DOTTORATO DI RICERCA IN
SCIENZE BIOMEDICHE dell’ETA’ EVOLUTIVA

CICLO XXVI

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CLINICAL ANALYSIS OF THE COMPLEX WORLD OF
INHERITED MULTIPLE AUTOIMMUNITY

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ABSTRACT

IPEX is characterized by early-onset severe autoimmune enteropathy, eczema and endocrinopathy. Increasing number of patients affected by multiple autoimmunity resembling IPEX, without FOXP3 mutations have contributed to define an IPEX-like phenotype. We set to identify clinical and laboratory indicators to better define this disease spectrum. Clinical characteristics of about 76 patients with immunodysregulation referred to us for suspected IPEX syndrome were analyzed: only 16 had FOXP3 mutations and in selected patients STAT5b, CD25, STAT1 and IL10 deficiency (IL10RA, IL10RB, IL10) have been ruled out. Immune mediated enteropathy, frequently associated with failure to thrive, was the key clinical feature. In patients without enteropathy, endocrinopathy and cytopenias are the main clinical manifestations. The age of onset is diverse: a group of patients presents diarrhoea within the first year of life and behave similarly to the IPEX cohort, another group had a delayed onset characterized by multiple autoimmune disease (skin and endocrinopathy being less predominant than to autoimmune cytopenias and infections), whilst a third group had only early onset diarrhoea with no other symptoms associated (IBD-like?). Elevated IgE and dysgammaglobulinemia are often reported.

Outputs from this study define disease subgroups facilitating precise molecular studies in order to better understand and delineate the mechanisms of tolerance failure in patients with complex autoimmune
disease. The final objectives would be definition of new disease entities and ultimately discovery of new genes involved in immune-dysregulation diseases.
INTRODUCTION

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX, OMIM 304790) syndrome is a rare monogenic, life-threatening, congenital immunodeficiency caused by defective peripheral immune tolerance, originally described by Powell et al. in 1982 [1]. The phenotype is characterized by early-onset severe autoimmune enteropathy with failure to thrive, eczematous skin rash and endocrinopathy, usually insulin-dependent diabetes mellitus (IDDM). Less common manifestations include immune thrombocytopenia (ITP), autoimmune haemolytic anaemia (AIHA), thyroiditis and nephropathy [2, 3]. Moreover, most of the patients suffer from infections, ranging from moderate recurrent infections of the upper respiratory or gastrointestinal tract, to invasive life-threatening episodes, including sepsis and meningitis [4–6]. It is still unclear if the significantly increased susceptibility to infections is related to their genetic defect or if this is secondary to other clinical features, such as decreased barrier function of the skin and gut, or to immunosuppressive therapy. Laboratory findings include increased IgE and eosinophil values and a variety of autoantibodies, which usually correlates with signs of pathology in the target organs; no main immunological defects have been associated with IPEX.

IPEX is caused by mutations in the forkhead box protein 3 (FOXP3) gene, located on chromosome Xp11.23 [2,7], which encodes a DNA binding protein that is a member of the forkhead (FKH) family of
transcription factors critical for the development and function of natural CD4+CD25+FOXP3+regulatory T cells (nTreg), that suppress the activation of peripheral auto-reactive T cells [4,5]. In addition to the loss of suppressive function, FOXP3 mutations seems also cause a high instability of the Treg cell compartment with a marked shift to the Th17 cell phenotype of bona fide nTreg cells [8], that means an inflammation-driven conversion from a regulatory to an effector (i.e.,IL-17-producing) phenotype of mutated Treg cells, which may directly contribute to the autoimmune damage in the target organs.

FOXP3 consists of 11 exons that encode a protein of 431 amino acids expressed primarily in lymphoid tissues (thymus, spleen, and lymph nodes), particularly in CD4+CD25+ regulatory T cells. The FOXP3 protein has several specific regions including a proline-rich domain at the N-terminus, a zinc finger and leucine zipper (both conserved structural motifs involved in protein/protein interaction) in the central portion, and a forkhead DNA-binding domain at the C-terminus. The severity of the disease does not always correlate with the absence of protein expression. Gene analysis revealed missense mutations (usually point mutations) in the majority of affected individuals, resulting in a normal or reduced level of expression of mutant protein with a normal percentage of CD4+CD25+FOXP3+ T cell detected by flow cytometry in the peripheral blood. Therefore, in IPEX patients FOXP3mutTreg cells are physically present but functionally impaired, and this is considered the primary cause of autoimmunity in IPEX.

IPEX is a severe condition requiring a prompt treatment with
corticosteroids and immunosuppressive drugs [5, 9], but only hematopoietic stem cell transplantation (HSCT) can offer a permanent cure [10, 11].

Increasingly, patients affected by multiple autoimmune diseases resembling IPEX, without FOXP3 mutations, have contributed to define an IPEX-like phenotype. Two other proteins related to FOXP3 function has been recognized so far: IL-2R alpha (CD25) and STAT5b.

The IL-2 receptor is formed by the α (CD25), β (CD122) and common γ-chain (CD132) subunits, and plays a vital role in maintaining the immune system. CD25, constitutively expressed at high levels by Treg, binds exclusively IL-2. Therefore Treg are able to respond to low concentration of IL-2 at all times, promoting the maintenance of FOXP3 expression by amplifying IL-2 signaling in a STAT5-dependent way [12].

Three unrelated patients with CD25 deficiency have been reported so far [13-16]. They presented with clinical features resembling IPEX patients, but with an increased susceptibility to viral infections (mainly chronic CMV infection) and developed autoantibodies, hepatosplenomegaly, lymphadenopathy and lymphocytic infiltrates in various organs. Notably, CD25 expression on the surface of patient’s CD4+ T cells was absent.

STAT5b is a common downstream effector of various cytokines (IL-2, -4, -7, -9, -13, -15, -21), growth hormone (GH) [17], erythropoietin, thrombopoietin, and granulocyte colony-stimulating factor signaling molecules [18]. STAT5b is a member of the signal transducer and
activator of transcription family of proteins, which regulate gene transcription in response to various cytokines and growth factors. STAT5b is a critical transducer of interleukin-2-mediated signals required to sustain FOXP3 expression in Treg and to maintain Treg cells themselves [19, 20]. Ten cases of STAT5b deficiency, inherited as an autosomal recessive disorder have been reported [21-23] Affected individuals present with severe growth failure after birth with normal levels of serum GH and low levels of IGF-1 and IGFBP-3, autoimmune disease, chronic infections, chronic lung disease, eczema and diarrhoea. Immunological work up revealed modest lymphopenia, low T cell counts (CD4+, CD8+), low NK cells and Treg dysfunction. In mice, deletion of STAT5a/b in CD4+ T cells results in marked reduction of Foxp3+ and CD4+CD25+ cells in both thymus and periphery; humans with STAT5a/b mutations display immune dysregulation associated decreased CD25 and FOXP3 expression [24, 25].

Moreover, in this contest, IL-10 and TGF-β have also an important role in immune homeostasis and are secreted both by dendritic cells and Tr1 cells, a subtype of regulatory T cells which do not express CD25, nor FOXP3 [26]. Glocker et al [27], postulate that mutations affecting IL10 and IL-10 receptor (IL10R1 and IL102) abrogate IL-10-mediated immunomodulatory signaling, strongly associated with hyperinflammation of the intestine. Patients presented with early-onset enterocolitis (within the first year of age) characterized by an aggressive course with recurrent abscesses and entero-cutaneous fistula, follicular rash and recurrent infections that were thought to be primarily associated with immune suppression. Immune parameters
including serum immunoglobulins, lymphocyte subsets, and neutrophil oxidative burst seems to be normal. Alterations in IL10 and its receptor genes would be associated with specific IPEX-like phenotypes.

Recently a gain of function mutations in STAT1, a key transcription factor mediating IFN-α /β signaling, has been found in patients presenting with multiple autoimmune disease, chronic candidiasis and, sometimes, cerebral aneurism [28].

Although these four conditions have been identified, most IPEX-like patients remain without a genetic diagnosis. In this study we analyze the data from 76 patients referred to us for genetic analysis, trying to identify clinical indicators that differentiate IPEX and IPEX-like patients in order to characterize disease sub-groups within the IPEX-like cohort. The recognition of specific patient cohorts will be essential to better address genetic analysis and eventually perform specific genetic studies to discover new possible molecules responsible for the disease.
Fig. 1: multiple signaling pathways converge for the induction of forkhead box P3 expression. IL-2-induced STATs bind directly to evolutionarily conserved regions in the FOXP3 locus and induce the expression of this gene. *Huehn J et al, Nature Reviews 2009* [29]
MATERIAL AND METHODS

Patients

Blood samples from 113 patients with immunodysregulation, coming from different countries, were referred to the NEUROFARBA Department, Section of Child's Health, University of Florence, Italian Study Group of IPEX, for genetic analysis from 2004 to 2013. Patient’s clinical and immunological information were collected from the referring physician who filled out a registration form. Data from 14 patients were unavailable or lacking; clinical presentation in 17 subjects was not consistent with IPEX disease. 6 patients had a different diagnosis at follow up update. In this study data from 76 patients (56 male, 20 female) were analysed.

The clinical diagnosis of IPEX/IPEX-like syndrome was based on the presence of severe enteropathy together with at least one of the following characteristics: (a) IDDM or thyroiditis; (b) severe eczema; (c) specific autoantibodies (anti-insulin, IA-2, GAD, enterocytes); (d) cytopenias; (e) other autoimmune manifestations (hypothyroidism, polyarthritis, vitiligo, etc). A small group of patients without enteropathy was included in the study because of the complex autoimmunity clinical pattern.

Clinical and immunological data, were analysed retrospectively, and included age of onset; features of autoimmune enteropathy including intestinal biopsy results; evidence of endocrinopathy such as IDDM and thyroiditis; cutaneous symptoms; haematological abnormalities
including AIHA, ITP and neutropenia; type of infections; renal involvement; lymphadenopathy and/or hepatosplenomegaly; general examination.

Gut biopsies, when available, were evaluated to differentiate patients with autoimmune enteropathy with villous atrophy (GvHD-like enteropathy) from patients with other gut damage (celiac-like disease, enteropathy with autoantibodies) [30].

**Molecular analysis**

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's recommendations. The entire coding sequence (including exon-intron junctions) of the genes FOXP3, IL2Ra, IL10/RA/RB, STAT5b, was amplified using standard polymerase chain reaction (PCR) conditions using a different Tm: FOXP3 60°C, IL2Ra 55°C, IL10/RA/RB 58°C, STAT5b 58°C. PCR products were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an automated ABI PRISM 3130 Genetic Analyzer (Applied Biosystems) and compared to reported reference cDNA sequence.

The Laboratory of Clinical Infectious Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda performed **STAT1** analysis [28].

In 69/76 patients (13 female, 56 male) the coding regions, including intron/exon junctions, of **FOXP3** gene were analysed and the diagnosis of IPEX syndrome, based on **FOXP3** mutation, was confirmed in 16
patients. Female patients were screened for IPEX at the beginning for understanding the possibility of X-inactivation. Those with the typical clinical and pathologic features of IPEX, negative FOXP3 gene analysis or female patients, were classified as IPEX-like.

*CD25* was investigated in 26 patients and 3 mutations were identified.

*STAT5b* and *IL10, IL10 RA, IL10RB* genes were analysed in 15 and 3 patients respectively, but no mutations were found.

*STAT1* was studied in 25 patients and one showed a mutation in the gene sequence [table I].
RESULTS

IPEX

Clinical and laboratory features

IPEX was diagnosed in 16 out of 69 patients analysed for FOXP3 mutation [table II]. Unfortunately clinical information concerning two siblings was not available. Enteropathy is the main clinical feature of presentation among IPEX subjects. Early onset severe watery diarrhoea was present in 11/14 (range of onset: 0-5 months); small bowel biopsies were compatible with patterns of autoimmune enteropathy in all.

3/14 presented an atypical IPEX syndrome: one developed delayed onset diarrhoea (11 years old) with acute enterocolitis and ulcerative mucosa at gut biopsy; another one had failure to thrive since birth without diarrhoea with endoscopic appearance of ulcerative, oedematous and hyperaemic gastric mucosa; the last one was diagnosed because of a sibling affected, but he did not present any clinical symptoms so far.

Moderate to severe eczema affecting mainly trunk, face and limbs, occurred in 12/14 patients within three months of life.

Contrary to what previously described, only 2/14 had neonatal IDDM; no other endocrinopathies were mentioned. AIHA and ITP were present in 2 patients respectively. Renal calculi were found in one patient, probably as a consequence of dehydration and electrolyte alterations.
One experienced nephritic syndrome, one had acute tubular necrosis and one developed clear cell renal carcinoma. Lymphadenopathy and splenomegaly were observed in 2 and 4 patients respectively.

Before immunosuppressive treatment was started, serious infection occurred in 7/14 patients: 4 had septic episodes, 2 had osteomyelitis, 2 patients were admitted to Paediatric Intensive Care Unit for respiratory distress. Upper respiratory tract infections were present in almost all patients.

Laboratory work up showed increased IgE levels in 11/14 patients; none had impaired immunological results.

**Treatment and outcome**

Treatment approach was mainly focused on limiting life-threatening gastrointestinal symptoms. At the beginning patients were treated as a case of allergic gastro-enteropathy and different kind of elemental milk were introduced. Changing of feeds did not cause any improvement in their symptoms so total parenteral nutrition was commenced in 10/14. Because of the worsening diarrhoea and not gaining weight, corticosteroids first (methylprednisolone/prednisone) and immunosuppressive drugs after (cyclosporine, tacrolimus, anti-TNFα antibody, rapamycin, mycophenolate) were introduced in 12/14 subjects in different steps with poor benefit in most of them. HSCT was eventually performed in 6 patients.

Two siblings did not receive any treatment because one had severe diarrhoea that improved within the first year of life and the other one enjoy good health until now.
Patients are all alive but three, two deceased before HSCT because of intractable diarrhoea and sepsis, one after HSCT because of sepsis.

**Molecular Analysis**

The genetic analysis of *FOXP3* revealed the presence of 12 different mutations in 16 patients. Four of them are intronic mutations that alter the splicing mechanism: one causes the skipping of exon 1 (c.210+1G>C) and three lead to the skipping of exon 7 (c.816+3G>C, c.816+2delT, c.816+5G>A). One is a deletion of a single amino acid in the leucin-zipper domain, due to an in-frame deletion of three nucleotides in exon 7. Seven mutations are single nucleotide alterations that cause single aminoacid changes, five of them are located in the forkhead domain of the protein that is involved in the DNA binding, nuclear localization and repressor activity.

**IPEX-LIKE**

IPEX-like subjects can be divided in three subgroups: the first (31/60 patients; 10 female, 21 male) included patients with severe enteropathy within six months of life, the second (22/60 patients; 6 female, 16 male) comprised patients with a delayed onset (range: 1-24 years), the third enclosed patients with multiple autoimmune manifestations except diarrhoea (7/60 patients; 4 female, 3 male) [Fig. 2].
IPEX-like early onset

Clinical features

In this cohort of patients two different subgroups of subjects can be identified: those with only gut disease and those with enteropathy and multiple autoimmununity.

7/31 had only early onset watery diarrhoea (range of onset: 0-2 months) not improving after changing feeds. In 6 patients gut biopsy showed findings of autoimmune enteropathy with villous atrophy.

24/31 presented with severe diarrhoea within six months of age. Gut biopsy showed GvHD enteropathy in 19/24; eosinophilic colitis in 2/24; the other 3 patients had moderate to severe chronic inflammation of mucosa. Most of them (20/24) had difficulties in gaining weight with consequent failure to thrive. Cutaneous involvement with eczema with varying degrees of severity was present in 16/24 patients; only 2 subjects had alopecia and one developed pemphigus bullous. A red nodular rash confluent with red lumpy areas was observed in one patient since the second day of life, skin biopsy showed a vasculitic form between Lupus Eritematosus and Sweet Disease. Two weeks after he developed arthritis of fingers. 2 other patients experienced transient arthritis. Endocrinopathy was present in 10/24 patients: IDDM and autoimmune thyroiditis were evident in 4 and 7 patients respectively. Among them, one patient had neonatal onset of both diseases, other 2 subjects experienced both at different age. 2 had GH deficiency, in one patient probably due to hypophysitis. Autoimmune cytopenias were manifested at different age in 11/24 patients: 7 had AIHA, 5 had ITP, 4
had autoimmune neutropenia. Among them, two patients developed cytopenia affecting all three-cell lines. Autoimmune hepatitis was diagnosed in 4/24; epatosplenomegaly was evidenced in 2; epatomegaly and splenomegaly alone in 1 and 2 subjects respectively. Mild lymphadenopathy was found in 5/24. Two Indian patients, with similar clinical manifestations, developed tubular nephropathy (kidney biopsy non available). Three patients had psychomotor impairment, one had cerebral atrophy and one had cerebral aneurysm with consequent cerebral hemorrhage.

Most of the patients (21/24) suffered from different kind of infections since infancy. Upper respiratory tract infections (mainly acute otitis media) were described in 9 patients. Low respiratory tract infections were reported in 10 patients: 4 bronchiolitis (RSV was detected in 2) and 8 pneumonia due to virus and bacteria. Deep infections affected 8 patients: 6 sepsis mostly as a consequence of *Staphilococcus Aureus*, *Enterococcal* and *Pseudomonas Aeruginosa* infections and one *HSV* encephalitis (the patient was limphopenic due to immunosuppressive treatment). 6/24 had gastroenteritis and 3 had perianal abscesses. *CMV* PCR was found positive in 4 patients.

**Laboratory results**

Among this cohort of patients, immunological parameters were almost unremarkable with the exception of dysgammaglobulinemia in 9 patients and lymphopenia in 5 patients. Two siblings, in particular, were lymphopenic with good distribution of naïve and memory B cells, normal immunoglobulin production and normal in vitro proliferation to
PHA. In particular the oldest one had low T cell number (CD3 414 cells/microL, normal range: 1000-2000 cells/microL; CD4 185 cells/microL, normal range: 500-1300/microL; CD8 198 cells/microL, normal range: 300-800/microL; NK 28 cells/microL, normal range: 100-700/microL) and slightly low B cell number (253 cells/microL, normal range: 200-500/microL). Interestingly them both did never receive any immunosuppressive treatment during their life. The cytokine assays, performed in the two siblings, showed an impaired response to LPS (inability to induce IL12 and IL10 production) and significantly impaired production of TNFα and IL6. These results would suggest a possible defect of monocyte and T cell function, with TLR pathway function impaired.

IgE levels were increased in 14 patients (range 109 - >2000 kU/l; normal range <100 kU/l). Anti-enterocyte antibodies were positive in 2 out of 9 analysed; on the other hand antinuclear antibodies and anti-smooth muscle were positive only in 3 patients respectively.

**Treatment and outcome**

Total parenteral nutrition was initially started in almost half of the patients. 10 patients received IVIG for both hypogammaglobulinemia and ITP. Because of intractable diarrhoea and autoimmune manifestation, 16 out of 31 were treated with corticosteroids and in 15 patients immunosuppressive therapy was necessary for controlling the disease. In 7 patients HSCT was performed.

In the first subgroup of 7 patients, 2 died because of intractable diarrhoea. Two patients with autoimmune enteropathy, improved
clinically and are actually in good health; another one gradually recover after bowel surgery. We can postulate that their clinical and histological presentation, consistent with IPEX, was in both cases the consequence of subsequent episodes of infectious diarrhoea on an immature gut setting.

In the second cohort, three patients died at 13 months, 18 months and 12 years of age respectively in consequence of HSCT complications, one patient died when he was 4 years old of brain hemorrhage due to cerebral aneurysm, one patient died at 8 months of age because of severe diarrhoea and one child died at 3 months of age due to central venous catheter sepsis.

**Molecular Analysis**

Among the 31 patients of the IPEX like early onset cohort, the genetic analysis of CD25 showed the presence of 3 different mutations in three patients. Two of them are single nucleotide variations localized in exon 4 not described previously and absent in a group of 200 control chromosomes analyzed (one has been recently published [16]). Both the alterations lead to the substitution of a single aminoacid located in the “sushi” domain of the protein. This region seems to be fundamental for IL2-IL2Rα interaction, thus the aminoacid change could modify/prevent IL2 binding. The third is a single nucleotide variation found in exon 2 leading to an aminoacid change that could be responsible of the alteration of the tertiary structure of the receptor.

Their clinical presentation, according with the cases described in literature, was characterised by intractable watery diarrhoea with
villous atrophy within the first month of life associated with mild to moderate eczema. Two of them developed autoimmune hypothyroidism, one had also neonatal IDDM. CMV PCR was found positive in two of them, as in most of the patients with CD25 deficiency. IgE level was normal or slightly increased. At flow cytometry analysis they showed absence of CD25 cell surface expression.

*STAT1* was studied in 12 patients and one had a gain of function mutation (unpublished data). The patient had multiple autoimmune manifestations and died because of brain haemorrhage due to cerebral aneurism. *STAT5b* and *IL10, IL10RA, IL10RB* were found wild type in 6 and 3 patients respectively.

**IPEX-like delayed onset**

**Clinical features**

The second group of IPEX-like patients had a delayed onset enteropathy (range: 18 months – 8 years old). Diarrhoea was the symptom at onset in 13 patients; the other 9 patients had different clinical presentations characterized by eczema, IDDM and infectious disease.

In this cohort half of the patients had severe-watery diarrhoea with villous atrophy in 8 of them at gut biopsy. On the other hand, 11 out of 22 had a moderate to severe diarrhoea with active inflammation at gut biopsy; prominent eosinophil infiltrate was characteristic in 5 of them. Virus detection on specimen showed CMV presence in 3/22, and HHV6 and EBV positive in 2 patients respectively. Failure to thrive was present in 16 patients; one had GH deficiency.
Cutaneous involvement included eczematous rash (10/22), alopecia, vitiligo and dystrophic nails. Endocrinopathy was the second most frequent manifestation, as it was present in 11 subjects: 7 had IDDM (in 4 onset was in the first 2 years of life) and 6 had autoimmune thyroiditis.

Autoimmune cytopenias included ITP and AIHA in 5 patients respectively (3 presented Evans syndrome), neutropenia in 2, and positive direct Coombs test alone in 2. Hepatic involvement was sporadic: 3 patients had autoimmune hepatitis and 3 subjects had hepatosplenomegaly. Lymphadenopathy was described in 5. Food allergy with skin PRICK test positive was found in 3 subjects. 4 patients out of 22 suffered from a different degree of nephropathy. Arthalgia was mentioned in 5 subjects. Neurodevelopmental delay was present in 3 patients and 2 experienced autoimmune cerebral vasculitis.

Almost all patients suffered from infectious disease; upper respiratory tract infections were frequently reported, in particular 5 patients had recurrent otitis media with *Pseudomonas Aeruginosa* detected at specimen. 12 patients suffered from low respiratory tract infections, 4 developed bronchiectasis. 6 had deep infections: *Klebsiella Pneumoniae, MRSA, Candida* were the most frequent microorganisms isolated from cultures. *CMV* was present in blood and stools in 6 patients.

**Laboratory results**

Immunological analysis demonstrated mild alterations (i.e., decreased percentage of TCRα/β cells, low B or T cell number with impaired PHA) in few patients probably as a consequence of previous
immunosuppressive treatment. Hypogammaglobulinemia was found in 9 patients; 2 subjects had very low/absent B cells but no *BTK* gene mutation at gene analysis.

IgE levels were increased in 5 patients. Anti nuclear autoantibodies were detected in 3 patients.

**Treatment and outcome**

Total parenteral nutrition was necessary in one third of patients because of malnutrition due to severe enteropathy; 14 patients were treated with immunosuppressive drugs with transient clinical improvement in some of them. 9 patients received IVIG for both substitutive therapy and ITP treatment. HSCT was performed in 4 patients and they are all doing well.

In this cohort of patients one died at 3 years old because of severe diarrhoea and another one died when he was 18 because of sepsis.

**Molecular Analysis**

The genetic analysis of gene related to IPEX-like syndrome did not show any mutation in this cohort of subjects. *CD25, STAT5b, STAT1* genes were studied in 12, 7 and 9 patients respectively, but all resulted wild-type. One patient resulted heterozygous for a sequence variation of *STAT5b* was found and functional studies have to be performed to investigate the existence of a correlation with the clinical manifestations.
Multiple autoimmunity without diarrhoea

A small number of blood samples came from patients affected by multiple autoimmunity that present at least two autoimmune diseases, except diarrhoea. The age of onset range from 1 to 8 years old. The main symptoms at onset were endocrinopathy and cytopenia. IDDM was present in 4 patients, autoimmune thyroiditis in 2 and primary hypoadrenalism in 2. 4 patients developed ITP, neutropenia and direct Coombs test were reported in 2/7 respectively. Cutaneous involvement included atopic eczema and alopecia in 2 subjects respectively. 3/7 had autoimmune hepatitis and one had idiopathic arthritis. Bowel involvement with celiac disease was found in 2 patients. 3 patients with hypogammaglobulinemia suffered from recurrent respiratory infections; no deep infections were reported. Organ specific autoantibodies were found positive in most of them. The patients are all alive and most of them under IVIG, steroids and immunosuppressive treatment to control symptoms.

Among this group genetic analysis of STAT5b was performed in 2 patients with GH deficiency and consequent failure to thrive; STAT1 was studied in 4 of them. All probands carried wild type sequence. In two patients with hypoadrenalism, associated respectively with nails dystrophy/retention of primary teeth/infections and thyroiditis/cytopenia, APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) was excluded by AIRE analysis.
Several mechanisms are involved in the maintenance of immune tolerance. Treg are fundamental for active immune suppression. Different types of Treg have been described over the past years with different mechanisms of action. FOXP3 is a transcription factor critical for the development and function of CD4+CD25+ Treg. nTreg arise from the thymus and constitutively express FOXP3 and CD25. They repress lymphocyte proliferation through cell contact and do not proliferate themselves [31]. Inducible Treg (iTreg) generate in the periphery from CD4 naïve T cells upon stimulation with IL2 and TGF-beta [26]. They transiently express FOXP3 and exert regulatory function depending on the cytokine milieu present in the periphery after antigen stimulation of dendritic cells.

IPEX syndrome is a X-linked recessive disease caused by mutations within the FOXP3 gene. Early onset of severe diarrhoea, failure to thrive, eczematous rash and IDDM are the hallmarks of this syndrome, but other autoimmune disorders, including autoimmune cytopenias, nephropathy, thyroiditis, can be detected during its natural course.

In this study we have analyzed data from 76 patients affected by complex autoimmunity resembling IPEX syndrome and referred to the NEROFARBA Department, Section of Child’s Health, University of Florence. 69 patients were analyzed for FOXP3 mutations: 16 were found to have molecular aberrations within the gene, therefore affected by IPEX syndrome, the remaining 60 patients have been classified as
IPEX-like. Comparing the two cohorts of patients, we try to identify indicators that may help to differentiate IPEX and IPEX-like diseases and consequently to characterize possible disease sub-groups within the IPEX-like cohort to ultimately narrow the disease spectrum of complex autoimmunity and to eventually suggest new genetic causes.

Since enteropathy is the unique characteristic shared by nearly all IPEX-like patients, we divided IPEX-like patients in two groups based on the age of onset of gut disease. Based on the fact that we recently found two patients with an atypical form of IPEX-syndrome (the former presented bowel disease at 11 years old, the latter had failure to thrive in the first infancy and abdominal pain with mild diarrhoea as a child), we also included in the study another small group of patients characterized by multiple autoimmunity except mention of diarrhoea so far.

Between the first two cohorts of patients, histology from gut biopsies showed results compatible with autoimmune enteropathy in the majority of the subjects with an early onset, mostly complicated by a serious and rapidly worsening disease course. Interestingly diarrhoea was the main symptom at onset in all patients presenting early in life, frequently associated with important failure to thrive. Compared to the life threatening diarrhoea of IPEX syndrome, the course of diarrhoea in this cohort was not always as severe: in some patients symptoms were well controlled by immunosuppressive treatment, others had a mild to moderate diarrhoea without consequence on growth and few patients recover within the first years of life. In these last, we can postulate that
villous atrophy is the result of breaking the balance between microbiota and immune tolerance in immature neonatal gut with consequent increased susceptibility to acute infectious disease. On the other hand, 7 IPEX-like early onset patients required HSCT as unique permanent cure and 6 died due to consequence of intractable diarrhoea.

In the second cohort of subjects symptoms at onset could be various (infections, enteropathy, IDDM). Intestinal bowel disease and common variable immunodeficiency were frequently taken into consideration as possible differential diagnosis because of delayed onset diarrhoea associated with chronic inflammation at gut biopsy in one, and autoimmunity (mainly cytopenia) coupled with hypogammaglobulinemia in the other.

As hallmark signs of IPEX syndrome, half of patients with early onset presents a kind of eczematous rash; autoimmune skin disease (i.e. alopecia, vitiligo) are otherwise prevalent in subjects with a delayed onset in the course of time.

Endocrinopathy seems not essential for identifying IPEX-like syndrome because it is equally present in all the three subgroups of patients.

Differently autoimmune cytopenias seem to be predominant in the FOXP3 wild type cohort. This observation is not surprising since AIHA and ITP are the most common autoimmune manifestations among primary immunodeficiency disorders with autoimmune features in general [32], although in IPEX-like they contribute to define the clinical phenotype and are more frequent when compared to IPEX syndrome.

Laboratory findings in the group of IPEX patients showed increased IgE
level, hypereosinophilia and positive anti-enterocyte antibody, when tested, consistently with the literature. In contrast, in IPEX like subjects IgE were found increased in 14 patients of the early onset group versus only 5 in the late one. Regarding other immunological indices, only few IPEX-like patients had positive organ-specific autoantibody with poor clinical correlation. Mild impairment of lymphocyte count was sometime reported, mostly in relation to heavy immunosuppressive treatment. Accordingly these patients had a significantly increased susceptibility to bacterial and viral infections. In patients with early diarrhoea onset it is reasonably postulated that severe infections could be also a consequence of decreased barrier function of the gut.

In the IPEX-like cohort genetic analysis was addressed to identify mutations in genes actually known to be involved in FOXP3 pathway. Studies were driven by clinical presentation, according to what described in literature.

It is remarkable that affected patients presented symptoms within few months of life. CD25 was found mutated in 3 patients with watery diarrhoea, villous atrophy and eczema started at one months of age with no expression of CD25 at flow cytometry, and a STAT1 mutation was identified in a patient deceased at four years of age for aneurysm rupture [table IIIa, IIIb].

Interestingly, recently we investigated the follow up of IPEX-like patients and we learnt about three new diagnosis of novel primary immunodeficiencies made by the referring centres. Two Pakistani brothers with early onset diarrhoea, recurrent severe respiratory
infections, perianal abscesses, hepatosplenomegaly and impaired response to LPS, were found to have a mutation in a gene that encodes for a key integrator of signaling pathways initiated by stimulation of death receptors, bacterial or viral infections, genotoxic stress and T-cell homeostasis. Further studies are still ongoing to better characterize the gene defect.

Exome sequencing performed in a 6 years old Slovenian girl with mild eczema, diarrhoea with EBV in gut, bacterial sepsis and viral infections, facial dysmorphism and psychomotor delay, showed a mutation in another gene involved in activating nuclear factor-κB (NF-κB) response in antigen-stimulated lymphocytes. NF-κB signaling plays a key role in inflammation and immunity. This mutation was not previously described and the Slovenian group is actually performing functional tests to understand the importance of the sequence mutated.

Finally, lipopolysaccharide responsive beige-like anchor (LRBA) gene mutation was detected by exome sequencing in a Turkish girl with delayed onset severe diarrhoea, autoimmune endocrinopathy and cytopenia. LRBA deficiency has been recently described as a novel immunodeficiency characterized by features of CVID, immune dysregulation, or both manifesting as early onset colitis with chronic diarrhoea and autoimmunity; additional reports and long term follow-up will delineate the full phenotypic spectrum of this disease [33, 34].

Giving the broad clinical spectrum of complex autoimmune diseases, it is intriguing to postulate that other regulatory mechanisms different than CD4+CD25+FOXP3+ regulatory T cells, impaired in IPEX disease,
could contribute in modulating IPEX disease course and determining IPEX-like phenotypes. In particular, a defective interaction within antigen presenting cells and CD4 naive T lymphocytes could cause alteration in T cell commitment and peripheral tolerance and mechanism impairing signaling for apoptosis could lead to autoimmune and autoinflammatory conditions.

In summary this study shows that, among subjects with clinical features of congenital/inherited immune dysregulation where IPEX is ruled out, we could identify three distinct groups. The first one is the “true” IPEX-like group, which comprises patients with severe early onset diarrhoea associated with other autoimmune manifestations (behaving similarly to the IPEX cohort in terms of clinical and immunological features). In those will be mandatory to study FOXP3 signaling pathway and eventually to analyze other genes involved in immune regulation (CD25, STAT5b, STAT1). The second group consists of patients affected by multiple autoimmune diseases: patients with delayed onset of more than 2 autoimmune manifestations. Immunological work up will be essential to exclude a combined primary immunodeficiency or a B cell disease (features often overlap with CVID, LRBA deficiency, ALPS, Omen Syndrome, DGS, etc.) or to contribute to address further genetic analysis. The third one includes subjects with immune-mediated enteropathy with no other symptoms associated, except perianal abscesses or enteric fistula. In these cases the presence of IL10, IL10RA, IL10RB gene mutations should be excluded and gut biopsy will help to explain pathogenesis (Innate immunity? IBD-like?).
In conclusion this preliminary study highlights the importance of severe autoimmune enteropathy as key clinical marker of immunedysregulation.

In addition, genetic analysis for *FOXP3* mutations should always be performed in male patients with symptoms indicative of IPEX syndrome, and in case of normal *FOXP3*, the other genes involved in FOXP3 function (i.e. *CD25* *STAT5b*) and, in selected patients, *STAT1*, *IL10*, *IL10RA-RB*, should also be considered if the phenotype is suggestive. Flow cytometry could be essential to address analysis of *CD25* when the expression is absent [Fig.3].

The final objectives would be definition of new disease entities and ultimately discovery of new genes involved in immune-dysregulation diseases, and exome sequencing will be of help in the near future.
TABLE I: Molecular analysis. Among 113 patients analysed, only 76 had clinical presentations and laboratory results consistent with IPEX Syndrome.
<table>
<thead>
<tr>
<th>Patient n.</th>
<th>Genetic studies</th>
<th>Age of onset</th>
<th>Symptoms at onset</th>
<th>Enteropathy (age of onset)</th>
<th>Skin disease</th>
<th>Endocrinopathy</th>
<th>Autoimmune Cytopenia</th>
<th>Others</th>
<th>Infections</th>
<th>Laboratory results</th>
<th>Gut biopsy</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.1040G&gt;A; p.R347H</td>
<td>Neonatal</td>
<td>IDDM and diarrhea</td>
<td>Severe diarrhea</td>
<td>Neonatal IDDM</td>
<td>FTT</td>
<td>Hepatopathy</td>
<td></td>
<td></td>
<td>Increased [g/l] (7320 KU/L)</td>
<td>aETC</td>
<td>Villous atrophy</td>
<td>TPN IS Insulin</td>
</tr>
<tr>
<td>2</td>
<td>c.816+5G&gt;A</td>
<td>Neonatal</td>
<td>IDDM</td>
<td>Severe watery diarrhea</td>
<td>Mild eczema</td>
<td>Neonatal IDDM</td>
<td>FTT</td>
<td></td>
<td></td>
<td>Villous atrophy</td>
<td></td>
<td>TPN Insulin</td>
<td>Died at 9 months for diarrhea</td>
</tr>
<tr>
<td>3</td>
<td>c.1007T&gt;C; p.L340T</td>
<td>2 weeks</td>
<td>Diarrhea</td>
<td>Watery diarrhea</td>
<td>Eczema</td>
<td>Neonatal IDDM</td>
<td>FTT</td>
<td>Neptiritis Syndrome Myopathy</td>
<td>Sepsis, osteomyelitis</td>
<td>Increased [g/l] (1278 KU/L)</td>
<td>Villous atrophy</td>
<td>HSCT</td>
<td>Alive</td>
</tr>
<tr>
<td>4</td>
<td>c.1157G&gt;A; p.M370I</td>
<td>1 week</td>
<td>Diarrhea</td>
<td>Severe diarrhea</td>
<td>Severe eczema</td>
<td>AIHA</td>
<td>FTT</td>
<td>Sepsis, Pneumonia</td>
<td></td>
<td>Increased [g/l] (2000 KU/L)</td>
<td>Villous atrophy</td>
<td>HSCT</td>
<td>Alive</td>
</tr>
<tr>
<td>5</td>
<td>c.1100G&gt;A; p.H370I</td>
<td>Neonatal</td>
<td>Diarrhea</td>
<td>Severe diarrhea</td>
<td>Mild eczema</td>
<td>AIHA, ITP, neutropenia</td>
<td></td>
<td></td>
<td></td>
<td>Villous atrophy</td>
<td></td>
<td>HSCT</td>
<td>Died post-HSCT</td>
</tr>
<tr>
<td>6</td>
<td>c.816+3G&gt;C</td>
<td>1 week</td>
<td>Diarrhea</td>
<td>Severe diarrhea</td>
<td>Eczema</td>
<td></td>
<td></td>
<td></td>
<td>FTT, hepatitis/oemegaly, lymphaedopathy</td>
<td>Sepsis, bronchiolitis</td>
<td>Increased [g/l] (1202 KU/L)</td>
<td>Villous atrophy</td>
<td>HSCT</td>
</tr>
<tr>
<td>7</td>
<td>c.759T&gt;C; p.L253P</td>
<td>2 months</td>
<td>Diarrhea</td>
<td>Watery diarrhea</td>
<td>Severe eczema</td>
<td></td>
<td></td>
<td></td>
<td>FTT, hepatitis/oemegaly, lymphaedopathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>c.979T&gt;C; c.5430C&gt;T; c.970-102C&gt;T</td>
<td>No symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>c.979T&gt;C; p.F324S</td>
<td>3 weeks</td>
<td>Diarrhea</td>
<td>Moderate to severe diarrhea</td>
<td>Moderate eczema</td>
<td></td>
<td></td>
<td></td>
<td>Acute tubular necrosis</td>
<td></td>
<td>Increased [g/l] (175 KU/L)</td>
<td></td>
<td>Alive</td>
</tr>
<tr>
<td>10</td>
<td>c.816+2delGT</td>
<td>5 months</td>
<td>Diarrhea</td>
<td>Severe diarrhea</td>
<td>Eczema</td>
<td>ITP</td>
<td>FTT</td>
<td>Renal carcinoma</td>
<td>Bronchitis, osteomyelitis</td>
<td></td>
<td>Villous atrophy</td>
<td>TPN IS</td>
<td>Alive</td>
</tr>
<tr>
<td>11</td>
<td>c.1150G&gt;A; p.A384T</td>
<td>1 month</td>
<td>Eczema</td>
<td>Severe diarrhea</td>
<td>Severe eczema, Aleupenia</td>
<td></td>
<td></td>
<td></td>
<td>FTT, Hepatitis/oesomegaly lymphaedopathy</td>
<td>Sepsis, recurrent respiratory infections</td>
<td>Increased [g/l] (1450 KU/L)</td>
<td>Villous atrophy</td>
<td>TPN</td>
</tr>
<tr>
<td>12</td>
<td>c.210+1G&gt;C</td>
<td>5 months</td>
<td>FTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FTT, Cow’s milk allergy, GH deficiency, Nephrocalcinosis and proteinuria</td>
<td>recurrent respiratory infections</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>c.1040G&gt;A; p.R347H</td>
<td>11 years old</td>
<td>Diarrhea</td>
<td>Severe diarrhea</td>
<td>Eczema</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Increased [g/l] (2070 KU/L)</td>
<td>aETC</td>
<td></td>
<td>Ulcerative gastroesclitis</td>
</tr>
<tr>
<td>14</td>
<td>c.1157G&gt;A; p.R380H</td>
<td>1 month</td>
<td>Diarrhea</td>
<td>Severe diarrhea</td>
<td>Moderate eczema</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Villous atrophy</td>
</tr>
<tr>
<td>15</td>
<td>c.571_755delGAG; p.E251del</td>
<td>No clinical data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>c.571_755delGAG; p.E251del</td>
<td>No clinical data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Diabetes** insulin dependent diabetes mellitus; **AIHA**, autoimmune haemolytic anaemia; **ITP**, idiopathic thrombocytopenic purpura; **FTT**, failure to thrive; **aETC**, anti-enterocyte antibody; **TPN**, total parenteral nutrition; **IS**, immunosuppression; **HSCT**, hematopoietic stem cell transplantation
Fig. II. Clinical manifestations of IPEX-like early onset (31 patients) versus IPEX-like late onset (22 patients).

FTT, failure to thrive; IDDM, Insulin-Dependent Diabetes Mellitus; AIHA, autoimmune haemolytic anaemia; ITP, immune thrombocytopenia; NP, neutropenia; URTI, upper respiratory tract infections; LRTI, lower respiratory tract infections; AIE, autoimmune hepatitis; ANA, antinuclear autoantibody; SMA smooth muscle autoantibody; aETC, anti-enterocyte antibody.
TABLE IIIa. Comparison between clinical characteristics of IPEX-like patients with CD25 deficiency reported in literature. Our group of research identified the CD25 gene mutation in pt 3.

<table>
<thead>
<tr>
<th></th>
<th>Pt 1 (Sharfe N et al. [13])</th>
<th>Pt 2 (Caudy et al. [14])</th>
<th>Pt 3 (OUR PATIENT) (Goudy K et al. [16])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial presentation</td>
<td>Infancy</td>
<td>Infancy</td>
<td>Infancy</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Outcome</td>
<td>Alive</td>
<td>Alive</td>
<td>Alive</td>
</tr>
<tr>
<td>Enteropathy</td>
<td>Chronic diarrhoea</td>
<td>Severe diarrhoea (protein losing enteropathy) Gut biopsy: villous atrophy, CMV pos</td>
<td>Severe diarrhoea Gut biopsy: villous atrophy, CMV pos</td>
</tr>
<tr>
<td>Skin</td>
<td>None</td>
<td>Diffuse eczema</td>
<td>Diffuse eczema Bollous pemphigoid Alopecia universalis</td>
</tr>
<tr>
<td>Infections</td>
<td>Bacterial infections</td>
<td>Persistent sinusitis</td>
<td>Cellulitis caused by Staphilococcus Au. Pseudomonas Ae</td>
</tr>
<tr>
<td></td>
<td>Persistent oral thrush</td>
<td>Recurrent otitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Candida esophagitis</td>
<td>Recurrent pneumonias</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chronic lung disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herpes Infections</td>
<td>CMV pneumonitis</td>
<td>CMV in gut/blood</td>
<td>CMV in gut/blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BBV in lymphonode</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CMV pneumonitis</td>
<td></td>
</tr>
<tr>
<td>Endocrine</td>
<td>None</td>
<td>Hypothiroidism</td>
<td>Hypothiroidism</td>
</tr>
<tr>
<td>Growth</td>
<td>Failure to thrive</td>
<td>Failure to thrive</td>
<td>Failure to thrive</td>
</tr>
<tr>
<td>Hematology</td>
<td>Iron deficiency anemia</td>
<td>AIHA</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neutropenia</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>Lymphadenopathy</td>
<td>Lymphadenopathy</td>
<td>Lymphadenopathy</td>
</tr>
<tr>
<td></td>
<td>hepatosplenomegaly</td>
<td>hepatosplenomegaly</td>
<td></td>
</tr>
<tr>
<td>Laboratory Results</td>
<td>Increased IgG levels</td>
<td>Increased IgG levels</td>
<td>Increased IgE</td>
</tr>
<tr>
<td></td>
<td>Low IgA</td>
<td>Absent CD25 expression</td>
<td>Absent CD25 expression</td>
</tr>
<tr>
<td></td>
<td>Low CD3+CD4+ cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>Bone marrow transplant</td>
<td>IVIG Immunosuppressive</td>
<td>Bone marrow transplant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>drugs</td>
<td></td>
</tr>
</tbody>
</table>
TABLE IIIb. Comparison between clinical characteristic of IPEX-like patients with STAT1 mutation described by Uzel G et al [28] and the patient of our cohort affected by the same disease.

<table>
<thead>
<tr>
<th></th>
<th>Pt 1</th>
<th>Pt 2</th>
<th>Pt 3</th>
<th>Pt 4</th>
<th>Pt 5</th>
<th>OUR PATIENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial presentation</td>
<td>Infancy</td>
<td>Infancy</td>
<td>Infancy</td>
<td>Infancy</td>
<td>Toddler</td>
<td>Infant</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Deceased</td>
<td>Yes (Invasive Fungemia)</td>
<td>Yes (Intracranial bleed)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes (Intracranial bleed)</td>
</tr>
<tr>
<td>LML</td>
<td>Ural, esophageal, fingernail; Candida parapsilosis candidemia (catheter related)</td>
<td>Limited oral mucosal</td>
<td>Ural, esophageal, fingernail</td>
<td>None</td>
<td>Limited oral mucosal</td>
<td>Cutaneous candidiasis</td>
</tr>
<tr>
<td>Skin</td>
<td>Generalized eczema with hyperkeratosis</td>
<td>Mild eczema</td>
<td>Mild eczema</td>
<td>Severe atopic dermatitis</td>
<td>Mild-to-moderate eczema</td>
<td>Atopic eczema</td>
</tr>
<tr>
<td>Synopulmonary Infections</td>
<td>Encapsulated bacteria; RSV bronchiolitis; bronchiectasis with Mycobacterium avium; Aspergillus pneumonia</td>
<td>Encapsulated bacteria; RSV bronchiolitis; bronchiectasis</td>
<td>Encapsulated bacteria; RSV bronchiolitis; bronchiectasis</td>
<td>Recurrent pneumonia; otitis, sinusitis, external otitis</td>
<td>Recurrent group otitis</td>
<td>Recurrent respiratory infections</td>
</tr>
<tr>
<td>Deep infections</td>
<td>MRSA; disseminated aspergillosis, with immunosuppression Candida parapsilosis candidemia</td>
<td>MRSA</td>
<td>None</td>
<td>None</td>
<td>Enterococcus species (catheter related)</td>
<td>None</td>
</tr>
<tr>
<td>Herpes Infections</td>
<td>HSV stomatitis</td>
<td>HSV skin infection</td>
<td>Herpetic zoster twice, CNS V &amp; V</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>CNS</td>
<td>Normal vasculature by MRI</td>
<td>Cerebral artery aneurysms, hemiparesis and secondary hydrocephalus</td>
<td>Cerebral artery aneurysms, CVAs</td>
<td>None</td>
<td>None</td>
<td>Cerebral artery aneurysm</td>
</tr>
<tr>
<td>Endocrine</td>
<td>IIUM, anti-thyroid antibody</td>
<td>IIUM, Hypothyroidism</td>
<td>IIUM</td>
<td>None</td>
<td>Hypothyroidism, GH insufficiency</td>
<td>Hypothyroidism</td>
</tr>
<tr>
<td>Growth</td>
<td>Short stature, Delayed puberty</td>
<td>Short stature</td>
<td>Short stature, Delayed puberty</td>
<td>None</td>
<td>Short stature, Delayed puberty</td>
<td>Short stature</td>
</tr>
<tr>
<td>Gastroenterology</td>
<td>Hepatosplenomegaly, Esophageal dysmotility, Protein losing enteropathy, Villous blunting</td>
<td>Hepatosplenomegaly, Lymphocytic and eosinophilic enteritis, Villous blunting, Recurrent intussusception</td>
<td>Hepatosplenomegaly</td>
<td>Protein losing enteropathy, Lymphocytic and eosinophilic enteritis</td>
<td>Chronic gastritis, celiac lymphocytic enteritis, Villous atrophy, Hepatosplenomegaly, Frequent episodes of watery diarrhea, Villous atrophy</td>
<td></td>
</tr>
<tr>
<td>Hematology</td>
<td>ITP, AIHA</td>
<td>None</td>
<td>None</td>
<td>Neutropenia</td>
<td>None</td>
<td>ITP, AIHA, neutropenia</td>
</tr>
<tr>
<td>Bone</td>
<td>Generalised osteopenia and osteoporosis</td>
<td>Generalised osteopenia and osteoporosis, Multiple bone fractures</td>
<td>Generalised osteopenia</td>
<td>Spina bifida occulta</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Laboratory Results</td>
<td>Low CD3+CD4+ cells, Low class switched memory B cells, Poor vaccine response</td>
<td>Low class switched memory B cells, Poor vaccine response</td>
<td>Low CD3+CD4+ cells, Low class switched memory B cells, Poor vaccine response</td>
<td>None</td>
<td>Low CD3+CD4+ cells, Poor vaccine response</td>
<td>None</td>
</tr>
</tbody>
</table>
Fig. 3. Diagnostic flow chart for IPEX/IPEX-like syndromes

Clinical features suggestive for IPEX

- Enteropathy +/-
- ≥ 1 AI manifestation

Male with normal/low FOXP3 expression

flow cytometry analysis of CD4+CD25+127low cells

Absent CD25 expression

CD25 gene analysis

FOXP3 gene analysis

FOXP3 wild-type or Female

Reconsider clinical presentation

STAT5b Gene analysis
- Low IGF1, normal GH
- Chronic lymphocytic interstitial pneumonitis
- Lymphopenia (low NK & T cells)
- Eczema
- Diarrhoea
- +/- AI disorders

STAT1 Gene analysis
- Chronic mucocutaneous Candidiasis
- Eczema
- Bacterial/viral infections
- Cerebral aneurysm
- Lymphopenia
- Hypogammaglobulinemia
- +/- AI disorders

IL10, IL10RA, IL10RB Gene analysis
- Severe early onset diarrhoea
- Perianal abscesses
- Enteric fistula

Other diseases?
LRBA, Omenn syndrome, WAS, DOCK8, STAT3, …
REFERENCES


