous liver and bone marrow infiltrates. In addition patient 1 had history of multiple delayed reactions to
various antibiotics. All patients showed reduced IL-10 and IL-17 cytokine responses. Furthermore patients 1 and 2 also showed highly elevated IL-12 production in response to LPS/IFNγ stimulation. This was associated with skewing of CD4 T cells towards Th1 phenotype and increased expression of ICOSL on monocytes (Figure 1).
Table 1 Clinical and genetic characteristics of 6 new patients with iCDS deficiency

<table>
<thead>
<tr>
<th>Family</th>
<th>Newborn 1</th>
<th>Newborn 2</th>
<th>Newborn 3</th>
<th>Newborn 4</th>
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LRU, lower respiratory tract infection; OM, otitis media; SM, meningitis; URU, upper respiratory tract infection; TB, tuberculosis.

Table 2 Immunological characteristics

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Conclusions:

ICOS deficiency is associated with defective T cell activation but at the same time enhanced stimulation of monocytes. The latter is likely to result from lack of ICOS/ICOSL interaction which might be necessary to provide negative feedback to limit monocytes activation.
AN INBORN ERROR OF IMMUNITY CAUSED BY BACH2 HAPLOINSUFFICIENCY


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Lymphocyte Cell Biology Section Molecular Immunology and Inflammation Branch- Biodata Mining and Discovery Section and Protein Expression Laboratory- National Institutes of Arthritis- and Musculoskeletal Diseases & MRC Centre for Transplantation, Bethesda and London, USA
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Lymphocyte Cell Biology Section Molecular Immunology and Inflammation Branch- Biodata Mining and Discovery Section and Protein Expression Laboratory- National Institutes of Arthritis- and Musculoskeletal Diseases, Bethesda, USA
6John Radcliffe Hospital- Oxford, Nuffield Department of Medicine, Oxford, United Kingdom
7National Institutes of Health, Laboratory of Molecular Immunology, Bethesda, USA
8Hammersmith Hospital, Department of Haematology, London, United Kingdom
9Hammersmith Hospital, Imperial BRC Genomics Facility, London, United Kingdom
10Merck & Co. Inc, Merck Research Laboratories, Boston, USA
11National Institutes of Health-, National Cancer Institute, Bethesda, USA
12University of Edinburgh, Centre for Genomic and Experimental Medicine, Edinburgh, USA
13Northern Centre for Cancer Care- Freeman road- Newcastle upon Tyne & John Radcliffe Hospital- Oxford, Department of Haematology & Nuffield Department of Medicine-, Newcastle upon Tyne and Oxford, United Kingdom

Background:

BTB And CNC Homology 2 (BACH2) a transcriptional repressive factor whose expression is regulated by a super-enhancer (SE) plays a key role in T and B lymphocyte differentiation. In B cells BACH2 inhibits premature expression of the plasma cell regulatory protein BLIMP-1 to facilitate isotype class switching and somatic hyper-mutation following antigen exposure. BACH-2 polymorphic variant have been associated with a number of autoimmune disorders.

Methods:

We describe an autosomal dominant syndrome of BACH2-related immunodeficiency and autoimmunity (BRIDA) in three patients from two independent kindreds, resulting from haploinsufficiency of BACH2.
Results:

Clinical history include recurrent sino-pulmonary infections, intestinal inflammation and immune investigations showed immunoglobulin deficiency reduced class-switched memory B and CD4+ CD4+FoxP3+Treg proportions and increased inflammatory CD4 Th1 responses. Two distinct BACH2 missense mutations identified by exome sequencing led to reduced BACH2 expression in CD4+, CD8+ and B lymphocytes, with increased BLIMP-1 mRNA. The BACH2 protein mutant (L24P) was insoluble, inhibited homomeric dimerization, and the E788K mutant formed cytoplasmic aggregates. Familiar linkage studies, cellular transfection assays and murine BACH2+/-model confirmed that BACH2 haploinsufficiency was most likely responsible for pathological finding observed in these patients. We also found we found that genes causing monogenic haploinsufficient diseases are substantially enriched for transcription factors, super-enhancer -architecture and heterozygous mutations.

Conclusions:

Results of this study extend the association of gene with SE architecture from diseases of polymorphic variants to Mendelian inheritance and suggest that investigation for BACH2 mutations should be considered in diagnostic work up of patients with primary antibody deficiency syndromes.
PARALLEL SESSION: ORAL ABSTRACT PRESENTATIONS

ESID7-0007

TBK1 / IKKE BLOCKADE INHIBITS MUTANT STING MEDIATED INFLAMMATORY RESPONSE IN PATIENT CELLS

M.L. Frémond1,2, C. Uggenti1, L. Van Eyck1, I. Melki1,2,3, V. Bondet4,5, N. Kitabayashi1, C. Hertel6,
A. Hayday6, B. Neven2,7, Y. Rose1, D. Duffy4,5,8, Y.J. Crow1,9, M.P. Rodero1

1IMAGINE Institut, Laboratory of Neurogenetics and Neuroinflammation - INSERM U1163, PARIS, France
2Hôpital Necker-Enfants Malades-APHP, Paediatric Hematology-Immunology and Rheumatology Department, Paris, France
3Hôpital Robert Debré-APHP, General Paediatrics- Infectious Disease and Internal Medicine Department, Paris, France
4INSERM, UMR 1223, Paris, France
5Institut Pasteur, Laboratory of Dendritic Cell Immunobiology, Paris, France
6ImmunoQure, AG, Düsseldorf, Germany
7IMAGINE Institut, Immunogenetics of paediatric autoimmune diseases - INSERM U1163, PARIS, France
8Institut Pasteur, Center for Translational Research, Paris, France
9University of Manchester, Manchester Centre for Genomic Medicine- Institute of Human Development Faculty of Medical and Human Sciences, Manchester, United Kingdom

Background:

Gain-of-function mutations in TMEM173 encoding STING (stimulator of interferon genes) underlie a novel type I interferonopathy, minimally responsive to conventional immunosuppressive therapies and associated with high childhood morbidity and mortality. STING gain-of-function causes constitutive over secretion of interferon (IFN). We determined the effects of a TBK1 / IKKE inhibitor (BX795) on IFN secretion and signalling in primary peripheral blood mononuclear cells (PBMCs) from four patients.

Methods:

PBMCs from STING patients were treated with BX795. The effect of BX795 on IFN pathways was assessed by western blot, an IFNβ reporter assay, IFNα quantification in cell lysates, STAT1 phosphorylation status and by RNA expression of IFN-stimulated genes (ISGs).

Results:

BX795 inhibited the phosphorylation of IRF3 and IFNβ promoter activity induced in HEKs by cGAMP or by genetic activation of STING. In vitro exposure to BX795 inhibited IFNα production in PBMCs of STING-mutated patients. In addition, BX795 decreased STAT1 phosphorylation and ISG expression independently of IFNα blockade.

Conclusions:

Our findings demonstrate the effect of BX795 on reducing type I IFN production and IFN signalling in cells from patients with gain-of-function mutations in STING (see figure). A combined inhibition of
TBK1 and IKKE therefore holds potential for the treatment of STING-mutated patients, and may also be relevant in other type I interferonopathies.
IDENTIFICATION OF NOVEL INNATE IMMUNODEFICIENCIES IN PATIENTS WITH VARICELLA-ZOSTER VIRUS ENCEPHALITIS

A. Hansen¹, M. Carter-Timofte², M. Christiansen³, S. Paludan², T. Mogensen¹,²,⁴
¹Aarhus University Hospital, Department of Infectious Diseases, Aarhus, Denmark
²Aarhus University, Department of Biomedicine, Aarhus, Denmark
³Aarhus University Hospital, Department of Clinical Immunology, Aarhus, Denmark
⁴Aarhus University, Department Clinical Medicine, Aarhus, Denmark

Background:

Most of the population in the western world is at some point infected with varicella-zoster virus (VZV), manifesting as chickenpox. The virus however, remains latent and can later reactivate to cause shingles. In a minority of individuals, the virus spreads to the central nervous system (CNS) causing encephalitis. So far it has remained largely unknown why some people develop CNS infection, whereas most of the infected population does not.

Methods:

We included ten adult patients, previously suffering from VZV CNS infection, to investigate the genetic etiology of the disease. We performed whole exome sequencing aiming at identifying rare and deleterious mutations. This was followed by stimulation of patient PBMCs with relevant ligands and infection with VZV to evaluate immunological function.

Results:

In six patients, we identified mutations in genes encoding cytosolic RNA-polymerase III, which transcribes dsDNA into dsRNA recognizable by the cytosolic receptor RIG-I. In general we found decreased induction of pro-inflammatory cytokines when PBMCs were stimulated with the RNA-polymerase III agonist poly(dA:dT) in patients compared to controls. Moreover, we found decreased induction of especially TNF-α and IL-6 upon stimulation with VZV as well as increased levels of the viral gene ORF63, suggesting increased viral replication in the patient cells.

Conclusions:

In this study, we have identified possible genetic etiologies in several patients, which we suggest contributed to the development of VZV infection of the CNS. This is strongly supported by results from our functional cell studies, which demonstrated impaired production of several antiviral and proinflammatory cytokines in patient cells harbouring these mutations.
DOCK8 IS REQUIRED FOR T REGULATORY CELL SYNAPSE FORMATION AND THE MAINTENANCE OF PERIPHERAL IMMUNOLOGICAL TOLERANCE

E. Janssen¹, S. Kumari², M. Tohme¹, S. Ullas¹, V. Barrera², J. Tas⁴, M. Castillo-Rama¹, R. Bronson⁵, S. Usmani⁷, D. Irvine², T. Mempel⁴, R. Geha¹

¹Boston Children’s Hospital, Medicine, Boston, USA
²Massachusetts Institute of Technology, Bioengineering, Cambridge, USA
³Harvard T.H. Chan School of Public Health, Bioinformatics, Boston, USA
⁴Massachusetts General Hospital, Center for Immunology and Inflammatory Diseases, Boston, USA
⁵Dana Farber Cancer Institute, Pathology, Boston, USA

Background:

DOCK8 is a membrane-anchored protein with guanine nucleotide exchange factor (GEF) activity for CDC42. Patients with DOCK8 deficiency have decreased numbers and impaired in vitro function of T regulatory (Treg) cells and generate autoantibodies, but seldom develop autoimmunity, possibly due to impaired T effector (Teff) cell function.

Methods:

Treg cells from Dock8⁻/⁻, Dock8⁻/⁻/⁻, and Foxp³/YFP-Cre/Dock8floox/flox mice were examined. Treg cell activation and suppressive function were measured by flow cytometry and microscopy. RNAseq and qPCR were used to compare the transcriptomes in DOCK8 deficient and sufficient Treg cells.

Results:

We show that Dock8⁻/⁻ mice have decreased numbers and impaired in vitro function of Treg cells, but do not develop autoimmunity. In contrast, mice with selective DOCK8 deficiency in Treg cells spontaneously develop lymphoproliferation, autoantibodies, and gastrointestinal inflammation, despite normal percentages of Treg cells and proper localization of Treg cells in lymph nodes. DOCK8 localized within the lamellar actin ring of the immune synapse (IS) in Treg cells. Dock8⁻/⁻ Treg cells had abnormal actin dynamics, decreased adhesiveness, formed an unstable IS with decreased recruitment of signaling molecules, and had impaired transendocytosis of CD86. Treg cells from Dock8⁻/⁻ mice also had an altered gene expression profile. Treg cells from Dock8⁻/⁻/⁻ mice, which express a DOCK8 S1827P mutant that lacks GEF activity, had a phenotype similar to Dock8⁻/⁻ Treg cells.

Conclusions:

These data suggest that DOCK8 GEF activity controls TCR-driven signaling, actin dynamics and IS integrity in Treg cells, and thereby the generation of tolerogenic antigen presenting cells and peripheral immunological tolerance.
HIGH INTERFERON-RESPONSE GENE SIGNATURES (IRS) AND OVERLAPPING CLINICAL FEATURES CHARACTERIZE SUBGROUPS OF PATIENTS WITH PRESUMED IFN-MEDIATED AUTOINFLAMMATORY DISEASES.

A. de Jesus\textsuperscript{1}, Y. Hou\textsuperscript{1}, L. Malle\textsuperscript{1}, S.W. Canna\textsuperscript{2}, A. Biancotto\textsuperscript{3}, S. Brooks\textsuperscript{4}, G. Montealegre-Sanchez\textsuperscript{1}, H. Kim\textsuperscript{5}, B. Marrero\textsuperscript{1}, Z. Deng\textsuperscript{4}, J. McElwee\textsuperscript{6}, R. Goldbach-Mansky\textsuperscript{1}, &. Autoinflammatory disease network\textsuperscript{7}  

\textsuperscript{1}National Institute of Allergy and Infectious Diseases NIAID/NIH, Translational Autoinflammatory Diseases Studies Unit, Bethesda, USA 
\textsuperscript{2}Children’s Hospital Pittsburgh/UPMC, Pediatric Rheumatology and Immunology/Mellon Institute for Pediatric Research, Pittsburgh, USA 
\textsuperscript{3}National Heart Lung and Blood Institute NHLBI/NIH, Center of Human Immunology CHI, Bethesda, USA 
\textsuperscript{4}National Institute of Arthritis- Musculoskeletal and Skin Diseases NIAMS/NIH, Translational Immunology Section, Bethesda, USA 
\textsuperscript{5}National Institute of Arthritis- Musculoskeletal and Skin Diseases NIAMS/NIH, Clinical Scholar’s Program- Office of the Clinical Director, Bethesda, USA 
\textsuperscript{6}Merck, Genetics and Pharmacogenomics GpGx, Boston, USA 
\textsuperscript{7}Translational Autoinflammatory Research Initiative TARI, NIH, Bethesda, USA 

Background:

Pediatric patients with early-onset autoinflammatory diseases (AID) who are mutation-negative for known genetic causes pose diagnostic and therapeutic challenges. A role for Type-I IFN in AID pathogenesis and as therapeutic target led to screening for an IFN–response gene signatures (IRS) and clinical, immunological and genetic characterization.

Methods:

63 patients with no known AID-causing mutations were assessed for IRS. Patients underwent clinical assessments, cytokine analyses, and genetic testing by whole exome (WES) or whole genome sequencing (WGS).

Results:

Of 63 patients tested, 36 had elevated IRS scores. Higher IRS scores were associated with higher serum levels of IP-10, MIG, MIP1a, MIP1b and SCF. Patients could be grouped into 8 phenotypically distinct groups, of which patients with interstitial lung disease (ILD), macrophage activation syndrome (MAS) and ultra-high IL-18 levels had a previously described 12-cytokine pattern (ref). We identified novel mutations in 6 genes known to be associated with immune-dysregulatory diseases and novel genes not previously known to cause autoinflammatory phenotypes. Among 7 patients with panniculitis, novel mutations in PSMB8, and PSMG2 revealed additional causes for CANDLE; a somatic \textit{de novo} mutation in \textit{TREX1} is a likely modifier of a complex interferonopathy.

Conclusions:
The assessment of patients with unknown AIDs and high IRS revealed 8 clinical, immunological and/or genetic subgroups. Functional assessment of novel disease-causing mutations in genes previously associated with immune-dysregulatory phenotypes and mutation in novel AID genes will help to characterize intracellular pathways that lead to Type-I IFN dysregulation. A previously observed cytokine signature may identify patients at risk for MAS independent of genetics.
PARALLEL SESSION: ORAL ABSTRACT PRESENTATIONS

ESID7-0208

IMMUNE DYSREGULATION IN COMMON VARIABLE IMMUNODEFICIENCY: PHENOTYPIC AND FUNCTIONAL IMMUNE PROFILING VIA MASS CYTOMETRY

E. Hsieh¹, D. Kong¹, R. Baxter¹

¹University of Colorado Denver, Immunology and Microbiology, Aurora- CO, USA

Background:

Common Variable Immunodeficiency (CVID) is a clinically and molecularly heterogeneous disorder characterized by hypogammaglobulinemia and failed vaccine responses, with several types of inflammatory complications. Given gaps in our understanding of the immunopathogenesis underlying these complications, identification of biomarkers capable of 1) predicting the development of specific inflammatory phenomena, or 2) identifying appropriate therapeutic targets, remains challenging. Our objective is to apply mass cytometry to deconvolve the different types of CVID complications, via phenotypic and functional characterization of immune dysregulated signaling pathways (T cell, B cell, and toll-like-receptor—TCR, BCR, TLR signaling).

Methods:

We have established a platform to examine simultaneously, at the single-cell level, phenotypic and functional characteristics of circulating lymphoid and myeloid cell subsets, by assessment of surface markers and activation status (ie. phosphorylation) of intracellular signaling proteins in CVID. Using peripheral blood samples from CVID patients with and without complications, we examined ex-vivo activation of TCR, BCR and TLR signaling pathways, by assessment of phosphorylation of proximal ITAMs (immunoreceptor tyrosine-based activation motifs, such as CD3ζ), Src family kinases (Lck), phospholipase Cy (PLCy) NF-κB, MAPK/Erk, and PI3K/Akt/mTOR pathways.

Results:

CVID patients with specific autoimmune/lymphoproliferative complications shared a unique multiparametric "signaling signature." Specifically, patients with granulomatous disease demonstrated defective TLR 7/8 signaling and increased TCR activation as compared to other CVID complications.

Conclusions:

The application of mass cytometry to the understanding of CVID immunopathogenesis holds promise to identify cellular and molecular mediators that initiate and propagate autoimmunity in CVID, providing novel candidate disease biomarkers to prognosticate these complications and direct therapeutic choice.
PARALLEL SESSION: ORAL ABSTRACT PRESENTATIONS

ESID7-0225

CELLULAR AND MOLECULAR CHARACTERIZATION OF DEFECTIVE THYMOPOIESIS IN MHC CLASS II DEFICIENCY

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1San Raffaele Scientific Institute, San Raffaele Telethon Institute for Gene Therapy SR-Tiget- Division of Regenerative Medicine- Stem Cells and Gene Therapy, Milan, Italy
2San Raffaele Scientific Institute, Pediatric Immunohematology and Bone Marrow Transplantation Unit, Milan, Italy
3Vita-Salute San Raffaele University, ., Milan, Italy
4Istituto di Ricerca Genetica e Biomedica- Consiglio Nazionale delle Ricerche, Milan Unit, Milan, Italy
5Humanitas Clinical and Research Center, ., Rozzano Milan, Italy
6Bambino Gesù Children's Hospital- University of Rome "Tor Vergata", University Department of Pediatrics, Rome, Italy
7Science and Technology Park Polaris, CRS4, Pula Cagliari, Italy
8University of Brescia, Department of Molecular and Translational Medicine- Pathology Unit, Brescia, Italy

Background:

Bare Lymphocyte Syndrome (BLS) is a rare primary immunodeficiency due to mutations in genes regulating the expression of Major Histocompatibility Complex class II (MHCII) molecules. MHCII deficiency results in impaired cellular and humoral immune responses, leading to severe infections and autoimmunity. The only treatment option is bone marrow transplantation, but success rate is limited. Therefore, development of new therapeutic strategies is needed.

Methods:

We took advantage of the Aβ0/0 mouse model of BLS to investigate the molecular and cellular mechanisms underlying defective thymopoiesis in this disease.

Results:

MHCII deficiency resulted in thymic structure perturbation, with reduced thymic epithelial cell (TEC) representation, especially in the medulla, where decreased frequency of Aire-expressing cells was found. These data were confirmed in a BLS patient thymic biopsy. CD4+ T-cell maturation resulted severely impaired, with few CD4+ T cells in thymus and secondary lymphoid tissues, mostly with activated/memory phenotype. Specific TEC transcriptome analysis through RNA-Seq on sorted cortical TEC (cTEC) and medullary TEC (mTEC) revealed an altered gene expression profile in Aβ0/0 mice, as compared to WT. We observed a dramatic reduction in the expression of genes involved in mTEC maturation in Aβ0/0 mTEC. As a consequence of the defective maturation of mTEC, we detected a strongly reduced expression of genes involved in central tolerance establishment, including tissue-restricted self-antigens, in Aβ0/0 mTEC.

Conclusions:
Our results reveal the impaired maturation of mTEC and the resulting defective establishment of central tolerance in MHCII deficiency. Further studies on TEC molecular alterations will be fundamental to develop new strategies for BLS treatment.
More than 1 g of cellular DNA is eliminated and degraded each day in humans. Given that microbial nucleic acid recognition serves as the major stimulus to an antiviral response, there is a fundamental requirement for such nucleic acid debris to be efficiently cleared - in order to limit the
misrepresentation of self nucleic acids as viral and the induction of interferon-mediated autoinflammation. The lysosomal endonuclease DNase II plays a central role in the clearance of nucleic acid generated through apoptosis and the phagocytosis of maturating erythroblasts.

**Methods:**

Screening for upregulated type I interferon signalling identified patients demonstrating an autoinflammatory disease characterized by severe but resolving neonatal anaemia, thrombocytopenia and hepatosplenomegaly, variably followed by later onset anaemia, recurrent fevers, membranoproliferative glomerulonephritis, liver fibrosis, hypogammaglobulinemia, arthropathy, insulin-dependent diabetes and skin vasculitis. We undertook exome sequencing, RNA and protein expression analyses and functional experimentation.

**Results:**

We identified biallelic mutations in *DNASE2*, encoding the endonuclease DNase II, associated with a loss of DNase II activity *in vitro* and in patient fibroblasts, which could be rescued by expression of wild-type protein. We recorded increased interferon alpha protein levels using digital ELISA, and enhanced interferon signalling by RNA-Seq and *ex vivo* assays. Constitutive up-regulation of phosphorylated STAT1 and STAT3 in patient cells was reduced by the JAK1/2 inhibitor ruxolitinib. A hematological disease transcriptomic signature and increased numbers of erythroblasts were also recorded.

**Conclusions:**

Neonatal anaemia and autoinflammation with enhanced type I interferon signalling is an autosomal recessive disease due to mutations in the human lysosomal endonuclease *DNASE2*. 
DOMINANT MUTATION IN TOPOISOMERASE 2-BETA CAUSES B CELL IMMUNODEFICIENCY

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Background:

Rare mutations that affect early stages of B cell development cause B cell immunodeficiencies and agammaglobulinemia.

Methods:

Here we studied two families with the autosomal dominant immunodeficiency with undetectable B cells and a rare combination of skeletal and urogenital defects, known as the BILU syndrome.

Results:

Exome sequencing led us to identify a novel heterozygous missense mutation A485P in the TOP2B gene that segregated perfectly with the clinical phenotype in four patients from the two unrelated BILU families. Topoisomerase 2-beta (TOP2B) is an enzyme that regulates DNA topology through the introduction of transient double strand breaks. TOP2B has been implicated in regulation of transcription during cell differentiation. We demonstrated that the newly found mutation A485P affects stability and catalytic activity of TOP2B. Bone marrow analysis of the BILU patient showed a complete absence of CD19+ B cell progenitors, but normal T cell and myeloid progenitors, suggesting that TOP2B is critically important for the early stages of B cell differentiation. To investigate the emerging role of TOP2B in B cell development we used mass spectrometry and characterised the TOP2B interactome. We demonstrated that in pro-B cells TOP2B is a part of a multi-protein complex that regulates epigenetic control of gene expression and that its inhibition abrogates expression of the key transcription factors known to regulate B cell fate.

Conclusions:

Our work discovered a novel dominant mutation in TOP2B that causes BILU syndrome and revealed a previously unrecognised role of TOP2B in cellular commitment to the B cell lineage.
DENDRITIC CELL AND MONOCYTE ANOMALIES IN HUMAN IKZF1 HAPLOINSUFFICIENT IMMUNODEFICIENCY

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Background:

Hematopoiesis is coordinated by transcription factors, which may act in multiple lineages and govern the expression of both differentiation and functional gene sets. Consequently, transcription factor mutations result in complex immunodeficiencies. Dendritic cells (DCs) are critical in the initiation and coordination of immune responses but receive limited attention in the analysis of primary immunodeficiencies. Heterozygous IKZF1 mutations were recently identified as the cause of an immunodeficiency syndrome characterized by progressive loss of B cells, hypogammaglobulinaemia, T cell subset skewing, recurrent infections and autoimmunity. Ikzf1 mutation affects murine dendritic cell development suggesting that anomalies of DCs potentially contribute to immune dysfunction in humans with IKZF1 deficiency. IKZF1 is also targeted for selective proteosomal degradation by the drug lenalidomide.

Methods:

Enumeration, phenotypic and functional analyses were performed on peripheral blood monocyte and DC subsets in twenty affected individuals from four kindreds with heterozygous IKZF1 mutations. Similar analyses were undertaken in cells with reduced IKZF1 protein levels resulting from in vivo or in vitro exposure to lenalidomide.

Results:

Loss of pDC, expansion of cDC1s and reduction in non-classical monocytes were consistent findings in patients with heterozygous IKZF1 mutations. Functional assays revealed a reduction in IFNa and IL-12 production. These findings were replicated in patients or cultures treated with lenalidomide. In vitro differentiation of DCs from CD34+ progenitors revealed a dose-dependent effect of lenalidomide on pDC development.

Conclusions:
This study confirmed the essential role of IKZF1 in human DC development and function and highlighted the contribution of DC and monocyte anomalies to primary immunodeficiency phenotypes.
Genetic analysis of a cohort of 221 patients with hyper IgE syndromes and chronic mucocutaneous candidiasis

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Background:

Hyper IgE syndromes (HIES) and chronic mucocutaneous candidiasis (CMC) constitute rare primary immunodeficiency syndromes with an overlapping clinical phenotype including recurrent fungal infections as well as recurrent pneumonias, skin abscesses and markedly elevated serum IgE levels in the case of HIES. During recent years a growing number of underlying genetic defects have been identified. The advent of next generation sequencing has since greatly facilitated the genetic work-up of these patients.

Methods:

We have analyzed a cohort of 221 HIES and CMC patients by targeted panel resequencing relying on Agilent HaloPlex and Illumina MiSeq technologies.

Results:

This approach has allowed us to identify a total of 74 mutations (34 previously described and 40 novel mutations) in 63 patients, which translates into a diagnostic hit rate of 28%. Specifically, mutations in AIRE, CARD9, DOCK8, IL12RB1, IL17RA, RLTPR, SPINK5, STAT1, STAT3 and ZNF341 have been detected. The average coverage of detected mutations in this study amounted to 933 reads. All
mutations could be confirmed by Sanger sequencing.

Conclusions:
The panel sequencing approach has allowed us to identify mutations in patients with atypical clinical presentations, highlighting the importance of a genetic diagnosis, as this may have important implications for counseling the patient regarding treatment, prognosis and family planning. In our experience the panel sequencing approach provided a cost-effective first-line genetic screening method. The achieved high coverage is one of the distinct advantages of panel sequencing over
whole exome and especially whole genome sequencing and is crucial to reduce errors, particularly in a diagnostic setting.
HETEROZYGOUS IKZF3 MUTATION IN PATIENTS WITH B CELL DEFICIENCY


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Background:

IKZF3 encodes AIOLOS, which is a member of Ikaros zinc finger protein family (IKZF). IKZF3 forms homo- and heterodimers and work as transcription factors involved in lymphocyte development. AIOLOS regulates pre B cell differentiation by suppressing pre-BCR component expression and regulating cell cycle.

Methods:

We identified heterozygous missense mutation in IKZF3 in a family of patients with B cell deficiency and B cell lymphoma by whole exome sequencing. Electrophoretic mobility-shift assay and structural analysis were performed for molecular characterization of the mutant protein. Knock-in mouse model of the patients' mutation was used to recapitulate phenotype of the patients. Transcriptome analysis of B cell progenitors in knock-in mouse was performed to identify targets of AIOLOS in B cell development. Genome wide DNA binding of AIOLOS and IKAROS was assessed to show interaction of mutant protein with its heterodimeric partner.

Results:

Mutant AIOLOS lost binding to its canonical consensus sequence and showed higher affinity to atypical motifs. Knock-in mouse of the patients' mutation reproduced human disease phenotype, showing B cell developmental blockade at pro B to pre B cell stage. Transcriptome analysis revealed down-regulation of the genes related to B cell development in the pre B cells of knock-in mouse. Moreover, DNA binding sites of Ikaros were altered in knock-in mouse harboring the patients' mutation.

Conclusions:

Here we report first cases of IKZF3 mutation in the patients with B cell deficiency. Our results indicate that the mutation in AIOLOS interferes IKAROS function, causing B cell developmental defects.
PLENARY SESSION 5: Late Breaking Oral Presentations

ESID7-0539

STING HYPERACTIVATION MOUSE MODEL (STING V154M +/-) IS CHARACTERIZED BY A COMBINED IMMUNODEFICIENCY DISEASE PHENOTYPE
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Background:
In humans, point mutations in the STING gene, such as V155M, lead to a severe autoinflammatory disease called SAVI (STING Associated Vasculopathy with onset in Infancy) characterized by vasculopathy leading to necrosis, pulmonary fibrosis and a lupus-like pathology.

Methods:
In order to better understand the pathophysiology of this disease, we generated a mouse model with the corresponding Sting mutation (V154M), using CRISPR/Cas9 technology.

Results:
Surprisingly, these mice develop a combined immunodeficiency disease (CID) with a significant decrease of peripheral B, T and NK cells. This defect seems to be present since the early stages of development. Indeed, in Sting V154M mice, this defect is already observed in Hardy fraction A of B-cell progenitors in bone marrow and at the double negative state (DN2) in thymus, and the size of thymus is significantly decreased, compared to control mice. In addition, the humoral response is impacted since there is almost no secretion of antibodies (total IgM and IgG). The results of in vitro experiments are in accordance with the T- and B-cell defects that we observed in vivo. Moreover, these mice show a significant expansion of the monocytes’ and granulocytes’ compartments in the periphery. Finally, as SAVI is an interferonopathy, we also checked the IFN production. Sting V154M mice are characterized by an IFN signature but their phenotype is independent of the IFN pathway, as the phenotype is not reversed in Sting V154M IFNAR KO mice.

Conclusions:
In conclusion, our results highlight a new and important role of STING in lymphocyte development.

Grant: ANR-14-CE14-0026
ROLE OF THE WISKOTT-SYNDROME PROTEIN (WASP) IN EARLY MARGINAL ZONE B CELL DIFFERENTIATION

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Background:

Patients with Wiskott-Aldrich syndrome (WAS), Was deficient and conditional B-cell Was-deficient mice lack marginal zone B-cell (MZB). Because Notch signalling is critical for MZB cell differentiation and Notch receptor endocytosis and endosomal trafficking can be postulated to be affected in the absence of the actin-regulatory function of WASp, we hypothesized that the MZB differentiation defects seen in WASp-deficient models may result from defective Notch signalling.

Methods:

To test this hypothesis, we crossed Was<sup>-<i>y</i></sup> and N2ICD mice to obtain animals in which the intracellular domain of the NOTCH2 receptor is specifically overexpressed in B-cells in a WASp-deficient background.

Results:

Analysis of splenic B-cells from these CD19<sup>cre</sup> NICD2 Was<sup>-<i>y</i></sup> mice showed clear increase of CD21<sup>hi</sup>CD23<sup>low</sup> MZB cells (24±1% of CD19<sup>+</sup> B-cells), over the levels observed in CD19cre Was<sup>-<i>y</i></sup> (1.8±0.4%) and even WT control mice (7.2±1.8%). By confocal microscopy, we confirmed that the newly generated MZB cells localized properly to the MZ (Figure 1A) and, contrary to what previously postulated, showed that their integrin-dependent retention in the MZ was conserved, since intravital injection of aL/a4-blocking antibodies led to their rapid and total disappearance in 4/4 tested mice.
Conclusions:

Therefore, we conclude that WASp is involved in the early stages of MZB cell differentiation. Mechanistic studies investigating the role of the temporal or B-lineage specific expression of WASp in MZB cell differentiation are ongoing.
INTERFERON-MEDIATED AUTOINFLAMMATION DUE TO A HOMOZYGOUS ACTIVATING MUTATION IN STAT2

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Background:

Interferon-α/β is a potent inflammatory mediator. Monogenic disorders of excessive IFN-α/β production (“type I interferonopathies”) are associated with deleterious inflammation. A key question is whether IFN-α/β is directly pathogenic in these disorders, or acts as a marker of disturbed cellular homeostasis.

Methods:

We investigated a 14-month-old child with excessive systemic inflammatory response to influenza A/Streptococcus pneumoniae co-infection in the context of intracranial calcification and developmental delay. He went on to develop haemophagocytic lymphohistiocytosis (HLH) with progressive neuroinflammatory disease despite treatment. We employed assays of IFN-α response in patient cells and performed lentiviral complementation of STAT2-deficient cells.

Results:

We detected persistent elevation of interferon-stimulated gene transcripts in whole blood. Targeted next generation sequencing identified a homozygous rare missense variant in STAT2. Patient cells were highly sensitive to IFN-α stimulation but IFN-α/β production was normal. Reconstitution of STAT2-deficient cells with mutant STAT2 recapitulated this cellular phenotype, consistent with hypermorphic behaviour. Data on the molecular mechanism will be presented.

Conclusions:
This is the first report of a gain-of-function mutation of STAT2 - a transcription factor that operates exclusively within the innate IFN signalling pathway. The resulting heightened cellular response to IFN-α/β is associated with significant tissue inflammation. This observation supports the hypothesis that IFN-α/β is directly pathogenic in monogenic type I interferonopathies, and might therefore be amenable to therapeutic blockade. HLH was a notable feature, which has not been observed in the context of excessive IFN-α/β induction, suggesting that the failure to restrain and/or terminate IFN-α/β signalling results in more serious immunopathology.
IFN-γ and CD25 drive unique pathological features during CD8 T cell hyperactivation in hemophagocytic lymphohistiocytosis

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Background:

The inflammatory activation of CD8 T cells can, when unchecked, drive severe immunopathology. Hyper-stimulation of CD8 T cells, through a broad set of initiating signals, can precipitate hemophagocytic lymphohistiocytosis (HLH), a life-threatening systemic inflammatory disorder. The mechanism linking CD8 T cell hyper-activation to pathology is complex, with excessive production of IFNγ and, more recently, excessive consumption of IL-2, proposed as complementary hypotheses.

Methods:

Here we formally tested the proximal mechanistic events through the genetic deletion of each pathway in a mouse model of LCMV-driven HLH using either Perforin/ IFNγ doubly knockout mice or Perforin knockout mice backcrossed with mice with constitutive CD25 depletion exclusively on CD8+ T cells.

Results:

We found a striking dichotomy between mechanistic basis of the hematological and inflammatory components of CD8 T cell-mediated pathology. The hematological features of HLH were completely dependent of IFNγ production, with complete correction following loss of IFNγ production, without any role for CD8 T cell-mediated IL-2 consumption. The mechanistic contribution of the immunological features, by contrast, were reversed, with no role for IFNγ production, but substantial correction following the reduction of IL-2 consumption by hyper-activated CD8 T cells.

Conclusions:

These results synthesize the mechanistic models of HLH pathology into a dichotomous disease process driven through discrete pathways. The synthesized model provides a new paradigm for understanding HLH, and, more broadly, the consequences of CD8 T cell hyper-activation.
RC3H1 NONSENSE MUTATION CAUSES A NOVEL IMMUNE DYSREGULATION SYNDROME ASSOCIATED WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Background:

Posttranscriptional regulation of RNA is crucial during the orchestration of innate and adaptive immune responses. The CCCH zinc finger protein Roquin1 coordinates the localization and decay of mRNA coding for key immune-regulatory molecules such as TNF and ICOS. The central role of Roquin1 in limiting immune responses is highlighted by the sanroque mutant mice in which a single missense mutation in Roquin1 unleashes severe immunopathology.

Methods:

Here, we present the case of a 16-year-old boy, first child of Belgian consanguineous parents. He was admitted to the hospital for chronic fever and ultimately diagnosed with hemophagocytic lymphohistiocytosis (HLH). Shortly after tapering the ciclosporin treatment, the patient was readmitted for a relapse of HLH.

Results:

Whole exome sequencing was performed and uncovered a homozygous novel nonsense mutation in the RC3H1 gene encoding Roquin1. Immunological work-up revealed expansion of mainly immature B-cells and an increase of both regulatory and Th17 T-cell populations. Similar to sanroque mice, serum levels of inflammatory cytokines (e.g. IFNg) and ICOS expression on T-cells were markedly elevated. Analysis of Roquin1 by immunoblotting revealed presence of full length Roquin1, albeit at strongly reduced levels. In contrast, low molecular weight products, presumably corresponding with cleavage products of Roquin1, were increased. Importantly, mutant Roquin1 failed to colocalize with P bodies, cellular organelles associated with mRNA degradation.

Conclusions:
Altogether, we report the first patient harbouring a nonsense mutation in the *RC3H1* gene with limited expression of a dysfunctional mutant Roquin1 protein. We postulate that the ensuing increased expression of ICOS predisposes to an immune dysregulation syndrome.
COMBINED IMMUNODEFICIENCY AND SELECTIVE CD8+ LYMPHOPENIA HAVING A NOVEL MUTATION IN LCK GENE

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Background:

The Lymphocyte-Specific Protien Tyrosine Kinase (LCK) gene is a member of the non-receptor protien tyrosine kinase (SRC) family of protein tyrosine kinases (PTKs) and the encoded protein founds in plasma associated with CD4 and CD8 proteins where it plays a key role in initial steps of the TCR signaling process by mediating phosphorylation of ITAM in the intracytoplasmic domains of CD3 lead to recruitment and activation of Zeta Chain Associated Protien (ZAP 70). Actually, LCK and ZAP70 functions and activities are tightly intertwined in T cell signaling.

Methods:

To report a 5 years old child with a novel frame shift mutation in LCK gene and a missense mutation in ataxia telangiectasia mutated (ATM) gene with clinical, laboratory and immunological phenotype goes with LCK gene defect rather than ATM.

Results:

The patient is a male Saudi child for consanguineous parents presented early in his age at 7 months old with chronic diarrhea, failure to thrive, abscesses and otitis media; found to have profound T-cell dysfunction and selective CD8+ Lymphopenia. Later, at the age of 3 years, he developed B-cell lymphoma. After stopping chemotherapy by 2 months, he got immunodysregulation, sclerosing cholangitis and minimal change nephrotic syndrome.

Conclusions:

Up to our knowledge, we are presenting a case of a novel frame shift mutation in LCK gene having a severe T-cell dysfunction with selective CD8+ lymphopenia similar to the immunological phenotype of ZAP 70 deficiency rather than the selective CD4+ lymphopenia seen in the previously reported cases of LCK protein defects.
ETHANOL EXTRACT OF MORINGA OLEIFERA LEAVES INHIBITS T-CELL PROLIFERATION

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Background:

Ethnopharmacological relevance: Moringa oleifera (family Moringaceae) commonly called Horseradish or tree of life is used in ethnopharmacology for immunity boosting.

Aim of the study: The objective of this study is to investigate the T-cell mediated immunity boosting property of the ethanol extract of the leaves of Moringa oleifera.

Methods:

Materials and methods: Toxicity profile was evaluated using trypan blue exclusion assay, and the probable mechanism of death evaluated by staining cells with annexin V and 7AAD. The effect of extract on proliferation was measured by staining with CFSE. The pattern of homing for CD3 and CD54 or CD28 activated T-cells in presence or absence of Moringa oleifera was monitored by estimating expression of CCR7.

Results:

Results: The result showed a concentration dependent significant increase in death by necrosis for inactivated Jurkat cells. Prestimulation with CD3 and CD54 or CD28 shifted the probable mechanism of death to apoptosis in a dose dependent manner. CFSE assay showed that inhibition of proliferation by Moringa oleifera was concentration dependent. The extract killed more than 50% of Jurkat cells at concentration higher than 80μg/ml. Expression of CCR7 was relatively higher in CD3 and CD28 prestimulated Jurkat than CD3 and CD54.

Conclusions:

Conclusion: The study suggests that the ethanol extract of Moringa oleifera leaves possesses positive immunomodulatory activity mediated via CCR7 expression. This finding justify the traditional use of Moringa oleifera extract in immunity boosting.
THE NEWLY TWO PATIENTS WITH DI GORGE SYNDROME IN AZERBAIJAN

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Background:

Introduction: Di George Syndrome (DGS) is a congenital severe T-cell immunodeficiency that is mostly associated with genetic defect - 22q11 deletion. DGS is characterized with congenital aplasia or hypoplasia of the thymus, facial dysmorphia, hypocalcaemia due to hypoparathyroidism.

Objective: Two our patients suffering from DGS have been identified in our Center. Initial manifestation in the first patient (2 month boy) was tetany and focal pneumonia. The dismorphic facial features of the patient include: micrognathia, hypertelorism, low-set ears. Chest radiography and US examination detected an absence of thymus shadow. The second patient (1 year old girl) also has aplasia of the thymus, multiple cardiac defects, cleft palate and congenital tetany. Both patients had repeated acute respiratory infections.

Methods:

Methods: Analyzing lymphocyte subsets through immune phenotyping, level of serum immunoglobulins was assessed by ELISA, NBT test has been applied for the detection of phagocytic activity. Diagnose was confirmed genetically in first patient: heterozygous variant in TBX1 gene deletion.

Results:

Results: Patients had a significant decreased CD3+cells, CD4+ and CD8+ subsets of T-cells, the quantity of NK cells in first patient was high, however, in second patient it was normal. B-cells in both patients were slightly increased. IgA and IgG levels were diminished and NBT test showed normal phagocytic activity. The replacement therapy with thymus hormones shows positive effect in the physical condition and immune indexes in both patients.

Conclusions:

Conclusion: Timely identification and diagnosis of this condition are based on the significant immunological changes and the results of the genetic analyses.
Background:

Common Variable Immunodeficiency (CVID) is a heterogeneous group of disorders characterized by impaired immunoglobulin production and dysregulation of immune system. Dysregulation may manifest as lymphoproliferative, granulomatous or autoimmune diseases, which occur in 25-30% of CVID patients. The underlying mechanisms have not been entirely revealed yet. Alterations, particularly in adaptive immunity, have been described, however, only few studies regarded T cell compartment were carried out. Therefore, we initiated our study to characterize predominant immune response (Th1, Th2 or Th17) and corresponding cytokine production and expression of associated markers (chemokine receptors and transcriptional factors) and to assess expression of activation markers in a cohort of CVID patients.

Methods:

PBMC (peripheral blood mononuclear cells) were isolated from whole peripheral blood using Ficoll-Paque gradient. After isolation PBMC were stimulated with ionomycine and PMA (Phorbol myristate acetate) for 6 hours including including protein inhibition by Brefeldin A. We measured expression and production of activation markers (CD69, CD154, HLA-DR), chemokine receptors (CXCR3, CRTH2, CCR6), transcriptional factors (T-Bet, GATA-3, ROR-gamma) and intracellular cytokine production (IFN-gamma, IL-5, IL-17) using standard flowcytometric protocols. All flowcytometric data were statistically analyzed.

Results:

Together 20 CVID patients with/without autoimmune complications were analyzed. We compared the results of CVID cohort to corresponding sex and age related cohort of healthy controls.

Conclusions:

We found skewing in the character of immune response and in expression of the assessed markers.
T Cell

ESID7-0106

AUTOANTIBODIES AND ACTIVATION OF THE CLASSICAL PATHWAY OF COMPLEMENT IN A GROUP OF IDIOPATIC CD4 LYMPHOPENIC PATIENTS

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Background:

Patients with Idiopathic CD4 Lymphocytopenia (ICL) have low numbers of circulating CD4 T cells and develop opportunistic infections, autoimmunity and papillomavirus-related dysplasia or neoplasias. CD4 lymphopenia is sometimes accompanied by low numbers of CD8 T cells and/or NK and/or B cells, suggesting the existence of etiologically different sub-categories of ICL. In the present work we tested whether complement plays a role in the disappearance of CD4 cells in ICL patients.

Methods:

We used flow cytometry to detect complement proteins C1q, C3, and C4c either directly ex vivo on PBMC from 74 ICL patients or after incubation of Healthy Control (HC) PBMC or lymphoid cells lines with patient’ sera.

We measured Complement Dependent Cytotoxicity (CDC) on HC PBMCs after incubation with patients’ sera.

ICL sera was depleted of immunoglobulins to assess their role in complement activation.

Results:

We found that PBMC from six ICL patients had C1q, C3, and C4c deposited on their T cells, pointing at the Classical Pathway (CP) of complement as the one triggered in vivo. We also found that the CP was activated by factors present in the patients’ sera since HC PBMC or lymphoid cell lines incubated with patients’ sera showed not only C3 deposition but also CDC. Furthermore, these effects were abrogated when depleting the sera from immunoglobulins, suggesting a crucial role of autoantibodies in the CP activation on ICL patients.

Conclusions:

Our work suggests the potential role of autoimmunity triggered by autoantibodies and complement activation as a new etiology of CD4 lymphopenia in a subgroup of ICL patients.
INDIVIDUAL RADIosenSITIVITY ASSESSMENT IN ATAXia TELAngIECTASIA FAMILIES

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Background:

Ataxia-telangiectasia (A-T) is an autosomal recessive neurodegenerative disorder characterized by radiosensitivity, genomic instability, and predisposition to cancer. The rarity of this disease, immense phenotype variation, disorders with similar features, and lack of definite laboratory test make definite diagnosis difficult. In addition, there is no rapid reliable laboratory method for identifying A-T heterozygotes, who susceptible to ionizing irradiation and have a higher risk of cancers, diabetes type 2, and atherosclerosis, for family screening and planning. This study aimed to measure individual radiosensitivity (IRS) in A-T patients and carriers in A-T families.

Methods:

With informed consent 4ml peripheral blood was collected from the 15 A-T patients, their parents, and 24 healthy controls with no family history of malignancy, diabetes type 2 or atherosclerosis. We used G2 breakage assay with G2-checkpoint abrogation with caffeine and SMC1pSer966 (pSMC1) in-cell colorimetric ELISA before and after irradiation for diagnosis and screening in A-T families.

Results:

Based on G2-assay and SMC1 phosphorylation, IRS of A-T carriers were significantly higher than healthy controls and significantly lower than in A-T patients. However, occasional overlaps were observed between controls and heterozygotes especially in ELISA method. In-cell ELISA in spite of rapidness and simplicity showed poor imprecision (22.49% coefficient of variation [CV]) in comparison to G2-assay (4.28%) for inter-day imprecision.

Conclusions:

It seems there is considerable heterogeneity of radiosensitivity among A-T heterozygotes, healthy controls and even A-T patients. However, this modified G2-assay can be proposed for individual radiosensitivity assessment within A-T families.
Background:

Since the beginning of HIV epidemic, opportunistic infections have been recognized as a common cause of complications of HIV infection, which lead patients to death. The relative frequency of these opportunistic diseases varies in different countries and even in different areas within the same country. The objective of this study was to assess the prevalence and association of opportunistic diseases and other co-morbidities in HIV sero-positive patients.

Methods:

The study was conducted in Dire-Dawa and the data was collected from 3,144 HIV sero-positive patients admitted to Dil-Chora Hospital from September 2006 to August 2011. A retrospective cross-sectional survey method was used to determine the prevalence and association of diseases. The data were analyzed using descriptive and inferential statistical tools such as frequency, percentage, and Pearson’s chi-square test.

Results:

The most prevalent disease among HIV sero-positive patients was oral candidiasis (36.7%) followed by recurrent upper respiratory infections (29.6%), and herpes zoster (28.3%). Oral candidiasis, herpes zoster, and amoebiasis were the most prevalent diseases in elderly patients. A statistically significant association was observed between CD4 level of patients and pulmonary tuberculosis, oral candidiasis, and pneumonia. Opportunistic diseases like papular pruritic eruption, pulmonary tuberculosis, recurrent pneumonia, recurrent upper respiratory tract infections and bacterial pneumonia continued to occur after ART.

Conclusions:

In general, this study indicated the variations in the distribution of diseases in different study areas and their associations with the CD4 level of patients. The health planners of the administrative council are recommended to devise mechanisms for prevention and control of the most frequent diseases.
CASE REPORT OF IMMUNODEFICIENCY 7, T-CELL RECEPTOR-ALPHA/BETA DEFICIENCY AND BCG VACCINATION.

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Background:

Severe combined immunodeficiency in Brazil is BCG-associated complications.

Methods:

Immunophenotyping by flow cytometry.

Results:

VGBG, female, first non-consanguineous daughter, was born on 02/10/2016 of cesarean delivery, with 40 weeks’ gestation, 3300g and 46cm. With 10 days of life, oral candidiasis and diaper dermatitis began, with progressive worsening of the back, neck and oral cavity without response to nystatin. At the age of 3 months, she was admitted to the hospital for low weight gain (14 g/day), pneumonia and disseminated moniliasis treated with itraconazole, rifampicin, ethambutol and isoniazid because she had the BCG vaccine at birth with local nodulation. Physical examination showed no adenomegaly or hepatosplenomegaly. Leukocytes 13,970 / mm³, neutrophils 10,000 / mm³, lymphocytes 2,235 / mm³ monocytes 558 / mm³; platelets 685,000 / mm³; TCD3⁺ 2,137 / mm³; TCD4⁺ 126 / mm³; TCD8⁺ 919 / mm³; CD3TCRαβ⁺ 28.6%; CD3 TCR γδ⁺ 74%; CD19 240 / mm³; NK 0.2%; NKT 0.8%. IgM 35.1 mg / dL; IgG: 200 mg / dL; IgA <6.5 mg / dL; IgE: <0.01 IU / mL; anti HBS titre (hepatitis B vaccine 3 doses) was 16 IU/mL. In the 30-day interval, she lost 350g of weight even breast-feeding and regular use of triple therapy for BCG vaccine infection and replacement of human IgG.

Conclusions:

BCG adverse reaction, candidosis, marked increase of γδ T cell and reduction of naïve δβ T cells, suggests the diagnosis of Immunodeficiency 7, TCR-alpha / beta deficient. This child at 10 months of age underwent haploidentic bone marrow transplantation with therapeutic success.
T Cell

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IDENTIFICATION OF RISK FACTORS FOR THE DEVELOPMENT OF INFECTIONS, AUTOIMMUNITY AND ALLERGY IN PATIENTS WITH DIGEORGE SYNDROME: A RETROSPECTIVE STUDY ON A LARGE COHORT

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Background:

Immunodeficiency in DiGeorge syndrome (DGS) is characterized by various degrees of T-cell deficiency, with multiple other factors contributing to its clinical phenotype. The aim of this study was to assess the impact of different factors in the development of infections, autoimmunity/allergy in partial DGS (pDGS).

Methods:

210 pDGS patients (M/F 1.15:1; mean age 9.97 ± 5.55) followed at Great Ormond Street Hospital from 2010-2016 were enrolled.

Results:

Low CD3% (<10th centile for age) was the commonest (67%) T-cell defect and IgM deficiency (IGMD) (<5th centile for age) the commonest humoral alteration (34%). Recurrent respiratory tract infections (RTI) and otitis media with effusion (OME) were identified in 69% and 37.1% of patients, respectively; multivariate binary logistic regression revealed their association with underlying conditions rather than immunodeficiency: in particular, RTI with gastroesophageal reflux (OR 2.59; 95% CI 1.25-5.38) and asthma/rhinitis (OR 2.93;95% CI 1.02-8.39) while OME with cleft palate (OR 2.96; 95% CI 1.43-6.14). However, immune defects including low CD3% (OR 4.06;95% CI 1.14-14.40) and IGMD (OR 3.03;95% CI 1.3-7.07) were commonest in patients with RTI requiring antimicrobial prophylaxis. Severe/atypical infections were observed in 8/210 patients, autoimmunity in 19/210 and allergy in 50/210 patients. Allergy was more prevalent in patients with IGMD (OR 5.12;95% CI 1.57-16.62).

Conclusions:

This study confirms the contribution of factors other than immunodeficiency in increased susceptibility to infections in pDGS. However, immunodeficiency should be considered when infections are frequent enough to require antibiotic prophylaxis. IGMD is the commonest humoral defect associated with increased allergy incidence and RTI requiring antibiotic prophylaxis.
EOSINOPHILIC GASTROENTEROPATHY COMPLICATING CD4+ IDIOPATHIC LYMPHOPENIA

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Background:

Idiopathic CD4+ lymphopenia [ICL] is a disorder of unknown cause, which may produce an immunodeficiency syndrome resembling AIDS, but there is likely a spectrum of causes, since the clinical phenotype is variable, and includes some patients who may go long periods with no opportunistic infection or other clinical signs of immunodeficiency. Other autoimmune inflammatory diseases have been associated with ICL [ITP, autoimmune hemolytic anemia, polymyalgia rheumatica, pemphigus vulgaris and pemphigus foliaceus, systemic lupus erythematosus, progressive multifocal leukoencephalopathy, primary sclerosing cholangitis, psoriasis, Sjögren's syndrome, alopecia areata, vitiligo, and perhaps sarcoidosis], but eosinophilic gastroenteropathy has not been previously reported.

Methods:

Below.

Results:

While half of cases of eosinophilic gastroenteropathy have not been food-induced disease, we here report a case of food-induced eosinophilic gastroenteropathy [as proven by a complete remission following institution of an elemental diet], which appears to have arisen as a complication of idiopathic CD4+ lymphopenia. The patient was initially seen by the Infectious Disease division for evaluation of pulmonary MAC infection, and the patient's gastrointestinal symptoms were initially attributed to mycobacterial infection. Endoscopic intestinal biopsies however demonstrated intense infiltrates of eosinophils, coupled with results of paracentesis which showed 70% eosinophils in ascitic fluid, establishing the diagnosis of eosinophilic gastroenteropathy. Serum IL-5 levels were only slightly elevated, and no elements of oligoclonality of T cells in the intestine could be shown. Allergen-specific IgE assays for food proteins subsequently shown to trigger blood eosinophilia by clinical oral challenge were negative.

Conclusions:

We conclude that the immunodysregulation produced by ICL may somehow predispose to eosinophilic gastroenteropathy.
Background:

The role of CD4 T cells in HIV has been overlooked for a long time, due to the elimination of those cells upon infection. However, in elite controller individuals the count of CD4 stays high, as they are able to control the viral load.

Methods:

We identify a CD4 T cell population specific from HIC individuals exhibiting a highly skewed TCR repertoire displaying high affinity and functionality and an amazing range of crossreactivity upon multiple HLA-DR molecules.

Results:

Using structural and functional approaches, we gain access for the first time to how CD4 TCR engages with an immunodominant HIV epitope presented by different HLA-DR molecules.

Conclusions:

The structural information revealed how a single TCR can crossreact onto so many different HLA molecules, and the role of the HIV epitope in driving this recognition. Our work provide some information on the role of CD4 T cells into the HIV infection.
Background:

Autosomal recessive Hyper-IgE Syndrome (AR-HIES) is caused mainly by mutations in DOCK8 (Dedicator Of Cytokinesis 8) gene, and is characterized by eczema, recurrent Staphylococcal and viral infections, lymphopenia and elevated serum levels of IgE.

Methods:

Here we describe three patients affected by AR-HIES but with clinical milder phenotype.

Results:

We performed genetic analysis that revealed homozygous or compound heterozygous DOCK8 mutations. We made a Western Blot assay on EBV-immortalized B-cells, and surprisingly we found a residual amount of protein in these patients, whereas classical DOCK8-mutated subjects display a total absence of the protein. This result was confirmed with a Real-Time PCR: these patients showed lower expression of DOCK8-mRNA compared to control, but higher than classical patients. We analyzed lymphocyte subpopulations of DOCK8 deficient patients by flow cytometry. All the patients displayed reduced number of memory B-cells, CD4+ naive T-cells and RTE (Recent Thymic Emigrants) CD4+. Moreover, two out of three subjects showed incomplete Th2 polarization as compared to typical DOCK8 deficient patients.

Conclusions:

In conclusion, we identified three DOCK8 deficient patients with clinical milder phenotype and residual expression of the protein. However, all of them have required hematopoietic stem cell transplantation.
Background:

Adenosine deaminase (ADA) deficiency is a rare cause of severe combined immune deficiency (SCID). This autosomal recessive genetic disorder typically leads to SCID with dysfunction of T, B, and natural killer (NK) cells (T-B-NK-SCID) that presents in the first few months of life. Herein, we report a 4-month-old boy presented with recurrent lower respiratory tract infections (LRTI) together with monoliasis.

Methods:

A 4-month-old boy presented with recurrent LRTI and persistent trush. He had hospitalization history in neonatal period because of sepsis and respiratory distress. Bronchoscopic examination revealed candidiasis on trachea and upper airways. On attendance, his vitals were as follows: heart rate 125/min, blood pressure 95/60 mmHg, respiratory rate 46/min. Breath sounds were course in both lungs and wheezing was heard during auscultation. Other system examinations were unremarkable. Laboratory examination revealed severe lymphopenia (white blood cell 3680/mm3, absolute lymphocyte count 100/mm3) and hypogammaglobulinemia (IgA <1 mg/dl, IgM 17 mg/dl, IgG 122 mg/dl). Anti-HIV, serum CMV DNA, EBV DNA levels were negative. Coronavirus NL63 was found to be positive from nasopharyngeal swab. Intravenous immunoglobulin (IVIG) treatment was applied due to severe lymphopenia and hypogammaglobulinemia. He was started on isoniazid, fluconazole and TMP-SMX prophylaxis and IVIG replacement twice a month. Molecular analysis revealed complete adenosine deaminase deficiency (ADA: 0 nmol/h/mg, normal: 26.4±10.0).

Results:

Adenosine deaminase enzyme replacement was also initiated. He was referred to a bone marrow transplantation center for curative treatment.

Conclusions:

Early diagnosis of SCID is vital, since infections can be life-threatening in these patients.
A 5 MONTH-OLD BOY WITH X-LINKED HYPER IgM SYNDROME PRESENTING INITIAL PNEUMOCYSTIS JIROVECI PNEUMONIA

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Background:

The classic hyper-IgM syndrome (HIGM) is the X-linked disease caused by mutation in the CD40 ligand gene on chromosome Xq26.3. Patients with HIGM present opportunistic or recurrent respiratory or gastrointestinal infections due to dysgammaglobulinemia during early childhood.

Methods:

A 5 month-old boy was admitted due to suddenly developed peripheral cyanosis for 1 day.

Results:

He was born with 39+6 gestational age and 4.49 kg (90th-97th percentile). On admission, he was showed peripheral cyanosis with tachypnea and coarse breathing sound without rale. Initial laboratory data showed Hb 16.5 g/dL, WBC 22,250/mm³ and 536,000/mm³ of platelet. Blood chemistry was normal with low C-reactive protein. Radiologic findings showed diffuse opacity of alveoli and interstitial inflammation. Pneumocystis jiroveci was isolated by bronchoscopic lavage. Other respiratory virus or mycoplasma study were negative. On immunologic studies serum IgG and IgA were low, but IgM was normal in his age. And, CD3 count was 2307/mm³ (37.3%), CD19, 3816/mm³ (61.7%), CD4, 528/mm³ (22.9%), CD8, 302/mm³ (13.1%), and CD3-CD56+ was 37/mm³ (0.6%). We identified new mutation of CD40 ligand to premature termination on exon 1 (c.89[6]; Leu32Serfs*17) by direct sequencing. His mother was shown as carrier, but his maternal grandmother and mother’s sister don't have the mutation. Now, we keep regular IVIG and prophylactic antibiotics for him and he is to take hematopoietic stem cell transplantation.

Conclusions:

This case was X-linked Hyper IgM syndrome from new mutation of CD40 ligand to premature termination on exon 1 (c.89[6]; Leu32Serfs*17).
GEOGRAPHICAL AND EVOLUTIONARY ANALYSIS OF THE CTPS1 INTRONIC VARIANT RESPONSIBLE FOR CTPS1 DEFICIENCY

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Background:

Cytidine 5' TriPhosphate 1 (CTPS1) deficiency results in a predominately T-cell mediated immunodeficiency associated with severe persisting viral infection, particularly with varicella zoster virus (VZV) and Epstein Barr Virus (EBV), as well as recurrent encapsulated bacterial infection. There is a high risk of extra-nodal lymphoma, associated with a high mortality rate. All 12 cases reported to date and a further case we have recently identified via the 100,000 Genomes Project have the same homozygous CTPS1 IVS18-1 G>C mutation and have ancestors originating from the North West of England.

Aims To date the origins and assess the distribution of the disease causing intronic CTPS1 variant.

Methods:

We studied the frequency of the causative CTPS1 variant across the UK using data from the UK biobank project and compared these data with exome and genome population databases worldwide. To assess the time of the occurrence of the variant we simulated the change of allele frequency under a recessive model and complex population demography.
**Results:**

Whilst the variant is more common in the UK than in other populations worldwide, the minor allele frequency in the North West of England is not higher than in other English locations. We estimate that the variant occurred around 9,000 years ago.

**Conclusions:**

Heterozygous carriers are spread throughout the UK, highlighting the importance of *CTPS1* genetic testing outside the North West of England in patients with severe VZV or EBV infection.

**Acknowledgements** This research has been conducted using the UK Biobank Resource (http://www.ukbiobank.ac.uk).
PLASMA CXCL13 LEVELS ARE INCREASED AND ASSOCIATE WITH FOLLICULAR HELPER T CELL SUBSET DISTRIBUTION AND ACTIVATION IN PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY AND AUTOIMMUNITY

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Background:

Common variable immunodeficiency (CVID) is the most prevalent symptomatic primary antibody deficiency. Autoimmunity (AI) is the most difficult feature to treat in CVID because of insufficient knowledge regarding its cause and the poor response of patients to therapy. CVID can be B- or T-cell mediated. In the latter case, follicular helper T cells (Tfh) and follicular regulatory T cells (Tfr), that respectively drive and regulate T cell-dependent humoral immunity in germinal centres (GCs), were shown to underpin CVID development. Because CXCL13 plasma levels are considered a blood biomarker that indicates GC activity, we analyzed its levels in patients with CVID+AI.

Methods:

We analyzed plasma CXCL13 levels and associated them with the frequency, subset distribution and activation status of circulating Tfh and Tfr in 11 patients (4 pediatric and 7 adult) with familiar CVID+AI of unknown etiology.

Results:

Plasma CXCL13 levels were found increased in patients with CVID+AI. Tfh and Tfr percentages were highly variable but within normal range and their frequencies did not correlate with CXCL13 plasma levels. Altered distribution of Tfh subsets (i.e. Tfh1/2/17 and highly functional Tfh) and heightened expression of PD-1 were seen in all patients. Interestingly, plasma CXCL13 levels showed a statistically significant inverse correlation with the Tfh2 subset.

Conclusions:

Elevated plasma CXCL13 levels were observed in patients with antibody deficiency. This evidence is in line with previous studies suggesting a possible Tfh-mediated mechanism in the clinical phenotype of patients with CVID and AI.
LONG-TERM ASSESSMENT OF IMMUNE SYSTEM HOMEOSTASIS IN PATIENTS WITH DIGEORGE SYNDROME

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Background:

Literature has shown that age-related T-cell number decline is slower in partial DiGeorge (pDGS). In this study we evaluated age-related T-cell compartment variations in pDGS.

Methods:

Scatter diagrams evaluated the variations of single parameters with age in 438 pDGS patients followed-up at Great Ormond Street Hospital from 2001-2016 and in 75 age-range matched controls.

Results:

Lymphocyte subset count and percentage were always lower in patients and declined with age more slowly in patients than in controls. The number of patients with a low CD3 count/percentage (<10th centile of the age) reduced with age; from 73 to 37% for count and from 85 to 54% for percentage. In a subgroup of patients with low CD3 count/percentage in the first years of life, more than 60% of the patients recovered the CD3 count/percentage defect by 18 years of age. Naive CD4 and CD8% were lower in patients and declined with age more rapidly in patients than in controls. Memory CD4 and CD8% though were higher in patients, increasing with age more rapidly in patients than in controls. T-cell receptor Vbeta spectratyping showed age-related deterioration. 9/18 patients (50%) aged >12 years versus 8/42 (19%) <12 years showed abnormal spectratyping. Abnormalities involved the CD8 compartment in most of the cases.

Conclusions:

In conclusion, this study confirms that the normal age-related decline of T-cell numbers in childhood is slower in pDGS and shows for the first time an age-related deterioration of the spectratyping, supporting the hypothesis of homeostatic peripheral expansion of T-cells particularly in the CD8 subset.
THE EVALUATION OF THE NUMBER OF TH17 LYMPHOCYTES IN PERIPHERAL BLOOD OF IMMUNE DEFICIENT PATIENTS WITH FUNGAL INFECTION

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Background:

- The determination of Th17 cell numbers in peripheral blood is a challenging test but might help identify patients with an underlying immunodeficiency that predispose to mucocutaneous fungal infection and evaluation of Th17 before the gene assay help us that which groups of immunodeficiency needs to be evaluated.

Methods:

- A case control study was done in 8 patients with fungal infection in Mofid Children’s Hospital that combined immunodeficiency ruled out. After Ethic committee confirmation and questionnaire filling, mononuclear lymphocyte was separated and incubated in culture medium with inomycine. Afterthat flowcytometery was done with antiCD3 and anti IL17 A usage. Result was analysed by spss-16.

Results:

- 5 patients were female. Their ages were 4 – 35 yrs. 3 patients had related parents. Two patients were a mother and her son. Sign and symptoms includes: colon candidias, candidial esophagitis, adrenal insufficiency and hypothyroidism respectively in 1 case, dermatitis, candidial vulvovaginitis and persistent thrush respectively in 2 cases, nail fungal infection and pulmonary infections in 3 cases. Serm Igs and LTT to PHA and BCG were normal in all of them. LTT to candidia was impaired in 3 patients. Flowcytometery results in 4 patients were available and showed normal CD3, CD4, CD8. TH 17 numbers were : 0.79 ± 0.18 in patient group and 3.63 ±1.01 in control group. (pvalue:0.001)

Conclusions:

With regard to high cost of genetic study, TH 17 assay in patient with fungal infections could be a good tool for narrowing differential diagnosis.
STUDY OF THE SIGNALING PATHWAYS OF T- AND B-CELL ACTIVATION IN CVID-SIGHT TO THE PATHOGENESIS OF DISEASE

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Background:

The most essential in pathogenesis in Common variable immunodeficiency (CVID) is an intrinsic dysregulation of the immune system leading to defective B-cell differentiation and function. The role of T-cell subpopulations in the mechanisms of the disease is poorly studied. The aim of this study is to examine the intracellular JAK/STAT and mitogen activated protein kinase (MAPK) signal pathways in lymphocytes of CVID patients.

Methods:

Flowcytometric analysis of spontaneous and interleukin induced expression of phosphorylated proteins- ERK 1/2, p38MARK, Stat3, Stat5 and Stat6 in CD4+, CD8+ and CD19+ lymphocytes in 10 patients and 10 healthy controls.

Results:

Results in patients in comparison with healthy controls showed the following statistically significant differences: increased spontaneous Erk activation of CD4+ T cells, increased IL-2/STAT5 induced activation and increased STAT5 activation index in CD4+ cells in combination with reduced spontaneous STAT5 activation of CD8+ lymphocytes, but increased STAT5 IL-2-dependent activation index in CD8+ T cells; decreased induced STAT3 activation and decreased STAT3 activation index in B lymphocytes; decreased induced STAT6 activation of CD8+ T cells and reduced STAT6 activation index CD8+

Conclusions:

The impairments we found could explain the cytokine Th1/Th2 disbalance in disease, and also alterations observed in CVID helper and cytotoxic T cells as well as B cells count, phenotype and function. Such a research approach in a larger group in combination with genetic analysis would have not only scientific but also clinical and therapeutic potential.

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NEW INSIGHTS ON THYMIC FUNCTION AND T CELL DEVELOPMENT IN PATIENTS WITH THYMIC DEFECTS

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Background:

Autoimmunity has been observed in several immunodeficiencies characterized by altered thymic development and defects in T-cell compartment, such as Di George Syndrome (DGS), Down Syndrome (DS) and CHARGE Syndrome. Thymus has an important role in maintaining central/peripheral tolerance; it is possible that an abnormal bulk of thymic tissue results in an incomplete thymic selection or that a reduced/altered AIRE expression results in the development of autoreactive lymphocytes. It is not known whether the immunodysregulation in these patients is more related to thymic alterations or to lymphocyte defects.

Methods:

We evaluated the thymic architecture and function (stroma and thymocyte development) and the peripheral blood (T lymphocytes, Tregs and Recent Thymic Emigrants) in DGS, DS, CHARGE Syndrome patients. We also investigated whether a possible impairment of AIRE expression could affect thymic selection leading to peripheral autoimmunity. Results were compared to age-matched healthy donors.

Results:

DGS patients showed lower frequency of T cells and Tregs, characterized by an activated phenotype and a reduced suppressive capacity in vitro. Histology of thymic tissue revealed several alterations. Immunodysregulation showed in DGS patients could be overlapped, for the most part, to that of CHARGE Syndrome patient. DS patients showed an altered thymocyte distribution and increased frequency of Tregs with impaired suppressive function in vitro. Immunohistochemistry and gene expression analyses showed abnormalities that highlight a possible accelerated aging.
Conclusions:

The analysis of thymus maturation showed an altered development of thymic compartments, with features peculiar for each syndrome. Thymic alterations could be correlated with the imbalances of immunity typical of these patients.
A NOVEL CASP8 MUTATION IDENTIFIED IN A PATIENT WITH LYMPHOPROLIFERATION, CAUSING IMPAIRED NFκB SIGNALING AND APOPTOSIS

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Background:

Caspase 8 is an intracellular cystein-aspartic acid protease, primarily involved in signaling leading to apoptosis. So far, only 4 patients with a loss-of-function missense mutation in the CASP8 gene have been described in the literature, suffering from severe lymphoproliferation and increased susceptibility to infections. Here we present another patient with lasting severe lymphoproliferation, in whom a novel homozygous missense mutation in the CASP8 gene was discovered.

Methods:


Results:

We identified a homozygous c.1276C>T mutation in the patient, which was present in heterozygous state in his father, mother and brother, who are all healthy. We show highly activated T-cells, with increased effector memory CD4⁺ T-cells and low naive CD4⁺ and CD8⁺ T-cells in the patient. TNF-α induced NFκB signaling was normal, whereas anti-CD3/28 induced NFκB signaling was decreased. Both anti-FAS induced caspase 3 cleavage and apoptosis were impaired.

Conclusions:

We identified a patient with severe lymphoproliferation and a novel mutation in the CASP8 gene. We verified the functional impact of the mutation using an array of tests, detecting decreased NFκB signaling, anti-FAS induced apoptosis and caspase 3 cleavage.
T Cell

ESID7-0286

SEVERE T-CELL DEFICIENCY IN A GIRL WITH ANHIDROTIC ECTODERMAL DYSPLASIA
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Background:

We report a case of severe T-cell deficiency in a girl with thymus aplasia and anhidrotic ectodermal dysplasia. The girl was born at 36 weeks of gestational age and showed complete cleft palate, rib malformation, muscle hypotonia and thin dry skin. Further pathologic findings were an abnormal hearing test and bilateral micropapilles.

Methods:

Since the girl failed to thrive and suffered from recurrent oral candidiasis, fever and pulmonary infections, further immunologic diagnostic was performed. Imaging revealed an absent thymus in MRI and ultrasound.

Results:

The patient had severely reduced T cell counts (122 CD4, 12 CD8, 916 B, 474 NK), CD8:CD45RA:CD31: 0.4 % of CD4+ cells) and absent proliferation to PHA. Immunoglobulin serum levels were normal, Genetic diagnostics excluded a microdeletion 22q11.2 SCID panel diagnostic was negative. Because of the association of thymic aplasia, cleft palate and ectodermal dysplasia, a TP63-mutation (EECUD-syndrome) was excluded. Array CGH revealed a duplication in 9q33.1q33.2. To evaluate the potential benefit of a thymic transplant OP9-DL4 culture of bone marrow stem cells was performed and confirmed normal capacity to generate T cells in vitro.

Conclusions:

Taken together, we present a case of a severe T-cell deficiency in combination with different accompanying somatic abnormalities. Thymus aplasia seems to be the underlying defect. The only detectable genetic aberration is a duplication 9q33.1q33.2. Whether this is related to this clinical syndrome is not clear. Thymus transplantation is currently considered for this patient.
Background:

Common variable immunodeficiency (CVID) is characterised by antibody production failure. T follicular helper (Tfh) cells are essential for antibody secretion. Tfh cell subsets vary in their helper functional efficiency. We postulated that Tfh cell subsets might be deranged in CVID.

Methods:

CVID patients were split into two groups: switched memory B cell high (smB+) and low (smB−). Samples from CVID and healthy donors (HDs) were studied by multicolour flow cytometry. pTfh cells were defined as CD3+CD4+CD45RA−CXCR5+ lymphocytes.

Results:

We observed that pTfh frequencies were significantly higher in both CVID groups compared to HDs. pTfh1 (CXCR3+ CCR6−) percentages were significantly higher in smB− CVID compared to smB+ CVID and HDs. Conversely, pTfh17 (CCR6+ CXCR3−) frequencies were significantly lower in the smB− CVID compared to HDs. The expression of ICOS in pTfh1 subset was significantly greater in smB+ CVID compared to smB− CVID and HDs. Similarly, the expression of ICOS in pTfh2 subset was significantly higher in smB CVID compared to HDs. PD-1ICOS pTfh1 and PD-1ICOS pTfh2 were significantly lower in both CVID groups compared to HDs. PD-1ICOS pTfh17 were lower in smB−CVID compared to HDs.

Conclusions:

pTfh cell subsets recognised for their optimal efficiency in providing B cell help are relatively reduced in CVID compared to HDs; this is specially so in the smB− CVID group. This may provide a mechanistic insight into the cause of antibody production failure.
COMPOUND HETEROZYGOUS IL-2RA MUTATIONS: IMPACT ON EFFECTOR AND REGULATORY T CELLS
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Background:
IL-2Rα/CD25 deficiency (OMIM 606367) is the underlying cause of a rare syndrome characterized by immunodeficiency associated with autoimmunity. CD25 is essential for maintenance and expansion of both T effector (Teff) and FOXP3⁺ T regulatory (Treg) cells. Our aim is to assess functional impairments affecting both cell subsets in a family of individuals carrying two different CD25 mutations not previously reported, and affected by predominant autoimmunity.

Methods:
Membrane CD25 expression was tested by flow cytometry in CD4⁺ T cells freshly isolated or upon TCR-mediated stimulation. STAT5 phosphorylation was assessed in response to IL-2, IL-7, or IL-15. Treg cells were sorted based on CD127⁻TIGIT⁺ phenotype, and either expanded and/or tested for suppressive activity.

Results:
CD25 expression was absent in Teff cells of individuals carrying a compound heterozygous CD25 mutation, but could be induced upon polyclonal stimulation. The percentage of FOXP3⁺CD127⁻ Treg cells was within the normal range. Teff and Treg cells presented impaired STAT5 phosphorylation in response to IL-2 stimulation, but a normal response to IL-7 or IL-15. Freshly isolated Treg cells presented low suppressive activity, whereas expanded Treg cells had restored suppressive capacity.

Conclusions:
Individuals carrying CD25 mutations had impaired functional responses of Teff and Treg populations. CD25 expression could be induced only upon stimulation, raising the question of whether these mutations could affect the stability of the protein, especially on Treg cells that physiologically require persistent CD25 expression. Studies are ongoing to determine the effect of these mutations on stability, expression and function of CD25 on multiple cell types.
DIGEORGE SYNDROME – THE EXPERIENCE OF A PORTUGUESE CENTRE
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Background:

DiGeorge Syndrome constitutes a spectrum of disorders due to a deletion in the region of 22q11.2 with wide phenotypic variation. The estimated incidence is 1/4000 persons. It may affect many organs and systems and present with a wide range of clinical manifestations.

Methods:

In order to characterize patients with DiGeorge syndrome, the authors reviewed the clinical records of the children and adolescents followed at the outpatient clinic of our Unit.

Results:

Eighteen children and adolescents were identified (13 males, 5 females; median age 15 years-old). Median age at diagnosis was 38 months (2 months to 11 years). Sixteen children presented a de novo 22q11.2 mutation. The diagnosis was made, in most of the cases, following investigation of cardiopathy which was present in 13 cases. Eight required cardiac surgery. More than 50% had the characteristic facial features, 2 had cleft palate and 3 had velopalatine insufficiency. Two presented severe neonatal hypocalcaemia. Thrombocytopenia was identified in 4 patients. Eleven had T-cell deficiency. Infections were more frequent in the first years of life. During the follow-up, 10 were found to have delayed psychomotor development and psychiatric disorders.

Conclusions:

The characteristic facial features can be missed at early ages, so it is specially important to have a high index of suspicion in newborns with heart anomaly or hypocalcemia, infants with recurrent infections or children with developmental delay. An early diagnosis is important to manage the related problems associated with this syndrome, improving the prognosis of the affected children and adolescents and allowing genetic counseling of the families.
NOVEL MUTATION IN A FAMILY WITH IKKB DEFICIENCY
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Background:
Inhibitor of κB Kinase β (IKKβ) deficiency is a recently described type of severe combined immunodeficiency. It is caused by loss of function mutation of IKBKB gene that impede the NF-κB transcription pathway.

Methods:
The clinical and immunological data of four patients diagnosed with SCID from two related Saudi families were collected. Whole exome sequencing was performed on the index case, a normal sibling and their parents to define the genetic etiology.

Results:
The patients had early onset (2-4 months of age) severe infections caused by viruses, bacteria, mycobacteria and fungi. They all demonstrated low absolute lymphocyte count (420-2680 cells/mm³) and hypogammaglobulinemia. Their lymphocytes fail to respond to PHA mitogen. Three patients succumb to their disease. One underwent hematopoietic stem cell transplantation (HSCT) from a fully matched sibling successfully, but still struggling with BCGitis. A novel homozygous nonsense mutation in IKBKB, c.850C>T (p. Arg284*) was identified in the patient. Interestingly, all patients have delayed umbilical cord separation. None of the eleven patients with IKKβ deficiency described in the literature had this except one Saudi patient with a different mutation. This feature has been commonly described in patients with leukocyte adhesion defects. In addition, 20% of IRAK4 deficiency patients, another protein contributing to NF-κB activation, found to have delayed cord separation with no clear underlying mechanism.

Conclusions:
IKKβ deficiency has high morbidity and mortality rate. Immune reconstitution with HSCT should be undertaken as early as possible. Delayed cord separation in CID patients may be a clue to IKKβ deficiency.
ANALYSIS OF GENE EXPRESSION PROFILE IN REGULATORY T LYMPHOCYTES IN CHILDREN WITH TRANSIENT HYPOGAMMAGLOBULINEMIA OF INFANCY AND SELECTIVE IGA DEFICIENCY


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Background:

Transient hypogammaglobulinemia of infancy (THI) is a primary immunodeficiency disease (PID) characterized by reduced serum IgG (and sometimes IgA) levels in early childhood. Selective IgA deficiency (SIgAD) is the most common PID in Caucasians, characterized by an undetectable serum level of IgA with normal IgM and IgG levels. The underlying, definitive basis for both disorders remains unknown. Our previous study, performed on children with THI and SIgAD showed significant differences in the number of circulating Treg cells between these groups. To determine the role of Treg cells in the pathomechanism of THI and SIgAD, gene expression profile analysis of isolated Treg cells was performed.

Methods:

Treg cells were isolated from peripheral blood mononuclear cells using magnetic sorting. The analysis of transcriptome-wide gene- and exon-level expression profiles was done with microarray technology using Clariom D assays (Affymetrix) and Transcriptome Analysis Console (TAC) Software.

Results:

Based on preliminary results, the significant differences in gene expression profiles as well as in alternative splicing between THI and SIgAD patients was observed. We found 122 statistically significant genes, up- or down-regulated in SIgAD in comparison to THI group and some of them seems to be involved in the transforming growth factor beta signaling pathway.

Conclusions:

At this stage, obtained data indicate the possible, important role of T_{reg} cells in the development of studied PID. Gene expression profiles of Treg cells is currently being evaluated and should be updated in the poster.
**THYMIC TRANSPLANTATION IN A FOXN1 DEFICIENT SCID PATIENT**

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**Background:**

Thymus transplantation is a promising therapy for infants born without thymic function. These patients lack T cell development and have a severe primary immunodeficiency.

**Methods:**

Alopecia, nail dystrophy, marked lymphopenia (800 cells/mm³) and absence of thymic shadow on radiograph were found in a newborn at Sassari’s Hospital and therefore a diagnosis of SCID was suspected.

**Results:**

She was transferred to our center to confirm the diagnosis. Immunological tests showed absence of T cells and immunological phenotype suggested a diagnosis of T-B+NK+SCID. Mitogen responsiveness was absent. Genetic analysis confirmed compound heterozygous mutations in FOXN1 gene.

The only lifesaving treatment is thymic transplantation. She was transferred to Great Ormond Street Hospital, London to undergo transplantation. After a few days in London, she developed a rash and an increase of T memory cells, indicating the potential development of an Omenn-like Syndrome. For this reason, she was treated with ATG and methylprednisolone. Thymic transplant surgery was performed at two months of age without complications. At days +20 after transplantation the baby returned to our center. Patient remained clinically well during her admission: no fever, infections, CMV, EBV and Adenovirus always negative.

Two months post-transplantation she shows low level of T cells (CD3+: 15 cell/µL; CD3+CD4+: 2 cell/µL; CD3/CD8+: 6 cell/µL), no mitogen response to PHA and CD3. This is expected as immunological reconstitution only starts later (5-6 month). She is well and at home.

**Conclusions:**

To our knowledge this is the successful therapeutic strategy that allowed the cure of 2 previously treated children affected by FOXN1 SCID (Markert ML et al. 2011. Blood 117:688-96) Bone marrow transplantation is not indicated.
IDENTIFICATION OF A NOVEL VARIANT IN CD40L IN TWO DISTANTLY RELATED INDIVIDUALS WITH A NON-CLASSICAL PRESENTATION ALLOWS RAPID SCREENING IN NEWBORN E. Staples¹, S. Grigoriadou², J. Stephens³, H. Baxendale⁴, H. Firth⁵, E. Gkrania-Klotsas⁶, D. Kumararatne⁴, K.G.C. Smith¹, H. Lango Allen³, J. Thaventhiran¹
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Background:

CD40L deficiency (X-linked hyper IgM syndrome) often presents with antibody deficiency but patients are also at risk of opportunistic infection.

The NIHR Bioresource- Rare Disease (BRIDGE) study has whole genome sequenced 912 individuals referred by Clinical Immunologists with suspected primary immune deficiency.

Methods:

Patients’ WGS data were screened for likely pathogenic variants in genes in the 2015 IUIS Phenotypic Classification for Primary Immunodeficiencies.

To assess CD40L protein expression CD4 T cells were separated using magnetic beads, then stimulated with CD3/28 beads overnight and CD40L expression measured by flow cytometry.

Results:

Two males aged 50 and 58, initially thought to be unrelated, were found to be hemizygous for the same novel c.98 T>A variant in the CD40LG gene, resulting in an I33N substitution in the transmembrane region of CD40L. Genomic data suggested 3% relatedness and family history revealed that their mothers were first cousins.

The two patients had both been diagnosed with CVID, though the 58-year old was reclassified as combined immunodeficiency after developing cerebral and retinal toxoplasmosis. The 50-year old had chronic active hepatitis, arthritis and thrombocytopenia. Both patients had normal IgM levels.

Following the molecular diagnosis the daughter of the 58-year old had a baby boy. He was reviewed by a Clinical Geneticist and Paediatric Immunologist and treated with cotrimoxazole prophylaxis until genetic and protein results excluded CD40L deficiency.

Conclusions:
Whole genome sequencing identified a novel causal variant in CD40L, linked two distantly related individuals and enabled rapid screening of a newborn male in this pedigree.
T Cell

ESID7-0397

AN UNUSUAL PRESENTATION OF ZAP-70 SCID WITH NORMAL CD8 T CELLS, ANTI-CYTOKINE AND ANTI-PLATELET ANTIBODIES AND A B CELL LYMPHOMA
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Background:

A seven month old male of Mennonite background presented with chronic dermatitis and bloody diarrhea from age 2 months, and weight loss. He had generalized lymphadenopathy and hepatosplenomegaly, atopic dermatitis and a petechial rash.

Methods:

Extended immune investigations were initiated.

Results:

Hemoglobin was 98 g/L, ANC was normal, ALC was markedly elevated at 13.3x10e9/L, and he had severe thrombocytopenia (platelets 1000x10e6/L). ALT and AST were greatly increased. IgG 24 g/L (normal 2.7-9.1) and IgA and IgM were also elevated. IgG showed an oligoclonal gammopathy. Previous CBCs were normal at age 2 months. Coombs was positive. There was no increase in platelets after repeated platelet transfusions. Lymphocyte subsets showed normal numbers of CD3(3496), CD4(2521), CD8(860), CD56(516) and CD19(1432) with reduced CD4+CD45RA+(32%) cells. There was no proliferation to mitogens or anti-CD3 but a strong response to IL-2. TCR Vβ distribution normal except for an increase in Vβ13.1. Microbiology demonstrated a severe CMV viremia. Further studies found antibodies to interferon-α and -ω and IL-12. A homozygous pathogenic mutation was found in ZAP-70 c.1624-11G>A. The patient was treated with rituximab, prednisolone, ganciclovir, foscarnet and romiplostin causing a decreased CMV viremia, mild increase in platelets, and some improvement in hepatitis. He then developed an enlarged cervical lymph node on biopsy shown to be a B cell lymphoma. He was treated with a MUD CMV positive bone marrow transplant.

Conclusions:

This patient exhibited an unusual ZAP-70 phenotype with abundant CD8 T cells, numerous autoantibodies to platelets, erythrocytes and anti-cytokine antibodies and the development of a B cell lymphoma.
IDIOPATHIC CD4 LYMPHOPENIA, CASE REPORT
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Background:
There are few reported cases with diagnosis of idiopathic CD4 lymphopenia, first case was reported in 1992. To reach this diagnosis, following criteria: CD4 lower 350 cells/μL or 20% of circulating lymphocytes, exclude deficiencies of OKT4, normal or increased number of CD8, CD19 and CD56, HIV negative and exclude other primary causes of lymphopenia.

Methods:
38-year-old female with keratoconus, during preoperative assessment for corneal transplantation, chest X-ray with mediastinal tumor, and plantar papilloma of a year evolution with a dimension of 5 cm on left foot, on this wise a study protocol was developed. Also she said weight loss approximately 12 kg in 8 months, nocturnal diaphoresis and asthenia. 12-2015: by mediastinoscopy, took ganglion sample, RFLP-PCR for Mycobacterium tuberculosis complex positive, tuberculosis treatment started, improvements were shown. 05-2016, febrile syndrome, asthenia rebooted, chest CT showed increase in mediastinal mass, histoplasm was requested, with positive by immunoflorescence.

Results:
Quantifications of serum lymphocytes showed recurrent lymphopenia, subpopulations (08-2016): leukocytes 5300mm3, neutrophils 63% (3657 cells/mm3), Monocytes: 5% (371 cells/mm3), lymphocytes 14% (742 cells/mm3), CD16+56: 14%, CD3: 71%, CD4: 39% (205 cells/mm3), CD8: 55%. Three quantifications of P24 antigen and qualitative determination of antibodies HIV 1-2: negative, it currently has 4 quantifications since 2015 until 2017, all negative

Conclusions:
Diagnosis of idiopathic CD4 lymphopenia should exclusion of other causes of lymphopenia, a situation that has hitherto been performed. It continues in study protocol in search of mutations. Currently, HPV, histoplasm and tuberculosis are considered diseases conditioned by the decrease of the cellular immunity of this patient.
T Cell

ESID7-0404

COMBINED IMMUNODEFICIENCY: CASE REPORT OF MUTATION IN MALT 1
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Background:

Term child, male, non-consanguineous parents, presenting with important cutaneous xerosis, diffuse erythema and severe eczema.

Methods:

Case report of a Severe Combined Immunodeficiency due to MALT 1 mutation

Results:

Family history of 2 siblings with similar eczema, malnutrition and recurrent infections dead at age of 5 and 8 months. Laboratory tests revealed hypogammaglobulinemia, severe neutropenia, normal serum albumin, normal lymphocyte immunophenotyping and bone marrow with dysgranulopoiesis. Human immunoglobulin replacement and antibiotic prophylaxis have been started at 3 months of age. There was a progressive failure-to-thrive, delayed neuropsychomotor development and chronic non-bloody diarrhea despite of neutropenia improvement after initial medical management. He was hospitalized for several times at the Immunology Service of the Hospital das Clínicas da Universidade Federal de Minas Gerais, sometimes with ICU admissions, due to poor weight gain, difficult-to-control infections and hydroelectrolytic disturbances. Genetic sequencing was finally performed by Whole Exome wich found a mutation in homozygous MALT1 (mucosa associated lymphoid tissue lymphoma translocation gene 1) located on chromosome 18, position p. Asp471His. Indicated Bone Marrow Transplantation, as a curative treatment, but patient died due to complications of the disease, before this happened.

Conclusions:

MALT-1 deficiency can lead to fatal complications as severe malnutrition and hidroelectrolytic disturbances needing prompt intervention to preserve patient life.
AN INHERITED STAT3 LEU225SER GAIN-OF-FUNCTION MUTATION IS ASSOCIATED WITH AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME LIKE DISEASE

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Background:

Patients with hyperactive or constitutively active STAT3 mutations share common clinical characteristics with patients suffering from autoimmune lymphoproliferative syndrome (ALPS) like disease. We report on an 8.5 year old boy, who first presented with generalized lymphadenopathy. Abdominal ultrasound showed multiple enlarged lymph nodes. Histologic examination revealed benign follicular and paracortical hyperplasia. Immunophenotyping of peripheral blood detected 6-10% double negative T lymphocytes. FAS mediated apoptosis was decreased. The presumptive diagnosis of ALPS was established.

Methods:

To analyze the cause of the disease whole-exome sequencing, Sanger sequencing and reporter assays were carried out.

Results:

Sanger sequencing excluded germline or somatic mutations in the genes FAS, FASLG or CASP10 typically associated with ALPS. Whole-exome sequencing of the patient and his parents identified a germline mutation (c.674T>C, p.Leu225Ser) in the transcription factor Signal Transducer And Activator Of Transcription 3 (STAT3) inherited from the asymptomatic father. The mutation was validated by Sanger sequencing. No other probable gene candidates were identified. The CADD-score (27.0) indicated a high likelihood of the Leu225Ser mutation being pathogenic. To analyze the impact of the identified mutations on the transcription factor function of STAT3, human embryonic kidney (HEK) 293 cells stably expressing STAT3 wildtype or the STAT3 variants Leu225Ser and STAT3 Lys392Arg were generated and dual-luciferase reporter assays carried out. Compared to wildtype STAT3, the patient derived Leu225Ser mutant was transcriptionally moderately increased active.

Conclusions:

We suggest that moderate hyperactivity of STAT3 Leu225Ser causes ALPS like disease in the presented patient, but lacks full penetrance in his father.
ADENOSINE DEAMINASE DEFICIENT SEVERE COMBINED IMMUNODEFICIENCY WITH HYPERTROPHIC CARDIOMYOPATHY

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Background:

Deficiency of adenosine deaminase (ADA), an enzyme in the purine metabolic pathway, leads to T-B-NK- SCID, as well as a variety of non-immunological manifestations such as involvement of the nervous and skeletal systems.

Methods:

We report 5 ADA-SCID patients, diagnosed between 2009-2016, homozygous for the mutation c.646G>A, p.216GLY>ARG, with hypertrophic cardiomyopathy (HCM), a previously unreported clinical feature.

Results:

The median age at diagnosis of ADA-SCID was 62 days (range 25-124 days). There was no known family history of cardiomyopathy and no evidence of generalised myopathy. Cardiomyopathy was a presenting feature in one patient, was present before definitive therapy in two patients and evolved during treatment with haematopoietic stem cell transplant (HSCT) (which included corticosteroids) in two patients. Four patients were treated with propranolol. All five patients demonstrated echocardiographic evidence of resolution of HCM after definitive therapy for their ADA-SCID (four were treated with HSCT, one with gene therapy).

Conclusions:

This case series suggests that HCM may be part of the ADA-SCID clinical phenotype, previously unreported in the literature, although the pathophysiology remains unclear. The potential effect of corticosteroids is important to consider, but was not given to all patients and thus is more likely to occur independently of corticosteroid use. While inherited forms of cardiomyopathy are seen in the Irish Traveller cohort, the phenotype differs from that seen in this case series and the cardiomyopathy typically does not resolve. We recommend a screening echocardiogram on infants newly diagnosed with ADA-SCID and that ADA-deficiency be considered in the differential diagnosis of infants with HCM.
T Cell

ESID7-0509

FAMILY HISTORY AND CANDIDIASIS: CLINICAL FEATURES THAT AFFECT THE AGE AT DIAGNOSIS OF SCID


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Background:
Severe combined immunodeficiency (SCID) is fatal unless treated timely with hematopoietic stem cell transplant (HSCT). Delay in diagnosis is common without newborn screening. Family history has been associated with earlier diagnosis.

**Methods:**

From 2005 to 2016, 147 SCID patients were referred to the Asian Primary Immunodeficiency Network (APIN). Patients with genetic diagnosis, age at presentation and age at diagnosis were selected for study. Features which potentially affecting age at and time to diagnosis were analyzed, including family history of early infant death (FH), candidiasis, BCG infections, CMV infections, failure to thrive, chronic diarrhea, recurrent infections and absolute lymphocyte count (ALC) below 3x10⁹/L. Data were in median and range. Statistical analysis was performed using Mann-Whitney U test and linear regression.

**Results:**

Eighty-three of the 147 patients were included in study. The commonest gene identified was *IL2RG*(n=57), followed by *RAG1*(n=6), *RAG2*(n=6), *JAK3*(n=5), *DCLRE1C*(n=3), *IL7R*(n=3), *RFXANK*(n=2) and *ADA*(n=1). FH was present in 29 patients. The commonest opportunistic infections was candidiasis *(n= 27)*, followed by BCG infections *(n=19)*. The median ALC was 1.05x10⁹/L(0.134-52.2x10⁹/L,n=70) with 62 patients’ ALC below 3x10⁹/L.

FH was associated with earlier age at presentation and diagnosis but not shorter time to diagnosis. Candidiasis was associated with later age at diagnosis. Candidiasis and recurrent infections were associated with longer time to diagnosis. Other features didn’t affect the age at and time to diagnosis.

**Conclusions:**

FH was useful to aid earlier diagnosis but was overlooked together with other SCID features by clinicians. Ultimately the only solution for earlier diagnosis is newborn screening.
PSTPIP1 controls immune synapse stability in human T-cells
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Background:

PSTPIP1 is a cytosolic adaptor protein involved with T-cell activation, differentiation, and migration. Upon cognate T-cell contact, PSTPIP1 is recruited to surface-expressed CD2, where it regulates f-actin remodeling. An immune synapse (IS) is thereby rapidly formed, consisting of TCR clusters surrounded by a ring of adhesion molecules including CD2.

Methods:

From genetic screening of primary immunodeficiency patients, we identified two mutations in PSTPIP1, R228C and T274M. We performed cellular assays using PSTPIP1 R228C and T274M patient and healthy control T-cells, and PSTPIP1 variant-transfected HEK293T and Jurkat T-cell lines.

Results:

These PSTPIP1 R228C and T274M patients presented with immunodeficiency without signs of auto-inflammation. The R228C patient had expansion of mostly naive phenotype T-cells and few memory T-cells; the T274M patient had 75% reduction in CD4 T-cells that were predominantly of memory subset.

We observed f-actin polymerization defects in both PSTPIP1 patient T-cells, most notably T274M. Capping of CD2-containing membrane microdomains was disrupted. Analysis of IS formation using Jurkat T-cell transfectants revealed a reduction in f-actin accumulation at the IS, again especially in T274M PSTPIP1 cells. Patient T274M T-cells migrated spontaneously at increased speed as assessed in a 3D collagen matrix, while TCR crosslinking induced a significantly diminished calcium flux.

Conclusions:

We propose that PSTPIP1 T-cell differentiation defects are caused by defective control of f-actin polymerization. A pre-activated polymerized f-actin status, as seen in PSTPIP1-T274M T-cells, appears particularly damaging. PSTPIP1 controls IS formation and cell adhesion, through its function as orchestrator of the f-actin cytoskeleton.
CASE SERIES OF PATIENTS WITH PRIMARY IMMUNODEFICIENCY DISORDERS AND IMPROVEMENT IN WARTS WITH SUBCUTANEOUS IMMUNE GLOBULIN

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Background:

Warts in patients with primary immunodeficiency disorders (PIDD) may be difficult to eradicate. Few reports of the utility of immunoglobulin in patients with PIDD and warts have been reported.

Methods:

Gamma globulin replacement is an essential and lifesaving treatment for patients with antibody disorders. Patients with PIDD may suffer from infections due to warts secondary to various defects in the immune system. We report three patients who were treated for antibody deficiency, who had concomitant improvement in warts while on SCIG used to treat their hypogammaglobulinemia and poor specific antibody titers. One patient had Common Variable Immunodeficiency (CVID), another DiGeorge Syndrome (DGS), and the third Ataxia Telangiectasia (AT). All had improvement in warts during this therapy.

Results:

A young adult with CVID and warts of her extremities had complete resolution of her warts within 3 months of starting SCIG 20% (Hizentra). Another patient with DiGeorge Syndrome, and poor specific antibody titers to polysaccharide vaccines, also had complete resolution of warts within 3 months of starting immune globulin replacement (1 dose IVIG and subsequent weekly SCIG 20%). One teenage patient with AT and disseminated, longstanding warts, who failed bleomycin injections, had near resolution of the warts within 5 months of starting SCIG 20%.

Conclusions:

Subcutaneous immunoglobulin replacement for hypogammaglobulinemia and poor specific antibody titers improved or led to complete resolution of warts in three patients with PIDD. Further studies are needed to further define the safety and determine the generalizability to other patient populations.
THERAPY

ESID7-0012

PRION SURVEILLANCE IN PRIMARY IMMUNODEFICIENCY PATIENTS EXPOSED TO UK-SOURCED IMMUNOGLOBULIN IN THE UK, 1996-2000
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Background:

Variant Creutzfeldt-Jakob disease (vCJD) is a very rare disease associated with an abnormal form of a naturally occurring protein, the prion protein, the presence of which can be detected in certain body tissues. Most cases of vCJD can be attributed to eating contaminated meat products, however infection may be spread through treatment with certain blood products. We aim to find out whether patients with primary immunodeficiency, who were treated with these blood products in the past, are affected.

Methods:

Patients with primary immunodeficiency who received UK-sourced immunoglobulin between December 1996 and December 2000 are eligible for inclusion in this study, with informed consent. By following these patients over several years and testing available tissue and, when a suitable test becomes available, their blood, this study can look for evidence of prionopathy.

Results:

Since 2006 when the study began, a total of 77 patients have been recruited from 16 centres across Britain. The participants have now been followed up for approximately 1250 person-years following first exposure. In this time no patients have shown any clinical features of vCJD or pathological evidence of abnormal prion protein.

Conclusions:

The results are reassuring but it is important to continue to monitor patients over the longer term, to consider the wider implications to patients and the public health.
Background:

Patients with secondary antibody deficiencies may need long-term substitution with immunoglobulins to prevent recurrent infections and irreversible complications. In addition to efficacy, tolerability of immunoglobulin products is a critical factor for treating these patients.

Methods:

In June 2011, Octagam® 5% and 10% strengths became available on the market with modified manufacturing processes to increase product safety. Three multicentre, non-interventional studies were (re-)started in Europe to examine the tolerability of octagam® in different indications. Presented is a subgroup analysis in patients with secondary immunodeficiencies (SID) relying on data generated between June 2011 and March 2014.

Results:

A total of 1367 patients (mean age: 66 years) with SID receiving 11,345 infusions with octagam® 5% or 10% were included in this analysis (mean of 8.3 infusions at 0.20 g/kg per infusion). Most patients had SID due to chronic lymphocytic leukaemia (CLL, 34.2%), non-Hodgkin lymphoma (NHL, 27.3%), multiple myeloma (MM, 15.7%), or other leukaemia (4.5%).

A total of 124 adverse drug reactions (ADRs) occurred in 1.1% of infusions (octagam® 5%: 0.4% [Study 1: 0.3%; Study 2: 2.5%]; octagam® 10%: 2.1% [Study 2: 1.4%; Study 3: 2.2%]); 10 of these were serious.

Efficacy was assessed by asking physicians to rate the influence of treatment on infection frequency/severity/duration and antibiotic consumption as “favourable”, “unchanged”, or “unfavourable”: 77.4-82.8% of assessments were “favourable”, 0.4-0.5% were “unfavourable” and the remaining “unchanged”. Free of infections were 83% of patients.

Conclusions:

This subgroup analysis shows that octagam® 5% and 10% are well tolerated and effective in patients with SID.
THERAPY

ARE PRIMARY IMMUNE DEFICIENCY DISEASE PATIENTS AT HIGHER RISK FOR MYELO SUPPRESSION EFFECT OF PROPHYLACTIC TRIMETHOPRIM-SULFAMETHOXAZOLE?
RETROSPECTIVE TWO GROUPS COMPARISON STUDY

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Background:

To compare and describe the myelo suppression effect of Trimethoprim-Sulfamethoxazole (TMP-SMX) used as a prophylactic in two groups: immune competent patients for urinary tract infection (UTI) indication, and primary immune deficiency (PID) patients

Methods:

A retrospective study of existing data for PID patients and UTI patients who received TMP-SMX as a prophylaxis dose in Qatar. Data about CBC results (WBC, Neutrophils, Lymphocytes, RBC, Hemoglobin, and Platelet counts) at baseline and at maximum myelosuppression observed during the period of TMP-SMX administration, were collected

Results:

A total of 64 patients were included in this study (41 PID patients, and 23 immune competent patients). There are a clear differences noticed in the percent of patients who developed clinical suppression (i.e. less than normal value for age) in Neutrophil count (60.9% vs. 21.7%), Hemoglobin (63.4% vs. 30.4%), Red Blood Cells (29.2% vs. 0%), and Platelet (26.8% vs. 4.3) in PID and immune competent patients, respectively. Around half of included PID patients (42%) had Neutrophil function /migration disorders. Thus, suppressions observed in this group were most likely due to TMP-SMX effect rather than disease itself.

Conclusions:

Primary immune deficiency (PID) patients are highly expected to develop myelo suppression secondary to TMP-SMX prophylaxis (especially neutrophil count) at higher rate comparing to immune competent patients. Future larger prospective study is required to confirm this association
ANALYSES OF EFFICACY AND TOLERABILITY IN PATIENTS WITH PRIMARY IMMUNODEFICIENCY DISEASES (PID) TREATED BY DIFFERENT MODES OF ADMINISTRATION OF IMMUNOGLOBULIN THERAPY DURING THREE CONSECUTIVE STUDIES

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Background:

Administration modes of Ig therapies for PID differ in pharmacokinetics, infusion parameters, and tolerability. We report the efficacy/tolerability data for 30 patients with PID who received all three modes of Ig therapy administration in sequence from IVIG to SCIG to fSCIG during consecutive clinical studies creating a unique opportunity to follow this cohort of patients across these modalities of therapy.

Methods:

In Study 1, patients received IVIG 10% every 3-4 weeks (~3 months), followed by weekly SCIG 10% (~12 months); in Study 2, patients were switched to hyaluronidase-facilitated SCIG 10% (fSCIG) every 3-4 weeks; then, in Study 3 (extension of Study 2), they then continued with the same fSCIG dose (for ~2.7 years in Studies 2 and 3).

Results:

Longitudinally, in 3 consecutive studies, the annualized rate per patient of validated acute bacterial infections and all infections, respectively, were low: IVIG (0.00/4.17), SCIG (0.09/3.68) and fSCIG (0.04/2.42). The rate of causally-related systemic AEs/pt-yr was lowest in patients receiving fSCIG (0.88) vs IVIG (5.60) and SCIG (1.93). The rate of causally-related local AEs/pt-yr was higher for fSCIG (1.57) compared to conventional SCIG (0.92). Mean IgG trough levels (g/L) at steady state were IVIG (10.64), SCIG (12.74), and fSCIG (10.26).

Conclusions:

Evaluation of the same patient cohort in three consecutive studies over more than 3 years demonstrated that all three modes of administration provided similar efficacy and relative rates of local and systemic AEs and tolerability as expected.
THERAPY
ESID7-0058

TREATMENT SATISFACTION DURING PIVOTAL CLINICAL TRIALS WITH THE NEW HUMAN SUBCUTANEOUS IMMUNOGLOBULIN (SCIG 20%) IN PATIENTS WITH PID WHO WERE PREVIOUSLY TREATED WITH IVIG

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Background:

Treatment satisfaction (TS) is an important consideration for Ig treatment. This analysis compared TS during the IVIG and CUVITRU (SCIG 20%) treatment periods of two pivotal phase 2/3 studies (North America [NA] and European [EU]) conducted in patients with PID treated with intravenous immunoglobulin (IVIG) therapy prior to study entry.

Methods:

During the studies, patients were treated with SCIG 20% for ~12 months following 3 months of treatment with IVIG. TS was assessed using the Life Quality Index (LQI; higher scores indicate greater satisfaction) instrument at the end of the IVIG period and after the completion of the SCIG 20% period among patients entering the studies from IVIG therapy. Wilcoxon Signed Rank test evaluated statistical significance.

Results:

Patients reported significant improvement in the Therapy Setting LQI domain relative to the IVIG therapy period after treatment with SCIG 20%, improving from 17.5 to 20.0 (P<0.001; n=46) and 18.0 to 21.0 (P=0.002; n=30) in the NA and EU study, respectively. Patients also reported improvements during the SCIG 20% period compared to the prior IVIG period in the Treatment Interference LQI domain in the NA study (36.5 vs 33.5; P=0.049; n=46) and the EU study (39.0 vs 34.5; P=0.016; n=30).

Conclusions:

After 12 months on the new SCIG 20%, patients reported improvements in treatment satisfaction in the Treatment Interference and Therapy Setting domains compared with IVIG. Clinicians may consider improved satisfaction in offering the new SCIG 20% to patients with PID currently treated with IVIG.
THERAPY

ESID7-0059

TREATMENT PREFERENCE ON THE NEW SUBCUTANEOUS IMMUNOGLOBULIN 20% (SCIG 20%) TREATMENT IN PATIENTS WITH PRIMARY IMMUNODEFICIENCY DISEASES (PID) IN EUROPE (EU)

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Background:

SCIG offers an opportunity for patients with PID to self-infuse at home, potentially reducing treatment burden and improving satisfaction. This analysis assessed treatment preference with CUVITRU, the new SCIG 20%.

Methods:

Forty-eight EU patients with PID treated with IVIG 10% for 3 months followed by SCIG 20% for ≥12 months within a phase 2/3 study were assessed for treatment preference with a questionnaire. Questionnaires were administered at the end of the study to evaluate preferences about treatment aspects using a 5-point Likert scale and included questions regarding patients’ preferred location of therapy and preference to continue the new SCIG 20%. Questionnaires were completed by their caregiver/parent (≤13 years) or patient (≥14 years).

Results:

The aspects of treatment with the highest proportion of ‘like’/‘like very much’ responses were “ability to fit treatment into my own schedule” (96%) and “ability to self-administer without medical supervision” (94%). Overall, 88% of all patients stated that they would prefer to receive SCIG 20% rather than other Ig treatments with 84% of younger (≤13 years) and 91% of older (≥14 years) patients preferring SCIG 20%. Home infusion was preferred by 88% of all patients.

Conclusions:

At the end of the study, both children and adults preferred to continue to receive Ig treatment using the new SCIG 20% product—overall, the vast majority (88%) of patients indicated they preferred the investigational therapy.
Background:

CUVITRU (SCIG 20%), a liquid preparation of highly purified human IgG, was evaluated for adverse events (AEs) and tolerability in two phase 2/3 studies in patients with primary immunodeficiency disease (PID) in Europe and North America.

Methods:

A pooled analysis of the rate of AEs and tolerability were assessed in patients with PID, ≥2 years, with IgG trough levels >500 mg/dL at screening who were treated with SCIG 20% for ~12 months subsequent to ≥3 months of treatment with IVIG (NA study) or IVIG or SCIG (EU study). Patients received weekly SCIG 20% infusions up to 60 mL/site and 60 mL/hr/site.

Results:

Overall, 91.8% (112/122) of patients aged 2-83 years who were treated with SCIG 20% completed the studies with only one discontinuation due to an AE (mild infusion site pain). Of the AEs causally-related to SCIG 20%, none were serious or severe. Combined data show that local AEs causally related to SCIG 20% were reported in 28.7% of patients, all of which were either mild or moderate, with a rate of 0.034/infusion and 1.8/patient-year. No treatment-related local AEs were reported in 71.3% (87/122) of patients or in 97.3% of infusions. Systemic AEs causally related to SCIG 20% were reported in 22.1% of patients at a rate of 0.025/infusion and 1.3/patient-year.

Conclusions:

The new SCIG 20% demonstrated a low rate of AEs and was well tolerated in patients with PID at infusion volumes of up to 60 mL/site and infusion rates of up to 60 mL/hr/site.
INTERIM RESULTS OF A POST-AUTHORIZATION SAFETY STUDY (PASS) ON THE LONG-TERM SAFETY OF FACILITATED SCIG 10% IN PATIENTS WITH PRIMARY IMMUNODEFICIENCY DISEASES (PID) IN EUROPE

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Background:

HyQvia (fSCIG), Ig 10% with human hyaluronidase (rHuPH20) to facilitate SC Ig infusion, is approved in the EU as a replacement therapy in adults, children, and adolescents (0-18 years) with PID, myeloma, and chronic lymphocytic leukemia. This study was initiated in EU in July 2014, to acquire additional data on the long-term safety of fSCIG and to assess the prescribed treatment regimens and administration in routine clinical practice.

Methods:

This ongoing prospective, non-interventional, open-label, uncontrolled, multicenter study was designed to evaluate the long-term effects of fSCIG in adult patients in routine clinical practice. Adult patients who are currently receiving or prescribed fSCIG are eligible. The treatment regimen is at the discretion of the attending physician; patients are followed according to standard clinical practice and anti-rHuPH20 antibodies are measured on a voluntary basis.

Results:

As of October 7, 2016, the safety analysis population included 62 patients (out of 86 enrolled) who had received ≥1 dose of fSCIG; fSCIG exposure duration was 66.7 patient-years. Overall, 104 non-serious AEs (excluding infections) were reported in 40 patients. None of the 45 patients with immunogenicity data developed positive binding (defined as titer ≥160) or neutralizing antibodies to rHuPH20. The annualized rates of hospitalization and of emergency room visits were less than 1 event per patient each. Most treatments were administered at home during the first (89.5%) and second year (91.5%) in the study.

Conclusions:
The interim analysis of this prospectively-collected data of fSCIG use in a clinical setting indicates that fSCIG is well tolerated.
REVIEW OF THE ONBOARDING EXPERIENCE AND TOLERABILITY OF THE NEW 20% HUMAN IMMUNE GLOBULIN FOR SUBCUTANEOUS ADMINISTRATION (SCIG 20%)
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Background:
Data from a phase 2/3 North American clinical trial provided an opportunity to understand the onboarding experience of the new SCIG 20% in patients with PID. This new highly-concentrated SCIG 20% allows for fast infusion rates and large infusion volumes/site that minimizes infusion time and the number of infusion sites.

Methods:
Associations between the rate of causally-related local AEs and the infusion parameters were evaluated in patients (3-83 years) receiving weekly SCIG 20% for ~1.3 years at volumes up to 60 mL/site and rates up to 60 mL/hr/site as tolerated.

Results:
Of the 77 enrolled patients, 53 (69%) had no previous SCIG-experience. Overall, 91% (67/74) of patients treated with SCIG 20% completed the study; none of the discontinuations (n=7) were due to causally-related systemic or local AEs. There was no association between the number of infusions with causally-related local AEs (0.4%, 1.4%, 1.1%, 0.3%, respectively) and the infusion volume/site (30-39, 40-49, 50-59, ≥60 mL/site, respectively). Median total infusion time was 0.95 hours. Overall, 72% of patients reached an infusion rate of 60 mL/h/site. Most (99.8%) infusions were completed without a rate reduction, interruption, or discontinuation due to associated tolerability concerns or AEs.

Conclusions:
Irrespective of relatively high infusion volumes/site and fast infusion rates, infusions of the new SCIG 20% product were well-tolerated during onboarding through the end of the study. The rate of local AEs was not associated with increasing infusion rates or volumes/site. The percent of patients who experienced causally-related local AEs during onboarding was low and decreased further over time.
Background:

Combined safety and tolerability data from two phase 2/3 studies of CUVITRU (SCIG 20%) in patients <18 years with PID in Europe and North America are presented.

Methods:

Children already receiving Ig replacement therapy (300-1000 mg/kg Q3-4W) ≥3 months with serum IgG trough level >500 mg/dL were included. Patients received weekly SCIG 20% infusions at volumes and rates up to 60 mL/site and 60 mL/hr/site, respectively.

Results:

Fifty pediatric patients aged <6 (n=6), 6-<12 (n=22), and 12-<18 (n=22) years with PID received 2624 SCIG 20% infusions for a mean treatment duration of 358.7, 371.6, and 375.6 days, respectively. No serious adverse events (AEs) that were deemed related to SCIG 20% occurred. All causally-related events were mild or moderate. Excluding one 13-year-old patient incurring 12/17 causally-related systemic AEs and 79/119 causally-related local AEs in his group, causally-related systemic AE rates/infusion (excluding infections) were 0.010, 0.003, and 0.005, and causally-related local AE rates/infusion (excluding infections) were 0.000, 0.039, and 0.036, respectively, for age groups <6, 6-<12, and 12-<18 years. Median infusion volumes were 14.0, 15.0, and 30.0 mL/site; median maximum infusion rates were 18.0, 20.0, and 30.0 mL/hr/site; and median infusion durations were 0.75, 0.78, and 1.05 hours, respectively for age groups <6, 6-<12, and 12-<18 years.

Conclusions:

These data confirm that pediatric patients with PID in Europe and North America tolerated SCIG 20% well with low rates of local and systemic AEs.
THERAPY

ESID7-0066

SIDE EFFECTS AND CONTRIBUTING FACTORS OF INTRAVENOUS IMMUNOGLOBULIN REPLACEMENT THERAPY IN PATIENTS WITH PRIMARY IMMUNODEFICIENCIES
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Background:

In our study, it was aimed to evaluate the side effects and their relations with contributing factors of intravenous immunoglobulin (IVIG) replacement therapy in patients with primary immunodeficiency.

Methods:

One hundred forty five immunodeficient patients receiving IVIG were evaluated during a 2-year period.

Results:

Side effects occurred in 44.8% of 145 patients. A total of 1214 infusions were recorded and 172 (14.2%) of these were associated with adverse reactions. The most frequently observed side effect was headache seen in 7.8 of all infusions. Headache accounted for 36.3 of immediate side effects and 77.7 of delayed side effects. As for immediate period, fever was the most common side effect. It was determined that side effect ratios were significantly increased in the presence of acute infectious disease. Although there was no statistically significant difference the incidence of adverse events gradually decreased from 25% to 11.7% with recurrent infusions. Premedication was given in the patients previously experienced side effects, and it was determined that 77 of the side effects taken under control with premedication. There was no significant correlation between the concentration of IVIG preparations and the ratio of side effect development. None of the cases developed a renal, hematologic or serious reaction.

Conclusions:

In our study, there was no serious reaction to IVIG. However, since rare serious life-threatening complications can be occurred, IVIG treatment indications should be well-defined and patients, patient relatives, and health care personnel should be informed about possible side effects of the treatment.
INTERIM RESULTS OF A POST-AUTHORIZATION SAFETY STUDY (PASS) ON THE LONG-TERM SAFETY OF FACILITATED SCIG 10% IN PATIENTS WITH PRIMARY IMMUNODEFICIENCY DISEASES IN THE US

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Background:

HyQvia (fSCIG), Ig 10% with human hyaluronidase (rHuPH20) to facilitate SC Ig infusion, was approved as a replacement therapy in adults with PID in the US. To acquire additional safety data on the long-term use of fSCIG and assess the prescribed treatment regimens and administration in routine clinical practice, a global PASS was initiated in the US in November 2015.

Methods:

An ongoing prospective, non-interventional, open-label, uncontrolled, multicenter study to assess local and systemic effects of fSCIG in adult patients within a routine clinical setting, including optional measurement of anti-rHuPH20 antibody titers. Patients aged ≥16 years with PID, who have been prescribed and/or have started fSCIG are eligible for enrollment. Patients are followed according to standard clinical practice and their treatment regimen is at the discretion of the treating physician.

Results:

As of October 31, 2016, 50 patients had been enrolled at 13 US study sites and 28 patients who had ≥1 documented fSCIG treatment entered into the database were included in these safety analyses; none of the 19 patients with immunogenicity data exhibited positive binding (titer of ≥1:160) or neutralizing antibody titers. Seven patients reported 8 SAEs (0.47 SAEs/patient-year) all assessed as unrelated to treatment; the rate of SAEs per infusion was 0.057 (8 SAEs/141 infusions). Only two patients (7.1%) experienced causally-related local AEs, one each, both mild.

Conclusions:

This prespecified interim analysis of the prospectively-collected data of fSCIG use in routine clinical practice indicates that fSCIG treatment is efficacious and well tolerated in patients with immunodeficiencies.
THYROID FUNCTIONS OF THE PATIENTS WITH PRIMARY IMMUNODEFICIENCY FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION


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Background:

Increased incidence of autoantibodies is detected following HSCTs. Thyroid antibodies accompanying thyroid dysfunction is reported to be frequent during the post-transplant period. Although the underlying pathogenesis has not been illustrated, it was considered to be associated with immunoreconstitution. In this study, we evaluated the effect of the thyroid autobody production on thyroid functions in line with immune-reconstruction process.

Methods:

This study consists of 28 PID patients, who underwent HSCT and followed-up at least twelve months in our clinic between years 2013-2016. Thyroid function tests and thyroid autoantibodies were studied every three months. Results were analyzed for correlation with HSCT and immune-reconstitution parameters.

Results:

The PID subtypes of the patients were as follows: 13 SCID, 8 CID, 3 DOCK8, 1 LAD, 1 CGD. The patients underwent HSCT at a median age of 10 (2-180mo) months. Post-transplant median follow-up time was 22 months. 70% of the HSCTs were MSD, 21% were haploidentical and 4% were MUD.46% of the cases did not receive conditioning, myeloablative and RIC was used in 25% and 28% of the cases respectively. During the follow-up, chronic GvHD occurred in 18% of the transplants. Although thyroid autoantibodies were documented in 85.7% of the patients, it did not lead to thyroid dysfunction. There was no correlation between autoantibody positivity and thymopoiesis or CD4 lymphocyte reconstitution. At follow-up time longer than 12 months autoantibodies disappeared.

Conclusions:

Although thyroid autoantibody presence is frequent during the post-transplant period, it is a benign clinical phenomenon, which does not lead to thyroid dyshormonogenesis.
NO BENEFIT OF SINGLE AGENT THERAPY WITH INTERLEUKIN SIX INHIBITOR TOCILIZUMAB IN TWO PATIENTS WITH ACTIVATING MUTATIONS OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION THREE

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Background:

We describe two patients with activating mutations of signal transducer and activator of transcription three (STAT3). Activating mutations result in both immune deficiency and autoimmunity. Interleukin 6 (IL-6) activates STAT3. Blocking IL-6 with humanized monoclonal antibody tocilizumab would in theory reduce activity of STAT3 and modify the phenotype. Both patients described have autoimmune disease refractory to conventional immunosuppressant therapy. Neither experienced clinical benefit from therapy with tocilizumab as a single agent.

Methods:

Case series

Results:

Patient one is an eleven-year-old girl with dermatitis and inflammatory bowel disease. Sequencing of STAT3 identified a novel heterozygous mutation (c.1708G>A, p.Asp570Asn) conferring gain-of-function. Her symptoms were refractory to single agent therapy with cyclosporin, tacrolimus and sirolimus. Treatment with single agent tocilizumab led to an initial marked improvement in her skin, but a deterioration in her gastrointestinal symptoms. The benefit was not sustained and tacrolimus was added. To date she remains stable on combination therapy.

Patient two is a five-year-old boy with generalized lymphadenopathy and splenomegaly, autoimmune thyroiditis and lymphocytic interstitial pneumonitis. Sequencing of STAT3 identified a heterozygous mutation (c.1904A>T, p.Gln635Leu) conferring gain-of-function. Sirolimus therapy resulted in diminution of lymphadenopathy and splenomegaly, which increased when he was switched to single agent tocilizumab. His respiratory disease was refractory to treatment and he progressed to an allogeneic haematopoietic stem cell transplant.

Conclusions:

We conclude that tocilizumab as a single agent has limited benefit in patients with activating mutations of STAT3. Its role in management of other manifestations of disease (eg autoimmune cytopenias) or as part of combination therapy requires more study.
THERAPY

ESID7-0100

TOLERABILITY AND EFFICACY OF A 16.5 % SUBCUTANEOUS IMMUNOGLOBULIN PREPARATION IN CHILDREN AND ADULTS WITH ANTIBODY DEFICIENCIES
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Background:
Patients with antibody deficiencies need long-term substitution with immunoglobulin G (IgG) for infection prophylaxis to prevent irreversible complications. Subcutaneous IgG replacement therapy by self-administration at home is well established as an alternative to the intravenous application and provides higher flexibility and independence to patients.

Methods:
Final results of a multicentre, non-interventional study (NIS) on the tolerability and efficacy of gammanorm® in patients with antibody deficiencies are presented. The study was conducted between 2004 and 2013 in Germany.

Results:
A total of 113 patients (61 adults / 52 children and adolescents) were enrolled in this study. The majority of patients (n = 108) were treated for primary antibody deficiencies like CVID (n = 59) and IgG subclass deficiencies (n = 30). The mean time in study per patient was 1.7 years. On average, the patients received a monthly dosage of 0.4 g/kg administered on 6.1 days per month.

Efficacy was assessed by analyzing the serum IgG levels before and during gammanorm® treatment. A mean increase of 2.1 g/l from 6.4 g/l at baseline to 8.7 g/l during treatment was observed for all patients. The IgG values remained stable during the whole observation period.

The severity of infections as well as the number of hospitalizations and days absent from school or work were reduced during treatment compared to the time before gammanorm® treatment.

Adverse drug reactions (ADR) occurred only in 10 out of 113 patients.

Conclusions:
The results show that gammanorm® is well tolerated and effective in all age groups of patients with antibody deficiencies.
PREVALENCE, INCIDENCE, AND MORBIDITY OF PATIENTS WITH SECONDARY IMMUNE DEFICIENCY (SID) IN THE UNITED KINGDOM: AN ANALYSIS OF TWO NATIONAL DATA SETS

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Background:
An analysis was performed of the prevalence and incidence of SID and its common causes and co-morbidities.

Methods:
Data was obtained from: 1) the National Immunoglobulin Database, which records volumes of immunoglobulin infused in the UK (excluding Wales) for commissioning purposes; 2) the UKPID Registry, which began collecting information on SID in 2011.

Results:
Initial findings reveal the number of SID patients in the National Immunoglobulin Database treated with immunoglobulins during 2015/16 was 1,512. Patient numbers increased by +51% compared to 2014/15, due in part to Scotland and Northern Ireland starting to contribute data. Use of the Database in England is long established; the increase in SID patients here was +34%, reflecting an increase in patients requiring immunoglobulin.

Mean annual totals administered per SID patient (during 2015/16) were 193 g for subcutaneous immunoglobulin and 321 g for intravenous immunoglobulin. The proportion of SID patients receiving subcutaneous immunoglobulin was just 9%, markedly lower than the proportion of PID patients receiving subcutaneous immunoglobulin (39%).

The UKPID Registry featured complete data on only 328 SID patients of whom 299 are known to be alive: 239 were on immunoglobulin replacement. Over the period 2009-2017 there were 652 separate infectious episodes. In the one year prior to data lock 120 individual patients had an infectious episode; 50% had an isolated infection recorded, with 6% having 4-7 infections in the same period. Antibiotics administered to 141 patients comprised long-term azithromycin (60/228), Septrin® (31/228), and nebulised antibiotic prophylaxis (5/228) with some individuals having more than one antibiotic and others receiving prophylaxis without infection episodes in the prior year.

Conclusions:
Antibiotic usage in this SID cohort was relatively high, perhaps reflecting the incidence of bronchiectasis, age, or other structural organ damage, and indicating that just as in PID, immune deficiency in SID can compromise health.
SID
prevalence
Background:

Common variable immunodeficiency (CVID) phenotype is relatively frequent in Kabuki Syndrome (KS). 10-20% of CVID patients develop an interstitial lung disease (ILD). The association of CVID and ILD is infrequent in KS and currently there are no therapeutic guidelines.

Methods:

We present a case of a patient with KS and severe ILD who benefitted from therapy with sirolimus.

Results:

A 13-year-old female with KS (no mutation in KMT2D and KDM6A genes) who developed signs and symptoms of CVID. Two years after diagnosis, she presented, completely asymptomatic, a steady increase of lactate dehydrogenase (LDH) levels and a higher consumption of immunoglobulins. No viral or bacterial infections were detected. A high resolution Chest scan (HRCT) showed marked interstitial affection: pulmonary nodules, and mediastinal, hiliar and axillary lymphadenopathies. Lung function test (LFT) showed carbon monoxide diffusion damage. PET-SCAN was done followed by a biopsy of a representative adenopathy, ruling out lymphoma and confirming benign lymphocytic proliferation (scarce CD20+ lymphocytes with preponderance of the CD3+ lymphocytes). CTLA4/FOXP3 expression was evaluated showing CTLA4 expression impairment, so treatment with sirolimus was started. One month later, immunoglobulins and LDH normalized; at 3 months, LFTs improved and at 7 months HRCT was completely normal. The expression of CTLA4 increased in FOXP3+ cells.

Conclusions:
This is the first case report showing that sirolimus might be beneficial in patients with ILD in the context of KS. The role of the defective T cell function in KS is still unknown.
Background:

Common variable immunodeficiency (CVID) is the most common symptomatic primary immune deficiency of the adulthood. Treatment is based on replacement therapy with human immunoglobulin administered by the intravenous (IVIg) and the subcutaneous (SCIg) route.

Methods:

We compared in a long-term period these two treatment modalities in patients with CVID, diagnosed according to ESID criteria. We described 32 patients, 16 treated with IVIg and 16 treated with 20%SCIg (Hizentra®). Patients were studied according to a standardized protocol.

Results:

Mean follow-up were 63 and 35 months in the IVIg and SCIg groups, respectively. All patients achieved and maintained serum protective IgG levels. Mean through IgG levels were 665 mg/dL in the IVIg group and mean steady IgG levels were 796 mg/dl in the SCIg group (p<0.01). No severe infections were observed, except in one patient with a complex phenotype treated with SCIg. Mean value of non-severe infections were 1.31 and 1.23 in the IVIg and SCIg groups, respectively. Mean hospitalization days were 14 days/year in the IVIg group, and 4 days/year in the SCIg group. According to the questionnaire, most of the patients referred (often or always): asthenia (50%), concern about the future (47%), fear of worse (44%), fear of been infected by others (44%), less autonomy (41%) and reported to feel a sick person (41%).

Conclusions:

Even if data regarding clinical efficacy were similar in the two groups, in SCIg patients we documented reduced hospitalization days, due to the home-self administration and significantly higher through IgG level.
FACILITATED SUBCUTANEOUS IMMUNOGLOBULIN: A MULTI-CENTER ITALIAN EXPERIENCE IN ADULT PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY (CVID)

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Background:

Treatment of CVID is based on replacement therapy with intravenous immunoglobulin (IVIg), subcutaneous immunoglobulin (SCIg) and more recently with facilitated subcutaneous immunoglobulin (f-SCIg, 10%Immunoglobulin plus recombinant human hyaluronidase).

Methods:

We described 28 patients (16 females/12 males), mean age 42 years-old, followed in three Italian centers with CVID diagnosed according to ESID criteria, enrolled from November 2014. All patients, except two, were previously treated with IVIg or SCIg. Twenty-one patients had a non-complicated phenotype, five patients had cytopenia (four autoimmune thrombocytopenia (ITP), one autoimmune hemolytic anemia (AIHA)) and two men had previous malignancy. All patients were treated with fSCIg according to a standardized protocol of infusion, evaluating efficacy and safety of treatment. Patients received monthly administration (range of dosage:20-40g), except in three cases complicated by cytopenia (20g every two weeks). The first three infusions were administered in hospital, thereafter they continued home-self-administration.

Results:

During the follow-up period, no severe infections were described, but only mild upper respiratory tract infections in eight patients, two of them treated with antibiotics. All patients achieved and maintained protective serum IgG levels. No systemic adverse reactions, nor hemolytic or thrombotic events were observed. We documented local reactions (redness, swelling) which disappeared during the first hours after the infusion. All patients reported satisfaction. In the cases associated with ITP we observed also a progressive increase in the platelets values meanwhile in the case with AIHA we were able to reduce prednisone daily dose.

Conclusions:

f-SCIg constitutes an effective, safe and well-tolerated alternative of replacement therapy in patients with CVID.
SENEQA: STUDY ON THE UTILISATION OF HYQVIA (10% NORMAL IMMUNOGLOBULIN AND RECOMBINANT HUMAN HYALURONIDASE) IN ELDERLY PATIENTS
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Background:
HyQvia is a dual-vial unit consisting of recombinant human hyaluronidase (rHuPH20) and 10% normal immunoglobulin (IgG) solution. IgG provides the therapeutic effect and rHuPH20 facilitates dispersion and absorption of the IgG, increasing its bioavailability. In the registration study, HyQvia was effective, safe, and bioequivalent to intravenous IgG at the same administration intervals, with fewer systemic reactions. HyQvia received market authorization in the European Union in July 2013 for the treatment of patients with primary (PID) and secondary (SID) immune deficiencies. We aimed to assess the suitability of HyQvia in elderly patients with PID or SID.

Methods:
The SENEQA study is a retrospective chart review of elderly patients in several institutions in The Netherlands and Germany. Collected parameters comprise patients’ disease characteristics, previous and current immunoglobulin G (IgG) replacement regimens, self-administered versus assisted infusions, infusion parameters, serum IgG levels, infections, and pharmacoeconomic parameters. Detailed information on the most recent HyQvia application is documented.

Results:
Patients are eligible for participation if they have provided written informed consent, are at least 65 years old, have PID or SID, and received at least 1 HyQvia infusion in the past. No explicit exclusion criteria have been specified to avoid selection bias. The goal is to enrol a minimum of 10 patients. Currently, 9 patients have been enrolled; the first enrolled in November 2016. Database lock is expected end of May 2017.

Conclusions:
SENEQA is expected to provide real-life data on HyQvia in elderly patients, complementing data from the controlled trial.
IMPACT OF BI-WEEKLY 20% SUBCUTANEOUS IMMUNOGLOBULINS INFUSION ON SERUM IGG AND INFECTIONS: A SUBCOHORT OF PEDIATRIC PATIENTS WITH PRIMARY IMMUNODEFICIENCIES FROM THE IBIS STUDY

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Background:

Replacement therapy with immunoglobulins (IgRT) is the standard treatment for patients with primary immunodeficiencies (PID). In order to minimize side effects and improve quality of life, many patients previously treated with intravenous Ig (IVIg) switched to subcutaneous Ig (SCIg). The IBIS study aimed at investigating the effects of double-dose Hizentra® (20% SCIG) administered bi-weekly (every 14±2 days), compared with previous IVIG/SCIG-based treatment, in PID patients. Here we present preliminary clinical and laboratory outcomes of the pediatric subcohort.

Methods:

In this multicentre study, 13 pediatric PID patients were observed for a 12-month retrospective period during IgRT (at least 3 months of weekly Hizentra® just before enrollment) + 12-month prospective period of bi-weekly Hizentra® treatment. Serum IgG trough levels, number of serious and other infections and days with antibiotics were evaluated.

Results:

Median age was 13 years (range: 2-16). IgG levels collected during the retrospective (833.8±175.7mg/dL) and the prospective (842.0±188.0mg/dL) phases were comparable. Only two episodes of serious infections were reported: pneumonia (retrospective period) and visceral abscess (prospective period). During the retrospective period, 29 episodes of other infections were recorded (being bronchitis the most frequent one) with 10/13 patients experiencing ≥1 infection. During the prospective phase, 35 infections were observed (pharyngitis was the most common) and 9/13 patients had ≥1 infective episodes. Mean durations of antibiotic therapies (per patient) were 14.7±15.7 and 13.7±21.8 days during the retrospective and prospective phases, respectively.
Conclusions:

In pediatric PID patients, Hizentra® administered bi-weekly resulted an effective and non-inferior option with respect to previous IVIG/SCIG-based treatment.
BACKGROUND:

Intravenous immunoglobulins (IVIG) are widely used for replacement of IgG in primary and secondary immune deficiencies. The selection of a commercial product taking into account its individual characteristics is not routinely recommended. However, in clinical practice, not rarely clinicians may need to individualize the therapy taking into account these characteristics. In the present work we present distinct case reports that support the need for individualization of IVIG products in selected settings.

METHODS:

Clinical and immunological characteristics of case reports.

RESULTS:

Case-1. A 19-year XLA patient with Echovirus-11 meningoencephalitis was treated with an experimental antiviral drug, high-dose IVIG and intrathecal immunoglobulin therapy. Batches of a selected product disclosing the higher titer of neutralizing antibodies against Echovirus-11 of the patient were used. Case-2. An adult male patient with IgG3 subclass deficiency and recurrent pneumonia despite conventional antimicrobial therapy and IVIG obtained a better outcome when he changed into a product having higher IgG3 titers. Monitoring of IgG3 levels demonstrated an increase after conversion. Case-3. A CVID patient with undetectable IgA on regular IVIG therapy disclosed an allergy-like reaction after an infusion. High titer anti-IgA antibodies were detected. The patient was changed to an IVIG product with lower IgA concentration. Case-4. A women in waiting list for heart transplantation disclosed intestinal lymphangiectasia, severe bacterial infections and low IgG levels (<100 mg/dL). Restriction of volume was recommended. A 10% IVIG product was selected.

CONCLUSIONS:

In selected cases or clinical conditions difficult to control we suggest that individualization of therapy using IVIG products with specific characteristics is necessary.
Background:

Rheumatoid Arthritis (RA) is the most common chronic inflammatory arthropathy. About 30% of patients are treated with biological therapy (BT). The optimization of BT doses in patients in clinical remission is a strategy used in clinical practice.

Our objective was to describe the characteristics of the patients and their disease who remain longer with optimized regimen of BT.

Methods:

Observational, descriptive, longitudinal and retrospective study in a cohort of 32 patients with RA, which performs treatment with reduced doses of BT.

We analyzed the characteristics of patients and their disease who remain for a longer period of time in optimization of BT dose. Results:

62.5% women, mean age at diagnosis: 42.6 years. The mean duration of RA at the start of TB was 98.63 months and the time to onset of BT reduction was 160.66 months. 75% was FR positive and 56.7% ACPA positive.

59.4% received etanercept, 21.9% adalimumab, 12.5% infliximab and 6.3% certolizumab.

11 patients discontinued dose reduction.

Men stay longer in reduced doses than women (83.3% vs 55%). Patients with negative ACPA and RF remained longer with reduced doses (69.2% vs 75%). No differences were observed in DAS28, duration of disease until the first reduction and number of previous BT performed until the reduced biological.

Conclusions:

Male and patients with ACPA and negative FR, remain longer with reduced doses. No differences were found between the two groups when comparing DAS28 prior to reduction of BT, disease duration and number of previous BT used.
DESCRIPTIVE STUDY OF STRUCTURAL DAMAGE EVOLUTION IN RHEUMATOID ARTHRITIS WITH OPTIMIZED DOSES OF BIOLOGICAL THERAPIES

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Background:

Rheumatoid Arthritis (RA) is the most common chronic inflammatory arthropathy. About 30% of patients are treated with BT. The optimization of biological Therapies (BT) doses in patients in clinical remission of the disease is a strategy used in clinical practice. The best method for assessing structural damage in daily clinical practice is the SENS method (simplified form of the Van der Heijde method).

The main endpoint was evaluate the evolution of the structural damage of the disease in a cohort of patients with RA receiving optimized doses of BT, according to the BT dose optimization protocol elaborated in our unity.

Methods:

Observational, descriptive, longitudinal and retrospective study in a cohort of 32 patients diagnosed with RA, who are treated at reduced doses of TB.

The evolution of structural damage was measured using the SENS method on hand radiographs.

Results:

62.5% are women, mean age at diagnosis of 42.6 years.

The duration of RA at the start of BT was 98.63 months and the biological treatment time at the beginning of the optimization was 160.66 months.

The 75% of the sample was FR positive and 56.7% ACPA positive. The 59.4% were with etanercept.

The mean of SENS score in hands before the optimization was 8.78. The mean of SENS score in patients who continued optimized at time of analysis was 10.67. There were no statistically significant differences.

Conclusions:
The results of our study suggest that optimized doses of BT in patients in clinical remission maintains the patient without significant progression of structural damage.
HEMATOPOETIC STEM CELL TRANSPLANTATION (HSCT) OUTCOME OF PID PATIENTS FOLLOWING REDUCED INTENSITY CONDITIONING WITH TARGETED BUSULFAN

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Background:

Hematopoietic stem cell transplantation (HSCT) is the curative therapeutic option in various primary immune deficiencies (PID). Due to the increase in survival rates the long term consequences and organ toxicities of conditioning regimes are of greater concern. Reduced intensity conditioning regime with targeted busulfan dosing was shown to be efficacious in high-risk patients with chronic granulomatous disease, who had chronic organ damages due to uncontrolled inflammation. We used the same protocol in patients with various diagnoses of PID being severely effected by chronic inflammation.

Methods:

A total of 12 patients who received a targeted dose busulfan conditioning regimen for HSCT, were enrolled. The selection of conditioning was based on clinical decision due to a high risk of developing transplant-related toxicity. Type of diagnosis, donor and stem cell source, pre-transplant organ damages, infections, engraftment, chimerism, and transplant-related toxicities were analyzed.

Results:

At a median follow-up time of 18 months, the overall survival was 100%. Following HSCT 10 out of 12 patients successfully engrafted. Primary graft failure happened in one while a secondary graft failure occurred in another patient at post-transplant 1 year. AUC for busulfan detected in 5 patients, in 3 of them cumulative busulfan levels were within 45-60 mg/L.hr.

Conclusions:

Targeted busulfan-based reduced intensity conditioning regimens before HSCT is associated with lower toxicity and safe, however AUC levels must be monitorized carefully for successful engraftment.
HAPLOIDENTICAL TRANSPLANTATION WITH DEPLETION OF CD3 AND CD19 LYMPHOCYTES CAN BE ALTERNATIVE FOR PATIENTS WITH SEVERE COMBINED IMMUNODEFICIENCY WITHOUT MATCH DONOR.
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Background:
Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for severe immunodeficiency. Despite growing number of volunteer donors in case of rare HLA haplotypes or urgent clinical situation haploidentical HSCT is the treatment of choice. Different methods of T-cell depletion had been used in haplo- setting. In this analysis we aimed to evaluate the efficacy of CD3 and CD19 depleted transplantation in children with immunodeficiency transplanted in two pediatric centers.

Methods:
Eleven patients with severe combined immunodefiency (SCID) underwent haploHSCT in 2 centers (Wroclaw, Lublin) in years 2006-2011. Median age of the children at the time of HSCT was 11 months (range 1 month- 3.2 years). Nine patients were male, seven donors were fathers. Conditioning regimens in all but one patient were chemotherapy based, one child was transplanted without conditioning. Graft was depleted of CD3 and CD19 lymphocytes in all centers using the same immunomagnetic method (CliniMacs, Miltenyi Biotec, Germany). Primary endpoint of the study was overall survival (OS), and secondary was immune-reconstitution.

Results:
All patients engrafted and achieved stable hematopoiesis. Median follow-up of the patients is 5.5 years. OS at 5 years was 73%. Three patients died from transplant related complications: 1.5, 3 and 8 months post HSCT. Infections (aspergillosis, CMV and mixed) and as a result multi-organ failure was causes of death. All surviving patients remain IvIG independent, without infections.

Conclusions:
Haploidentical stem cell transplantation with CD3/19 depletion in patients with SCID can be an alternative for children lacking match donor in reasonable time.

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ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II DEFICIENCY: CASE REPORT

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Background:

Major histocompatibility complex class II (MHC II) deficiency is a rare autosomal recessive immunodeficiency disorder, comprising approximately 5% of all severe combined immunodeficiency (SCID) cases. Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only available curative treatment. Nevertheless, in patients with MHC class II deficiency is associated with a lower survival rate than of HSCT performed for other primary immunodeficiencies.

Methods:

Case report

Results:

We present three patients with MHC II deficiency who underwent HSCT at a single center and their evolution following hematopoietic stem cell transplantation. One of them had in utero diagnosis and the transplant was performed after birth. Stem cells were derived from an HLA-identical related donors in two patients (one of which received two transplants) and unrelated cord blood donor in the third patient. Conditioning was with myeloablative regimens. Graft failure occurred in two children, one of whom underwent a second SCT and died of overwhelming CMV infection. Patient with identical related donor and early diagnosis had complete engraftment and he is currently doing well without acute GVHD. The patient with the Cord Blood transplant lost its graft 6 months after transplantation and is currently awaiting second transplant.

Conclusions:

Allogeneic HSCT is the only therapeutic option available for patients with MHC class II deficiency. Allogeneic HSCT is linked with poor prognosis even where an HLA-identical donor is available because it is complicated by a high rate of engraftment failure. A higher success rate could be seems if early diagnosis and transplantation are performed before presenting infectious processes.
THERAPY

ESID7-0202

A PHASE 3, RANDOMISED, CROSSOVER STUDY OF THE PHARMACOKINETICS, SAFETY, AND TOLERABILITY OF GAMMAPLEX® 10% AND GAMMAPLEX® 5% IN SUBJECTS WITH PRIMARY IMMUNODEFICIENCY DISEASES

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Background:

This phase 3, multicentre, open-label, randomised, crossover bioequivalence trial evaluated the pharmacokinetics, safety, and tolerability of intravenous immunoglobulins (IVIGs) Gammaplex 5% and Gammaplex 10% in subjects with primary immunodeficiency diseases (PID).

Methods:

Subjects aged >16 years (n=33) received 5 Gammaplex 5% infusions followed by 5 Gammaplex 10% infusions, or vice versa, on a 21- or 28-day dosing schedule. Children (age 2-15 years; n=15) received 5 Gammaplex 10% infusions only. For this analysis, the adult cohort included those subjects ≥18 years old (n=31); paediatric subjects were <18 years old (n=17) in line with European Medicines Agency (EMA) age definitions.

Results:

Bioequivalence of Gammaplex 10% and 5% at the 28-day dosing interval in adults was shown based on the Gammaplex 10%:5% ratio of area under the absolute immunoglobulin G (IgG) concentration vs time curve values. Throughout the study, total IgG trough levels on Gammaplex 10% and 5% were well maintained, with values generally >600 mg/dL (minimum level for study inclusion). Tolerability was comparable and acceptable in all subjects treated with Gammaplex 10% and 5% at the dosing regimens and infusion rates used in this study.

Conclusions:

This comparison of 5% and 10% IVIG products in PID subjects demonstrated bioequivalence of Gammaplex 10% and 5% at the 28-day dosing interval. The Gammaplex 10% formulation was well tolerated in adult and paediatric PID subjects. Based on the results from this bridging study, Gammaplex 10% could be expected to have a therapeutic effect similar to the licensed Gammaplex 5%.

Funding: Bio Products Laboratory
ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION IN PATIENTS WITH PRIMARY IMMUNODEFICIENCIES IN A SINGLE CENTER IN KOREA: ELEVEN-YEAR EXPERIENCE

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Background:

Patients with primary immunodeficiency diseases (PID) are vulnerable to life-threatening infection and allogeneic hematopoietic cell transplantation (HCT) has led to the improvement in their survival.

Methods:

We report the results of HCT performed in patients with PID for eleven consecutive years from 2006 to 2016 at Samsung Medical Center, Seoul, Korea.

Results:

Twenty six recipients with PID were identified; CGD (n=11), WAS (n=4), SCID (n=3), familial HLH (n=2), LAD type 1 (n=1), SCN (n=1), hyper IgM syndrome (n=1), and undifferentiated PID (n=3). Donor types were matched sibling (n=4), matched unrelated (n=8), umbilical cord blood (UCB) (n=10), and haploidentical (n=4). Nine patients (34.6%) received HCT during the former half period and 17 patients (65.4%) during the latter half period.

Five patients experienced graft failure (GF), while 4 of them were eventually engrafted after additional HCT. The estimated 5-year overall survival rate was 79.1%. Six patients (23%) died and the causes of death were sepsis (n=3), chronic GVHD (n=2), and diffuse alveolar hemorrhage (n=1).

Conclusions:

In conclusion, candidates for PID patients have been increasingly identified for allogeneic HCT in Korea and the majority of them could be cured by HCT. Establishment of systematic registry of PID patients for HCT is needed.
UNDERSTANDING PRIMARY IMMUNE DEFICIENCY (PID) CONDITIONS

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Background:

Although rare, PID needs great attention both in the management of infections, pain, support and quality of life services in PID such as Selective IgA Deficiency and Severe Combined Immunodeficiency, B-cell disorders and selective Immunoglobulin.

Methods:

Retrospectively reviewing clinically collected data, targeting some 40 - 60 PID conditions in children from January 2016 - December 2017. Inclusions: age range from 01-16 on the general pediatric ward and the outpatient; considering incidence and prevalence. The purpose of the study is understanding the maximum outcome possible with whatever resources and options available. Exclusions include children above 20 years and comorbidities especially HIV.

Results:

Because of the high HIV, poverty and malaria prevalence, in the community, it is complex especially with limited resources, professionals and facilities to manage or follow-up these kids. It becomes more complex when other comorbidities a prevalent disease like malaria, HIV or other is diagnosed alongside PID

Conclusions:

Most of the families already too poor cannot afford for care since there is no insurance, thereby often visiting the street drugstores, fail appointments, get frustrated and even mystify PIDs. Complete study outcome available at the end of the study and the study outcome is hoped to influence our current approach of care, support and management of PIDs.
THERAPEUTIC PATIENT EDUCATION: CAN IT BE AN EFFECTIVE TOOL TO IMPROVE PRIMARY IMMUNODEFICIENCIES PATIENTS’ QUALITY OF LIFE AND TREATMENT COMPLIANCE?
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Background:

Primary Immunodeficiencies (PID) can affect many different areas of the individual’s life and cause poorer quality of life. Therapeutic Patient Education (TPE) helps patients to acquire or maintain the skills needed to manage their life with a chronic disease.

Aim: to quantify the health related quality of life (HRQoL) and emotional health in children and adults with PID before and after that TPE has been introduced.

Methods:

Both PID children and adults have been assessed with the HRQoL questionnaires SF-36, GHQ-12 (adults) and CBCL, Peds QL 4.0 (both children and their parents) at beginning (T0) and after 12 months (T1) of TPE programme (monthly individual psychological counselling and group support).

Results:

HRQoL questionnaires have been administered to PID patients (mainly CVID): 31 adults, 12 children and 12 couples of parents. At basal assessment (June 2016), the general personal health is perceived lower than general Italian health population. The 64.5% of PID adults resulted to have a high level of distress and high levels of emotional difficulties; 55% were females, aged 30-50 years and diagnosed for PID longer than 5 years before. CBCL and Peds QL-4.0 questionnaires show that the PID children and their parents do not perceive the disease as the same. Kids perceive a greater impact of the disease than their parents on their HRQoL. T1 questionnaires will be re-administered after 12 months.

Conclusions:

Having a right knowledge, management and adaptation to chronic disease since childhood, could lead to a lower impact on the quality of life in adulthood.
MANAGEMENT OF CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA IN CHILDREN: INSIGHTS FROM THE PROSPECTIVE FRENCH COHORT OBS’CEREVANCE

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Background:

The available literature data do not allow establishing second-line strategies in chronic childhood immune thrombocytopenic purpura (ITP). The objective of this study is to describe real-life management of unselected children with chronic ITP.

Methods:

French patients of less than 18 years of age at initial diagnosis with chronic primary ITP registered in real time from 2004 in the national prospective observational cohort OBS’CEREVANCE were studied. Collected data include patients’ characteristics, details on treatments and ITP status over time.

Results:

This analysis included 385 children from 27 hematologic paediatric centres: 173 males and 212 females, ITP initial diagnosis from 1990 to 2014, median follow-up 5.9 years (2.0 – 25.1). In 46% of them “watch and wait” attitude or first-line treatments were used. For 54% of them, second-line treatments were used (one rank (n=120), up to 7 ranks (n=90)), mainly splenectomy (n=107), rituximab (n=60), hydroxychloroquine (n=54), azathioprine (n=39), TPO receptors agonists (n=18), mycophenolate mofetil (n=15) with a changing face of practices over the last 15 years. No death was
recorded. Ten years after initial diagnosis, 80% of children did not achieve a sustained complete remission, whatever the strategy is.

Conclusions:

Several lessons are available from this wide observational cohort. The practices are heterogeneous. A good clinical outcome can be obtained in 46% of children with limited first-line attitude. Regarding second-line therapies, the use of splenectomy is in decline. To delay or avoid it, licensed old treatments may still have a place. Our on-going national studies will precise the benefit-risk balance of each strategy.
SUCCESS USE OF SUBCUTANEOUS IMMUNOGLOBULIN (SC) IN PEDIATRIC PATIENTS WITH PRIMARY IMMUNODEFICIENCIES IN COLOMBIA HOSPITAL INFANTIL UNIVERSITARIO DE SAN JOSÉ BOGOTÁ

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Background:

Primary immunodeficiencies are diseases of genetic origin characterized by structural, anatomical, functional deficiency, quantitative or qualitative damage in production of antibodies, alteration by defect or excess in function, signaling, transcription, signal translation, communication of intracellular and intercellular responses of different components of immune system. It mainly affects pediatric age and prevails in male sex. Characterized by recurrent infections, inflammation, autoimmunity and increased risk of cancer.

Methods:

We included 5 pediatric patients who had been treated with SC-Beriglobine P® over a period of 2.1 years (January 2015 - February 2017) in a tertiary hospital in Bogotá. A single-center, retrospective observational study was conducted.

Results:

Overall 5 patients were included (3 males, 2 females), with median age of 8.3 years, diagnosed with Hypogammaglobulinemia (2), Specific Antibodies Defect (4), Hyper IgE (1) and Ataxia Telangiectasia (1). Four shared two different diagnostics. A total of 191 individual treatments were recorded, with a median dose of 7,200 mg and a median follow-up time of 1.66 years. Five adverse events (AE) were record for a rate of 1 event per 1.39 patient years. One case was secondary to application (edema - erythema). No serious AE were associated with Beriglobine P®. None patient required hospitalization.

Conclusions:

Since 2013 we have worked with a "personalized follow up". We adjusted individual doses to reach optimal clinical response and minimize AEs. We found this personalized treatment approach to be successful for both patient and their families.
GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF) IN TREATMENT OF SEVERE DISSEMINATED BCG-INFECTION IN A PATIENT WITH SEVERE COMBINED IMMUNODEFICIENCY (SCID)

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Background:

Disseminated BCG infection is a life-threatening complication in vaccinated SCID patients, with high mortality rate. It presents a treatment challenge and often defines prognosis.

Methods:

We report a boy diagnosed with XSCID (IL2RG mutation c.563 A>C) at the age of 9 months who was vaccinated with BCG at birth and developed disseminated BCG infection involving skin, liver, spleen, bones and brain. After two weeks of antimycobacterial therapy that included rifampicin, amikacin, ethambutol, levofloxacin and isoniazid, the patient still had fever, inflamed local lesions, intoxication and high C-reactive protein.

Results:

Therapy with GM-CSF was added to potentially increase monocyte/macrophage Mycobacterium killing. The dose was increased up to 35 mcg/kg with target monocytes count 0.8 x10⁹/l or higher. Patient’s general condition and lesions significantly improved after 10 weeks of combination therapy which allowed us to perform hematopoietic stem cell transplantation from unrelated donor.

Conclusions:

Although there is no direct evidences of the role of GM-CSF in achieving control over BCG infection in this SCID patient, based on the previous anecdotal reports and our own experience we conclude that GM-CSF therapy should be considered in a combination therapy of generalized BCG infection in SCID.
SUBCUTANEOUS IMMUNOGLOBULIN REPLACEMENT THERAPY IN PRIMARY AND SECONDARY HYPOGAMMAGLOBULINEMIA: A RETROSPECTIVE SINGLE CENTRE STUDY OF 203 PATIENTS

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Background:

Subcutaneous immunoglobulin (SCIg) replacement is an effective, safe and well-tolerated treatment approach in primary Ig deficiencies (PID), but no extensive data are available on their use in secondary hypogammaglobulinemia (SID).

Methods:

In this retrospective, single center study we evaluated efficacy and safety of SCIg treatment in a cohort of 205 patients with PID and SID. Serum IgG trough levels, SCIg dosage and infusion schedule, adverse events and infection rate were compared between PID and SID. Different SCIg formulations were used.

Results:

PID patients presented CVID (69), Agammaglobulinemia (2) and IgG subclass deficiency (8). SID patients included 38 NonHodgkin Lymphomas, 55 B-CLL, 19 MultipleMyeloma and 12 immune-mediated disorders. Mean duration of SCIg treatment was 33.5±19.5 months (44.1±23.5 in PID, 26.7±12.3 in SID). In both groups, SCIg appeared to be effective in replacing Ig deficiency and reducing the infection rate. No serious adverse events were registered. SCIg monthly dosage was significantly lower in SID than in PID (p <0.01). Moreover, SID patients used longer between-infusions intervals than PID patients. Finally, in anti-CD20-related SID a small number of patients was able to discontinue SCIg replacement therapy after recovery of spontaneous IgG production.

Conclusions:

This is, to our knowledge, the biggest single center cohort of SCIg-treated PID and SID patients ever described. Results suggest that safety and effectiveness of SCIg is similar in PID and SID, irrespective of the mechanisms underlying IgG depletion. Moreover, in SID a lower IgG dosage is required and replacement does not need to be lifelong, with clear pharmacoeconomic implications.
SUCCESSFUL ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN AUTOSOMAL RECESSIVE HYPER IgE SYNDROME

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Background:

The hyper IgE syndrome due to DOCK 8 deficiency has a poor long-term prognosis. The hematopoietic stem cell transplantation (HSCT) remains the only curative treatment.

Methods:

We report the case of a nine-year old girl who initially presented with an allergy to cow's milk proteins at 18 months of age. She later began to suffer from recurrent episodes of respiratory infections complicated by bronchiectasis. At the age of 8 years she developed onychomycosis, and persistent diffuse skin lesions of molluscum contagiosum. She had elevated total IgE level and hyper eosinophilia. The diagnosis of HIES by DOCK 8 deficiency was confirmed. HSCT was performed from her fully matched brother.

Results:

The patient was conditioned with Fludarabine and intravenous Busulfan. Cyclosporine A and methotrexate were given for graft versus host disease (GVHD) prophylaxis. Neutrophil engraftment occurred on day+14. She had also developed on day + 21, signs of acute cutaneous GVHD grade I with spontaneous resolution. Transient aggravation of cutaneous lesions occurred from day + 30 to + 43. Skin lesions completely disappeared from 2 months after HSCT. Complete stable post-transplant donor chimerism was obtained from day +30. Nine months after HSCT the patient is infection free, with no signs of GVHD, no skin lesions, normal eosinophil cell count and Ig E level.

Conclusions:

HSCT with myeloablative conditioning was curative in this patient with DOCK8 deficiency, although long-term follow-up will be needed to determine whether correction of the hematopoietic compartment is sufficient to protect patients from infection and cancer.
CASE OF SYSTEMIC TOXOPLASMOSIS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN A PATIENT WITH WISKOTT-ALDRICH SYNDROME (WAS)

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Background:

Systemic toxoplasmosis is a rare life-threatening complication of HSCT.

Methods:

Eleven years old boy with no previous history of toxoplasmosis was diagnosed with WAS based on typical symptoms and WAS gene mutation c.560-1G>A and underwent HSCT from haploidentical donor with TCRab/CD19 depletion of graft. Flu/Treo/Mel conditioning regimen and posttransplant GVHD prophylaxis with tacrolimus were used.

Results:

Engraftment was achieved on day +11. Starting at day +42 patient developed sustained fever nonresponsive to massive antibacterial and steroid therapy. On day +47 retinal (by ophthalmoscopy) and brain (by MRI) lesions were detected. Prior to HSCT a small undefined brain focus located at the same area was identified via MRI. PCR analysis of intraocular and cerebro-spinal fluids revealed Toxoplasma gondii (Toxo) DNA. Clindamycin and co-trimoxazole therapy was started, yet the patient developed progressive encephalitis and pneumonia (positive Toxo DNA in bronchial lavage). Pyrimethamine/sulfadiazine were added. Due to severe inflammatory syndrome (fever, high levels of CRP, skin rash (skin biopsy - positive for Toxo DNA with no evidences of GVHD) therapy with tocilizumab and ruxolitinib was started.

On day +112, as CD4+ numbers reached 400/mcl, antimicrobial and anticytokine therapy was reduced and eventually stopped on day +131 with resolution of symptoms of toxoplasmosis and clearance of microorganism.

Currently on day +180 the patient has a complete immune recovery and is symptoms-free.

Conclusions:
Combination of specific antimicrobial therapy with IL-6 and JAK-1,2-tyrosine-kinases inhibitor proved to be effective in post-HSCT systemic Toxo infection with no hindrance to immune reconstitution.
Background:

Common variable immunodeficiency (CVID) patients have reduced diversity of the gut microbiota, increased plasma lipopolysaccharide (LPS) levels and increased systemic inflammation compared to healthy controls. We hypothesized that rifaximin, an oral non-absorbable antibiotic that decreases LPS levels in liver failure, may alter gut microbial composition and thereby LPS levels and systemic inflammation in CVID.

Methods:

Adult CVID patients (aged 21-69, 63% women) were randomized 1:1 (n=20 in each group) to rifaximin (550mg bid) versus no study treatment for 2 weeks. The primary and secondary end-points were intra-individual (alpha) diversity in stool samples and plasma levels of LPS, soluble (s) CD14, sCD25, and selected cytokines, respectively, measured at 0, 2 and 8 weeks.

Results:

Compared with no treatment, rifaximin use was associated with a significant decrease in alpha diversity during the first two weeks followed by an increase to baseline six weeks after rifaximin was terminated (figure 1, p=0.027). A significant difference between the three time points was shown for 17 gut bacterial taxa. However, plasma levels of LPS (P=0.67), sCD25 (P=0.27), sCD14 (P=0.95), IL6 (P=0.56), IL8 (P=0.39) and TNF (P=0.82) were not significantly changed by rifaximin treatment. One patient experienced a moderate adverse event probably related to rifaximin use.
Conclusions:

A 2-week course of rifaximin significantly modulated microbial alpha diversity, but had no effect on LPS or systemic inflammatory activation in CVID.

Figure 1: Comparing alpha diversity (phylogenetic diversity) in stool samples before (week 0), after (week 2) and at follow up (week 8) of a 2-week rifaximin course versus no treatment.
SUCCESSFUL HEMATOPOIETIC STEM CELL TRANSPLANTATION IN A CHILD WITH INTERFERON GAMMA RECEPTOR 1 DEFICIENCY AND DISSEMINATED BCG DISEASE

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Background:

Patients with complete Interferon-gamma receptor-1 deficiency (IFNgR1) usually die of disseminated mycobacterial infections in childhood in absence of hematopoietic stem cell transplantation (HSCT).

Methods:

we report the case of a five-year-old boy with IFNgR1 deficiency who initially presented with a disseminated BCG disease at the age of 40 days cured after 25 months of treatment. BCG disease relapsed at the age of 3 years with persistent fever, hepatomegaly, splenomegaly pulmonary micronodules, and disseminated lymphadenopathies. Insufficient improvement was noted despite two years of treatment. Histological and microbiological tests confirm the persistence of BCG disease. HSCT was performed from his fully matched older sister.

Results:

The patient was conditioned with Fludarabine, intravenous Busulfan and Anti-lymphocyte serum. Cyclosporine A and Mycophenolate Mofetil were given for graft versus host disease (GVHD) prophylaxis. Isoniazid and Rifampicin were discontinued; he received ethambutol and ciprofloxacin. Neutrophil engraftment occurred on day+27. Undocumented fever occurred on day +20. He had no signs of acute GVHD. All lymphadenopathies, disappeared completely from two months post HSCT. Stable, donor hematopoietic chimerism was obtained from one month post HSCT. Analysis of T and B lymphocyte subsets showed a satisfactory immune reconstitution from 3 months after HSCT. Currently, four months after HSCT, the patient is still alive without any health problem.

Conclusions:

Patients with IFNgR1 deficiency should ideally receive transplants before developing refractory mycobacterial infections. In this case, despite active BCG disease, the use of a transplant from an HLA-identical sibling and myeloablative conditioning regimen had provided a satisfactory result with a complete recovery of BCG disease.
STEINERT MYOTONIC DYSTROPHY SYNDROME AND HYPOGAMMAGLOBULINEMIA: NOT A CASUAL COMBINATION

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Background:

A 55 years old man suffering from Steinert myotonic dystrophy (MD) came to our attention for symptomatic hypogammaglobulinemia with recurrent airway infections. MD is an autosomal dominant inherited disorder related to the dysfunction of MDPK gene on chromosome 19q13.3. MD is characterized by progressive muscular weakness and atrophy, myotonia, involvement of the central nervous system, eyes, heart and endocrine systems. Hypogammaglobulinemia is common in these patients. The defect concerns IgG, especially IgG1 subclass.

Methods:

Blood tests showed 8.6% gammaglobulin, 4.63 g/L IgG. Secondary causes of hypogammaglobulinemia were excluded. Spirometry and HRCT showed mild-severe restrictive respiratory deficit, bronchiectasis and bronchiolectasis respectively. We started subcutaneous Ig replacement therapy but early after, because of functional limitation in fine manual movements and the unavailability of a caregiver, we switched to IVIg therapy. Nevertheless pre-infusional IgG level remained under 5 g/L.

Results:

The reason why this patient still had respiratory infections, despite the appropriate therapy, is IgG hypercatabolism due to the dysfunction of FcRn which increases IgG half-life, protecting them from intracellular degradation. Therefore we tried facilitated subcutaneous Ig (fSCIG) therapy and now IgG levels are stable at the protective level of 6 g/L.

Conclusions:

Understanding the pathogenesis of hypogammaglobulinemia in MD was essential to provide the right therapy. Thanks to hyaluronidase, bioequivalence to IVIg and convenient timing of administration of fSCIG, we obtained stable IgG levels with reduction of airway infections and stability of spirometry results.
THERAPY

ESID7-0390

MANAGEMENT OF CHRONIC MUCOCUTANEOUS CANDIDIASIS WITH JAK INHIBITOR IN 12YEAR OLD BOY
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Background:

We present a case of JAK inhibition used to manage STAT1 gain-of-function chronic mucocutaneous candidiasis (CMC) in 12year old boy with, whose disease progressed despite long term symptomatic treatment with antifungal drugs, prophylactic antibiotics and immunoglobulin treatment.

Methods:

We report on the clinical and laboratory effect of oral administration of JAK1/2 inhibitor (ruxolitinib), dosing, follow-up scheme and adverse effects in this patient, who represents, to our knowledge, first reported paediatric case of CMC managed with JAK inhibition.

Results:

We aim to describe the clinical course of our patient's symptoms and map the associated hematological, biochemical and immunological parameters during and post-treatment with ruxolitinib.

Conclusions:

Autosomal dominant STAT1 GOF mutation is the most common cause of inborn CMC with relatively poor clinical outcome, mainly due to infectious, autoimmune, malignant complications and aneurysms, as well as consequences of high resistance to azoles necessitating long-term administration of more toxic antifungal drugs. HSCT in CMC has so far delivered unsatisfactory results. The search for new therapeutic approach is warranted.

(Specific result will be delivered based on the treatment outcome which is currently undergoing)
INFECTION RATES ASSOCIATED WITH DIFFERENT ROUTES OF ADMINISTRATION IN PRIMARY IMMUNE DEFICIENCY TREATMENT

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Background:

Gammaglobulin treatment for primary immune deficiency (PIDD) is effective in intravenous (IV) and subcutaneous (SC) routes of administration (ROA). These different ROA have differing reported infection rates. This study’s goal was to assess real world infection rates in IV and SC PIDD patients in a US claims database.

Methods:

We used the Pharmetrics Plus commercial claims database to identify prevalent PIDD patients from 1/2012-12/2014. Patients’ first PIDD claim was their index date with at least 6 months pre and 18 months post index enrollment and were identified as IV-treated (Gamunex-C, Gammagard, and Privigen) or SC-treated (Hizentra). To study stable patients, we assessed patients who did not switch therapy, but with minimum adherence required (2 months with doses pre, 6 months with doses post). Patients were Greedy matched on Elixhauser conditions, age, and gender. Infections were identified using ICD-9 codes and antibiotic prescriptions.

Results:

1,123 PIDD patients were identified: 766 IV and 357 SC. 143 remained in each group post-match. No significant differences existed in any infection assessments: average infection rates (8.3 SD 12.9 for IV and 7.7 SD 13.7 for SC, p=0.46), proportion of patients with an infection (87.4% IV, 88.8% SC, p=0.71), days supply of antibiotics (119.7 days (SD 177.1) IV versus 100.6 (SD 147.7) SC, p=0.32). The odds of having a post-period infection were also nonsignificant with SC patients having 14% greater odds (OR=1.14, 95% CI- 0.56-2.32, p=0.71).

Conclusions:

When comparing prevalent commercially insured PIDD patients using IV or SC ROA, no significant differences in infection rates over an 18 month follow up existed.
AN INTERVENTION WITH INTRAVENOUS IMMUNOGLOBULIN TO MODIFY IgG HYPOGAMMAGLOBULINEMIA IN HEART RECIPIENTS WITH SEVERE INFECTION IS ASSOCIATED WITH LOWER RATES OF DEATH

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Background:

Secondary IgG hypogammaglobulinemia (HGG) is a risk factor of severe infection in heart recipients. Single center studies (distinct induction protocols; various centers around the world); a multicenter prospective study; evaluation of reproducibility of IgG testing among centers and a metanalysis support the usefulness of this biomarker. Interventional studies evaluating the effect of the modification of the risk factor are necessary. We evaluated the impact of therapeutic intervention of intravenous immunoglobulin (IVIG) in heart recipients with severe infections and HGG on clinical outcomes.

Methods:

Retrospective analysis of prospectively collected data of 233 patients in a single center. 91 patients that developed severe infections in the post-HT period and were found to have HGG (serum IgG<600 mg/dL), received non-specific IVIG in addition to conventional antimicrobial therapy with the aims of contributing to control of infection, secondary prevention of infection and normalization of IgG (IgG>750 mg/dL). IVIG was administered up to three months after infections were resolved (negative bacterial culture or CMV DNAemia). 142 heart recipients from the same center, who where not treated with IVIG, were analyzed as controls.

Results:

Both groups were comparable in terms of demographic and clinical variables. IVIG treated recipients disclosed a lower rate of death (p=0.006). In multivariate regression analysis, IVIG use (RH 0.29, 95\%CI 0.12-0.73, p=0.0085) and use of non-cytolitic induction (anti-CD25) vs cytolitic (ATG) (RH 0.22, 95\%CI 0.10-0.48, p=0.0002) remained in the final model as protective factors.

Conclusions:

Personalized immunoguided intervention of IVIG in heart recipients with severe infections and HGG is associated with better outcome.
18F-FDG PET/CT FOR EVALUATION OF TREATMENT RESPONSE IN A DISSEMINATED MYCOBACTERIAL INFECTION DUE TO INTERLEUKIN-12 RECEPTOR β-1 (IL-12Rβ1) DEFICIENCY

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Background:

IL-12Rβ1 deficiency predisposes to infections by intracellular pathogens. Diagnosis, treatment and follow-up of these infections remain challenging; whilst 18F-FDG PET/CT arises as a useful tool.

We report the case of a patient with IL-12Rβ1 deficiency a disseminated mycobacterial infection. Multiple combinations of antimycobacterial agents and IFN gamma were used. Serial PET/CT scans allowed monitoring treatment response and supporting therapeutic decisions.

Methods:

Case Presentation

6-year-old girl with IL-12Rβ1 and disseminated mycobacterial infection by M. genavense (intestinal, mesenteric and retroperitoneal involvement), identified by positive bacilloscopy and Mycobacterium complex PCR from intestinal biopsy without antimicrobial susceptibility data and negative cultures.

Results:

Empirical anti-mycobacterial treatment (oral rifampicin, ethambutol, clarithromycin, levofloxacin for 4 months; later modified to oral rifampicin and clarithromycin and intravenous ciprofloxacin and amikacin for 9 months) combined with subcutaneous IFN-gamma-1b (50-80 mg/m² 3 times a week) were administered without clinical response.

Persistence of active infection despite previous treatment (administered for 13 months) was confirmed by PET/CT. Intravenous clarithromycin, amikacin, ciprofloxacin, linezolid and cefotixin were started with IFN-gamma-1b (200-250mg/m² 3/week), with improvement verified by PET/CT 3 months after.

In the following 24 months 6 PET/CT scans were performed for monitoring treatment response. Third PET/CT, performed 14 months after last treatment course, showed worsening of infection and rifampicin was added. Improvement and normalization of PET/CT was seen 5 months after. The clinical and radiological improvement allowed progressive discontinuation of the treatment with no further relapses.

Conclusions:
Management of mycobacterial infections is a challenge in patients with primary immunodeficiencies. PET/CT might be an useful tool for diagnosis and evaluation of treatment response in those scenarios.
LONG-TERM FOLLOW-UP AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN A PATIENT WITH S32I MUTATION IN IKBALPHA
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Background:
Gain of function mutation in nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (IkBalpha) cause autosomal dominant ectodermal dysplasia with immune deficiency (AD ED-ID).

Methods:
Here we describe for the first time a 20 years follow-up post HSCT in a patient with AD ED-ID due to de novo mutation in IkBalpha (p.S32I).

Results:
Full donor chimerism in T cells, B cells, and monocytes was achieved however B-cell phenotype and function was impaired and patient still require Immunoglobulin replacement therapy (IgRT). Currently, the patient appear in overall good general condition however he continued to suffer from respiratory tract infections, chronic sinusitis with nasal polyposis, chronic bronchopneumopathy and a K. Pneumoniae bacteraemia when IgRT was temporarily discontinued. Recurrent viral infections have also been observed including 3 episodes of shingles and persistent planar warts. Non-infectious complications accounted for two episodes of aseptic meningitis following IVIG, an isolated inflammatory coxofemoral arthritis. During the follow-up, we observed a fluctuating increase of transaminase and gamma- GT without any plausible etiology despite several investigations performed, suggesting that intrinsic factors could be involved. Growth pattern remains at the 3rd percentile for weight and 3rd to 10th for height and normal pubertal development was recorded. Regular school attendance was achieved.

Conclusions:
These data show that HSCT might be a treatment option for IKBA mutated patients but it is not fully curative because of residual defects in architectural non hematopoietic cells. Therefore, other therapeutic strategies could be necessary to improve the long-term outcome of these patients.
SEVERE EPISTAXIS DUE TO NASAL TELANGIECTASIA IN A BOY WITH ATAXIA TELANGIECTASIA

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Background:

Ataxia telangiectasia (AT) is a rare autosomal inherited disorder with progressive ataxia, cutaneous and ocular telangiectasia, an increased risk for malignancies due to chromosomal instability and varying degrees of immunodeficiency. Telangiectasia mostly occurs in eyes and sun exposed skin areas. However, mucosal bleeding due to telangiectasia within the vesical bladder has been reported.

Methods:

We, for the first time, report massive epistaxis in a 12 year old boy with AT due to multiple endonasal telangiectasias.

Results:

Bleeding was refractory to a combined tamponade but could successfully be controlled using laser vaporization.

Conclusions:

Telangiectasias in mucous membranes have to be considered in AT patients with bleeding events. In particular, epistaxis in patients with AT should prompt for ENT examination to diagnose and treat endonasal telangiectasia.
THERAPY

ESID7-0488

BRONCHIECTASIS SEVERITY INDEX SCORE PREDICTS LONG TERM MORTALITY IN PATIENTS WITH PRIMARY ANTIBODY DEFICIENCY SYNDROMES

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Background:

Chronic lung disease is an important cause of death in patients with common variable immune deficiency (CVID). Although bronchiectasis was not found to a significant cause of death in a Northern Europe cohort its clinical course is unpredictable and radiological findings do not always reflect disease activity. Two recent bronchiectasis severity scores; FACED and Bronchiectasis Severity Index (BSI) predict long-term mortality in patients with non CF-bronchiectasis. There is little consensus on how lung disease should be monitored in patients with primary antibody deficiency syndromes.

Methods:

BSI and FACED scores at time of presentation were obtained from 63 patients with CVID and 32 patients with specific polysaccharide antibody deficiency (SPAD) on IgG replacement therapy attending a single centre tertiary respiratory infection service.

Results:

CVID and SPAD patients had a mean age of 42 and 48 respectively. Mean overall follow up was 11.7 years with 10 patient deaths. Mean FACED and BSI score were 3.76 and 8.22 respectively for CVID and 5 and 14.5 for SPAD. Proportions of patients categorised as severe bronchiectasis were 22% (FACED) and 43% (BSI) for CVID, and 34% (FACED) and 66% (BSI) for SPAD. BSI scores were significantly higher in CVID and SPAD patients who died compared to survivors. Pseudomonas aeruginosa positive sputum cultures were associated with significant reduction in FEV1, higher BSI and reduced survival after 20 years.

Conclusions:

FACED and BSI scores vary widely in CVID and SPAD patients. Use of bronchiectasis severity scores should be considered in future studies of clinical outcomes in primary antibody deficiency patients.
THERAPY

ESID7-0501

BILATERAL LUNG TRANSPLANTATION IN A CVID PATIENT WITH BRONCHIECTASIS AND GLILD

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Background:

Although CVID patients are mainly affected by pulmonary infections, up to one-third develop a granulomatous-lymphocytic interstitial lung disease (GLILD) which is associated with a poor prognosis due to chronic respiratory failure. Currently, there is no consensus for the management of GLILD in CVID.

Methods:

We report on a 47 yrs old male CVID patient with end-stage lung disease due to bronchiectasis and complicated by GLILD, receiving lung transplantation (LTx).

Results:

The patient had been lately diagnosed with CVID at the age of 30. Afterwards, despite intravenous immunoglobulin replacement therapy having being started, frequent lung infections led to the development of extensive bronchiectasis and progressive decline in lung function. In late 2014 oxygen therapy had been started. The patient had been referred to our centre in early 2015 and after the switch to subcutaneous immunoglobulin replacement therapy a comprehensive reevaluation highlighted an overlaid GLILD on bronchiectasis (radiologic evidence only). Unfortunately the lung function was already too compromised and LTx seemed the only valuable option.

On september 2016, following antithymocyte globulin induction, patient received orthotopic bilateral LTx and maintenance therapy with cyclosporine, mycophenolate mofetil and prednisolone. The histopathological evaluation confirmed the presence of GLILD on the explanted lungs.

The early post-operative course was complicated by mild acute cellular rejection. At present the lung function is normal and the IgG trough level is higher, despite using the same replacement dosage than before LTx.

Conclusions:

To the best of our knowledge this is the first reported case of LTx in CVID with bronchiectasis and GLILD.
Background: This study provides the data of the experience of two-years fSCIG administration in Greek patients with PIDs.

Methods:

55 patients (male/female: 34/21, median age: 40y, range: 11-78) were enrolled, including 48 with CVID, 5 with XLA and 2 with HIGM. Prior fSCIG, 16 patients were receiving IVIG, 33 switched from another subcutaneous supplement (withdrawn due to regional/national financial reasons), 2 were alternately receiving either IVIG or SCIG, and 4 newly-diagnosed patients had not received treatment. The median duration of previous treatment was 171.0 months (range: 0-481.0), the median monthly Ig dosage was 32.0gr (range: 0-64.0), and the mean (±SDEV) IgG trough levels were 749.1 (±227.1) mg/dL.

Results:

960 infusions were recorded; the median monthly Ig dosage was 35.0gr (range: 15.0-60.0) and the mean (±SDEV) IgG trough levels were 859.3 (±161.9) mg/dL. The commonest adverse events were locally (edema: 94.5%, redness: 89.1%); the systemic events, including itching (21.8%), fatigue (14.5%), drowsiness (10.9%), headache (10.9%), fever (5.5%), nausea (5.5%), diarrhea (9.1%) and atopy (1.8%) were reversible into 48-72h after infusion. Only one XLA patient interrupted fSCIG treatment after 7 infusions, due to severe skin toxicity. Bacterial infections were displayed by 17 patients and an increase of fSCIG dosage was necessary for 4 patients, due to lower IgG trough levels. The majority of patients (90.9%) declared a clear preference of fSCIG due to longer infusion intervals and less needle involvement.
Conclusions:

fSCIG has been proved to be an effective treatment option for the majority of patients with PIDs receiving immunoglobulin replacement treatment.
SWITCHING FROM IVIG TO SCIG. MAY THE INCREASE OF IGG TROUGH LEVELS BE WORTH?

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Background:

Higher immunoglobulin G (IgG) trough levels (>800 mg/dl) are currently recommended for PID patients receiving IG replacement. Subcutaneous immunoglobulin (SCIG) has shown to increase patients' quality of life. Several studies have demonstrated a progressive increase in IgG levels after switching from intravenous (IVIG) to SCIG. We conducted a descriptive study to determine whether switching from IVIG to SCIG replacement therapy impacts on IgG levels in PID patients and clinical outcome.

Methods:

We conducted a retrospective study from November 2006 to June 2017 on PID patients receiving SCIG to compare IgG trough levels 12 months before and after switching from IVIG to SCIG.

Results:

We included 17 patients (median age: 7; IQR: 11.7-2.9; 10 males; bronchiectasis 8/17). The most common diagnosis were CVID (10) and XLA (3). Median dose was 480mg/kg/month on IVIG while 411mg/kg/month on SCIG therapy (p=0.079). An increase of 22% in median IgG trough levels was demonstrated (712 mg/dl, 869 mg/dl, respectively (p=0.016). Average IgG trough levels were higher in patients with bronchiectasis either on SCIG or IVIG therapy. No serious infections were reported in patients receiving SCIG therapy and adverse events were less common than with IVIG (1 vs 5 serious AE, respectively).

Conclusions:

Switching from IVIG to SCIG allows higher IgG trough levels that seem to be associated with a better lung prognosis in pediatric PID patients. When appropriate levels are reached with IVIG dose may even be reduced to decrease the economic cost associated with SCIG. Relationship with pulmonary outcome is currently being evaluating.
Working Party - Registries

ESID7-0085

APDS ESID REGISTRY: FOCUS ON AUTOIMMUNITY AND IMMUNE DYSREGULATION
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Background:

The APDS registry was initiated to better define the natural history of APDS, to document disease evolution and the impact of different treatment strategies, with the aim of identifying predictors for outcome. The registry is supported by Novartis, GSK and UCB and may also be used to offer patients participation in clinical trials with selective p110δ inhibitors.

Methods:

Patients with genetically confirmed APDS are registered with a retrospective case report form, followed by prospective 6-monthly follow-up, allowing longitudinal clinical and biological assessment. The data collected to date were reviewed, with focus on features of autoimmunity and immune dysregulation.

Results:

29 APDS1 and 17 APDS2 patients were registered by March 2017. Patients had a median age of 18 years (range 6-47). 3 registered patients have so far been recruited into p110δ inhibitor trials. Seven patients had autoimmune haemolytic anaemia, associated with thrombocytopenia in one and with neutropenia in another patient. Two patients had autoimmune hepatitis and 2 had arthritis. Seborrheic dermatitis was reported in 2, and eczema in 6 patients. Eight patients suffered from inflammatory bowel disease. The onset of these autoimmune manifestations in relation to onset/severity of other disease manifestations such as lymphoproliferation and infections, as well as the relationship to the immune phenotype and the treatment history, will be presented at the congress.

Conclusions:

The APDS ESID registry is a powerful tool to document the natural history of APDS and to investigate relationships of clinical manifestations, offering the opportunity for patients to enrol in novel treatment protocols.
THE NEW REPORTING TOOL FOR THE ESID ONLINE REGISTRY: EASY TO USE DATA VISUALIZATION

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Background:

As operator and maintainer of the ESID registry we often are confronted with querying data in order to answer research questions. Often we end up in sending out raw data which researchers may visualize by their own depending on individual needs. Especially in cases of repeating tasks and for presentation purpose we see a potential to save resources and add functionality by offering a new reporting platform.

Methods:

In order to save resources on both sides we designed a toolkit which can visualize such requests. Therewith we offer a tool which can perform statistical evaluations and offer visualization on predefined questions. Repeating tasks can be instantly requested on a periodically refreshed database and include data from all contributing sub-databases, like UK-PID.

Results:

Every user with an existing valid registry account can access the reporting tool with the same credentials as a web-platform. There are two categories of statistical evaluations: one contains general questions on the whole registry data of all centers. The other contains evaluations on the associated center of the user. Additionally, more complex statistical methods can be implemented as the system allows us to implement data analysis in R. Furthermore, the generated diagrams can be downloaded.

Conclusions:

The reporting tool is a platform for data visualization and evaluation. We started with a limited amount of statistics. In future, we will expand the available reports and provide statistical evaluations based on research questions formulated by registry users.
OUTCOMES OF CHILDREN WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS GIVEN ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ITALY

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Background:

Patients undergoing allogeneic Hematopoietic Stem Cell Transplantation (HSCT) for Hemophagocytic Lymphohistiocytosis (HLH) are a population with specific peculiarities, warranting special considerations about timing of HSCT, donor choice and conditioning regimen.

Methods:

We present the largest cohort of HLH patients undergoing HSCT. Included in the analysis were 109 patients undergoing 126 transplant procedures between 2000 and 2014 in centers associated with the Italian Pediatric Hematology Oncology Association (AIEOP).

Results:

Genetic diagnosis was FHL2 (32%), FHL3 (33%) or other defined disorders known to cause HLH (20%). Donor for first transplant was an HLA-matched sibling for 25 patients (23%), an unrelated donor for 73 patients (67%) and a partially matched family donor for 11 patients (10%). Conditioning regimen was busulfan-based for 61 patients (56%), treosulfan-based for 21 patients (20%) and fludarabine-based for 26 patients (24%). The 5-year probability of overall and event-free survival were 71% and 60% respectively. Death was mainly due to transplant-related mortality (TRM), while 12 out of 14 patients suffering rejection/relapse were salvaged with a subsequent transplant. Use of HLA-partially-matched family donor and use of peripheral blood stem cells were associated with adverse
outcome in univariate analysis, while only the former variable remained significant in multivariate analysis. Active disease at transplantation did not significantly affect prognosis.

Conclusions:

These data suggest that active disease should not preclude transplantation, which should be performed preferably using either bone marrow or cord blood cells. Since HLA-haploidentical HSCT in patients with HLH is currently associated with unsatisfactory outcomes, innovative approaches are warranted.
BCG-RELATED INFLAMMATORY SYNDROME (IS) IN SEVERE COMBINED IMMUNODEFICIENCY (SCID) PATIENTS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) WITH TCRab/CD19 DEPLETION OF GRAFT – SINGLE CENTER EXPERIENCE

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Background:

TCRab/CD19 depletion is a promising technology of graft engineering with potential reconstitution of innate immunocompetent cells.

Methods:

From 2012 to 2017 17 patients with SCID who were BCG vaccinated at birth received HSCT with TCRab/CD19 depletion of graft in our center. Median age at diagnosis - 5.3, at HSCT - 7.25 months.

Results:

3 of 17 patients developed BCG-related IS early after HSCT. In 2 patients (1 after conditioned and 1 – nonconditioned HSCT, both had well controlled systemic form of BCG infection before HSCT) IS presented at days +4 and +12 after HSCT with fever, local symptoms exacerbation and laboratory signs of macrophage activation syndrome (MAS) in one of them. One patient without signs of BCGitis before HSCT after conditioned HSCT at day +38 developed IS with symptoms of systemic BCG infection and MAS.

Escalation of antimycobacterial therapy was not effective. In 2 patients IS was controlled with anticytokine therapy (1 – short course of IL-6 inhibitors, 1 prolonged IL-1 inhibitors), 1 patient did not respond to IL-1 inhibitors and required high doses of steroids. 2 patients eventually died of sepsis due to profound lymphopenia after prolonged immunosupression, 1 – alive at 3.5 years after HSCT with no clinical problems.

Conclusions:

We hypothesize that BCG-induced graft TCRgd and NK cells cell activation leads to cytokine release and development of extensive poorly controlled IS in BCG-vaccinated SCID patients.
Background:

Early thymectomy is routinely performed in infants undergoing surgical correction of congenital heart defects. Immunological changes have been described after early thymectomy, but the long-term clinical consequences are unknown. The aim of this study was to investigate the association between early thymectomy and risks of immune related diseases.

Methods:

The study is a nationwide population-based cohort study using the Medical Birth, Cause of Death and National Patient Registers in Sweden. We identified 5664 individuals born in 1973-2009 thymectomized before five years of age. For each individual ten age and sex matched general population controls as well as 2276 surgery controls who had undergone early cardiac surgery, not involving thymectomy, were included.

Results:

Compared to the surgery controls thymectomized individuals were at increased risk for hypothyroidism (aHR 3.03; 95%CI 1.17-7.83), type 1 diabetes (aHR 3.16; 95%CI 1.08-9.21) and both viral (aHR 1.40; 95%CI 1.30-1.50) and bacterial (aHR 1.26; 95%CI 1.11-1.43) infections. Compared to the general population, increased risks were detected for hypothyroidism (aHR 4.94; 95%CI 3.27-7.46), juvenile idiopathic arthritis (aHR 1.85; 95%CI 1.11-3.09), rheumatic diseases (aHR 1.89; 95%CI 1.00-3.57), celiac disease (aHR 1.96; 95%CI 1.42-2.72), cancer (aHR 1.61; 95%CI 1.07-2.43), infections (aHR 3.18; 95%CI 3.07-3.30) and asthma (aHR 1.84; 95%CI 1.64-2.07) in thymectomized individuals.

Conclusions:

In conclusion, early thymectomy is associated with increased risks of autoimmune diseases, cancer as well as infectious diseases. The study implicates an important role for the human thymus in post-natal life and suggest caution during early cardiac surgery and, if possible, avoidance of total thymectomy.