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Review Article

Common Variable Immunodeficiency (CVID) and LRBA Mutation: Different Clinical Phenotypes and Effects of Hematopoietic Stem Cell Transplantation (HSCT) in Three Children

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Abstract

LPS responsive beige-like anchor protein (LRBA) deficiency is leading to autoimmune clinical symptoms. Within clinical symptoms of LRBA deficiency are lymphoproliferation, interstitial lung disease and organomegaly suggesting ALPS or hypogammaglobulinemia. Autoimmunity and hypogammaglobulinemia leading to infections are diagnosed as common variably immunodeficiency (CVID). The course of disease is different from mild to severe life-threatening. The early onset of chronic refractory diarrhea (VEO-IBD often precedes hypogammaglobulinemia and diagnosis of CVID. LRBA deficiency was showed in 3 our patients with final diagnosis of CVID. Two of them (siblings) demonstrated early onset diarrhea at the beginning, hypogammaglobulinemia, juvenile rheumatoid arthritis and severe nervous involvement (flaccid tetraparesis) in course of disease. The third patient was initially diagnosed as ALPS due to lymphoproliferation, splenomegaly, thrombocytopenia and hypogammaglobulinemia. Therapy included immunoglobulins in regular substitution, immunosuppression according clinical symptoms with haematopoietic stem cells transplantation (HSCT) as final treatment after diagnosis of LRBA deficiency. However, despite of immune system reconstitution, there were no complete resolving of clinical symptoms. The course of CVID with LRBA in our patients support indications for early introducing of genetic study including LRBA deficiency suggesting more adequate therapy of these patients.

Keywords : LRBA mutation; CVID; juvenile arthritis; chronic diarrhea; Crohn-like disease; hemolytic anemia; thrombocytopenia; tetraparesis; HSCT

Abbreviations

ASD - atopic skin disease
CVID - common variable immunodeficiency
CTLA-4 – cytotoxic T lymphocyte antigen-4
GvHD – graft versus host diseases
GLILD - granulomatous lymphocytic interstitial lung disease
LRBA - LPS responsive beige-like anchor protein
HSCT - haematopoietic stem cells transplantation
HA - hyperalimentation

IVIG - intravenous immunoglobulins
JRA – juvenile rheumatoid arthritis
LIP - Lymphocytic interstitial pneumonia
MDS – myelodysplastic syndrome
MTX - methotrexate
MRD - matched related donor
MUD - matched unrelated donor
6MP - 6-mercaptopurine
MMF – mycofenolate mofetile
NSAIDS - non-steroid anti pain drugs
SCIG - subcutaneous immunoglobulins
TPN - total parietal nutrition
VEO-IBD – very early onset of inflammatory bowel disease

Introduction

Common variable immunodeficiency (CVID) characterises variety of clinical phenotypes from mild form consisting hypogammaglobulinemia and recurrent infections with poor response to therapy associated with low production of specific antibodies to vaccines up to serious symptoms of autoimmunity including haemolytic anaemia, thrombocytopenia, organomegaly and lymphadenopathy with severe clinical course [1]. The study of possible genetic background of CVID showed different disorders with mutation in LPS responsive beige-like anchor protein (LRBA) described first time in 2012 year as one of them in patients with severe autoimmunity in CVID. Majority of LRBA deficiency is discovered within CVID patients, however, there are patients with LRBA deficiency and typical clinical symptoms without immunodeficiency. Gen *LRBA* is located in 4q31.3 position with 57 exons coding LRBA cytosolic protein (2851 amino acids residues) involved in protection of CTLA-4 protein present in T lymphocytes from lysosomal degradation. LRBA is playing regulating of CTLA-4 trafficking in T regulatory lymphocytes. Patients with LRBA deficiency showed deeply reduced CTLA-4 level due to rapid degradation in lysosomes [2-5]. LRBA is widely expressed within tissue, however, immune cells need activation to show expression of LRBA protein. In patients with LRBA deficiency, the number and function of T regulatory lymphocytes (Treg), class switched B memory cells and plasmablasts are reduced. Moreover, responsible for CVID with deficiency of antibodies production may be combination of disturbed differentiation and maturation of B lymphocytes including memory B cells, low number of plasma blasts and plasmocytes. In LRBA deficiency, Treg in phenotype show, the markedly decreased expression of FOXP3, CD25, CTLA-4, what resembles aberrant and reduced function of Treg cells. Moreover, in peripheral blood, the number of circulating follicular Treg is lower than in healthy control. The autophagy defects in LRBA patients lead to perturbation of mucosal homeostasis and autophagosomal processing of intracellular bacterias within mucosal cells, what might be relate to gastrointestinal syndromes e.g. chronic diarrhea in small children, colitis and Crohn-like disease in older ones, as one of pathomechanisms [2]. The reduced autophagy process is involved in B cell differentiation and increased apoptosis described in LRBA deficiency may be a possible mechanism. The disturbances in differentiation of B cells were shown in culture of B cells from LRBA patients with anti CD40/IL-4 and CD40/IL-21 stimulation [2]. Autophagy is required for the formation and maintenance of long-lived plasma cells pool in periphery. The higher apoptosis is associated and is leading to decrease of memory B cells and plasmablasts number. These deregulations within immune system supported the role of LRBA in autophagy/apoptosis process [2,6]. The observed combination of low number of Treg, increased autoimmune clones within mature naive B cells and increased number of CD21 low B cells found in LRBA deficient patients are considered as mechanisms of dominating autoimmunity within clinical symptoms in LRBA deficiency [4-7].

The patients with CVID and LRBA deficiency demonstrate in clinical phenotypes, recurrent infections with prolonged course and weak response to therapy, low level of specific antibodies after vaccines and hypogammaglobulinemia. In LRBA deficiency penetration of gene is high, almost full, however, the clinical phenotypes are different with severe course and fatal outcome in part of patients. Typical clinical symptom in majority of patients, are chronic diarrhea and enteropathy resistant to aggressive therapy, including prolonged systemic steroids and immunosuppression, noted as early as first year of life. In part of patients, diarrhea, thrombocytopenia are preceding hypogammaglobulinemia and diagnosis of immune deficiency with LRBA gene mutation [1,2,8]. Within group of 7 patients with LRBA - 3 of them died at age 4, 14 and 16 years of life from the uncontrolled enteropathy, colitis and complication of immunosuppressive therapy. However, within these patients were siblings (boy and girl) diagnosed as LRBA deficiency. Boy showed severe clinical course with enteropathy, thrombocytopenia, organomegaly and recurrent infections. Girl was without any clinical symptoms during time of observation [8]. In cohort of 31 LRBA deficiency patients majority (19 patients) presented severe autoimmunity, hypogammaglobulinemia, organomegaly, respiratory infections and chronic diarrhea. Within this cohort in 7 patients autoimmunity was associated with joints, similar number of patients showed neurological symptoms. These last included 1 case of myasthenia, 2 patients with cerebral granulomas, 2 patients with atrophy of optic nerve [2].

The most common autoimmune symptoms are hematologic (haemolytic anaemia, thrombocytopenia, leukopenia), however, diabetes, rheumatoid arthritis, Crohn-like colitis and interstitial lung disease (LIP, GLILD) are described [1-4,8,9].

Therapy and HSCT

Hypogammaglobulinemia typical for CVID is corrected with immunoglobulins infused intravenously or, more frequent, subcutaneously as home therapy comfortable for patients. Substitution of immunoglobulins usually is continued from time of CVID diagnosis up to time of HSCT procedure suggested as therapy for LRBA deficiency. Concomitant infections required prolonged and wide spectral antibiotics, antimycotic therapy. More difficult are autoimmune phenomena and diseases based of these mechanisms. The basic and typical therapeutic approach is systemic use of steroids as systemic usually for long time. In haematological symptoms like haemolytic anaemia, thrombocytopenia steroids are used in high dose intravenously 3-5 consecutive days followed with lower dose administered orally. In case of steroid intolerance or severe side effects of such therapy, the classical immunosuppression is introduced. For jejunal autoimmunity (colitis, Crohn disease, Crohn- like chronic inflammation, enteropathy) cyclosporine A, anti-TNF monoclonal antibodies and classic immunosuppression e.g. azathioprine, sulfosalazine are ordered in different schedules for individualised (“patient tailored”) therapy [1].

As supplementary, the modified diets, vitamins, probiotics, albumins, and other preparations are used to support gain of weight and prevent the effects of malabsorption and symptoms of malnutrition. The clinical condition of patient, elimination of acute and chronic infections are important factors for prognosis of HSCT procedure effects. As routine for HSCT procedure, the first step included searching for matched family donor (related e.g. siblings) with the exclusion of carriers the genetic mutation without clinical symptoms. In majority of patients, the unrelated donor is used for HSCT due to lack of related donor. The protocols of conditioning before HSCT are different, often based on busulfan, fludarabine, ATG. After HSCT in LRBA patients the course of immune system reconstitution, possible complication as GvHD are similar to post-transplantation period in other patients treated with HSCT procedures [2,8-11].

The alternative therapy, is based on regular substitution CTLA-4-Fc fusion IgG1 immunoglobulin (abatacept) in patients with CTLA-4 and LRBA deficiency. The mechanism of this monoclonal antibody is based on replacement of CTLA-4 and restore inhibitory function of T cell activation. The effects of abatacept used of this therapy in CTLA-4 and LRBA deficiency with rheumatoid arthritis symptoms were matter of time. Abatacept therapy in a cohort of 22 patients diagnosed as LRBA deficiency, showed in 16 of them, completely controlled the chronic diarrhea, lymphoproliferation, improvement of autoimmune cytopenias as well. The most effective abatacept schedule was every or every other week administration regularly. There were no serious side effects. However, within this group 2 patient with severe course of disease were relatively resistant to abatacept therapy [4,6,12].

In our group of children diagnosed as CVID with autoimmunity in three of them showing severe course of disease the deficiency of LRBA was diagnosed. The clinical profile of symptoms was different, even between siblings with similar LRBA deficiency and CVID.

Case Presentation

Patient 1

A girl was born as healthy full term baby. She developed chronic diarrhea from 3rd months of life. Modifications of diet and elimination of milk protein and enzyme did not improve clinical course of diarrhea. Prolonged steroid therapy slowly resolved symptoms. In biopsy of duodenum and in endoscopy, the small granular lymphoproliferation and prolonged gastritis with ulcerations were noted. During two first years of life therapy was based on immunosuppression, steroids, eliminating diets, TPN. These symptoms were suggesting very early onset inflammatory bowel disease (VEO-IBD), however, 20 years ago this diagnosis was not commonly used [11]. In immunological tests - antibodies typical for celiac disease were absent, immunoglobulins, number of T, B lymphocytes and NK cells were within normal value for age. In following years symptoms of Crohn-like disease and gastritis were controlled with continued therapy, however, in endoscopy inflammation with granular lymphoproliferation proved with histology were present. In 6 year of life, the episode of thrombocytopenia associated with purulent paradental and gingival inflammation was cured with high dose of immunoglobulins. Two years later exacerbations of Crohn-like disease was severe with symptoms of malabsorption and malnutrition. In therapy immunosuppression (6-MP) and budesonide were again introduced. In immunological test decreasing level of IgG with low number of B memory cells were noted. Diagnosis of CVID with autoimmunity was established followed with regular immunoglobulins substitution. The following two years showed decreasing number of B lymphocytes and NK cells, Crohn-like symptoms treated with immunosuppression modified due to leukopenia as severe side effect. Slowly remission of gastrointestinal symptoms was obtained slowly. Immunoglobulins substitution stabilised IgG level within normal value, however, number of B lymphocytes and NK cells gradually decreased. The flaccid paralysis started without infections symptoms from ptosis of right eyelid without improvement after steroid and antibiotics therapy. In next week general feeling of weakness was claimed by patients and lack of control of legs including stable standing and walking were noted. In differential diagnosis - Guillain-Barre syndrome, viral or bacterial encephalitis, demyelinating process were excluded based on cerebrospinal fluid assay and neurological diagnosis of severe axonal-sensitive polyneuropathy was suggested. Aggressive therapy with high dose steroids, high dose immunoglobulins, plasmapheresis procedures were without effect. This introduced therapy is a standard for Guillain-Barre syndrome, in this child thought as atypical due to with immunodeficiency, nor fulfilling the typical criteria. Problems with swallowing leading to pneumonias suggested bulbar involvement in paretic process and force to PEG surgery. The neoplastic lymphoproliferation was excluded base on lymph node biopsy. MRI showed enlargement of spleen and liver. In 2020 year, genetic study showed LRBA mutation. Symptoms of paralysis were without improvement despite of therapy and rehabilitation. The massive muscles atrophy and contractures progressed. After parents agreement for HSCT procedures, family study of MHC determinant showed matched sister as potential related hematopoietic stem cells donor. The HSCT procedure with MRD was performed in our patient in 13 years of age.

HSCT procedure and follow up.

Conditioning was based on TreoFluTT protocol (treosulfan, fludarabine and thioTEPA) during 7 days before transplantation. Prophylaxis of GvHD included: Cyclosporine A, MMF, alemtuzumab, steroids. Post-transplantation period was without serious infections with slow reconstitution of haematopoiesis, slow improvement of motoric function specially upper extremities, inhibition of muscles atrophy. Therapy included antibiotics, red blood cells and platelets transfusions, immunoglobulins infusions according to standard procedures after HSCT, modified and adjusted to patients symptoms. After 3 months at home, she suddenly developed symptoms of gastrointestinal massive bleeding with pain. In laboratory tests antinuclear antibodies were present. Gastroscopy was without source of bleeding in stomach. The episodes of life-threatening massive bleeding with clinical symptoms of shock were noted two times within month. Colonoscopy showed multiple regions with mucous bleeding in colon and jejunum. The life-saving therapy included red blood cells, platelets and plasma transfusions, inhibitors of proton's pump, antibiotics, steroids. The possible effect of autoimmunity, infection with CMV and Klebsiella pneumoniae, Enterococcus faecium VRE, Candida parapsilosis before HSCT and GvHD were considered as cause of gastrointestinal bleeding. In following months improvement of motor function was noted, however, she still demonstrates paraplexis of legs. Gastric tube and central catheter were removed. Immunosuppressive therapy was stopped. The reconstitution of haematological and immune system is protecting her from infections. She is under Transplantology Outpatient follow up examined every 6 months.

Patient 2

This patient is a brother of patient 1. His history started in first year of life with chronic bleeding diarrhea, resulting underweight, malnutrition and symptoms of atopic skin disease. Therapy with steroids, modify diet was helpful but diarrhea did not resolve completely. In next year exacerbation of diarrhea, food allergy resulted with protein loosing enteropathy, malnutrition, inhibition of growth, general oedema, ascites. In therapy albumin infusions, steroids, inhibitors of protons pump, TPN with central catheter were used. The improvement was obtained, however, in next year exacerbation of diarrhea was like before despite of prolonged steroid and modify diet in therapy. Protein loosing enteropathy, oedema, malnutrition were again treated with high dose steroids. After two years of steroid therapy serious side effects were present including osteoporosis, Cushing's symptoms in 4 years old boy. The immunological assay showed IgG level below normal value for age with IgA and IgM within normal value. Steroids were reduced and azathioprine was introduced with established diagnosis of Crohn's disease. The following assay showed immunoglobulins level within normal value for age, proportions of lymphocytes T subpopulations, numbers of T lymphocytes, activated T lymphocytes and B lymphocytes were within normal value. Therapy with azathioprine and steroids improved clinical state of patient and in following months immunosuppression was suspended in summer. However, in late autumn the exacerbation of Crohn's symptoms was noted with protein loosing enteropathy, diarrhea and, for the first time, swollen joints (hip, knee, ankles and elbow). The juvenile rheumatoid arthritis (JRA) was diagnosed as co-existent symptoms to Crohn's disease. Therapy was switched to methotrexate (MTX), non-steroid anti-inflammatory drugs (NSAIDS) and low dose of steroids as therapy for

JRA in patient with other chronic disease (patient-tailored therapy). In next year symptoms JRA were present without remission despite of therapy. pancreatic enzymes and modified of abdomen enlargement of lymph nodes, infiltrations of jejunum walls ("thicker") was seen. Laboratory tests showed increase of liver enzymes level, high amount of calprotectin in stool, hypogammaglobulinemia (IgG, IgM), decrease of B cells number. From this time diagnosis of CVID was established (8 years and 4 months of age) with substitution of immunoglobulins in consequence. After LRBA mutation showed in sister, the genetic study was undertaken with results indicating present LRBA mutation. The immunosuppressive therapy (MTX, sulfasalazine, steroids) was continued, however, symptoms of JRA were persistent without remission. Severe clinical course of JRA, Crohn's disease lead to underweight, inhibition of growth and poor general condition of patient. Moreover, the immunoglobulins used in dose 0.8-1.0 g/kg b.w. resulted in IgG level close to lower limit or even below limit for age, the number of T and B lymphocytes were below normal value. The HSCT procedure was considered and accepted, so the stem cells transplantation was undertaken for patient 9.5 years old. Conditioning included busulfan, fludarabine and campath (BuxFlu Campath) followed with transplantation of hematopoietic stem cells from matched unrelated donor (MUD). The follow-up after HSCT procedure was uneventful with supplementation of steroids (due low level of cortisol), immunoglobulins and GM-CSF. Prophylaxis of GvHD was based on cyclosporine and MMF with good toleration. He was discharged home after 34 days post HSCT with regular checking in Transplant ology Outpatient. One year and half patient claimed pain of joints (knee, ankles and fingers). The reactivation of JRA was diagnosed with cyclosporine and steroids as therapy, however, without improvement, so etanercept was introduced as second line of therapy. The examination of patient showed osteoporosis, enlargement of spleen, changes of jejunum like in food intolerance, swelling joints with pain, limitation of movement and contractions. Therapy with etanercept released joints symptoms, however, shortly after ceased the etanercept therapy left knee was painful and swollen again. Currently, patient is on continued etanercept therapy, steroids due to secondary hypocortisolaemia, antibiotics and antiviral prophylaxis. He is under multi-specialists care – rheumatology, endocrinology, transplantology, immunology and gastrology.

Patient 3

The leukopenia and thrombocytopenia with haemorrhagic diathesis at age 3 years 8 months treated with steroids and immunoglobulins intravenously with good response was the first symptom of disease. In laboratory fresh infection with common viruses were excluded, immunoglobulins were within normal value for age. The next episode of thrombocytopenia was 4 months later. In laboratory tests immunoglobulins were within normal value, bone marrow aspiration showed normal haematopoiesis without lympho- or myelo-proliferation. In assay of lymphocytes in peripheral blood small number of double negative T cells were noted. Together with autoimmunity, enlargement of lymph nodes, spleen and liver the clinical initial diagnosis of ALPS was suggested. The results of laboratory tests showed normal expression of Fas and FasL, low percentage of double negative TCR α/β T cells, what did not support this diagnosis. In this time HRCT of lungs showed changes indicating possibility of LIP/GLILD. Therapy with steroids and MMF was effective and lungs infiltrations resolved (13). The next episode of thrombocytopenia and haemolytic anaemia were half of year later treated successfully with steroids, immunoglobulins and MMF. The laboratory tests showed hypogammaglobulinemia. Based on this, the diagnosis of common variable immunodeficiency (CVID) with autoimmunity was suggested with regular substitution of immunoglobulins beginning with intravenous form followed with subcutaneous form. In next year, despite of regular substitution of immunoglobulins (0.5-0.7g/kg b.w.) episodes of thrombocytopenia were noted, in clinical features enlargement of spleen was present. In therapy MMF was changed to rapamycine. In 5 years old patient, the results of genetic study showed LRBA mutation followed with consideration of HSCT as curative procedure. Up to the time of HSCT procedure with unrelated donor (MUD), patient was treated with sirolimus, steroids (in episodes of thrombocytopenia exacerbations) and regular immunoglobulins infusions. In USG of abdomen enlargement of spleen (megaspleen), liver, lymph nodes were persistently present. Two months before planned HSCT procedure patient showed severe diarrhea without detectable pathogen (viral, bacterial or mycotic) in stool. Basic therapy (steroids, MMF (instead of rapamycine), immunoglobulins) was continued with mesalazine, hepatoprotection, albumin, pancreatic enzymes and modified diet due to diarrhea. Within month diarrhea slowly improved but did not resolve completely. Patient was transferred to HSCT procedure with central catheter, splenomegaly, lymphadenopathy, chronic diarrhea, dispersed small infiltrations ("regions of consolidations") in lungs. In laboratory tests the blood parameters, immunoglobulins level, lymphocytes subpopulations and numbers, liver, thyroid and thrombotic parameters were within normal value. The conditioning before HSCT was based on Treo Flu TT schedule followed with unrelated donor's bone marrow transplantation. The reconstitution of neutrophils, platelets (>20000/ul) was noted between 23 to 26 days after HSCT with Cyclosporine A and steroids as GvHD prophylaxis. The next serious problem was the reactivation of EBV infection. To prevent possible induction of lymphoproliferation of infected B cells, rituximab was added into therapy for the elimination of B lymphocytes. EBV infection was under control, however, hypogammaglobulinemia was most serious side effect of rituximab therapy. In this period of therapy infusion of immunoglobulins were sporadic according clinical symptoms. However, following assay showed low level of immunoglobulins without tendency to increase and normalisation, what indicated requirement for regular substitution of immunoglobulin. Now, patient is in good condition without symptoms of disease, on regular substitution of immunoglobulins. The patient is under Transplantation Outpatient for follow up.

Data	Patients 1 R.A.	Patient 2 R.M.	Patients 3 K.O.
Age of onset	3 months	3 months	3 years 8 months
CVID diagnosis	8 years	7 years	4 years 7 months
Genetic study	13 years	9 years	5 years
Gender	girl	boy	boy
Clinical diagnosis	Gastritis, Crohn-like disease,	ASD, Crohn disease protein loosing enteropathy, malnutrition, JRA	ALPS (excluded), Autoimmune thrombocytopenia, haemolysis, LIP,
Clinical symptoms	Chronic diarrhea with blood in stool, thrombocytopenia, malabsorption, enteropathy, flaccid tetraparesis,	Chronic diarrhea, malnutrition, enteropathy, multi joints inflammation, osteoporosis,	Recurrent episodes of thrombocytopenia, splenomegaly, cytopenia, lymphocytic pneumonia, general lymphadenopathy, chronic diarrhea

Endoscopy/biopsy	In gastric and colon: infiltrations with neutrophils, duodenum: diffused fine lymphogranular proliferation, Marsh 3c villous depletion	Joint fluid: lymphocytic infiltration, high level of cytokines, Jejunum: infiltrations and inflammation	Lymph node and lung: infiltrations with lymphocytes mainly T cells – exclusion of lymphoproliferation (HD, NHL), TBC infection, diagnosis of LIP
Therapy	Steroids systemic (high dose), budesonid, azathioprine, 6MP, antimycotic, antibiotics, TPN, HA (PEG), IVIG, plasmapheresis (central catheter),	Steroids systemic, 6MP, MTX, NSAPD, sulfasalazine, TPN, antibiotics (periodically)	Steroids systemic, IVIG (high doses), antibiotic, antimycotics, MMF, (central catheter)
Immunoglobulin substitution	Since CVID diagnosis 0.4-0.8g/kg b.w. IVIG/SCIG, 1.0-2.0g/kg b.w. IVIG in tetraparesis	0.4-0.8g/kg b.w. as regular, during Crohn disease and JRA – 1.0 g/kg b.w.	Regular for CVID – 0.4-0.6g/kg b.w.,1.0-2.0g/kg b.w. for AIH and thrombocytopenia
Final therapy	HSCT from MRD	HSCT from MUD	HSCT from MUD
Outcome	Reconstitution of immune system, paraparesis (legs) with slow improvement,	Reconstitution of immune system, reactivation of JRA symptoms (therapy – anti-TNF, CsA, steroids)	Reconstitution of immune and haematological system, reactivation of EBV infection (rituximab therapy), secondary hypogammaglobulinemia, regular substitution of immunoglobulins
Time of follow up	2 years 2 months	2 years 1 month	2 years 11 months

Table 1: Patients characteristics

Family member	Genetic results	Comments	
Mother R.B.	c.2449C>T, g.145203 C>T	Pathogenic	a
Father R.Sz.	c.7404_7405delGA, g.694378_694379delGA	Pathogenic	a
Patient 1 R.A.	c.2449C>T; p.Gln817, c.7404_7405delGA, p.Asp2502GInfsTer40	Heterozygotic Pathogenic	c
Patient 2 R.M.	NM_006726.4 c.[2449C>T] [7504_7505delGA] NP._006717.2 p. [Gln817 Ter] [p.Asp2502GInfsTer40]	CVID8 OMIM#614700	b
Sister 1 R.K.	LRBA exons 20 and 51 without changes	No pathogenic variants	a
Sister 2 R.K.	c.7504_7505delGA, g.694378_694379delGA p.Asp2502GInsfTer40	pathogenic	a
Brother 1 R.Ł.	cDNA.7748_7749delGA, g.694378_694379delGA	Pathogenic	a
Patient 3 K.O.	Variant: c.2836_2839 del.	Pathogenic	c

Table 2: Genetic study LRBA in family of patient 1 and 2 (siblings) and patient 3

Laboratory:

a. – Diagnostic Laboratory, Department of Clinical Immunology, University Children Hospital in Kraków

b. – Medical Pediatric Laboratory, Laboratory of Immunopathology and Genetics Clinical Department of Pediatry, Oncology and Hematology in Łódź

c. – Warszawa ?

Data	Patient 1 before in course IVIG/SCIG	Patient 2 before in course IVIG/SCIG	Patient 3 before in course IVIG/SCIG
Immunoglobulin			
IgG – g/l (L-low)	5.49,(L) 8.61,	4.52,(L) 4.53,(L)*	4.71(L) 8.92
IgA – g/l	1.09, 0.74,	1.12, 0.64,(L)	0.32 0.40
IgM – g/l	0.67, 0.39	0.27,(L) 0.19,(L)	0.32 (L) 0.72
IgE - (IU/ml)	119.0 23.0		
T lymphocytes: total/ul	2020 1390	1096 (L)	819 (L) 1459
CD3 (/ul)	1279 1243	753	721 1226
CD4 (/ul)	853 838	355	508 788
CD8 (/ul)	426 349	343	156 336
CD3/HLA-DR (/ul)	264	102	180 (H)

TCRa/b TCRg/d (%)(%) T reg (%)	61, 2		94.2, 2.5 7.1 9.6
B lymphocytes: CD19 (/ul) B memory cells: Class switch (/ul)	345 56 (L) 23 (L)	55 (L)	16 (L) 102 (L) 5 (L) 24 (L)
NK cells: (/ul)	386 84 (L)	288	82 (L) 102 (L)
Other important data: ASCA IgA U/l, IgG U/l ANA (screening)	23.2 negative	Negative negative	negative
Complement level: C3c g/l C4 g/l	1.16, 0.21	1.35 0.24	1.21 1.24 0.07 0.05
Adhesive molecule	ND	ND	present
Fas, FasL expression	ND	ND	normal
T cells double negative	ND	ND	within normal range

*enteropathy, IVIG – 1.0g/kg b.w.

L – value below normal

H – value above normal

Table 3: Immunological laboratory data

Discussion

Presented siblings showed similar symptoms of chronic, severe, therapy refractory diarrhea as first signal of gastrointestinal involvement based on autoimmunity. Hypogammaglobulinemia occurred later, suggesting diagnosis of CVID with autoimmunity. Both patients were diagnosed as LRBA deficiency, however, the clinical profile was different with severe JRA in boy and neurological involvement demonstrated as flaccid tetraparesis in girl. The genetic results of LRBA gene showed different variants with heterozygotic pathogenic mutation in girl and homozygotic pathogenic mutation in boy. The other family members showed variations registered as pathogenic without development of clinical symptoms of disease. The severity of clinical course with secondary symptoms supported decision of HSCT as curative procedure. Differences between siblings in clinical course are visible after HSCT – reactivation of JRA in boy, slow resolving of flaccid paralyse symptoms without new events in girl. Our patient 3 was initially diagnosed as ALPS due to suggestive clinical symptoms - lymphoproliferation, spleen enlargement and autoimmunity. According to this initial clinical diagnosis patients was treated with rapamycine as typical apoptosis-inducing therapy in ALPS. The results of laboratory tests (expression of Fas, FasL and double negative T lymphocytes TCR α/β) did not fulfilled criteria of ALPS, so diagnosis of ALPS was excluded. The overlapping of ALPS symptoms and CVID autoimmune symptoms were suggested indicating requirement for detailed and precise differential diagnosis to identify ALPS patients due to apoptosis-inducive therapy with rapamycin (14,15). Nowadays, the clinical symptoms suggesting ALPS but with monogenic background is defined as ALPS-like syndrome for differentiation from classic, typical ALPS, based on apoptosis disorders. ALPS-like syndrome is associated with other immune disorders diseases as additional combination of clinical symptoms often improved with rapamycin therapy (5,6).

LRBA deficiency– clinical problems – JRA, neurological symptoms

Prevalence of JRA in children with diagnosis of CVID is about 10-15% depending of studied cohort of patients. Within small group of CVID and LRBA deficiency number of patients with symptoms of JRA were about 10 (9). All described JRA patients were treated with steroids, immunosuppression (MTX, CsA) and monoclonal antibodies (infliximab, etanercept, abatacept (2 patients) and immunoglobulins what suggest rather severe course with difficulties in the induction of remission. Described patient with LRBA diagnosis at least was cured with successful procedure HSCT after 15 years of therapy with steroid, immunosuppression, rituximab with severe side effects (anemia, leukopenia, chronic enteritis) partially depending of therapy, partially due to LRBA deficiency [9]. The course of JRA in our patient was not very severe, however, other symptoms (malnutrition, inhibition of growth, muscles atrophy) were serious and difficult to be controlled and corrected, deteriorating quality of his life. HSCT was no fully successful, as JRA symptoms recurrent require prolonged therapy.

Neurological symptoms within CVID patients are rare, mainly manifest as infections or autoimmune diseases involving central and peripheral nervous system. Autoimmunity present in CVID facilitate development of Guillain-Barre syndrome after infections, axonal sensorimotor polyneuropathy or symptoms associated with cerebral vasculitis [2,16]. Our patient developed severe flaccid paralysis (diagnosed as general polyneuropathy) without preceding or present infection. So far, such severe symptoms and life-threatening course and in CVID patient with LRBA deficiency was not reported. HSCT procedure inhibited further development of neurological symptoms and give us the opportunity to improve patient symptoms with rehabilitation and supportive therapy.

Abatacept as supportive therapy

Therapy with abatacept is usually prolonged as replacement for deficient CTLA-4 protein with inhibitory activity. Response to this therapy is different depending of CVID-LRBA clinical profile – the best results were noted in chronic diarrhea (remission in 78.5% of patients) and lymphoproliferation (remission up to 80% of patients). Moreover, in studied cohort were patients resistant to abatacept therapy. Summarising these study, abatacept is supportive therapy but not curative. Regular use of abatacept let to tapered doses of immunosuppression and steroids, what were important for patients with steroid side effects after prolonged therapy [4,5,8,12]. In our patients diagnosis of LRBA deficiency was established late in disease with severe manifestation, what forced us to HSCT procedure as life-saving therapy in possible shortest time.

HSCT as curative therapy but non always successful

HSCT procedure is believe to exchange immune system and to corrected causative pathomechanisms for clinical symptoms of LRBA deficiency, like in other primary immunodeficiency including combined severe combined

types. HSCT procedure is often used for haematological patients (aplastic anemia, MDS, acute leukemia, relapsing lymphomas) with success in majority cases. The immune system reconstitution is longer for adaptive immune system then for innate immunity. Full reconstitution of haematopoietic system, including immune system, especially B ontogeny may take up to 2 years, even longer. Moreover, these observations are based on patients with other indication for HSCT than immune deficiency. The assay of functional reconstitution of B cells are based on vaccine response (specific antibodies production) and class-switched memory B cells number [17]. In patients diagnosed with primary immune deficiencies involving B cell function the secondary hypogammaglobulinemia after HSCT is observed in some of them. Moreover, this hypogammaglobulinemia is associated with defective reconstitution of B cell ontogeny and as persistent, requires immunoglobulins substitution for years, probably, for life. Children with primary immunodeficiency with significantly low level of immunoglobulins, after HSCT had a slower recovery of immunoglobulins production developing secondary hypogammaglobulinemia [18].

Previous observations of HSCT results in patients with LRBA deficiency indicated HSCT as curative procedure with complete resolving of clinical symptoms in majority of them. However, the decision about HSCT procedure is associated with severe course of deficiency e.g. recurrent severe infection, refractory immune cytopenias, chronic interstitial lung disease, severe gastrointestinal problems with malnutrition and severe neurological complications. In a cohort of 24 patients treated with HSCT - 7 showed complete resolving of symptoms, 5 - partial remission and 5 patients reached good clinical response, however, some symptoms were still present. All survived patients after HSCT from this cohort showed improvement of clinical state and 70,6% of them were without further therapy. Unfortunately, 7 patients died due to transplant-related mortality within 3 months after procedure (graft failure, multiorgan failure, acute GvHD, refractory GvHD, pre-existing severe infections, thrombotic microangiopathy). The better results after HSCT are when this procedure is performed early, within first 3 years after onset of LRBA related symptoms comparing to patients with longer course of disease [10,19]. Patients without HSCT, on conventional treatment (immunosuppression) or without therapy due to small symptoms or symptomless course of deficiency, are alive in 82.7% (43/52) for long time observation (from 1 to 35 years) [19]. Our patients demonstrated severe clinical course, longer than 3 years, however, there are alive with good clinical improvement (partial remission) on regular immunoglobulins substitution due to secondary hypogammaglobulinemia (patient 3) and on rituximab therapy due to recurrent JRA (patient 2). The combination clinical symptoms of LRBA deficiency, conditioning regimen, donor type or age at HSCT, are not predicting outcome of HSCT performed as therapy life-saving and resolving from disease symptoms [2,10,12,19].

Comments and problems

Common variable immunodeficiency is most common humoral immune deficiency in children older than 4 years of age. Usually, introducing of regular substitution of human immunoglobulin is inhibiting infections and improved patient's state in reasonable number of patients. More problematic are patients with story of autoimmune symptoms (e.g. thrombocytopenia, celiac disease) preceding hypogammaglobulinemia and diagnosis of CVID. Patients diagnosed as CVID showing severe clinical course, developing co-existent symptoms (autoimmune or autoinflammatory) are in minority, however, they are most problematic and need multi specialistic care, individualised, patient-tailored therapy. Now, it is obvious, that all children with initial diagnosis of immune deficiency should have genetic study helping in precision of diagnosis. Moreover, the clinical phenotypes are overlapping in many symptoms leading to different diagnosis before proper one of CVID. In our patients diagnosis of ALPS-like disease was made base on clinical phenotype before diagnosis of CVID with LRBA deficiency. The therapy of patient with immune deficiency as basic disease and many co-existent symptoms is very difficult. The medical team for such patients included gastrologist, rheumatologist, hematologist and other. The combination of clinical symptoms considering immune deficiency with autoimmunity as basic disease, is like puzzle with many unknown pieces e.g. reaction to therapy, side effects and drugs interactions. It is why managing such patients makes a big challange for doctors and makes medicine still fascinating.

Conclusions

The genetic study of CVID cases with severe course affecting multiple organs (early onset diarrhea, autoimmune disorders, lymphoproliferation) should include CTLA-4/LRBA genes mutations.

HSCT procedure is indicated as curative therapy, however, risk of persistence some of disease symptoms should be considered.

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