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ORIGINAL PAPER

What the pediatrician should know about different types of severe combined immunodeficiency: a single-center experience

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ABSTRACT

Introduction: Severe combined immunodeficiency (SCID) is the most serious inborn error of immunity, lethal if untreated. There are three SCID subtypes: typical (TS), atypical (AS) and Omenn syndrome (OS). The goal of this study is to characterize clinical and immunogenetic aspects of patients with different aforementioned types of SCID diagnosed in a single center and an attempt to select factors improving diagnosis in the areas without newborn screening.

Material and methods: This is a retrospective evaluation of the clinical course and hematological, biochemical, immunological and genetic tests leading to SCID diagnosis in 42 children.

Results: Median age of diagnosis was 4.4 months, higher for AS (8 months, $p < 0.05$). Median diagnostic delay (DD) was 2.5 months (4.7 months in AS children). Suspicion of the disease was raised by pediatricians (in 44.4% of all children), hematologists (19.4% of all children, 26.3% of AS children, with DD of 20 months), or dermatologists. The first symptoms were infections (64%), rash (22.2%), chronic diarrhea (5.6%), anemia (5.6%) and lymphopenia (2.8%). At the time of diagnosis, 79.5% of patients presented with lymphopenia. The median of recent thymic emigrants was 0.2%, 2.1%, 0.3% in TS, AS and OS, respectively. The most frequent pathogenic variants were found in the following genes: IL2RG (23.8%), RAG1/2 (16.6%), JAK3 (14.3%), ADA (11.9%) and IL7RA (4.8%). The overall survival rate was 69% in all children.

Conclusions: The diagnosis of SCID was established significantly later in the case of AS than TS and OS. The type of first symptoms does not differ between particular types of SCID. Lymphopenia is the most common symptom and the most often underestimated laboratory sign of SCID.

KEY WORDS:

specialist, Omenn syndrome, lymphopenia, immunoglobulin M, atypical SCID.

INTRODUCTION

Severe combined immunodeficiency (SCID) is the most profound inborn error of immunity affecting cellular and humoral immunity. If left untreated, the disease leads to death in the first years of life, mainly due to infections, including opportunistic ones. Affected infants appear healthy at birth however usually develop life-threatening bacterial, viral, fungal or opportunistic infections, persistent diarrhea and failure to thrive within the first weeks

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and months of life. The clinical manifestations of SCID include recurrent upper and lower respiratory tract infections caused by common pathogens with an atypical course and complications such as:

- chronic rhinitis,
- recurrent, chronic lower respiratory tract infections, e.g. caused by adenoviruses or parainfluenza viruses,
- urinary tract,
- diarrhea.

The most dangerous are infections caused by opportunistic pathogens (i.e. *Pneumocystis jiroveci*, cytomegalovirus – CMV, or fungi):

- recurrent thrush,
- symptomatic CMV infection with lung, liver, central nervous system involvement,
- aspergillosis with systemic or organ involvement (lung, liver, spleen, central nervous system, bones).

Routine immunization with live attenuated vaccines such as BCG (*Bacillus Calmette-Guérin*) vaccine or rotavirus are the cause of local or disseminated infection.

Following all infections infant's physical development is inhibited.

The next red flags are signs including non-infectious presentation (skin rashes, lymphadenopathy, hepatomegaly, splenomegaly) and abnormalities in diagnostic tests (i.e. lymphopenia, but also lymphocytosis, eosinophilia, elevated IgE, thymus absence and/or skeletal abnormalities) and also a family history of infants' or young children's deaths or immunodeficiencies. Depending on the clinical symptoms and laboratory test results, there are three SCID subtypes: typical, atypical (leaky) and Omenn syndrome (OS) as shown below in diagnostic criteria [1]. Early diagnosis, before the onset of severe infections and organ damage, is possible in children with a positive family history or through newborn screening (NBS) and is crucial for overall survival [2]. The awareness of SCID and early recognition of the first symptoms are extremely important in the case diagnosis without access to NBS or positive family history. The aim of this study is to characterize patients diagnosed with different types of SCID according to clinical and immunogenetic phenotypes in a single center and to select the factors that could help improve SCID diagnosis in the areas without NBS.

MATERIAL AND METHODS

PATIENTS

This study included 42 Caucasian children (16 girls and 26 boys), aged 6 days to 48 months, diagnosed with SCID in the Department of Immunology, Children's Memorial Health Institute, Poland, between 2010 and 2023. The clinical course as well as hematological, biochemical, immunological and genetic tests leading to SCID diagnosis were evaluated retrospectively. None of the patients received NBS for SCID, however 6 had a positive family history.

QUANTITATIVE AND FUNCTIONAL ASSESSMENT OF CELLULAR AND HUMORAL IMMUNITY

Lymphocyte subsets

Analysis of lymphocyte subsets was performed using commercially available BD Multitest six color cocktails of antibodies and Trucount tubes (Becton Dickinson, cat. No. 644611). The assessed lymphocyte subset panel included T-cells (CD3+/CD45+), cytotoxic T-cells (CD3+CD8+/CD45+), helper T-cells (CD3+CD4+/CD45+), NK-cells (CD16+CD56+CD3–/CD45+), and B-cells (CD19+/CD45+). Their distribution and absolute cell counts were determined using the lyse-no-wash approach. The staining procedure was performed according to the manufacturer's instructions. Identification of lymphocyte subsets was performed according to standard procedures. The absolute number of individual subsets was calculated based on the proportion of the respective cell subpopulation and absolute lymphocyte count. Results have been compared to normal age-related reference ranges. Recent thymic emigrants (RTE) – CD31+CD45RA+/CD3+CD4+, within T-helper cell population were analyzed separately, and results compared to local reference ranges [3].

Lymphocyte transformation test

Peripheral blood mononuclear cells were cultured in Roswell Park Memorial Institute 1640 medium supplemented with 10% heat inactivated fetal bovine serum, in 3 replicates: without mitogen (control), with phytohemagglutinin (PHA), and with anti-CD3 monoclonal antibody, to evaluate different mechanisms of T-cell stimulation [4]. Incorporation of 3H-thymidine into proliferating lymphocytes was used to measure the lymphoproliferative response to tested stimulants [5]. Mean counts per minute (cpm) and standard deviation were calculated for 3 replicates for cultures with or without the tested stimulants. The stimulation index (SI) measuring the response of the tested cells in relation to unstimulated controls was counted to normalize the intra- and inter-individual variations in the background of the cell proliferation. The obtained results have been compared to normal values PHA (≥ 16000 cpm, SI: ≥ 65) and anti-CD3 (≥ 15000 cpm, SI: ≥ 60).

Immunoglobulin class G (IgG) and M (IgM) were determined by nephelometry on automated BN ProSpec and Atellica® NEPH 630 Siemens Healthineers (since 2019) using high specificity monoclonal antibodies, and results were compared to local reference ranges [6].

MOLECULAR DIAGNOSTICS

The molecular diagnostics included whole gene direct Sanger sequencing or next-generation sequencing (NGS) for the primary immunodeficiency gene panel with confirmatory Sanger sequencing in the targeted region,

performed in different genetic laboratories (Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands; Department of Medical Genetics, Medical University of Warsaw; MedGen Medical Centre, Warsaw, Poland).

DIAGNOSTIC CRITERIA

The Primary Immune Deficiency Treatment Consortium criteria were used to make the diagnosis of SCID: typical SCID (TS), leaky/atypical SCID (AS) and OS [1], as follows:

1. Typical SCID:

- absence or a very low number of T-cells ($< 0.05 \times 10^9$/l) and pathogenic gene variant or no alternate explanation for low T-cell count and < 20% of CD4+ T-cells have naïve cell surface markers (or undetectable/low T-cell receptor excision circles – TRECs),
- or the presence of T-cells of maternal origin (transplacentally acquired maternal engraftment – TME).

2. Leaky/atypical SCID:

- two or more of: reduced number of CD3+ T-cells ($0.05–1.0 \times 10^9$/l for children < 2 years of age, $< 0.8 \times 10^9$/l if aged 2–4 years, $< 0.6 \times 10^9$/l any age); oligoclonal T-cells; < 20% of CD4+ cells are naïve (or abnormal TRECs),
- pathogenic gene variant or reduced proliferation (response to PHA, anti-CD3, or anti-CD3/CD28 < 50% lower limit of the reference range),
- and does not have: other SCID subtype, combined immunodeficiency with known genotype, thymic disorder, other disorder with low T-cell numbers.

3. Omenn syndrome:

- generalized skin rash plus absence of TME,
- pathogenic gene variant, however we also included 3 patients without pathogenic variants due to limited access to genetic diagnostics,
- > 80% of CD4+ T-cells have CD45RO+ memory phenotype,
- two or more of: eosinophilia ($> 0.8 \times 10^9$/l), elevated IgE, abnormal TRECs, lymphadenopathy, hepatomegaly and/or splenomegaly, oligoclonal T-cells.

The exclusion criteria were congenital athymia, as well as combined immunodeficiencies with known genotype, and other disorders with low T-cell counts [1, 7].

STATISTICAL ANALYSIS

Statistical analysis was carried out using the TIBCO Statistica version 13.3 software (TIBCO Software Inc., Santa Clara, CA, USA). The data distribution was not normal which was confirmed with the Shapiro-Wilk normality test. The data comparison between the three groups was carried out using the Chi^2 Pearson test for nominal data and the Kruskal-Wallis test for ordinal and quantitative variables. Statistically significant results were estimated at $p < 0.05$.

RESULTS

Between 2010 and 2023, SCID was diagnosed in 42 children (16 females and 26 males), including: 17 TS, 19 AS, and 6 OS. In 36 children, diagnosis was made after the onset of symptoms at a median age of 6 months, ranging 2–48 months. The median age of first symptoms was 2 months. Six children had a positive family history and were diagnosed mostly in the first 2 weeks of life, before the onset of symptoms. Typical SCID was diagnosed in 17 children: in 5 with a positive family history and in another 12 after the onset of symptoms. Atypical SCID and OS were diagnosed in 19 and 6 children, respectively. In children with AS, the first symptoms appeared later, at a median age of 4.9 months and were diagnosed significantly later, at a median age of 8 months. Median diagnostic delay (DD) was 2.5 months: 2.4 months in TS, 1.7 month in OS and 4.7 months in children with AS (Table 1). Children were referred for further diagnostics mainly by pediatricians (44%). Hematologists suspected SCID in approximately 20% of all patients, and in more than 25% of children with AS. About 60% of patients with OS were referred by dermatologists (Table 2). The diagnostic delay varied depending on the referring specialist and was longer for patients referred by hematologists: median 20 months (Table 3).

The first manifestations were infections (64% of children with symptomatic SCID and 83% of children with symptomatic TS), rash (22.2% of all symptomatic children and 80% of children with OS), chronic diarrhea (5.6%), anemia (5.6%) and lymphopenia (2.8%); no statistically significant differences between SCID subtypes were found (Table 2).

INFECTIONS

The most commonly reported complication was local or disseminated BCG infection following the mandatory vaccination within the first 48 hours of life. Over 50% of vaccinated patients (18 out of 32) experienced BCG-related complications, including 5 (16%) who developed disseminated BCG infection. Children required anti-tuberculosis treatment in accordance with Polish and European guidelines [8, 9]. This infection occurred more often in TS than in AS and OS: 83%, 44% and 25%, respectively (Table 2).

Other bacterial and fungal infections, including opportunistic ones, were less common (Table 2). Gastrointestinal infections occurred sporadically and were not included in the analysis.

NON-INFECTIOUS SYMPTOMS

Skin rash remained the first and main symptom in 8 patients. Exfoliative erythroderma with eosinophilia was the first manifestation in 4 infants, and 3 of them,

TABLE 1. Demographic features and laboratory results in typical severe combined immunodeficiency (12 symptomatic and 5 with a positive family history), atypical severe combined immunodeficiency and Omenn syndrome (1 with a positive family history)

Parameters	SCID, median (quartiles: Q1; Q3)	TS, median (quartiles: Q1; Q3)	AS, median (quartiles: Q1; Q3)	OS, median (quartiles: Q1; Q3)	*p*-value
Age of first symptoms (months)	2.0 (0.93; 6)	2.0 (1.2; 4.7)	4.9 (1.7; 8)	0.9 (0.3; 1)	0.0181
Age of diagnosis (months)	4.4 (2.5; 8.8)	3.0 (0.5; 7.5)	8 (4; 20.2)	2.3 (1.8; 2.8)	0.0011
Diagnostic delay (months)	2.5 (1.2; 7.1)	2.4 (1.3; 3.4)	4.7 (1.1; 19)	1.7 (1.6; 1.8)	0.2798
IgM [g/l]	0.23 (0.09; 0.74)	0.19 (0.07; 0.26)	0.61 (0.22; 1.85)	0.14 (0.05; 0.51)	0.0160
PHA [SI]	3.3 (1.1; 11.1)	1 (1; 1.7)	11.1 (5.6; 20.7)	8.8 (5; 12.5)	0.0001
Anti-CD3 [SI]	6.9 (1.3; 16.1)	1.1 (1; 1.4)	15.3 (9.3; 37.5)	11.05 (4.95; 15.2)	0.0005
RTE (%)	0.95 (0.2; 3.5)	0.2 (0; 1.3)	2.1 (0.6; 5.9)	0.3 (0.1; 0.5)	0.1387

AS – atypical severe combined immunodeficiency, IgM – immunoglobulin class M, OS – Omenn syndrome, RTE – recent thymic emigrants, PHA – phytohemagglutinin, SCID – severe combined immunodeficiency, SI – stimulation index (references values of SI for PHA were ≥ 65 and for anti-CD3 ≥ 60), TS – typical severe combined immunodeficiency

ultimately diagnosed with Omenn syndrome, were referred by the dermatologist quite quickly – median DD of 1 month. The other 5 children with skin rash were diagnosed after the onset of infection, although 4 of them (80%) had lymphopenia in an earlier assessment.

Severe anemia, mostly autoimmune hemolytic anemia (AIHA), was the reason for the hematological evaluation and treatment in 7 patients. All of them also presented with lymphopenia, but it was the reason for further immunological assessment only in 2 of them (age of diagnosis – AD, 2–5 months; DD 1–2 months). The other 5 children were diagnosed with AS later, when severe infections appeared: recurrent pneumonia in 4 children, and toxoplasmic retinitis leading to loss of vision in 1 patient (AD 3–48 months; DD 2–42 months). Among them, 4 children had a high IgM level (ranging 1.85–6.83 g/l). In 3 of them, genetic diagnostics showed the same homozygous variant in the *RAG1* gene (c.256_257delAA), known as the Vistula watershed variant [10].

Isolated lymphopenia led to the suspicion of SCID only in 1 patient, meanwhile at the time of SCID diagnosis, 80% of patients presented with lymphopenia in the blood count, which was significantly more profound in children with TS and AS than with OS (Table 2). Neutropenia and eosinophilia were less common, occurring in 36% and 31% of children, respectively.

IMMUNOLOGICAL AND IMMUNOGENETIC ASSESSMENT

The diagnosis of TS was made in 17 children according to the Primary Immune Deficiency Treatment Consortium [1], based on a T-cell count below 50 cells/ml and the exclusion of other causes of severe T-lymphopenia. Recent thymic emigrants were measured only in 3 patients due to a very low T-cell count (Table 1).

Atypical SCID was diagnosed in 19 patients, with the count of T-lymphocytes below 1000 cells/ml in 18 of them, RTE < 20% in 13/15 patients (median 2.1%), reduced lymphoproliferation in response to PHA < 50% of the lower limit of normal in 12/13 patients (median 11.1 SI, normal value > 65 SI) and reduced proliferation in response to anti-CD3 < 50% of the lower limit of normal in 10/13 children (median 15.3 SI, normal value > 60 SI) (Table 1).

Omenn syndrome was diagnosed in 6 children with generalized rash and the count of T-lymphocytes in the range of 106–80.943 cells/ml. Recent thymic emigrants were determined in 5 patients, all of them having values below 5%. Median lymphoproliferative response to PHA measured as SI was 8.8, and to anti-CD3 – 11.05 (reference values: 65 and 60, respectively). All 6 children with OS had eosinophilia, 4 out of 5 with elevated IgE levels. Three patients without a genetic diagnosis were included in this group due to limited genetic diagnostic possibilities.

Both IgG and IgM levels were below age reference values in 17.1% of patients, more often in TS – 23.5%, than in AS or OS – 11.1% and 16.7%, respectively. The IgM alone was below normal only in 45% of children, normal in 32.5% and elevated in 22.5%. High IgM was more frequently observed in children with AS (38.9%) than in typical forms (6.3%) or OS (16.7%), but no significant differences were found (Table 2).

Genetic diagnosis was available in 31 patients and revealed the most common pathogenic variants in the following genes: *IL2RG* (10 patients), *RAG1/2* (7 patients in total), *JAK3* (6 patients) and *ADA* (5 patients) (Table 2).

Thirteen out of 42 analyzed patients passed away until the end of 2024. Deaths occurred in one third of symptomatic patients and in 17% of patients with a positive family history. Four children succumbed before hematopoietic stem cells transplantation (HSCT) as a result of severe and overwhelming infections, and 9 died after HSCT: 5 of them due to infections and 4 due to non-infectious complications.

TABLE 2. Demographic features and laboratory results in typical severe combined immunodeficiency (12 symptomatic and 5 with a positive family history), atypical severe combined immunodeficiency and Omenn syndrome (1 with a positive history)

Parameters	SCID	TS	AS	OS	*p*-value (Pearson's χ^2 test)
Gender, *n/N* (%)					
Female	16/42 (38.1)	5 (29.4)	7 (36.8)	4 (66.7)	0.2681
Male	26/42 (61.9)	12 (70.6)	12 (63.2)	2 (33.3)	
Referring physician, *n/N* (%)					
Pediatrician	16/36 (44.4)	9/12 (75)	6/19 (31.6)	1/5 (20)	0.0126
Hematologist	7/36 (19.4)	1/12 (8.3)	5/19 (26.3)	1/5 (20)	
Dermatologist	3/36 (8.3)	0/12 (0)	0/19 (0)	3/5 (60)	
Anesthesiologist	4/36 (11.1)	1/12 (8.3)	3/19 (15.8)	0/5 (0)	
Infectious disease specialist	1/36 (2.8)	1/12 (8.3)	0/19 (0)	0/5 (0)	
Nephrologist	1/36 (2.8)	0/12 (0)	1/19 (5.3)	0/5 (0)	
Gastroenterologist	2/36 (5.6)	0/12 (0)	2/19 (10.5)	0/5 (0)	
Pulmonologist	1/36 (2.8)	0/12 (0)	1/19 (5.3)	0/5 (0)	
Cardiologist	1/36 (2.8)	0/12 (0)	1/19 (5.3)	0/5 (0)	
First symptoms, *n/N* (%)					
Pneumonia	11/36 (30.6)	4/12 (33.3)	7/19 (36.8)	0/5 (0)	0.1908
Aphthous stomatitis	2/36 (5.6)	2/12 (16.7)	0/19 (0)	0/5 (0)	
Encephalitis	2/36 (5.6)	1/12 (8.3)	1/19 (5.3)	0/5 (0)	
Recurrent respiratory tract infections	6/36 (16.7)	2/12 (16.7)	4/19 (21)	0/5 (0)	
BCGitis	1/36 (2.8)	1/12 (8.3)	0/19 (0)	0/5 (0)	
Chronic diarrhea	2/36 (5.6)	1/12 (8.3)	1/19 (5.3)	0/5 (0)	
Sepsis	1/36 (2.8)	0/12 (0)	1/19 (5.3)	0/5 (0)	
Skin rash	8/36 (22.2)	1/12 (8.3)	3/19 (15.8)	4/5 (80)	
Anemia	2/36 (5.6)	0/12 (0)	1/19 (5.3)	1/5 (20)	
Lymphopenia	1/36 (2.8)	0/12 (0)	1/19 (5.3)	0/5 (0)	
Infections, *n/N* (%)					
CMV	6/40 (15)	2/17 (11.8)	3/17 (17.6)	1/6 (16.7)	0.8842
BCG	18/32 (56.3)	10/12 (83.3)	7/16 (43.8)	1/4 (25)	0.1408
PJP	7/41 (17.1)	3/17 (17.6)	3/18 (16.7)	1/6 (16.7)	0.9966
Fungal etiology	13/34 (38.2)	8/13 (61.5)	5/15 (33.3)	0/5 (0)	0.0325
Bacterial etiology	20/39 (51.3)	5/17 (29.4)	10/16 (62.5)	5/6 (83.3)	0.0382
Lymphopenia, *n/N* (%)	31/39 (79.5)	15/17 (88.2)	14/16 (87.5)	2/6 (33.3)	0.0097
Neutropenia, *n/N* (%)	14/39 (35.9)	6/17 (35.3)	7/16 (43.8)	1/6 (16.7)	0.4977
Eosinophilia, *n/N* (%)	12/39 (30.8)	1/17 (5.9)	5/16 (31.3)	6/6 (100)	0.0001
IgM, *n/N* (%)					
Low	18/40 (45)	9/16 (56.3)	5/18 (27.8)	4/6 (66.7)	0.1375
Normal	13/40 (32.5)	6/16 (37.5)	6/18 (33.3)	1/6 (16.7)	
High	9/40 (22.5)	1/16 (6.3)	7/18 (38.9)	1/6 (16.7)	
IgG, *n/N* (%)					
Low	17/41 (41.5)	8/17 (47.1)	7/18 (38.9)	2/6 (33.3)	0.9697
Normal	19/41 (46.3)	7/17 (41.2)	9/18 (50)	3/6 (50)	
High	5/41 (12.2)	2/17 (11.8)	2/18 (11.1)	1/6 (16.7)	
Low IgG + IgM	7/41 (17.1)	4/17 (23.5)	2/18 (11.1)	1/6 (16.7)	0.6209

TABLE 2. Cont.

Parameters	SCID	TS	AS	OS	*p*-value (Pearson's χ^2 test)
Genetic defect, *n*/*N* (%)					
IL2RG	10/42 (23.8)	7/17 (41.2)	3/19 (15.8)	0/6 (0)	0.0695
JAK3	6/42 (14.3)	3/17 (17.6)	2/19 (10.5)	1/6 (16.7)	
ADA	5/42 (11.9)	2/17 (11.8)	3/19 (15.8)	0/6 (0)	
RAG1	3/42 (7.1)	0/17 (0)	3/19 (15.8)	0/6 (0)	
RAG2	4/42 (9.5)	0/17 (0)	3/19 (15.8)	1/6 (16.7)	
IL7RA	2/42 (4.8)	2/17 (11.8)	0/19 (0)	0/6 (0)	
Lig1	1/42 (2.4)	0/17 (0)	0/19 (0)	1/6 (16.7)	
Lack	11/42 (26.2)	3/17 (17.6)	5/19 (26.3)	3/6 (50)	

AS – atypical severe combined immunodeficiency, ADA – adenosine deaminase, BCG – Bacillus Calmette-Guerin, CMV – cytomegalovirus, IgG – immunoglobulin class G, IgM – immunoglobulin class M, IL2RG – interleukin 2 receptor subunit γ, IL7RA – interleukin-7 receptor a chain, JAK3 – Janus Kinase 3, OS – Omenn syndrome, PJP – pneumocystis jiroveci pneumonia, RAG1 – recombination-activating gene 1, RAG2 – recombination-activating gene 2, SCID – severe combined immunodeficiency, TS – typical severe combined immunodeficiency

TABLE 3. Severe combined immunodeficiency suspicion by specialist

Referring physician suspecting SCID diagnosis	Number of patients, *n* (%)	Age of diagnosis in months median (range)	Diagnosis delay in months median (range)	Number of patients TS/AS/OS
Pediatrician	16 (44)	5 (2–37)	2 (0–19)	9/7/1
Hematologist	7 (19)	21 (2–48)	20 (1–42)	1/5/1
Dermatologist	3 (8)	3 (2–3)	1 (1–2)	0/0/3
Gastroenterologist	2 (6)	16 (14–19)	9 (6–13)	0/2/0
Anesthesiologist/neonatologist	4 (11)	7 (4–9)	1 (0–1)	0/4/0
Cardiologist	1 (3)	4	1	0/1/0
Infectiologist	1 (3)	8	1	1/0/0
Pulmonologist	1 (3)	8	7	0/1/0
Nephrologist	1 (3)	15	13	0/1/0
All	36 (100)	6 (2–48)	2 (0–42)	12/19/5

AS – atypical severe combined immunodeficiency, OS – Omenn syndrome, SCID – severe combined immunodeficiency, TS – typical severe combined immunodeficiency

DISCUSSION

Severe combined immunodeficiency should be diagnosed as early as possible, as there is a greater chance of more effective treatment before severe infection and/or irreversible organ damage occur [2]. Patients with a family history of SCID are more likely to be diagnosed early. In our group, the survival rate of such infants was 83%, while the survival rate of the symptomatic patients was only 67%. We have observed progress in diagnostics for the last 3 decades. Comparing an earlier report (1993–2006) by Wolska-Kuśnierz [11] and our results, it is clear that in the later period both more patients (42 vs. 23) and at a younger age were diagnosed. The median age of SCID diagnosis decreased by 7–4.4 months, while the median DD was shortened by 5–2.5 months. It is worth emphasizing that the availability of immunological tests, including flow cytometry as well as genetic methods, has improved. In the earlier period, diagnosis was performed exclusively using the Sanger method and genetic confirmation was obtained only in 57% of patients (5 *IL2RG*, 3 *RAG1*, 3 *RAG2*, 1 *IL7RA*, and 1 *NHEJ1*). Currently, thanks to NGS, 74% of patients have genetic confirmation. However, there are still some goals to be achieved in terms of SCID diagnosis improvement.

It is known that early diagnosis is achievable with NBS for SCID. In Poland, there is no NBS program for SCID at the moment [12]. In a pilot Polish-German cross-border NBS study, among Polish infants, no cases of SCID were found, however 1 child with combined immunodeficiency and 1 with agammaglobulinemia were detected [13]. T-cell receptor excision circles-based screening for all newborns resulted in increased detection of infants with SCID and severe T-lymphocytopenia in many European countries [12]. In countries with no NBS program, SCID can be suspected either after the onset of symptoms or following a family history, which is extremely important in SCID patients [14]. That is why educating pediatricians and other specialists

about the first signs of SCID and positive family history is an important element of early diagnosis.

Severe combined immunodeficiency most frequently was suspected by pediatricians (up to 75% in the typical form), hematologists (over 25% in the atypical form) and dermatologists (60% of patients with Omenn syndrome). The diagnostic delay was shorter among patients referred by dermatologists, and concerned only a limited number of OS. It can be explained by a very severe clinical course including skin lesions not responding to treatment. The longer delay in diagnosis among patients referred by hematologists may result from a milder course (in most children with the atypical form) and presence of other causes of lymphopenia such as adverse drug reactions. There are no literature data on DD in SCID depending on the referring specialist. It is worth noting that currently the suspicion of the disease is established on the basis of NBS in many countries [12, 15].

In our cohort, recurrent, chronic or atypical infections were the first symptoms in 69% of all the children. The most common infection in this group of patients was BCG infection with a usually mild clinical course. This complication occurred significantly more often in children with TS than in those with AS and OS. The absolute counts of NK-cells did not influence the frequency of the local BCG reaction, but they seemed to play a role in protecting SCID patients against disseminated BCG complications, as reported previously by Bernatowska *et al.* [16] in another SCID group in Poland.

Retrospective analysis showed that 80% of all our SCID patients had pre-existing lymphopenia detected in blood count, but lymphopenia was the reason for SCID suspicion in only one patient. Lymphopenia is an important symptom of SCID and should not be underestimated if detected in an infant, even one who appears healthy. Flow cytometry analysis makes it possible to detect SCID before life-threatening symptoms and organ complications appear. Turkish neonatologists point out the need for diagnosis of SCID in newborns with lymphopenia, defined as a lymphocyte count below 3,000 cells/ml in a prospective study of 2,000 newborns [17]. The diagnosis of SCID should be considered in all infants with lymphopenia, particularly in the absence of a NBS program. It is extremely important when accompanied by the hypoplasia/absence of the thymus or bone dysplasia on chest X-ray. However, the absence of lymphopenia and normal immunoglobulin levels do not rule out the diagnosis of SCID. For example, children with *IL2RG* SCID might have normal lymphocyte counts in peripheral blood, but only B-lymphocytes are detected by flow cytometry, as was the case with one of our patients. Another explanation for lymphocytosis is transplacental maternal engraftment or oligoclonal lymphoproliferation in OS.

Immunoglobulin measurement rarely helps with the diagnosis of SCID. The immunoglobulin class G concentration might be within the reference values in the first months of age, because of its maternal origin. In our group, low IgG levels were detected only in 41.5%. Since low IgA can be found in healthy infants and young children up to 4 years of age, they are of limited diagnostic value. As already presented in the results, high IgM levels were observed in some symptomatic patients with no significant differences between all subtypes of SCID.

Both elevated IgM and IgG have been found in patients with *RAG* deficiency, autoimmunity and hyperinflammation in AS, OS and combined immunodeficiency. In *RAG*-deficient patients, impaired V(D)J recombination process leads to skewed repertoire of T- and B-cell receptors [18]. Additionally, the checkpoint in both T- and B-cell peripheral and central tolerance is broken and allows for the survival of cells with self-reactive specificity [19, 20]. High IgM levels were also reported by other authors. In a SCID group from India 19% had higher IgM levels [21].

The frequency of SCID genes varies in different populations. Pathogenic variants in the *IL2RG* gene are most common in our group of children (24%), as well as in the US population – 30%, German and Dutch population – 20% each [1, 22, 23], and dominant in the Chinese and Southeast Asian population [24, 25] – 53–73% of defects genetically confirmed. In the populations of Oman, Iran and Turkey, mutations in the *IL2RG* gene constitute less than 10% of identified SCID genes, while the most common genotype is *RAG1/2*, probably due to the higher incidence of consanguineous parents [26–28]. *RAG1/2* genes are also the most common causes of SCID in India, Italy, Serbia, and Montenegro [16, 29, 30].

CONCLUSIONS

In the study group, SCID diagnosis was established significantly later in AS than in TS and OS groups. Bacterial infections are significantly more frequent in children from the OS and AS groups, while fungal infections among TS children. Lymphopenia is the most common and most often underestimated SCID symptom. Almost 90% of children with TS and AS had lymphopenia before diagnosis, and SCID should be considered in all infants with lymphopenia, as it could have shortened DD. This is especially important if lymphopenia is accompanied by other cytopenia (e.g. AIHA) or dermatitis. High IgM concentration was observed in over ¼ of SCID patients. Further studies on larger groups are needed to clarify whether this could be a prognostic factor.

The most common gene variants causing SCID in our study were: *IL2RG* (23.8%), *RAG1/2* (16.6%), *JAK3* (14.3%), *ADA* (11.9%) and *IL7RA* (4.8%).

The survival rate was 69% in the presented group during the study period, with a lower rate in symptomatic than asymptomatic children – 67% and 83%, respectively – which confirms a better prognosis in case of earlier diagnosis.

It is extremely important to disseminate the awareness of SCID among pediatricians and other specialists, including hematologists, dermatologists and neonatologists. The milestone improving prognosis, survival and quality of life of both patients and families of that special group of inborn errors of immunity is to implement NBS for T-cell and B-cell lymphopenia in Poland.

DISCLOSURES

1. Institutional review board statement: The study was performed according to the Declaration of Helsinki Guideline on Biomedical Research Involving Human Subjects and was approved by the Bioethics Committee at the Children's Memorial Health Institute (Decision No. 30/KBE/2023).
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National experience with adenosine deaminase deficiency related SCID in Polish children

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Introduction: Deficiency of adenosine deaminase (ADA) manifests as severe combined immunodeficiency (SCID), caused by accumulation of toxic purine degradation by-products. Untreated patients develop immune and non-immune symptoms with fatal clinical course. According to ESID and EBMT recommendations enzyme replacement therapy (ERT) should be implemented as soon as possible to stabilize the patient's general condition, normalize transaminases, treat pulmonary proteinosis, bone dysplasia, and protect from neurological damage. Hematopoietic stem cell transplantation (HSCT) from a matched related donor (MRD) is a treatment of choice. In absence of such donor, gene therapy (GT) should be considered. HSCT from a matched unrelated donor (MUD) and haploidentical hematopoietic stem cell transplantation (hHSCT) are associated with worse prognosis.

Material and methods: We retrospectively evaluated the clinical course and results of biochemical, immunological and genetic tests of 7 patients diagnosed in Poland with ADA deficiency since 2010 to 2022.

Results: All patients demonstrated lymphopenia affecting of T, B and NK cells. Diagnosis was made on the basis of ADA activity in red blood cells and/or genetic testing. Patients manifested with various non-immunological symptoms including: lung proteinosis, skeletal dysplasia, liver dysfunction, atypical hemolytic-uremic syndrome, and psychomotor development disorders. Five patients underwent successful HSCT: 3 patients from matched unrelated donor, 2 from matched sibling donor, and 1 haploidentical from a parental donor. In 4

patients HSCT was preceded by enzyme therapy (lasting from 2 to 5 months). One patient with multiple organ failure died shortly after admission, before the diagnosis was confirmed. None of the patients had undergone gene therapy.

Conclusions: It is important to diagnose ADA SCID as early as possible, before irreversible multi-organ failure occurs. In Poland HSCT are performed according to international immunological societies recommendations, while ERT and GT are less accessible. Implementation of Newborn Screening (NBS) for SCID in Poland could enable recognition of SCID, including ADA-SCID.

KEYWORDS

adenosine deaminase (ADA) deficiency, SCID - severe combined immunodeficiency, ERT (enzyme replacement therapy), HSCT = hematopoietic stem cell transplant, lymphopenia

Introduction

Adenosine deaminase (ADA) deficiency is a rare inherited disorder of purine metabolism leading to life threatening severe combined immunodeficiency (SCID), otherwise known as ADA-SCID (1). The disease is inherited in an autosomal recessive manner, with incidence 1:200 000 - 1: 1 million births, and accounts for 10-20% of all SCID cases. The accumulation of deoxyadenosine, a metabolic substrate of ADA enzyme, causes abnormalities in immune cells development and functions and has systemic impact, mostly on skeletal development (costochondral abnormalities, skeletal dysplasia), cerebral (cognitive and behavioral defects), as well as hepatic and pulmonary functions (1, 2).

Most affected infants present with severe opportunistic infections, failure to thrive and developmental delay during their first 6 months of life. Undiagnosed or left untreated children die in their first year of life.

The hematopoietic stem cell transplantation (HSCT) from matched related donor (MRD) represents a successful treatment option with high survival rates and excellent immune reconstitution, and is an accepted treatment of choice. Two other therapeutic options are available: enzyme replacement therapy (ERT) and gene therapy (GT) (1).

This study reviews the diagnostic and therapeutic approach to ADA-SCID.

Materials and methods

This study included 7 Caucasian children 2 girls and 5 boys, now aged from 6 months to 13 years, diagnosed with ADA-SCID in Poland since 2010 to early 2022. Five patients were diagnosed in the Department of Immunology, Children's Memorial Health Institute (CMHI), Warsaw, two others – in the Department and Clinic of Pediatric Oncology, Haematology and Bone Marrow Transplantation, Wroclaw Medical University, Wroclaw, Poland and in the Department of Paediatrics, Haematology and Oncology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, respectively. We retrospectively evaluated the clinical course and results of biochemical, immunological, and genetic tests leading to the diagnosis of ADA-SCID. Basic hematological, biochemistry, and immunological results, including immunoglobulin level, lymphocyte proliferation test, and flow cytometry results were recorded.

Adenosine deaminase (ADA) activity was measured in patients' erythrocyte lysate and expressed in nmol/h/mg Hb (3).

Molecular diagnostics involved whole gene direct Sanger sequencing in 3 cases, and next-generation sequencing (NGS) for primary immunodeficiency gene panel with confirmatory Sanger sequencing to targeted region in 4 patients.

Evaluation of lymphocyte subsets was performed using commercially available BD Multitest six color cocktails of antibodies and Trucount tubes (Becton Dickinson, cat. no. 644611). The assessed lymphocyte subset panel included T cells (CD3+/CD45+), cytotoxic T cells (CD3+CD8+/CD45+), helper T cells (CD3+CD4+/CD45+), NK cells (CD16+CD56+CD3-/CD45+), and B cells (CD19+/CD45+). Their distribution and absolute cell counts were determined using the lyse-no-wash approach according to manufacturer's instructions (4). The absolute number of individual subsets was calculated based on the proportion of the respective cell subpopulation and absolute lymphocyte count.

ERT was carried out in 4 patients in the Department of Immunology, Children's Memorial Health Institute, Warsaw. One patient received polyethylene glycol-conjugated bovine ADA, intramuscularly at a weekly dose of 20 U/kg and 3 patients were treated with elapegademase, at a dose 0.4 mg/kg a week in 1-2 doses. Six patients underwent HSCT in three Polish centers.

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The study was performed in accordance with the Declaration of Helsinki on Biomedical Research involving Human subjects. The study was approved by the Bioethics Committee at the Children's Memorial Health Institute (Decision No. 21/KBE/2022).

Results

The diagnosis of ADA SCID was made in all 7 patients at the median age of 4.5 months (ranged 7 weeks to 27 months). None of the patients had positive family history or was detected in the Polish-German transborder area "RareScreen" newborn screening project (5). According to available data between 2010 and February 2022 SCID was diagnosed in 64 patients in Poland. The prevalence of ADA-SCID among all SCID was 10.9%. The first symptoms appeared during the first month of life in 4 patients (57%). Pneumonia was observed in all patients, in 4 of them during first months of life (2 weeks to 3 months of age), while in 3 others after the first year of life. Lymphopenia was diagnosed in 2 patients (28.5%) and failure to thrive in 2 other children (28.5%). Only 2 patients (P6, P7) demonstrated skeletal abnormalities on the chest X-ray as scapular spurring with flaring and cupping of rib ends (Figure 1).

Elevated transaminases were observed in total in 5 patients (71%), in 3 of them at diagnosis (43%). In patient P5, transaminase activity was slightly elevated already at the time of diagnosis, but significant increase in the value of transaminases (Alanine Aminotransferase - ALT 331 U/l, Aspartate Aminotransferase - AST 902 U/l) was observed while waiting for the ERT.

Delay in diagnosis ranged from 1.5 to 15 months (Table 1). Chronologically, first 3 patients were diagnosed after first year of life and the next 4 -in the first 6 months of life (Table 1)]. In patient P3, SCID was not suspected until at the age of 15 months due to severe nephrological symptoms i.e. atypical hemolytic uremic syndrome (aHUS) and arterial hypertension. At the time of the diagnosis the patient demonstrated multiple opportunistic infections (cytomegalovirus infection, *Pneumocystis jiroveci* and *Absidia* pneumonia) and advanced atrophic changes in the Central Nervous System (CNS) found in the brain magnetic resonance imaging. He died prior to establishing ADA-SCID diagnosis and was not treated with ERT or HSCT (6).

We confirmed viral infections in 4 patients (57%): 2 had cytomegaloviral infection, 1 respiratory syncytial virus (RSV), and 1 adenoviral (ADV) infection, 1 varicella zoster virus (VZV), and herpes simplex (HSV) (Table 1). Fungal infections were suspected in 4 more patients apart from patient 3. In 3 patients *Candida* infection was suspected due to the presence of fungal metabolites in serum (mannan or beta-D-glukan), in 1 patient *Aspergillius* (galactomannan in the serum), and in 2 patients

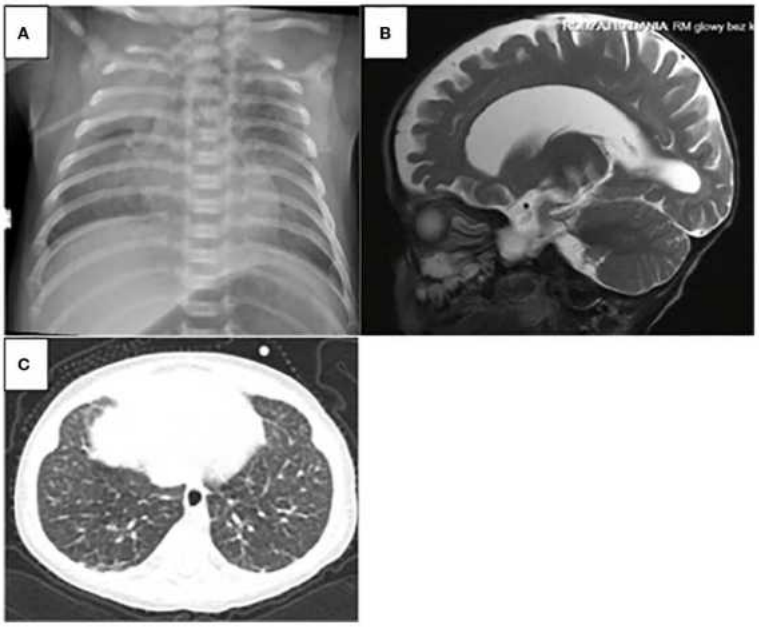


FIGURE 1
ADA-SCID sypmtoms: **(A)** Chest X-ray: scapular spurring with flaring and cupping of rid ends in the patient 6. **(B)** atrophic changes in the CNS were found in the brain magnetic in the patient 3 **(C)** computed tomography of the chest as ground-glass opacities with interlobular and intralobular interstitial thickening in patient 5.

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TABLE 1 Age of onset of symptoms and diagnosis.

Pt	Sex	Lymphopenia	Neutropenia	Hypertransaminasemia	Pneumonia	Failure to thrive	Hepatomegaly	Bone dysplasia	Infections	Age at SCID diagnosis	Age at ADA diagnosis
P1	F	15 mo	13 mo	15 mo	6 mo	15 mo	No	No	No	13 mo	21 mo
P2	M	15 mo	15mo	15 mo	2x	x	16 mo	No	2.5y CMV, Asp	27 mo	27 mo
P3	M	14 mo	15 mo	15 mo	15 mo	since birth	15 mo	No	3 mo, VZV 9 mo HSV 15 mo CMV, PJP	15 mo	16 mo
P4	M	2.5 mo	2.5 mo	2.5 mo	3 mo	since birth	6 mo	No	No	3 mo	6 mo
P5	F	7 w	7 w	7 w	7 w	2 m	No	No	Local BCG 5 mo Can	2 mo	3 mo
P6	M	2 w	2 w	3 w	2 w	no	No	2 mo: X-chest	Local BCG 2 mo RSV, Can	7 w	8 w
P7	M	5 w	5 w	7 w	5w	no	No	2 mo: X-chest	5 w PJP, ADV Local BCG 3 mo, Can	6 w	7 w

ADA, adenosine deaminase; ADV, adenovirus; Asp, Aspergillus spp; BCG, Bacille Calmette-Guerin; Can, Candida spp; CMV, cytomegalovirus; F, female; HSV, herpes simplex virus; M, male; Mo, months; PJP, Pneumocystis jiroveci pneumonia; RSV, respiratory syncytial virus; Pt, patient; SCID, severe combined immunodeficiency; w, weeks; VZV, Varicella Zoster Virus.

Pneumocystis jiroveci infection (positive specific IgG, chest x-ray image) (Table 1). Local Bacillus-Calmette-Guerin (BCG) complication was observed in 3 patients. In 2 of them local inflammation at the site of vaccination without lymphadenopathy developed between 3rd day and 4th week after initiation of ERT preliminary. In P5 the first signs of BCG complication were observed after 4 weeks of ERT and disappeared after starting treatment with two tuberculostatic drugs: isoniazid and rifampicin (Figure 2).

Laboratory tests showed lymphopenia in all patients, affecting subpopulations of T, B and NK cells (Table 2).

The diagnosis was made by measurement of ADA activity in 4 patients and in 6 patients confirmed by genetic testing. ADA activity in red blood cells (RBC) was very low in 3 patients (<1%) and low (4.9%) in one patient, who was tested 5 days after RBC transfusion (Table 2).

Genetic tests revealed the presence of 7 mutations in 6 patients. The most common variant (c.302G> A) was detected in 3 patients: in P3 and P4 as homozygous mutation while in P5 as heterozygous. The next common one (c.956_960delAAGAG) was found in 2 patients (P5, P7) in heterozygous genotype. In the rest of patients other variants were found (Table 2). In patient P2 no mutation was found, but testing was done only by Sanger.

ERT was administered to 4 patients for the period of 6 to 23 weeks with normalization of the lymphocyte count and transaminases activity (Figure 3).

HSCT was performed during the period of 2010-2022 in 6 patients: 2 MSD, 3 MUD, 1 haploidentical parental donor. All patients are alive at the time of preparing of this manuscript. The conditioning regimens in 5 cases were used in accordance with successive ESID recommendations. In two cases the regimen included treosulfan, fludarabine, and thiotepa.

None of the patients was referred for gene therapy (GT), which is available only in two centers across Europe. According to Polish regulations, the patient should obtain an agreement of the Ministry of Health for that treatment. Among five patients of CMHI one had a MSD, one died before any treatment and, only one agreed to undergo GT, however the parents eventually refused, due to complicated family situation. Two other patients decided to go for HSCT.

Discussion

The diagnosis of ADA SCID was established after typical symptoms appeared in the first months of life. The reported patients most frequently suffered from dyspnea with radiographical pneumonia, unresponsive to conventional treatment and leukopenia. Leukopenia and dependence on oxygen therapy stimulated the diagnostic work up for a primary immunodeficiency.

Pulmonary manifestation was demonstrated on imaging studies as ground-glass opacities with interlobular and intralobular interstitial thickening (Figure 1). These findings might be associated with infections, but they also may result from pulmonary alveolar proteinosis (PAP) frequently observed in the patients with ADA deficiency. Lung biopsy or bronchoalveolar lavage that could confirm pulmonary proteinosis were not performed, but improvement was observed with ERT treatment, with subsequent disappearance of dyspnea or weaning of oxygen therapy in two patients (P5, P6). According to the literature, reverse of PAP is observed after enzyme replacement therapy or after transplantation (7).

Although skeletal manifestations of ADA SCID, like scapular squaring and spurring and cupping of the costochondral junctions were not the first manifestation in Polish patients, they were observed in 2 patients after establishment of the diagnosis (8). Hypertransaminasemia was observed in the of patients. Patient P5 demonstrated significant increase in the value of transaminases during the period preceding initiation of ERT. Considering that hepatic failure can be rapid in patients with ADA-SCID, urgent treatment was



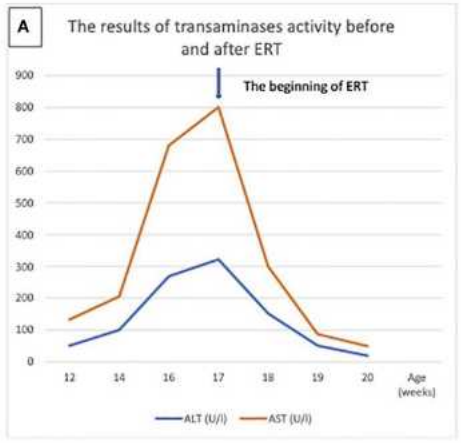
FIGURE 2
Local BCG disease in patient 5: **(A)** before enzyme replacement therapy (ERT) normal BCG vaccination scar **(B)** 4 weeks of ERT, at the site of the BCG injection small spot appeared and turned into a blister, treatment with two tuberculostatic drugs: isoniazid and rifampicin has started **(C)** 6 weeks of ERT, a crusty scrab formed, which healed into a small scar.

TABLE 2 Laboratory and clinical data at diagnosis.

Pt	WBC (K/µl)	ALC (K/µl)	AST (U/l)	ALT (U/l)	IgG (g/l)	IgA (g/l)	IgM (g/l)	CD3 (cells/ml)	CD4 (cells/µl)	CD8 (cells/µl)	CD19 (cells/µl)	CD16+56+CD3- (cells/µl)	RTE (%)	ADA activity (nmol/mg Hb/h)	Gene defect
P1	2,64 (L)	0.21 (L)	85 (H)	59 (H)	3.39 (N)	0.08 (N)	0.315 (N)	65 (L)	42 (L)	21 (L)	14 (L)	70 (L)	Nd	N	c.80T>A(;)646G>A p.Ile27Asn(;) Gly216Arg
P2	2 (L)	0.58 (L)	180 (H)	141 (H)	3.84 (L)	0.41 (N)	0.39 (N)	714 (L)	107 (L)	618 (L)	24 (L)	25 (L)	5.4 (L)	0.63 (L)	Not detected
P3	1.9 (L)	0.34 (L)	56 (H)	21 (N)	4.75 (N)	<0.06 (L)	<0.04 (L)	50 (L)	1 (L)	49 (L)	1 (L)	11 (L)	0 (L)	Nd	homozygous mutation c.302G>A p.Arg101Gln
P4	1.56 (L)	0.17 (L)	225 (H)	38 (N)	3.9 (N)	0.4 (N)	1.11 (N)	39 (L)	33 (L)	2 (L)	42 (L)	17 (L)		Nd	homozygous mutation c.302G>A p.Arg101Gln
P5	1.7 (L)	0.25 (L)	133 (H)	51 (N)	6.92 (H)	0.36 (N)	1.32 (H)	158 (L)	71 (L)	73 (L)	3 (L)	64 (L)	9.8 (L)	0.109 (L)	c.302G>A(;) 956_960delAAGAG p.Arg101Gln(;) Glu319Glyfs
P6	3.12 (L)	0.14 (L)	67 (N)	31 (N)	8.54 (4 days IVIG)	0.07 (N)	<0.04 (L)	35 (L)	31 (L)	4 (L)	2 (L)	5 (L)	0 (L)	4.9 (5 days after transfusion) (L)	c.363-3C>G(;) 778G>A p.(?)(;)Glu260Lys
P7	1.22 (L)	0.04 (L)	67 (N)	29 (N)	2.1 (L)	<0.07 (N)	0.08 (L)	27 (L)	20 (L)	7 (L)	3 (L)	22 (L)	0 (L)	0 (L)	c.956_960del(;)del ex 2: chr20: 44636227-44636288 p.Glu319Glyfs(;)

ADA, adenosine deaminase; ALC , absolute lymphocyte count; ALT, alanine transaminase; AST, aspartate aminotransferase; CD, cluster of differentiation; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; L, below norm; N, normal; Nd, not done; H, above normal; RTE, Recent Thymic Emigrants; CD31+CD45RA+/CD3+CD4+; WBC, white blood cells.

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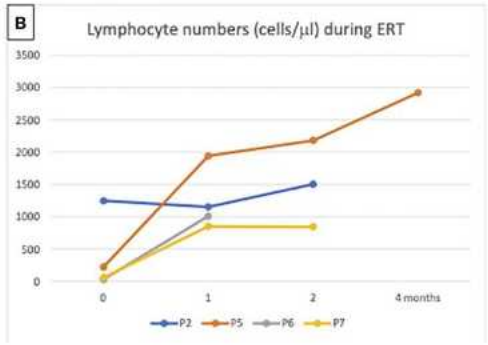


FIGURE 3
Enzyme replacement therapy (ERT): **(A)** Aminotransferases normalization in patient 5 after ERT, the arrow indicates the beginning of treatment, **(B)** Lymphocyte numbers in patient P2, P5, P6 and P7 after 1, 2 and 4 months ERT.

required (9). RBC transfusions, intended to provide the child temporarily with external source of ADA enzyme, and reduction of antibiotic prophylaxis were not effective in this patient. Significant improvement was achieved after initiation of ERT (Figure 3).

One of the reported patients (P2) showed mild phenotype with lung and liver function abnormalities but no life-threatening infections. Symptoms in this patient related to recurrent pneumonia showed milder course than in patients diagnosed with SCID, but hepatosplenomegaly, and hypertransaminasemia were present, and the child was consulted by pulmonologist and gastroenterologist before final diagnosis was established. Diagnosis of leaky ADA-SCID in patient P2 was based on criteria published by Shearer et al. (10), defined as lymphopenia CD3 T cells below 1000 cells/µl for the age up to 2 years, absence of maternal engraftment, and less than 30% of lower limit of normal T cell function (as measured by response to phytohemagglutinin (PHA). Delay in the diagnosis was 12 months from first symptoms, which emphasizes the need for multi-specialist teams taking care of children with rare diseases.

Another challenge in the reported group, was to initiate the right diagnostic approach in patients with atypical symptoms. In patient P3, recurrent diarrhea from the first month of life, failure to thrive and delayed psychomotor development were not sufficiently indicative of IEI. Even infections with VZV (at 3 months) and HSV (at 9 months) were not associated with immunodeficiency. Atypical hemolytic uremic syndrome (aHUS), respiratory failure, requirement for peritoneal dialysis and plasmapheresis, finally led to initiation of the diagnostic procedure for an inborn error of immunity. At the age of 15 months, when the diagnosis of SCID was finally established, the patient had already signs of multiple organ failure (MOF), advanced atrophic changes in the CNS, severe arterial hypertension, cytomegalovirus infection, and *Pneumocystis jiroveci* and *Absidia* pneumonia. Diagnosis of ADA-SCID was established *post mortem* (6).

Another issue that needs addressing here, is the post-BCG vaccination disease in an IEI patient (11). In Poland, vaccination against tuberculosis is obligatory in the first two days of life in all newborns, unless they have contraindications. For this purpose, locally-produced BCG Moreau strain vaccine is used, a descendant of the Brasilian BCG Moreau substrain with superior clinical safety profile. Serious complications in IEI patients are only observed in SCID, especially associated with low NK cell numbers, and patients with Mendelian susceptibility to mycobacterial diseases (MSMD). NK cells are thought to have a protective role probably due to IFN-γ production (11). However, none of our patients with ADA-SCID demonstrated a disseminated BCG disease, with local manifestations observed in total in 3 patients, one before ERT and two after initiation of ERT and with an increase in the number of lymphocytes, treated with antimycobacterial double therapy (isoniazid, rifampicin) (Figure 2; Table 3).

Currently, ADA-SCID diagnostics in Poland is quite easily accessible and not expensive. In cases when typical clinical manifestations are accompanied by poor flow cytometry results suggesting ADA-SCID diagnosis, the activity of the ADA enzyme in red blood cells is evaluated, and results confirmed by molecular methods. Before 2010, for various reasons the delay in the diagnostic process was far too long, leading frequently severe complications as in case of first patients from the described group, diagnosed at the age between 16 and 27 months, or fatal outcome without diagnosis. Therefore, the exact number of ADA SCID patients in Poland before 2010 remains unknown. The prevalence of ADA-SCID among all SCID diagnosed between 2010 and 2022 was 10.9%. According to the Statistics Poland reports during this period 50-88 infants' deaths were noted yearly due to different infections (Archive on the website: https://stat.gov.pl/en/topics/statistical-yearbooks/statistical-yearbooks/demographic-yearbook-of-poland-

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TABLE 3 ERT and HSCT characteristics and outcomes.

Pt	Age at ERT onset	ERT duration (weeks)	Age at HSCT (mo)	Donor	Type of conditioning	CD34 +/kgx10^6	Clinical complications	Post-HSCT follow up (month)	Last lymphocyte value (K/ul)	Last engraftment (CD3, CD19, PMN)	BCG treatment
P1	n.a.	n.a.	16	MSD	none	6.55	None	140	2860	1897/261/4480	n.a.
P2	28 mo	12	33	MSD	Bu-Flu	4.28	hypertensive crisis, FUO, CMV	131	2350	1746/413/1450	n.a.
P3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
P4	no	no	7	MUD	myeloablative, toxicity reduced (TREO+FLUD)	4.04	no	72	1900	1362/218/3750	n.a.
P5	4 mo	23	9	MUD	BuxFlu	4.6	mucositis III stage, hypertension	46	4600	chimerism 12-2021 - 88%	INH+RMP
P6	11 w	6	4	Hapl	Treo-flu-tiotepa	18.72	mucositis aGVHD st.2, autoimmune hemolytic anemia, patient retransplanted from the same donor (08.2022)	18		n/a	INH+RMP
P7	10 w	9	4,5	MUD	Treo-flu-tiotepa		aGVHD stage 2, autoimmune hemolytic anemia	3	600	475/0/2370	INH+RMP

aGVHD, acute graft versus host disease; BCG, Bacille Calmette-Guerin; CD, cluster of differentiation; CMV, cytomegalovirus; ERT, enzyme replacement therapy; FUO, fever of unknown origin; HSCT, hematopoietic stem cell transplantation; INH, isoniazid (isonicotinic acid hydrazide); mo, months; MSD, matched sibling donor; MUD, matched unrelated donor; n.a., not applicable; RMP, rifampicin; w, weeks.

2021,3,15.html). It can't be excluded that some undiagnosed SCID patients were among them.

The newborn screening program for SCID could enable identification of such infants. In Polish-German transborder NBS study (TREC and KREC assays) among 101 012 tested newborns (37 025 from Poland and 63 987 in Germany) two cases of atypical SCID/CID (both in Germany), two cases of autosomal recessive agammaglobulinemia (homozygous mutations in IGLL1 gene – one in Germany, one in Poland), one case of Nijmegen breakage syndrome (in Poland) were diagnosed. No ADA-SCID patient was detected, probably because of small group of newborns.

Standard curative option of ADA-SCID treatment, HSCT from MSD, is offered in Poland for children with HLA-matched sibling as a first choice (12). Based on data on HSCT performed between 1981 and 2009 in 16 centers worldwide, an overall survival rate (OS) in ADA-SCID depends on type of HSCT, being 86% for MSD, 81% for MFD, 66% for MUD and 43 for Haplo (13). Recent studies on large groups of patients: 131 ADA-SCID diagnosed in 1982-2017 (14) and 152 patients with SCID, including 43 ADA-SCID treated in 2006-2014 (15), showed higher OS in patients transplanted from MSD/MFD under 3.5 months of age and without active infections. According to the European Society for Immunodeficiencies (ESID), the European Society for Blood and Marrow Transplantation (EBMT), and European Reference Network on Rare Primary Immunodeficiency Autoinflammatory Autoimmune diseases (RITA), gene therapy should be considered in absence of a matched family donor (16). This therapeutic modality does not require donor search and it is associated with lower incidence of complications, including absence of graft versus host disease (GvHD) and procedure-related mortality. Limited availability and lack of reimbursement are the major obstacles for GT in Poland - it is available in only very few centers in Europe, but not in Poland, and the cost is extreme. Besides, longer duration of the procedure and time to achieve immune reconstitution are important clinical drawbacks. Frequency of therapy failure is similar: 10-20% in MSD HSCT and 5-20% in GT, but procedure-related mortality is 5.6 and 0% respectively (12). Some authors recommend considering GT as a first-line treatment, even if MSD is available, as this procedure abrogates any risk of alloreactivity (17). However, since treosulfan-based conditioning was introduced results for MUD, and Haplo HSCT are comparable with MSD in some centers and therefore recommended when there is no access to GT (18). GT is not readily available in Poland due to lack of specialized treatment center, as well as high cost of the procedure and need to run ERT until GT is carried out.

ERT is an effective treatment that has been used for over 3 decades. It should be implemented as soon as possible to stabilize the patient's general condition, detoxify the organism from accumulating purine metabolites, normalize transaminases, as well as treat pulmonary alveolar proteinosis and bone dysplasia. ERT is recommended only as an interim therapy before curative treatment, such as GT or HSCT, even if such therapy is available to improve patient's condition and prevent ongoing neuro- and hepatotoxicity (9). Recommended ERT duration ranges from few months to around 2 years, as ERT is associated with several adverse effects, including development of malignancies (often EBV-related lymphomas, mostly after 8-10 years of treatment), and ultimate failure due induction of enzyme neutralizing antibodies in about 10% of patients (1). An increase in B cell numbers is evident within the first month of treatment, and in T lymphocytes after 2-4 months, as was seen in our patients (19).

Costs of the drug and lack of reimbursement due to lack of drug registration in Poland and the European Union are the major obstacles in conducting ERT. As a consequence shortages in availability of ERT have been recorded in the past resulting in limited number of patients treated for short time.

Conclusions

Despite low numbers of ADA-SCID patients diagnosed in Poland, an advancement in early diagnostics was observed due to an increase in physicians' experience and improved availability of molecular diagnostics. Diagnosis in younger patients gives a chance for more effective treatment and prevention of irreversible damage.

ERT was effective in the analyzed cases, but high costs greatly limit access to the drug. Patients have restricted access to novel methods such as gene therapy, due to high cost and limitation to only few treatment centers.

Implementation of NBS for SCID in Poland could enable recognition of SCID, including ADA-SCID.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Bioethics Committee at the Children's Memorial Health Institute. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

ND-L, MP designed the concept of the manuscript. ND-L, BP, NB, MU, MP wrote the manuscript with contribution from all co-authors. BP did the immunological studies. ES did the adenosine deaminase activity. ND-L, KB-S, NB, EB, BW-K, KK, SK, AD, JG, MU, MP contributed to clinical data collection and

critical review. All authors contributed to the article and approved the submitted version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL ARTICLE

BCG Moreau Vaccine Safety Profile and NK Cells—Double Protection Against Disseminated BCG Infection in Retrospective Study of BCG Vaccination in 52 Polish Children with Severe Combined Immunodeficiency

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Abstract

Objectives The aim of the study was to estimate the rate of adverse reactions to live BCG Moreau vaccine, manufactured by Biomed in Poland, in severe combined immunodeficiency (SCID) patients.

Material The profiles of 52 SCID patients vaccinated at birth with BCG, hospitalized in Children's Memorial Health Institute, Warsaw (CMHI), in the years 1980–2015 were compared with those of 349 BCG-vaccinated SCID patients from other countries analyzed by Beatriz E. Marciano et al. in a retrospective study (Marciano et al. J Allergy Clin Immunol. 2014;133(4):1134–1141).

Results Significantly less disseminated BCG infections (10 out of 52 SCID, 19%) occurred in comparison with Marciano study—119 out of 349, 34% ($p = 0.0028$), with no death in patients treated with SCID anti-TB drug, except one in lethal condition. In our study, disseminated BCG infection was observed only in SCID with T-B+NK- phenotype and significantly lower NK cell counts ($p = 0.0161$). NK cells do not influence on the frequency of local BCG reaction. A significantly higher number of hematopoietic stem cells transplantations (HSCT) were performed in CMHI study ($p = 0.0001$). Anti-TB treatment with at least two medicines was provided.

Conclusion The BCG Moreau vaccine produced in Poland, with well-documented genetic characteristics, seems to be safer than other BCG substrains used in other regions of the world. Importantly, NK cells seem to play a role in protecting SCID patients against disseminated BCG complications, which NK- SCID patients are more prone to. HSCT and TB therapy could be relevant due to the patients' survival and the fact that they protect against BCG infection.

Keywords Primary immunodeficiencies · BCG Moreau vaccine · disseminated BCG infection · NKcells

Introduction

BCG vaccination administered at birth or shortly after birth is included in vaccination schedules in countries with a high prevalence of tuberculosis (TB). At present, three different BCG vaccine substrains, Danish 1331, Tokyo 172–1, and Russian BCG-I, are recommended by the World Health Organization (WHO) as International Reference Reagents (IRR). These three substrains constitute the major proportion of BCG vaccine production worldwide as they are supplied by

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the United Nations Children's Fund (UNICEF) [1–3]. An additional substrain, Moreau-RJ, was approved as a WHO IRR by the WHO Expert Committee on Biological Standardization meeting in 2012 [1, 2]. In general, the majority of locally produced BCG vaccine substrains have not been well-characterized. In terms of efficacy, no BCG substrain was found markedly superior to other strains and there is no global consensus on the choice of an optimal BCG substrain for general use [3–5].

Differences in reactogenicity between vaccines can be observed, and they relate to particular substrains. In 1955, the highly reactogenic BCG Danish vaccine was replaced in Poland by the locally produced BCG Moreau vaccine, a descendant of Brazilian BCG Moreau substrain. The BCG Danish 1331 vaccine and the Pasteur substrains have generally been considered reactogenic [6–10]. The mean risk of osteitis following BCG vaccination varies from country to country, with some reports indicating low incidence (e.g., 0.02 per million in Japan) and some presenting it as high (e.g., 1000 per million in Sweden and Finland) [5, 7–9]. In 1971, a dramatic rise in the incidence of osteitis observed in Sweden and Finland coincided with the replacement of the Gothenburg substrain of the BCG vaccine produced by the Swedish BCG Laboratory, based on the same Danish 1331 substrain [8]. In 1978, it was replaced by the BCG Glaxo substrain, which caused lower incidence of BCG osteitis in Finland and in Britain [6–8].

On the basis of the results obtained by Zapaśnik-Kobierska and Stopnicka, lymphadenitis complications in newborns vaccinated with BCG Moreau, BCG Danish 1331, or BCG Pasteur in the vaccinated cohort were found to stand at 0.3%, 2.4%, and 4.9%, respectively [11]. Furthermore, trials performed in Poland in 1972 revealed that the BCG Pasteur vaccine produced by the Lublin manufacturer was less reactogenic than that originally produced in France (18.6% versus 28.4%) [10]. Compared with the Pasteur substrains, the Tokyo and Moreau substrains are rarely associated with a high incidence of adverse events [5, 9, 10]. A recently published randomized clinical trial of a BCG vaccine produced with the Danish substrain 1331 SSI showed a higher incidence of lymphadenitis than expected, with a regional lymphadenitis rate of 6.1 per 1000 vaccinated [12]. Lymphadenitis following immunization with the BCG Moreau vaccine in Poland in the years 1994–2000 was estimated to be 0.2 per 1000 vaccinated. It was reported that the incidence rate of suppurative lymphadenitis in Poland was lower in comparison with other countries [13].

The frequency of fatal BCG disseminated infection is estimated at approximately 0.06–1.56 cases per million doses of vaccine administered, typically in primary immunodeficiency disease (PID) [14–34]. The majority of BCG disseminated infections have been reported in the literature in patients highly susceptible to mycobacterial diseases, well-defined SCID patients and those with Mendelian susceptibility to mycobacterial diseases (MSMD) [15–27]. The fatal outcome of BCG disseminated infection has also been reported in other PID less prone to BCG infection, such as chronic granulomatous diseases (CGD), hyper-IgE syndrome (HIES), X-linked hyper IGM syndrome (HIGM), nuclear factor (NF)-κB essential modifier (Nemo), and GATA2 deficiency [15, 18, 21, 22, 27–29]. One of the biggest groups of PID patients with BCG disseminated infection was retrospectively analyzed by Casanova et al. [14–16]. A multicenter, retrospective study comprising the data obtained from 28 centers in 17 different countries has recently been published by Marciano et al. This is the largest study published to date, involving 349 SCID patients vaccinated with BCG vaccine during the first months of life [35].

The aim of the present study was to estimate the frequency of complications and the risk associated with BCG vaccination in SCID patients hospitalized in the Department of Immunology, CMHI in Warsaw, over the period of 35 years, and to compare the results with those described in the retrospective study of SCID patients vaccinated with a variety of BCG vaccines containing different substrains used worldwide [35].

Material and Methods

As many as 1727 patients were diagnosed with PID in the Department of Immunology, CMHI, Warsaw, between 1980 and 2015. A total of 52 Caucasian origin SCID patients, born in Central-Eastern Poland, were vaccinated with BCG at birth. The incidence of BCG-associated infections following vaccination with the BCG Moreau vaccine produced by Biomed, Lublin, Poland, was analyzed in a group of SCID patients susceptible to BCG infection. BCG disseminated infections were diagnosed based on clinical, microbiological, and histopathological findings following ESID diagnostic criteria [19] (https://esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria). Localized BCG infection was referred to as a lesion inside the inoculation site (> 10 mm) and/or lymphadenitis limited to the region of the inoculation site while disseminated BCG infection was defined as a persistent process spreading over two or more regions beyond the inoculation site. The *Mycobacterium tuberculosis complex* molecular analysis was conducted using the PCR (MTD Gen-Probe) test and the analysis of *mycobacterium* culture was performed in a BACTEC 460 Tb or MGIT 960 system. Mutation analysis in PID patients was performed at the Department of Immunology, Erasmus MC in Rotterdam, Netherlands, in the Center for the Study of Primary Immunodeficiencies, Assistance Publique, Hopitaux de Paris, Necker Hospital, 75,015 Paris, France. Absolute numbers and percentages of circulating B and T subsets CD19/CD20, CD3, and natural

killer cells (NK-CD56/CD16) were assessed and compared with reference values established in age-matched groups of healthy children [36]. Statistical analyses were performed using STATISTICA v 10.0 and Microsoft Excel v 2007. Quantitative variables were characterized by the arithmetic mean of standard deviation or median or max/min. Qualitative variables were presented as counts and percentages. In order to check if a quantitative variable derived from the population of normal distribution, the Shapiro-Wilk test was used. Statistical significance of differences between groups (unpaired variables model) was processed with the Student *t* test or Mann-Whitney *U* test. The Chi-squared test and Fisher's exact test for independence was used for qualitative variables. In order to determine dependence, strength and direction between variables, correlation analysis was used by determining the Pearson or Spearman's correlation coefficients. In all calculations, the statistical significance level of $p = 0.05$ was used. The statistical analysis of the retrospective study of 52 SCID patients hospitalized at CMHI was compared with that of 349 SCID patients investigated by Beatriz E. Marciano and co-authors [35].

Ethics Statement

This study was approved by the Bioethics Committee of CMHI—resolution no 51/KBE/2017, 06 September 2017.

Results

In 52 SCID patients diagnosed in CMHI, the mean age at which first SCID symptoms occurred was 3.3 months (in the range of 1.0–24.0), the age of SCID diagnosis was 13.5 months (in the range of 1.0–63.0). In 19 out of 52 cases, the diagnosis with SCID was carried out at the age of 7–12 months, which was comparable with 94 patients ($p = 0.1510$) from the Marciano study.

Disseminated BCG infection occurred in 10 SCID patients out of 52 vaccinated with BCG, twelve localized complications were present in both group, separately or together with disseminated BCG infection. (Table S1). Disseminated BCG infection was diagnosed in 10 SCID patients with TB+NK- phenotype, in 6 with IL2RG mutation, in one patient with JAK3 deficiency and in 3 with unknown mutations. Disseminated BCG infection was not present in the group of TB-NK+SCID patients, including 16 patients with RAG1/2, 2 patients with IL7RA and 2 with Artemis mutation, 1 patient each with MHC II, ZAP-70, and Cernunnos, and in 10 patients with unknown mutations (Table S1). Among SCID patients, the clinical manifestation of disseminated BCG infection was recognized as meningitis in 1 patient, bronchopneumonia in 4, disseminated skin infection in 3, single skin abscess in 2, and osteomyelitis and/or pathological fractures occurred in 6 patients. Infiltrations in spleen and liver abscess were observed in 5 patients. No brain abscess, lymphadenitis, and other localization of BCG infection were observed. The infection was confirmed with the positive polymerase chain reaction (PCR) for the *Mycobacterium tuberculosis* complex and culture in 8 out of 10 patients. Following the diagnostic criteria for disseminated BCG infection, possible diagnosis of disseminated BCG infection was established in two patients with clinical symptoms and typical histopathologic changes with granulomas formation diagnosis [19] (https://esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria). In three infants, SCID diagnosis was established before 1985, when they were admitted in the terminal stage to the intensive care unit. Two of them died soon in the course of multiple organs insufficiency, postmortem examination found granulomas, which is the full field diagnosis criteria of possible form of disseminated BCG infection [19] (https://esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria). The third one, with a positive family history (death of 5 infants, boys) was admitted because of respiratory insufficiency and hepatosplenomegaly. Bronchopulmonary lavage samples were tested for *Mycobacterium tuberculosis* complex; positive PCR and culture results led to the diagnosis as a definitive form of the disease [19] (https://esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria). He died after 4 months, despite the administration of 5 anti-TB medications in a course of overwhelming BCG infection. The HSCT procedure was not considered because of serious condition of this child.

Disseminated BCG infections in 10 (19%) SCID patients hospitalized at CMHI constitute a significantly lower score in comparison with 119 (34%) in the Marciano study ($p = 0.0028$). However, in 12 (24%) SCID patients with localized BCG, the rate was higher, but not significantly, compared with the Marciano study's 60 (17%) ($p = 0.2417$). Mortality caused by BCG-associated complications in the Marciano cohort of SCID patients was higher, but not significantly: 46 (13%) deaths out of 349 ($p = 0.1724$). In the CMHI cohort, 3 (5%) deaths occurred before 1985.

The median absolute number of T cells at the time of SCID diagnosis in patients with disseminated BCG-associated complications was found to be insignificant ($p = 0.0915$), while the absolute number of B cells was significantly higher ($p = 0.0126$) and the number of NK cells was significantly lower ($p = 0.0161$) in the group with BCG complications in the CMHI study (Table S2).

Disseminated BCG-associated complications in the CMHI study were found only in 18 out of 52 SCID patients with a low number of NK cells ($p < 0.000001$), localized complications were observed in both groups; insignificantly higher in NK+SCID patients ($p = 0.4859$) (Table S3).

Dual drug anti-TB therapy (isoniazid and rifampicin) was administered to 17 patients (33%) with local BCG

complications. In 7 out of 10 SCID patients, BCG disseminated infection was successfully treated in all of them with 4 or 5 anti-TB drugs (isoniazid, rifampicin, ethambutol, ciprofloxacin, and aminoglycoside). In our study, the mean age at HSCT was 21.4 m. (a range of 4.0–96.0 m.). HSCT was administered in 43 patients (83%) in total vs 190 (54%) SCID patients in the Marciano study, the number being significantly higher in the CMHI study ($p = 0.0001$). Fifty-eight out of 349 SCID patients were diagnosed in Brazil, constitute the largest cohort of this study [35]. Similarly, fewer HSCT procedures were performed in SCID patients in Brazil, in 24 (46%) than at CMIH, in 43 (83%) ($p = 0.0001$). Polish and Brazilian cohorts were vaccinated at birth with a locally produced BCG Moreau vaccine [18, 19, 35].

In 7 SCID patients with BCG disseminated infection HSCT procedure was introduced: in 1 an unconditioned transplant from the matched related brother was done, in 4 patients HSCT from no HLA-matched related donor was performed, in 2 -HSCT from HLA-matched unrelated donor was done. In all 6 patients reduced-intensity conditioning was used, partial ablation with chemotherapy, serotherapy, antithymocyte globulin. This conditioning regimen prevented graft rejection and graft versus host disease (GVHD) with a satisfying stem cell engraftment, in one patient transient, moderate course of this disease was observed. Mixed chimerism concerning various cell lines was observed in 4 infants and is still present in one. Antimycobacterial treatment was discontinued within 8–15 months in all 7 treated patients, which coincided with immunological reconstitution. In 2 post-HSCT patients local and disseminated reactivation of BCG occurred within 3 months after transplantation. One of the patients was treated with 2 anti-TB drugs because of local BCG infection. He began to lose weight and a subfebrile condition developed. Infiltrations in spleen, liver, pathological fracture of the right hand third and fourth finger distal bones and disseminated skin abscess appeared. During the next 15 months, clinical improvement and full chimerism occurred. In the other patients with local BCG infection following HSCT, treatment with 2 anti-TB drugs was discontinued within the next few weeks. Enlargement and infiltration in the occultation site was observed and slowly dispersed during the 8 months of therapy with 3 anti-TB drugs.

Discussion

In the last three decades, numerous studies on BCG complications in PID patients have been published by national and regional or local centers. The cumulative study of 349 patients with SCID from 17 countries vaccinated with BCG constitutes the biggest record published to date [35]. The present paper compares the clinical course of disseminated BCG infection in 52 vaccinated SCID patients hospitalized at CMHI, Warsaw, with that documented in the study of Marciano et al. [35].

Reactogenicity of BCG Substrains

Throughout the years, a great number of publications on BCG-associated complications in PID patients susceptible to this infection have reflected the differences in the reactogenicity of various BCG vaccines produced with different substrains [14–34]. A summary of the earliest national retrospective studies performed in France between 1951 and 1994 reported 121 disseminated BCG infections in PID patients [15]. French studies conducted between 1974 and 1994 noted the deaths of 8 out of 16 SCID patients vaccinated with the BCG Pasteur substrain and 2 other patients vaccinated with the Glaxo and Copenhagen substrains, respectively [16]. A single-center French study conducted in the years 1970–1992 reported the occurrence of disseminated BCG infection in 10 out of 28 patients vaccinated with BCG in the group of 117 investigated PID patients [16]. In Canada, routine vaccination at birth with the BCG Pasteur Merieux Connaught Vaccine has been limited to some First Nation communities. In the years 1993–2001, four infants with SCID and one with HIV infection, vaccinated before the age of 3 months, developed disseminated BCG infection and subsequently died [30]. A retrospective study conducted between 2007 and 2012 in China presented the clinical outcome of PID patients vaccinated at birth with locally produced vaccines obtained from four different manufacturers using the BCG Danish substrain for production [21]. Fourteen out of 74 patients in this study, including 32 well-defined PID patients, developed disseminated BCG infection, two of SCID patients died. In the Czech Republic, 9 out of 12 SCID patients vaccinated at birth with a locally produced BCG Danish substrain vaccine developed disseminated BCG infection; five of them subsequently died [31]. Previous, as well as recent studies, demonstrate that the BCG Danish and Pasteur Merieux Connaught substrains are the most reactogenic substrains in both healthy and immunocompromised individuals [7–27]. Even in the study by Marciano et al., the Danish substrain gave the greatest, but not significant number of disseminated BCG complications [35].

The safety profile of the Polish Moreau BCG vaccine in SCID in comparison with other substrains used worldwide has been documented.

In Africa, where the BCG Danish substrain vaccine is widely used, the risk of disseminated BCG infection is several hundred-fold higher in HIV-infected infants compared to the documented risk in HIV-uninfected children [1, 37, 38]. Since 2004, WHO has not recommended BCG vaccination to neonates with HIV infection, vaccination should be delayed until anti-retroviral therapy has been initiated and they are immunologically stable [39, 40].

Polish BCG Moreau Substrain Vaccine

The Moreau BCG substrain used in vaccine production in Poland is a descendant of the parent *Mycobacterium bovis* BCG Moreau Rio de Janeiro substrain [41–44]. A retrospective study of a large cohort of Brazilian SCID patients vaccinated at birth with the BCG Moreau/Rio de Janeiro substrain vaccine manufactured in Brazil was published by Mazzuchelli et al. [20]. The majority of those patients were included in the Marciano et al. publication [35]. Out of 60 SCID patients vaccinated with BCG, 39 presented complications related to the BCG vaccine and disseminated BCG infection was diagnosed in 29 out of 60 (48.3%) [20]. By contrast, in our study disseminated BCG infection was observed only in 10 (19%) out of 52 SCID patients vaccinated with the Polish BCG Moreau substrain vaccine. Since various BCG substrains have been associated with different reactogenicity, genetic variations might also be involved in the level of potential BCG complications [42–44]. Krysztopa-Grzybowska et al. evaluated the genomic stability of the BCG Moreau substrain of the seed lots used in BCG vaccine production in Poland, demonstrating the reproducibility of genetic profiles [42–44]. The authors found that a defect in the biosynthesis of PDIM/PGL in the BCG Moreau substrain might reflect the lower level of reactogenicity of this vaccine. Additionally, unlike the Rio de Janeiro parent BCG substrain, the BCG Moreau substrain used in Poland does not harbor a deletion in *Rv3887c*, a region involved in the membrane transport protein which constitutes part of the ESX-2 type VII secretion system. The role of *Rv3887c* is not well known, but homology with the components of the ESX-1 secretion system might influence immunogenicity [45]. These findings suggest that the distribution of the BCG Moreau substrains for their subsequent use in vaccine production in different parts of the world might influence the divergent microevolution of the BCG Moreau substrain used in Brazil and Poland [42–45]. The documented genetic characteristics of the BCG Moreau vaccine produced in Poland and its good safety profile, as compared to other BCG vaccine substrains, are important attributes which should warrant its inclusion in the Expanded Programme on Immunization (EPI). Different BCG vaccine substrains have been obtained as a result of genetic changes which occurred during repeated subculture in different countries. Genetically divergent BCG vaccine substrains might be associated with different protective efficacy against tuberculosis, different rates of adverse events and the necessity of anti-TB treatment.

Therapy in SCID

Immunological reconstitution by HSCT and treatment of mycobacterial infection are the key elements of successful therapy in SCID patients. HSCT as the major type of intervention affecting the survival rates in patients with SCID was also confirmed by Marciano et al. [35]. In the SCID patients investigated in our study, significantly fewer cases of disseminated BCG infection were observed in comparison with the Marciano et al. study, which could be related to a significantly low number of HSCT procedures implemented in SCID patients in the latter [35]. The authors observed that considerably fewer HSCT procedures were implemented in the Brazilian cohort of the study. A separate study of 70 Brazilian SCID patients confirmed that fewer than half of the patients (42%) received HSCT; the mortality rate was as high as 35.5%, caused by BCG disseminated infection in at least 29% of cases [20]. BCG complications are the serious health problem in all SCID worldwide and are an indication for immediate anti-TB treatment and implementation of HSCT. Early diagnosis of the symptoms is crucial; without diagnosis and implementation of relevant treatment, the disseminated BCG disease may be the cause of death. BCG complication following HSCT is relatively rare. In all 7 patients with BCG disseminated infection, HSCT together with anti- TB therapy was attempted as the only available curative treatment, administered regardless of the occurrence of: related, unrelated donors, conditioned or unconditioned transplant, full chimerism or mixed chimerism. The practical recommendation resulting from our observations is not to discontinue anti-tuberculosis treatment in the post-transplant period, despite the increasing parameters of cellular immunity. Discontinuation of anti-TB treatment may take place when the clinical symptoms of BCG infection subside. What is critical in the treatment of both local and disseminated complications of BCG infection is the early implementation of at least two anti-TB drugs administered as soon as local BCG complications appear [19] (https://esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria). Anti-TB therapy is continued after HSCT, when local or disseminated symptoms are present or reactivation of BCG infection occurred, usually a few weeks after transplantation. Isoniazid and rifampicin constitute a base treatment in local complication, if disseminated process occurred after other anti –TB drugs were administered. In our group of SCID with BCG – associated complication, treated with anti-TB drugs, full recovery was observed, with no relapse during long-lasting observation, except one patient from early 80s in whom anti-TB treatment was ineffective, because of the severity of the overwhelming BCG infection.

Immune Defense Against *Mycobacterium bovis* BCG Infection

The immune response to *Mycobacterium tuberculosis* infection involves a complex cellular process which is not fully understood. Protection from disseminated disease in children is associated with a Th1-type response involving CD4+ T cells which produce interferon-gamma (IFN-γ). The study by Marciano et al. revealed that a lower number of T cells in SCID patients increased the probability of them developing BCG-associated complications. In our study, the number of T cells did not have

an impact on the occurrence of disseminated BCG in any type of patient diagnosed with SCID (Table S1,2). The number of B cells in SCID does not play a role in the defense against pathogens, since B cells, if present, do not produce antibodies [17].

NK cells can provide the primary source of IFN-γ production during blood exposure to the *Mycobacterium bovis* BCG substrain and thus contribute to the non-specific beneficial effects of BCG vaccination [46]. The production of IFN-γ by NK cells following direct contact with *Mycobacterium bovis* BCG in the absence of accessory cells has also been observed by other investigators [47]. The IL-12/IFN-gamma axis is crucial for protective immunity against Mycobacterium in humans and mice. The relative contribution of various human blood cell subsets and molecules to the production of or response to IL-12 and IFN-gamma showed that monocytes were probably the main producers of IL-12, and that NK and T cells produced similar amounts of IFN-gamma [47]. IFN-γ production by CD56 cells reflects the major function of NK cells, also presenting the immunotherapeutic benefits observed for *Mycobacterium bovis* BCG against malignancies [48]. It has also been demonstrated that BCG revaccination of adults with latent *Mycobacterium tuberculosis* infections induces long-lived BCG-reactive NK Cell responses [49]. The investigation of our 18 patients with SCID *RAG1* and *RAG,* and TB-NK+ revealed that the presence of NK cells may provide a defense against the development of disseminated BCG infection, which confirms the important role of NK cells in the defense against *Mycobacterium bovis* BCG infection. However, Marciano study shows that patients with and without BCG- associated complications were not significantly different in natural killer cell number [35].

Our findings demonstrate that the non-specific immune response evoked by NK cells protects against the overwhelming mycobacterial infection not only in RAG1/2 SCID deficiency patients, but also other SCIDs with the presence of NK cells (Table S1). In a multicenter study, in SCID patients with a recombination-activating gene RAG1/2 mutation, vaccinated at birth with BCG vaccines containing a variety of *Mycobacterium bovis* substrains, only local BCG complications appeared. In all but one patient, the absolute number of NK cells was within the normal range of 0.2–1.2 × 10E9/L [50]. However, BCG disseminated infection can rarely occur in TB-NK+ SCID patients with RAG1/2 mutation and patients vaccinated with the BCG Moscow-368 vaccine and the BCG Sofia SL 222 vaccine manufactured in Bulgaria, derived from the Moscow-368 substrain and used in Ukraine since 2014 [51]. The important role of innate immune response to mycobacterial infection has also been demonstrated in a group of newly described PID, particularly those with gain-of-function STAT1 mutation, where decreased STAT1-mediated IFN -γ responses impair immunity against mycobacterial infection [34, 52].

In our study, disseminated BCG infection was observed only in SCID patients with the IL2RG and JAK3 deficiency (T-B+NK-) (Table S1). Defects impairing cellular immune response, phagocytic function, and IFN-γ production are associated with BCG complications. Our findings demonstrate the major role played by the number of NK cells in preventing BCG complications as compared to the number of T cells.

In summary, for more than 50 years of the BCG Moreau vaccine being produced in Poland, with its confirmed genetic changes, it has proven to possess a well-documented safety profile, recently also confirmed in our paper, in comparison with the Rio de Janeiro parent BCG substrain and to the Danish and Pasteur substrains [41–44, 49, 50]. It was confirmed by significantly fewer number of complications with disseminated BCG in our SCID patients and in other less susceptible patients in comparison with different BCG vaccines substrains used worldwide [18, 19, 28, 33, 49, 50]. Our findings show that lower T cell numbers do not have a decisive effect on the spread of disseminated BCG infection, in contrast to the findings of Marciano et al., who demonstrated significantly low T cell numbers to be predictors of such a serious infection [35]. Furthermore, statistically higher NK cell counts in our study group were revealed to provide essential protection against disseminated BCG infection to all SCID patients.

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Compliance with Ethical Standards

This study was approved by the Bioethics Committee of CMHI—resolution no 51/KBE/2017, 06 September 2017.

Conflict of Interest The authors declare that they have no conflict of interest.

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The Clinical and Genetic Spectrum of 82 Patients With *RAG* Deficiency Including a c.256_257delAA Founder Variant in Slavic Countries

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Background: Variants in recombination-activating genes (*RAG*) are common genetic causes of autosomal recessive forms of combined immunodeficiencies (CID) ranging from severe combined immunodeficiency (SCID), Omenn syndrome (OS), leaky SCID, and CID with granulomas and/or autoimmunity (CID-G/AI), and even milder presentation with antibody deficiency.

Objective: We aim to estimate the incidence, clinical presentation, genetic variability, and treatment outcome with geographic distribution of patients with the *RAG* defects in populations inhabiting South, West, and East Slavic countries.

Methods: Demographic, clinical, and laboratory data were collected from *RAG*-deficient patients of Slavic origin via chart review, retrospectively. Recombinase activity was determined *in vitro* by flow cytometry-based assay.

Results: Based on the clinical and immunologic phenotype, our cohort of 82 patients from 68 families represented a wide spectrum of *RAG* deficiencies, including SCID ($n = 20$), OS ($n = 37$), and LS/CID ($n = 25$) phenotypes. Sixty-seven (81.7%) patients carried *RAG1* and 15 patients (18.3%) carried *RAG2* biallelic variants. We estimate that the minimal annual incidence of *RAG* deficiency in Slavic countries varies between 1 in 180,000 and 1 in 300,000 live births, and it may vary secondary to health care disparities in these regions. In our cohort, 70% ($n = 47$) of patients with *RAG1* variants carried p.K86Vfs*33 (c.256_257delAA) allele, either in homozygous ($n = 18$, 27%) or in compound heterozygous ($n = 29$, 43%) form. The majority (77%) of patients with homozygous *RAG1* p.K86Vfs*33 variant originated from Vistula watershed area in Central and Eastern Poland, and compound heterozygote cases were distributed among all Slavic countries except Bulgaria. Clinical and immunological presentation of homozygous *RAG1* p.K86Vfs*33 cases was highly diverse (SCID, OS, and AS/CID) suggestive of strong influence of additional genetic and/or epigenetic factors in shaping the final phenotype.

Conclusion: We propose that *RAG1* p.K86Vfs*33 is a founder variant originating from the Vistula watershed region in Poland, which may explain a high proportion of homozygous cases from Central and Eastern Poland and the presence of the variant in all Slavs. Our studies in this cohort of *RAG1* founder variants confirm that clinical and immunological phenotypes only partially depend on the underlying genetic defect. As access to HSCT is improving among RAG-deficient patients in Eastern Europe, we anticipate improvements in survival.

Keywords: *RAG1*, *RAG2*, primary immunodeficiency, geographic distribution, incidence, Slavic children

INTRODUCTION

Recombination-activating gene 1 (*RAG1*) and 2 (*RAG2*) encode lymphoid-specific proteins that are expressed during the early stages of T-cell and B-cell development and initiate the process of V(D)J recombination by introducing DNA double-strand breaks (DSBs) for recognizing millions of possible antigens (1). Genotype–phenotype correlation is strong, as null variants of *RAG1* and *RAG2* genes result in the T-B- severe combined immune deficiency (SCID) phenotype, whereas hypomorphic *RAG* variants have been associated with distinct clinical entities including Omenn syndrome

(OS) and combined immunodeficiency with granuloma and/or autoimmunity (CID/G-AI) with herpesvirus infections and lymphoproliferation (2–5) and with selective polysaccharides antibody deficiency (6). Furthermore, in the era of widespread next-generation sequencing, RAG deficiency is being identified among adults with variants of antibody deficiencies with a frequency of 1 in 500 patients (7).

The *RAG1* and *RAG2* genes are polymorphic. Described clinical phenotypes are associated with a variety of variants including non-sense, frameshift, in-frame deletion or insertion, and missense variants of the *RAG1* and *RAG2* genes that affect various domains of the proteins (1). Among numerous *RAG* variants, some of them were observed in a Jewish population with a high rate of consanguineous marriages (8). In our previous report of 11 OS patients from the East Slavic regions, we described the high rate of c.256_257delAA (p.K86Vfs*33) in the *RAG1* gene ($n = 4$, 50%) (9). This variant was also observed in Polish and Serbian patients with OS and SCID phenotypes (10–13), which suggests a founder effect.

Currently, there is no published systematic evaluation of Slavic patients with *RAG* deficiency. In this report, we aim to describe the genetic landscape of *RAG* deficiency by estimating the incidence, genetic diversity, clinical and immunological presentation, and survival rate of a large cohort of Slavic patients. In addition, we focus on *RAG1* p.K86Vfs*33 as a candidate founder variant among Slavic patients by studying the geographic distribution of allele and genotype frequencies of *RAG1* p.K86Vfs*33 mutation among patients in major Slavic populations.

MATERIALS AND METHODS

Patients and Kindreds

Patients with pathogenic *RAG* variants were recruited retrospectively for this study through extensive collaboration with clinical immunologists who collected the data of national primary immunodeficiency (PID) registries from *East Slavs* (Russia, Belarus, and Ukraine), *West Slavs* (Poland, Czech Republic, and Slovakia), and *South Slavs* (Serbia, Slovenia, Montenegro, Croatia, and Bulgaria). The patients were divided into ethnic groups (East, West, and South Slavs) according to their country of origin.

Ethics Statement

Informed consent forms were signed by the parents as requested and approved by the institutional review boards of various institutions involved. The protocol of study was approved by the institutional review board of Belarusian Research Center for Pediatric Oncology, Hematology, and Immunology (IRB0012-2015).

Study Design

A detailed questionnaire was completed by the physicians including demographic data (gender, country, place of birth, and year of birth), variants, and clinical data (age at disease manifestation, age at diagnosis, clinical and immunologic phenotype, and outcome).

Assignment to Phenotypic Subgroups

Corresponding clinicians from each country assigned patients to one of the following subgroups—SCID, OS, atypical SCID (AS), or combined immunodeficiency (CID)—on the basis of clinical and immunologic phenotype, and age at manifestation.

The Primary Immunodeficiency Treatment Consortium (PIDTC) diagnostic subdivides SCID into three categories: typical SCID, atypical SCID, and OS, based on total T-cell count, lymphocyte proliferation, presence of maternal T cells, characteristic phenotypic features, and gene defects. CID was classified by late and mild clinical presentation according to published criteria (4, 14). Roifman et al. distinguished CID from SCID based on a total CD3+ T-cell count of >500/μl (15). The 2019 ESID criteria for diagnosis of CID requires a symptomatic patient (infections, immune dysregulation) or history of affected family members with immune phenotype of two of the four parameters (low CD3 or CD4 or CD8 T cells, low naïve CD4 and/or CD8 T cells, elevated γδ T cells, and reduced proliferation to mitogen or TCR stimulation). This ESID 2019 criterion does not discuss underlying genetic defects for CID patients (16). The IUIS 2020 classification does list genetic defects for CID but fails to include *RAG* deficiency in this category (17).

To estimate the number of newborns with *RAG* variant in each country, the information about affected siblings in families with *RAG* variants was collected through hospital or personal records of affected families.

Mapping of Variants

Mapping was performed by the *ArcGIS 10.5* program on the map of Central–Eastern Europe and Russia.

RAG1/RAG2 Sequencing

Gene sequencing was performed using standard techniques (panel-based, Sanger) in local laboratories in Europe. The reference DNA and protein sequences for *RAG1* are from NIH RefSeq NM_000448.2 and NP_000439.1 and those for *RAG2* are from NM_001243785.1 and NP_001230714.1, respectively.

Measurement of Recombination Activity

For single-allele mutation, assay is based on a v-Abl *Rag1/Rag2*$^{-/-}$ pro-B cell line containing a single pMX-INV integrated cassette (18).

TRECs and KRECs

TRECs and KRECs quantity was measured by RQ-PCR using plasmid standards, and ALB was taken as an internal control according to manufacturer instructions.

Statistical Analysis

Allele frequencies were compared by chi-square test or Fisher's exact tests. Kaplan–Meier curve was computed for survival post-hematopoietic stem cell transplant (HSCT). $p < 0.05$ was considered significant. Statistical analysis was performed using GraphPad Prism version 6.0 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Population Demographics

We retrospectively collected and studied 82 patients with RAG deficiency from 67 families in 12 countries, born in the period of 1992–2018. Thirty-five of 82 (43%) patients were reported previously (9–13, 19–23). Distribution was even between males ($n = 40$, 49%) and females ($n = 42$, 51%). The median age at diagnosis of *RAG* was 1.3 years (range, 1 day to 24 years).

At the time of manuscript submission (March 2020), 43 of the 82 patients died (52%, 8 out of 43 after HSCT). All patients alive received HSCT (ranging from 1 to 18 years) except three females with LS/CID presentation from Poland (42_f), Bulgaria (54_f), and Czech Republic (55_f) who are alive without transplantation at the ages of 3, 6, and 25 (**Supplementary Tables E1** and **E2** in this article's Online Repository).

The largest group of patients ($n = 40$, 48.8%) was West Slavs from Poland ($n = 29$, $n = 1$ from Lithuania, Polish origin), Czech Republic ($n = 8$) and Slovakia ($n = 2$). The second largest group was East Slavs ($n = 30$, 36.5%) from Russia ($n = 24$), Ukraine ($n = 4$), and Belarus ($n = 2$). The smallest group was South Slavs ($n = 12$, 14.6%) from Serbia ($n = 6$), Slovenia ($n = 3$), Croatia ($n = 1$), Montenegro ($n = 1$), and Bulgaria ($n = 1$).

Clinical, Immunological, and Genetic Phenotypes

All patients have genetically confirmed pathogenic *RAG1 or RAG2* variants. *RAG1* variants were detected in 67 patients in 54 families (82%) and *RAG2* variants were detected in 15 patients from 13 families (18%). Nineteen children (23%) out of 16 families and 8 children (10%) out of 7 families were homozygotes while 47 patients (59%) out of 39 families and 8 patients (10%) out of 6 families were compound heterozygotes for *RAG1* and *RAG2* variants, respectively (**Supplementary Tables E1** and **E3**).

Clinical phenotypes, as determined by attending clinician following immunological and clinical guidelines, included SCID ($n = 20$), OS ($n = 37$), AS ($n = 21$), and CID ($n = 4$) with highly mixed immune phenotypes (different numbers of T cells in all groups and domination of B+ phenotype in AS group); however, *in vitro* relative recombinase activity showed good correlation between genotype and clinical cathegories. (**Figure 1C**, and **Supplementary Tables E1** and **E3** in this article's Online Repository). Four (10%) out of 41 vaccinated SCID patients with RAG1/2 variants developed BCGosis infection, and 12 (3%) BCGitis were noted. As expected, the AS group had increase frequency of autoimmune cytopenias and history of herpesvirus infections (**Figure 1A,B** and **Supplementary Table E2**).

Patients With RAG1 Variants

Patients with RAG1 variants are described in **Supplementary Tables E1** and **E2**. Most patients presented with OS (46%) (31 children from 26 families) followed by AS (28%) (19 patients from 16 families), SCID (19%) (13 patients from 11 families), and CID (6%) (4 patients from 4 families).

Patients diagnosed as having OS and SCID were characterized by classical clinical and immunologic phenotype (**Supplementary Table E1**). Their clinical phenotype allowed for diagnosis before the age of 1 year. On the other hand, the median age at the time of diagnosis in AS and CID was 4 years (± 6.02 years) and ranged from 5 months to 24 years (**Supplementary Tables E1** and **E2**).

In the AS group, infectious complications were notable for high prevalence of CMV infection ($n = 9$, 42%) in both localized ($n = 2$, retinitis) and systemic forms ($n = 7$). Among patients who received BCG vaccination, only a fraction (4 of 18, 22%) developed BCG infections (five with BCGitis and one with BCGosis) (**Supplementary Table E2** and **Figure 1A**).

Autoimmune findings in the AS and CID group were observed in 12/19 and 4/4 of patients (**Supplementary Table E2**), with autoimmune cytopenia being most prevalent in AS ($n = 11/19$, 58%) including autoimmune hemolytic anemia ($n = 8$), isolated immune thrombocytopenia (ITP) ($n = 2$), and multiple lineage cytopenia ($n = 2$). Autoimmune colitis was diagnosed in 9% (2/19) of AS patients with autoimmunity. In the CID group, one patient had skin granuloma, one developed vasculitis, and one had vitiligo (**Supplementary Table E2** and **Figure 1B**).

Immunological phenotype of the AS group is summarized in **Supplementary Table E1**. Curiously, high fraction of the patients had preserved fraction of B cells (>65% in 13 of 19 patients) at the time of diagnosis. However, 2 of 7 (28%) patients had complete absence of KRECs. This suggests abnormal B cell maturation even in the setting of preserved fraction of total B cells. Four patients presented with normal IgG level, and one had IgA deficiency at the age of 4 years that progressed to agammaglobulinemia with age.

In this study, we additionally determined the recombination activity of patient's RAG1/RAG2 mutant proteins. Of the *RAG* variants, 38 variants were unique, including frameshift ($n = 7$), non-sense ($n = 4$), and missense ($n = 27$), and 23 *RAG1* mutations were new in our cohort, and for all of them, recombination activity was determined (**Supplementary Tables E1** and **E2**). We had the possibility to demonstrate that mutations associated with SCID and OS had lower residual activity than mutations detected in patients with less severe clinical presentations (AS and CID) (**Figure 1C**). Nevertheless, genotype–phenotype correlation was less evident in our cohort; we have not found significant differences between OS/SCID and AI/CID in clinical and laboratory markers. Among the patients with homozygous *RAG1* p.K86Vfs*33 (c.256_257delAA) variant ($n = 18$), the clinical and immunological presentation was highly diverse, 4 patients presented as OS, 1 as SCID, and 13 as AS (**Supplementary Tables E1** and **E2**); these findings were supported by **Supplementary Table E1** Pasic et al. (12). This is suggestive of the strong influence of other genetic and/or epigenetic factors in shaping the final clinical and immunological phenotype.

RAG1 Variants

Homozygous *RAG1* variants were detected in 18 families, but consanguinity was identified only in 4 (22%) [3 families from Poland (2a_m, 2b_m; 39_f; 41a_m, 41b_m) and 1 from Serbia (44a_m, 44b_f, 44c_m)].

The most frequent genetic *RAG1* anomaly among patients of all groups and in 10 out of 11 countries was *RAG1* p.K86Vfs*33 (c.256_257delAA) (**Supplementary Table E1**). This

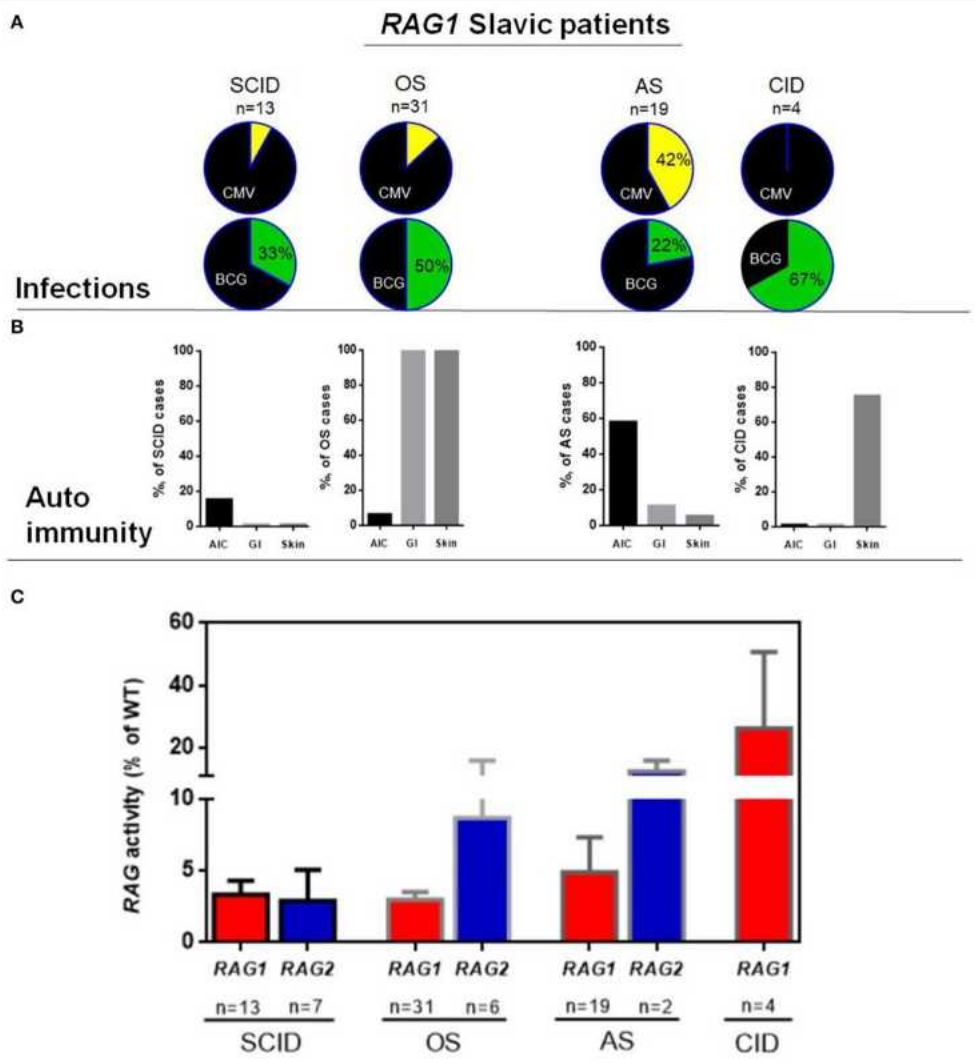


FIGURE 1 | Characteristics of RAG Slavic cohort. **(A)** Infectious complications in *RAG1* patients, upper row CMV in four groups, lower row BCG complications among patients, who received BCG vaccination. **(B)** Frequency of autoimmunity in 4 groups of *RAG1* deficiency. **(C)** Recombinase activity of *RAG1/RAG2* variants for the 82 patients divided into four major clinical presentations for *RAG1* and three clinical presentations for *RAG2*.

deletion occurred in one or both alleles in 22 out of 30 patients with OS, in 7 out of 13 SCID patients, and in 16 out of 19 patients with AI and 2/4 CID phenotype. Because of the enrichment of the p.K86Vfs*33 variant among all cohorts, we calculated the frequency of this allele among East, West, and South Slavic patients (**Figures 2A–F**) and their families (data not shown). Nine families had two or three children with immunodeficiency, and therefore, we conducted a separate analysis of families and analyzed frequency between countries counting one case per family.

Patients of South and West Slavic origin demonstrated the highest frequency of c.256_257delAA allele (**Figures 2C,E**). We analyzed the relative frequency of this predominant allele among Slavic patients and families. The differences in the allele frequency of c.256_257delAA frequency among the patients ($\chi^2 = 8.4$; $p = 0.015$) and the families ($\chi^2 = 22.8$; $p < 0.0001$) of the East/West/South Slavs proved to be statistically significant. In pairwise comparisons, the West Slavic population had a higher relative frequency of this allele than the East Slavic (families $p = 0.0006$; patients $p = 0.006$), but it did not differ from the South Slavic population (families $p = 0.14$; patients $p = 0.81$).

Besides the analysis of different Slavic cohorts, we calculated the contribution of each country to the overall frequency of *RAG1* c.256_257delAA allele and showed the highest occurrence in Poland (**Figure 2D**). *RAG1* c.256_257delAA allele (p.K86Vfs*33) was present in over 50% of *RAG1*-deficient patients from Serbia,

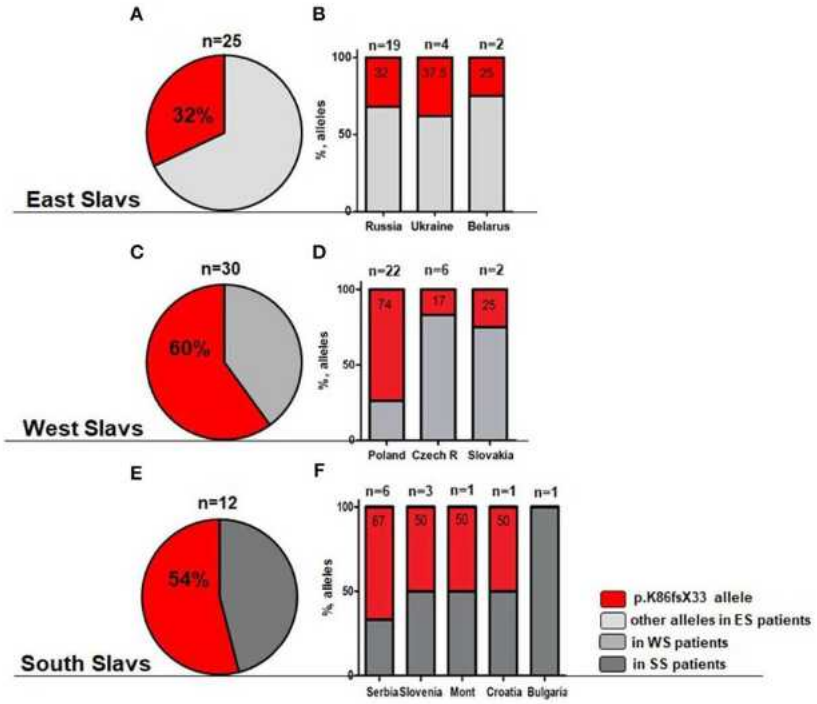


FIGURE 2 | Overall frequency of c.256_257delAA allele (p.K86Vfs*33) among all cohort of *RAG1* patients. **(A,B)** Light gray—East Slavs (ES); **(C,D)** Gray—West Slavs (WS); **(E,F)** Dark gray—South Slavs (SS); **(B,D,F)** Percentage of p.K86Vfs*33 allele in studied countries. In Montenegro and Croatia, only one patient was studied.

Slovenia, Montenegro, and Croatia, but extrapolating this finding to the whole South Slavic population is premature because only one patient was from Croatia and Montenegro (**Figure 2F**).

Among East and South Slavs, we observed only two families (one from Ukraine and one from Serbia) where p.K86Vfs*33 was detected in a homozygous constitution, but consanguinity was proved only in the Serbian patients. The Polish cohort of families showed 12 homozygous genotypes among 18 *RAG1* positive families, with only three cases of proven consanguinity.

Due to the high frequency of the *RAG1* p.K86Vfs*33 variant in Poland and homozygous genotypes, we studied its geographical distribution in West, East, and South Slavic populations. Our findings are illustrated on the map of East Europe and Russian Federation based on the patient's place of origin (one patient from each family) (**Figure 3**).

We studied the distribution of *RAG1* p.K86Vfs*33 variant in 11 out of 13 Slavic ethnic groups and it was widespread and found in 10 out of 11 countries except Bulgaria (**Figure 3**). The occurrence of p.K86Vfs*33 was observed from Ljubljana (Slovenia) in the West to Vladivostok in the East and from Moscow in the North to Cetinje (Montenegro) in the South (**Figures 3**, **4**). One patient who originated from Slovenia was born in Austria (Graz), but after delivery, he was treated in the homeland of Slovenia (**Figure 3**).

The geographic center of the presumed origin of the *RAG1* p.K86Vfs*33 variant corresponds to the Vistula watershed area of Central and Eastern region in Poland in which the variant was the most frequent (**Figure 3B**) and suggestive of shared origin for the p.K86Vfs*33 alleles in all Slavic populations.

Due to a small number of cases, gradual decrease in the prevalence of p.K86Vfs*33 among East and South Slavs could not be determined, but it is clearly shown that heterozygous families are spread among other Slavic countries from the Polish area of homozygosity (**Figure 3**).

The broadest area of East Slavs in Russia is located mainly in the European part of the Russian Federation (25). In our cohort of patients from Russia, the majority (11/19) is concentrated on the territory west of the Ural Mountains, but 8 patients from 5 families were born in Siberia and the Russian Far East, and all inherited our studied variant (**Figure 4**). This finding does not contradict our assumption that p.K86Vfs*33 has a Slavic origin. According to the map of the resettlement of peoples in Russia, most Russians live in the central part or in the south and northwest of Russia as well as in the Ural region. To the east of the Ural Mountains, Russians live predominantly near the southern border of Russia, which is shown in **Figure 3** and in **Figure 4** as a light gray area on the white map of the Russian Federation.

Patients with RAG2 Variants

Patients with RAG2 variants were detected only in three countries among West (Poland and Czech Republic) and East Slavs (Russia) (**Supplementary Table E3**). Among 15 patients from 13 families, 7/15 presented as SCID, 6/15 presented as OS,

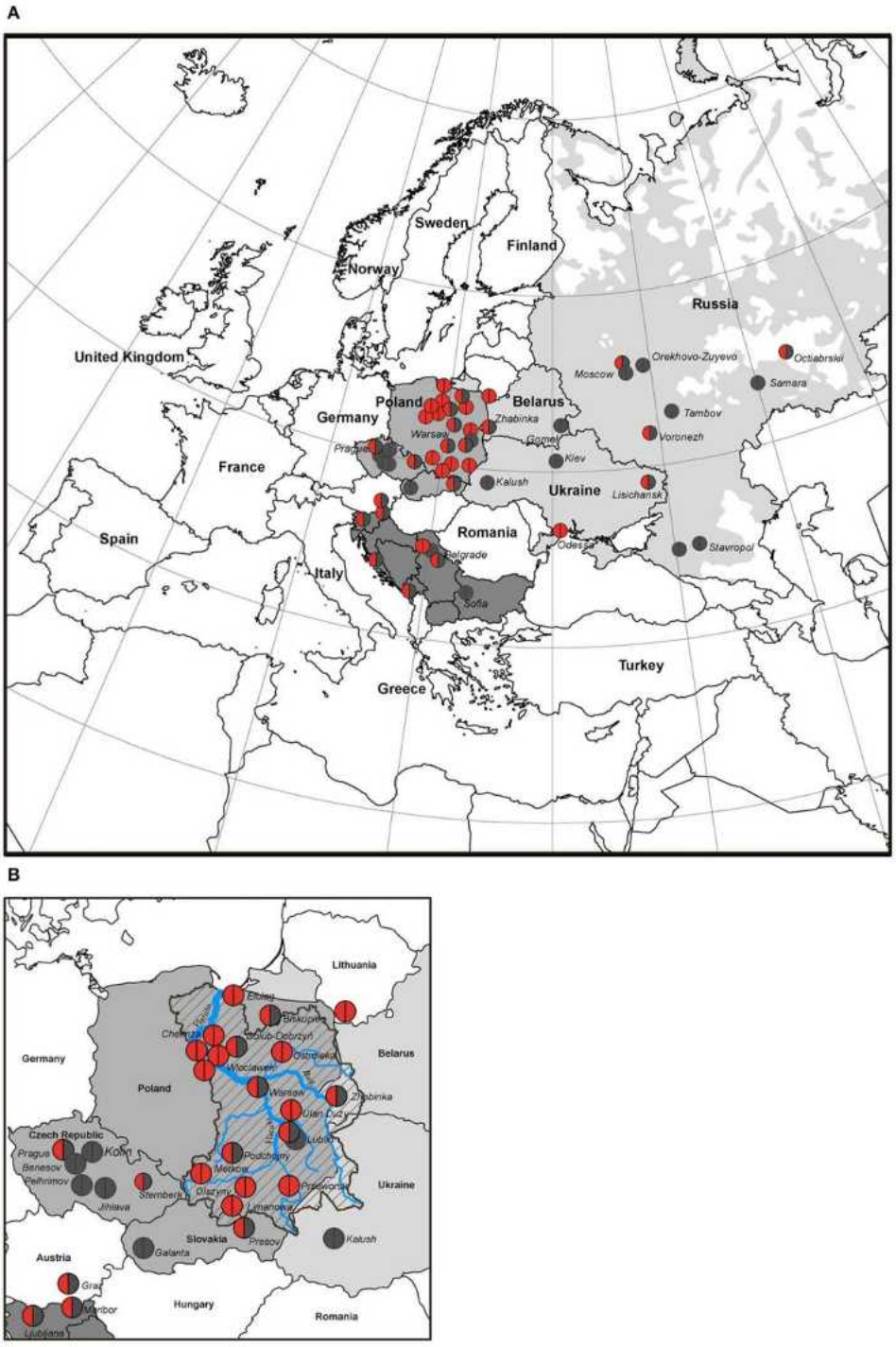


FIGURE 3 | Distribution map of families with *RAG1* p.K86Vfs*33 variant in Slavic countries (50 of 55 families). **(A)** The birthplace of the patients is indicated by the location of the circles; homozygous p.K86Vfs*33 variant is represented by red circles; heterozygous p.K86Vfs*33 variant is half red/half gray, and other variants are gray. **(B)** Map of the Western Slavs; the Vistula River basin is overlaid on the map of Poland. The blue line is the Vistula River, a zone marked by an oblique black line—the Vistula River basin, the geographic area coincides with the region of the largest concentration of families where patients with p.K86Vfs*33 homozygous variants were born.

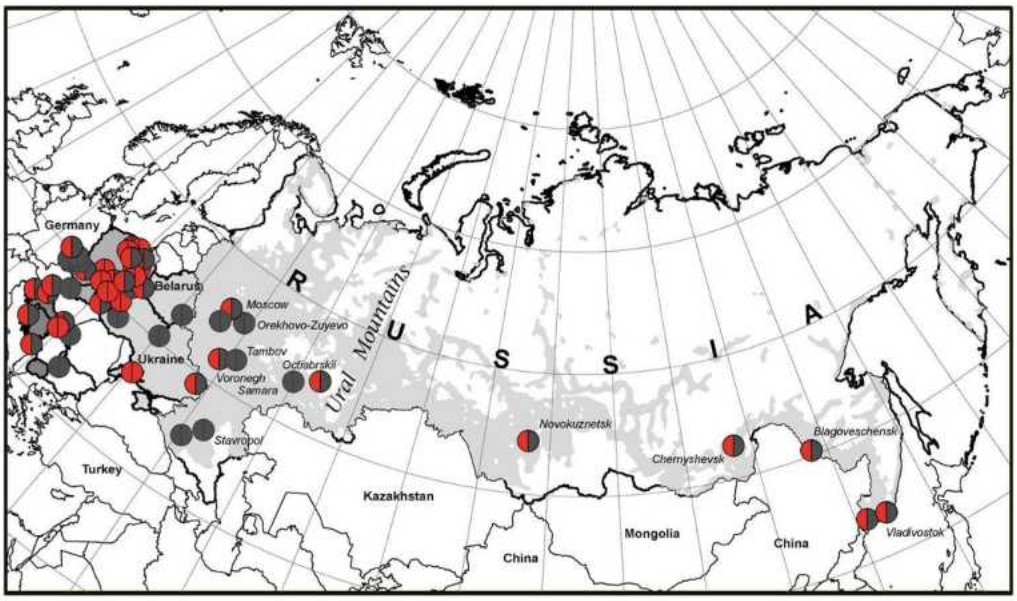


FIGURE 4 | The geographic picture of the p.K86Vfs*33 distribution among East Slavs. Light gray zone on the map of Russia is the territory of predominant Russian settlement (24).

and 2/15 presented as AS. Two Polish females manifested as atypical SCID/CID and one had a sister with SCID (8a_ f). In one Russian family, one male presented as OS (3b_ m) and one presented as SCID (3a_ m).

Females with late manifestation of *RAG2* variant presented with encephalitis, HSV, bronchitis, pneumonia, neutropenia and pneumonia (8b_f), local BCGitis, and failure to thrive (12_f).

Interestingly, the obtained data showed that 53% (8/15) of patients had homozygous variants in the *RAG2* gene, but consanguinity was established only in one family history (1_m). Despite the marked diversity of the genotypes, there are some repeated variants, p.Y434H in Russian, p.R229Q in Polish and Czech children (in homo and in heterozygous state), and p.W453R only among Polish patients. However, an obvious dominating region among variants was not established due to a small number of the studied cases.

The *RAG2* variants in Slavic cohort showed a wide range of recombination activity (**Supplementary Table E3**). Mutations associated with severe combined immune deficiency and OS had lower activity than those detected in patients with atypical SCID presentations (**Supplementary Table E3** and **Figure 1D**). Our data support genotype–phenotype correlation in *RAG2* deficiency in Slavic children (18).

The Average Annual Incidence of Slavic Patients With *RAG* Deficiency and Survival

We examined the incidence of all types of *RAG* deficiency together (*RAG1/RAG2* genotype; OS/SCID/AS/CID phenotype) in each country during different periods. The average incidence of *RAG* deficiency was calculated based on the number of all diagnosed patients and their confirmed siblings and the number of live births in studied countries during the period from the year of the first registered patient in the country to the last one (**Table 1**). The average 10-year incidence was estimated by the number of all patients of Slavic origin diagnosed since 2008 to 2017 in each country (**Table 1**, last column).

The obtained data showed that frequency of diagnosed *RAG* deficiency ranged from 1 per 75,000 in Slovenia to one per 1,100,000–1,600,000 live births in Russia and Ukraine.

A wide range in frequency among Slavic countries suggest underdiagnosis of atypical late-onset cases. We compared the two largest *RAG* cohorts from Russia ($n = 24$) and Poland ($n = 30$). In further analysis, we graphically illustrated the number of diagnosed patients in the two countries (the age at diagnosis was added to the year of birth) and showed that Russia achieved great success in identification of *RAG* patients in the last 8 years (**Figure 5A**, lower). Exponential growth (**Figure 5A**, upper) was observed; 19 out of 24 *RAG* patients were diagnosed from 2011 to 2017, and their geography covered the entire country (**Figure 4**). Poland, as the largest West Slav country, uses advanced immunologic diagnostics services and one to three patients have been confirmed approximately each year since 1999 (**Figure 5B**).

Despite an increased awareness for SCID and OS variants and improved access for HSCT in most Slavic countries, the mortality rate of *RAG* patients is still high in Eastern Europe. We studied relative mortality rate among OS/SCID/CID groups of patients with *RAG* deficiency (**Figure 5C**—ES, **Figure 5D**—WS, and **Figure 5E**—SS). Neither comparison of transplanted patients in two groups (WS and ES) as biggest cohorts revealed

TABLE 1 | Average annual incidence of *RAG* deficiency in Slavic countries.

No	Country	Number of patients with RAG	Number of families	Whole period	Sum of newborns for the period	The average annual incidence frequency	Number of patients in 10 year period*	Sum of newborns for the 10-years period	The average 10-years incidence*
EAST SLAVS									
1	Russia	24	18	2002–2017	26 557 598	1:1 106 566	18	17 553 840	1:975 213
2	Ukraine	4	4	2004–2013	4 834 300	1:1 208 575	3	4 795 200	1:1 598 400
3	Belarus	2	2	1998–2009	1 145 223	1:572 611	1	1 127 193	-
WEST SLAVS									
4	Poland	28	23	1999–2017	7 201 693	1:257 203	11	3 904 235	1:354 930
5	Czech Republic	8	8	1993–2016	2 395 156	1:299 395	6	1 126 990	**1:187 832**
6	Slovakia	2	2	2007–2015	515 219	1:257 609	2	572 776	1:286 388
SOUTH SLAVS									
7	Serbia	6	3	1992–2011	1 529 226	1:254 871	1	671 049	-
8	Slovenia	3	3	2003–2013	223 206	**1:74 402**	1	212 986	-
9	Montenegro	1	1	1995	9 492	-	0	76 785	-
10	Croatia	1	1	2010	43 361	-	1	411 277	-
11	Bulgaria	1	1	2013	66 578	-	1	715 571	-

*Bold values indicate the highest average frequency of RAG deficiency. *10-year period is 2008–2017.*

any statistically significant difference in survival (**Figure 5F**). Additionally, we compared the results of HSCT before and after 2011, due to changing the protocols in last years and we were able to show that survival of East Slavic patients became much better after 2011 and reached the same percentage as in West Slavs (**Figures 5G,H**). We suggest that poor outcome in HSCT before 2011 (three out of five died after HSCT) in East Slavs was due to delayed diagnosis in AS/CID patients and associated complications (chronic infections and immune dysregulation) (**Supplementary Figure E1C**). Patients with OS were transplanted with a high rate of survival in ES/WS/SS (**Supplementary Figure E1B**) and with SCID in ES/WS countries (**Supplementary Figure E1D**).

DISCUSSION

Several cohort studies with international collaborators are published, and a recent review of literature enumerate the number of published cases with *RAG* deficiency over 400 (2, 4, 7–9, 13, 14, 23). Among the population with high rate of consanguity, founder variants for *RAG* genes were proposed among the Amish and in the Middle East (26–28).

In this study, we present the largest geographically defined cohort of patients with *RAG* deficiency. Our patients are predominantly of Slavic origin from 11 European countries retrospectively collected in a 27-year period (1992–2018). In our current and previous study of patients with *RAG1* p.K86Vfs*33 (c.256_257delAA), we confirmed that different clinical phenotypes can been found in patients with identical variants even within the same family (9, 13). Family studies for homozygous *RAG1* p.S480G and *RAG2* p.M459L were also reported in consanguineous marriages of Arabic descent (29, 30). Furthermore, similar non-familiar findings were reported with patients with *RAG1* c.519delT (p.E174SfsX27) (13). Among all these reports, our study with 18 homozygous patients *RAG1* p.K86Vfs*33 (c.256_257delAA) is the largest in the literature within a geographically linked population.

The data from California's SCID newborn screening identified *RAG1/2* variants in 28.6% of SCID/OS cases, with an incidence of about 1:250,000 (31). In populations with domination of consanguineous marriages, *RAG* deficiency was diagnosed in 48% (21/44) of Turkish (32) and 51% (19/37) of Iranian SCID patients (33). The incidence of *RAG* deficiency is difficult to establish, due to the highly variable phenotype, late age of manifestation of hypomorphic variants, and different level of diagnostics capacities in different countries (5). Recently, it was identified that RAG deficiency among adults with a variety of antibody abnormalities occurs with a frequency of 1 in 500 patients with any variant of antibody deficiency (7). That is much higher than the expected number of *RAG1/2* SCID or OS cases. Based on our data reported in this study, we determined that the minimal incidence of *RAG* deficiency varied from 1:189,000 in the Czech Republic to 1:1,200,000 in Ukraine; the average was 1 per 190,000–300,000 live newborns.

It is still unclear if all *RAG* patients with AS/CID phenotype (with residual recombination activity) can be detected by newborn screening (NBS) (14). Retrospective testing of NBS card showed absence of TRECs in case reports (34). Current observation showed that KREC level can support and underlying *RAG* deficiency is relevant and further justifies the consideration of KREC in universal NBS for SCID and AS/CID. Our retrospective data suggest that most of the patients with late-onset forms of *RAG* deficiency (CID) are likely missed, but the level of discovery of SCID/OS patients is reasonable in most Slavic countries. There is a great need for early identification of these patients before infectious and autoimmune complications occur. The availability of next-generation sequencing and the

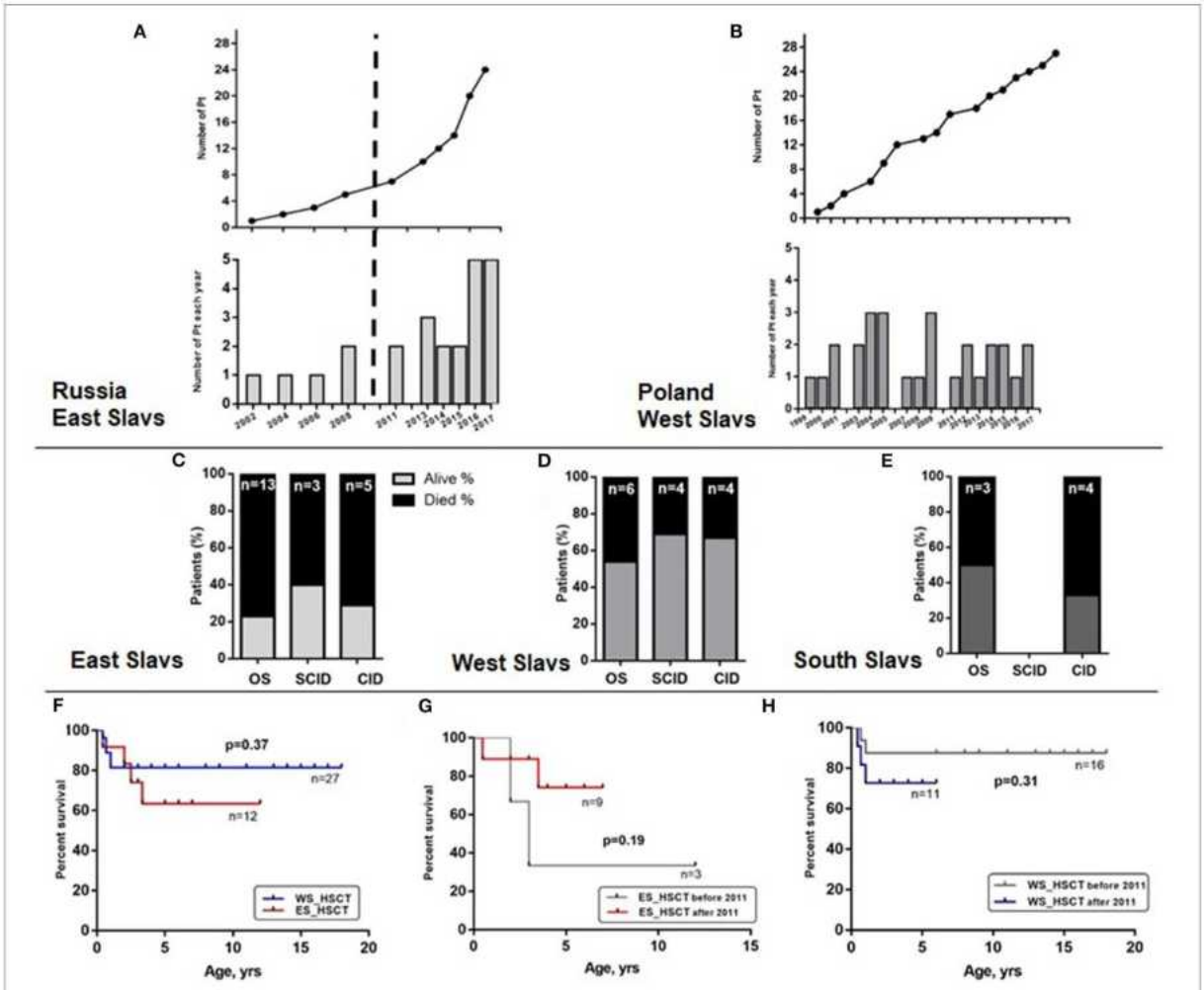


FIGURE 5 | Identification and survival of Slavic patients. Number of patients diagnosed in Russia **(A)** and Poland **(B)**. Percentage of dead patients (black region) among clinical groups of patients with *RAG* variants and East **(C)**, West **(D)**, or South Slavic **(E)** origin. Overall survival displayed as Kaplan–Meier survival curve of WS and ES patient groups with HSCT **(F)**. Overall survival curve of ES **(G)** and WS **(H)** patient transplanted before and after 2011.

access to HSCT service make it possible to improve the outcome for this group.

In the present study, 27% of patients were classified by attending physicians as atypical SCID. This phenotype was first described by Schuetz C. et al. in 2008, which they named "atypical/leaky SCID." During the last 10 years, there is growing awareness of these patients (2, 4, 7, 9, 13, 19, 20, 22, 30). Distinct clinical features include inflammatory and/or autoimmune complications. Autoimmune cytopenias (AIC) were the most frequent in our cohort, found in 58% (11/19 *RAG1* patients with AS phenotype). In the general population, ITP is the most common AIC. This is comparable to published data (23, 35). Regarding immunological features, although one third of the RAG1 patients had absence of very low B cell count, over two thirds of the cases had presence of low to normal B cells and immunoglobulins that progressive decline with age. Of note, many of our patients did not have full immune evaluation.

Slavs are the largest ethno-linguistic group in Europe (25, 36). In 10 out of 11 countries, the majority of patients carried p.K86Vfs*33 in RAG1 in homo or heterozygous state. Based on our data reported in this study, we established the geographic center of the origin of this specific RAG1 allele; it corresponds to the Vistula watershed area in Central and Eastern Poland (**Figure 3B**). Since SCID (Bernatowska E. unpublished data) and *RAG2* patients are evenly diagnosed in Poland, the lower frequency of absence *RAG1* variant p.K86Vfs*33 in the Western part of Poland may be likely related to distance from the

geographic center of the founder variant or due to lower medical care in this region. Together, these data suggest that it is most likely that the founder *RAG1* variant p.K86Vfs*33 is of a Slavic origin.

Location of the Slavic homeland prior to their great expansion in the fifth to sixth centuries is one of the key questions of European history. Although it is assumed that prehistorically the original habitat of Slavs was Asia, from which they migrated in the third or second millennium BC to populate parts of Eastern Europe, a debate concerning the European homeland of Slavs seems to remain unsolved. Different theories concerning the Slavs' geographic origin based on archaeological, anthropological, and/or linguistic data have been formulated. One places the cradle of Slavs in the watershed of the Vistula and Oder rivers (present-day Poland), and the other locates it in the watershed of the middle Dnieper (present-day Ukraine) (32). The obtained data strengthen the hypothesis that the Slavs could originate from the watershed of the Vistula due to the high number of families with homozygous variant from that region (**Figure 3B**). However, at present, it is impossible to deny that the variant could be found in the watershed of the middle Dnieper due to low number of cases and low incidence of *RAG* diagnostics in the Ukraine. Additionally, the only homozygous variant among East Slavs was found in the Ukraine (Odessa), where the parents denied their consanguinity. Collectively, these data indicate that p.K86Vfs*33 could be established as a "Slavic" *RAG1* variant or a founder variant.

Population migrations in Europe have led to the distribution of ethnic groups and cultures, and consequently to genetic mixing. Migrations, together with other factors, have also determined the prevalence of genetic variants among other populations (25, 36). In the GnomAD database, this deletion was found in heterozygous state in six people of European population (non-Finnish) of 282,486 individuals [https://gnomad.broadinstitute.org/variant/11-36595109-TAA-T]. Reviewing published cases, we established that patients with homo- and heterozygous p.K86Vfs*33 *RAG1* variants were detected in European cohorts (2, 13, 37–40). A recent Turkey study reported one patient with homozygous p.K86Vfs*33 in *RAG1* among the cohort of 44 SCID patients (32) and a SCID infant with EBV-positive B-cell lymphoma of the liver with homozygous p.K86Vfs*33 was diagnosed in Austria (40). It should be noted that in all these previous studies, the ethnic origin of the patients was not documented.

Our study highlight that a founder RAG1 variant is frequently present in Eastern Europe, especially in the watershed area of the Vistula. As cases accumulate in a specific region in Poland, screening of carriers of pathogenic RAG variants may be of high importance and could be offered in case of parental interest to reduce the incidence of RAG deficiency in this population. Our study underscores the importance of region of birth and ethnic background in genetic diagnosis. Further studies are needed to explore the carrier frequency of p.K86Vfs*33 in Central and Eastern Poland, as well in other Slavic countries.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

Informed consent forms were signed by the parents as requested and approved by the institutional review boards of various institutions involved. The protocol of study was approved by the institutional review board of Belarusian Research Center for Pediatric Oncology, Hematology and Immunology (IRB0012-2015). Written, informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SS designed the research, collected data, interpreted, analyzed the results and wrote the manuscript. DV prepared the maps. YY and TK studied RAG activity. MS-P, YR, BW-K, ND-L, BM, OP, PS, TF, TM, RF, AS, MS, TA, GM, PC, KK, SKo, TJ, KD, AG, MP, EN, SKa, KB-P, BB, ER, TV, AP, HG, MD, IM, AB, LC, LK, MG, JR, AM, EP, CG, and AL-P provided patient's information. JW, AS, TF, LN, OA, NK, and MB guided the writing of the manuscript. All authors have read and approved the contents of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.00900/full#supplementary-material

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Case report: Severe combined immunodeficiency with ligase 1 deficiency and Omenn-like manifestation

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DNA ligase I deficiency is an extremely rare primary immunodeficiency with only 6 patients reported in the literature. Most common manifestations include radiosensitivity, macrocytic anemia, lymphopenia with an increased percentage of gamma-delta T cells, and hypogammaglobulinemia requiring replacement therapy. Two-month-old girl with delayed development, T-B-NK+ SCID, and macrocytic anemia presented features of Omenn syndrome. Whole exome sequencing revealed two novel, heterozygous variants (c.2312 G>A, p.Arg771Gly and c.776+5G>T, p.Pro260*) in the LIG1 gene (NM_000234.1). Hematopoietic stem cell transplantation from a fully matched unrelated donor was performed at the age of 4 months using GEFA03 protocol. Mixed donor-recipient chimerism was observed, with 60-70% chimerism in the mononucleated cell compartment and over 90% in T-lymphocyte compartment, but autologous myeloid recovery. Stable CD4+ and CD8+ T-cell counts above 200/μL were achieved after 2 months, but the patient remained transfusion-dependent. Despite satisfactory immunological reconstitution, the second transplantation due to constitutional hemolytic defect has been considered. In light of possible re-transplantation, an issue of optimal conditioning protocol with sufficient myeloid engraftment is important. For the first time Omenn syndrome is described in a compound heterozygote carrying two the novel variants p.Arg771Gly and p.Pro260* in the LIG1 gene. Patients diagnosed with SCID and Omenn syndrome showing macrocytic anemia, should be screened for DNA ligase I deficiency.

KEYWORDS

ligase 1, immunodeficiency, Omenn-like, hematopoietic stem cell transplantation (HCST), Immunoglobilins

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Introduction

DNA ligase I deficiency is an extremely rare autosomal recessive primary immunodeficiency, caused by mutations in *LIG1* gene located on chromosome 19. As result, Okazaki fragments are improperly catalyzed during cell replication and single-strand DNA damage repair. The disease is associated with a diverse spectrum of clinical symptoms beginning in infancy or early childhood (1). An increased susceptibility to infections, macrocytic anemia, lymphopenia, increased percentage of γδ T cells, hypogammaglobulinemia requiring replacement therapy, and increased sensitivity to DNA damaging agents have been reported in all patients (2–4). Most of the only six reported cases demonstrated normal mental development. Growth retardation or failure to thrive and delayed or absent sexual maturation have been observed in two patients. Phenotype of one of the patients with delayed sexual maturation resembled Bloom syndrome and the patient died at the age of 19 years due to lymphoma (2). Fibroblasts from this patient showed an increased sensitivity to DNA damage caused by alkylating agents and ionizing and UV radiation (5, 6). Five remaining patients had normal physical and mental development (4). Severe combined immunodeficiency (SCID) was diagnosed in two cases and treated with allogeneic hematopoietic stem cell transplantation (HSCT) with reduced intensity conditioning regimen (3). Omenn syndrome was suspected in one patient, but skin biopsy did not confirm the diagnosis (3, 4).

Case description

A female infant was born at 35 weeks of gestation due to the first uncomplicated pregnancy of 28 y.o. mother by vaginal delivery with birth weight 1950 g and the 10 minutes Apgar score 6. Parents were healthy, non-consanguineous. After birth she demonstrated respiratory problems and hepatosplenomegaly. Blood tests revealed macrocytic anemia since the first day of life, with hemoglobin concentration 6.9 G/dL at birth date [normal range for the age, (N: 14.9-23.7 G/dL], mean corpuscular volume (MCV) 133 fL [N: 100-125 fL], thrombocytopenia in the first week of life (minimum platelet count $49x10^3/\mu L$), and lymphopenia since the third day of life (4-16%; 0.2 – $1.3x10^3/\mu L$). Due to respiratory failure probably of non-infectious cause, she was treated with a non-invasive positive pressure ventilation for one day, with nasal continuous positive airway pressure for the next 3 days. Infection markers and microbiological diagnostics were negative but newbborn was treated with empirical antibiotics (ampicillin and amikacin). Due to anemia, the child received multiple transfusions of irradiated, filtered, packed red blood cells and 10 doses of erythropoietin. Platelet concentrate was transfused once due to thrombocytopenia. Intravenous immunoglobulins were administered twice without serum immunoglobulins level tests (see the timeline in Figure 1). The girl was discharged from the neonatal unit at 32 days of age, but after a week, at the age of 5 weeks, she was re-admitted due to anemia (Hb 7.3 G/L) and lymphopenia $0.7x10^3/\mu L$) with erythematous papular rash not observed in neonatal period. Vitamin B12 and folate deficiency have been excluded. Flow cytometry revealed deep lymphopenia with T lymphocyte count 30 cells/μL, CD19+ B lymphocytes 93 cells/μL, and NK CD3-CD56.CD16+ 196 cells/μL. Hypercellular bone marrow with sparse erythroblastic and a reduced lymphocytic line, without signs of malignant proliferation, were found in bone marrow biopsy (Figure 2A). Based on the obtained laboratory results severe combined immunodeficiency was suspected.

The infant was admitted to the Immunology Department, Children's Memorial Health Institute, Warsaw, at the age of 8 weeks, in moderate general condition. The patient showed generalized erythematous-small lobular skin eruptions, with exfoliation located mainly on the face (Figure 2B), conjunctivitis of *Staphylococcus aureus* origin, and enlarged



FIGURE 1
The timeline of case report.

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cervical and inguinal lymph nodes of up to 1 cm diameter. The size of spleen and liver were within the age-related normal ranges. The body length was below 3 percentile in relation to percentile growth charts for preterm infants at gestational age 32-37 weeks, but the body weight and head circumference were proportional to length. Systemic antibiotic therapy, palivizumab, and intravenous immunoglobulins (IVIG) have been introduced to treatment, while anti-viral, anti-fungal, anti-*Pneumocytis jiroveci* pneumonia (PJP) prophylaxis was continued. Due to anemia, the girl required red blood cell transfusions approximately every 2 weeks. During hospitalization, mild but chronic diarrhea and erosions around the anus were observed with a transient rotavirus positive stool result. Immunological tests were repeated and based on results severe combined immunodeficiency with decreased number of T, B and NK cells was diagnosed (Table 1).

Omenn syndrome was suspected due to erythematous-exfoliative skin lesions, peripheral lymphadenopathy, eosinophilia (7020/µl), despite low concentration of IgE (<2.00 kIU/L), and oligoclonal distribution of TCR Vβ chain (Figure 3A). Feto-maternal chimerism was excluded. Fibroblasts cultured from skin biopsy showed an intermediate sensitivity to ionizing radiation (Figure 3B) in comparison to fibroblasts from a patient with radiosensitive SCID due to Artemis deficiency. Histopathological evaluation of skin biopsy was not carried out due to small surface of typical skin lesions present mainly on head and face. Combined prednisone and cyclosporine A immunosuppressive treatment resulted in gradual improvement of skin condition and reduction of eosinophilia.

Whole exome sequencing of the patient's DNA was performed. The libraries were prepared using Twist Human Core Exome Plus Kit (Twist Bioscience) and sequenced on Illumina platform with a 100x mean mapped read depth. The results of genomic testing revealed presence of two rare heterozygous variants in the *LIG1* gene (NM_000234.1): a missense variant of uncertain significance c.2312 G>A, p.Arg771Gly with high pathogenicity scores, including DANN score (7) reaching 0.9995, and a likely pathogenic splice variant c.776+5G>T, p.Pro260*. In order to verify whether the c.776 +5G>T variant affected mRNA splicing, direct cDNA sequencing was executed. It was found that the splice-variant resulted in an insertion of 16 nucleotides in intron 9, leading to a premature protein synthesis termination at position 260 (Figure 3C). Direct sequencing of DNA of the child and her parents in search of the presence of both variants of *LIG1* was subsequently performed (Supplementary Table 1). It was found that each of the parents was a carrier of a single *LIG1* mutation, which confirmed that both *LIG1* variants are located in the proband on separate alleles. Datasets are submitted to the European Variation Archive (EVA) repository (https://www.ebi.ac.uk/eva/?eva-study=PRJEB*****) and are publicly accessible under project number PRJEB56316, accession numbers ERZ14199911 at EMBL-EBI.

Due to the immunological features of severe combined immunodeficiency, the girl was referred for HSCT from an

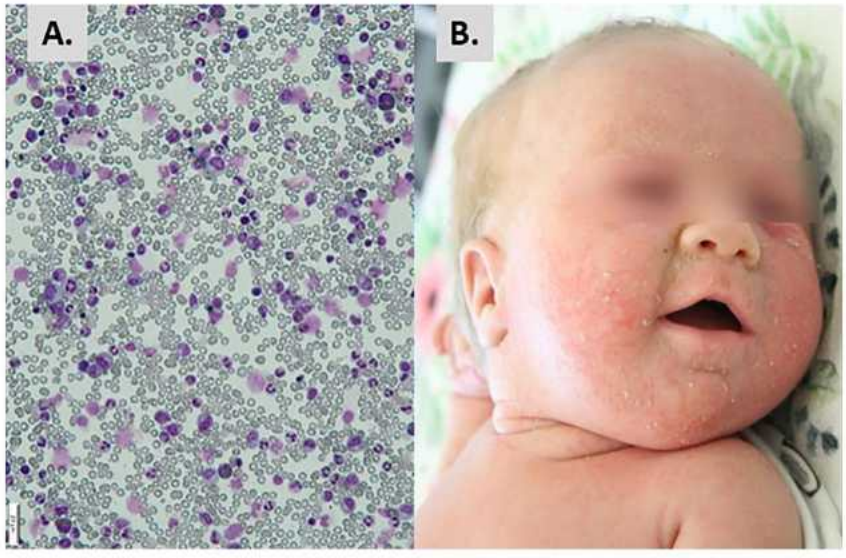


FIGURE 2
Initial presentation and diagnostic hallmarks. **(A)** Hypercellular bone marrow with suppressed erythroblastic lineage. **(B)** Erythematous-exfoliative skin lesions.

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TABLE 1 Laboratory results at the time of diagnosis.

Parameter	Result	Normal range for the age
CD45+	313 cells/µl	(3800-8100 cells/µl)
CD3+	106 cells/µl	(2200-5500 cells/µl)
CD3+CD4+	87 cells/µl	1400-4200 cells/µl
CD3+CD8+	5 cells/µl	400-1400 cells/µl
CD19+	107 cells/µl	700-1800 cells/µl
CD56+	84 cells/µl	200-980 cells/µl
CD31+CD45RA+/CD3+CD4+	0%	50-74%
γδT%	18,9%	3,2-4,9%
IgG	5.96 g/l (1 day after IVIG)	3.36-10.5 g/l
IgM	<0.04 g/l	0.21-0.51 g/l
IgA	<0.07 g/l	<0.06-0.07 g/l
IgE	<2.00 kIU/L	
Mitogen response tests		
PHA	1705 ± 56 SI 11.0	>16000 cpm >65
anty-CD3	2494 ± 159 cpm, SI 16	>15000 cpm >60
Pansorbin	20646 ± 224 cpm SI 133	>2000 cpm >5
Microbiology		
Nasal swab	*Staphylococcus aureus MSSA, Klebsiella pneumoniae*	
Throat swab	Physiological flora	
Rectal swab	Physiological flora	
Fecal culture	Physiological flora, *Salmonella, Shigella* negative, Norovirus, adenovirus negative, rotavirus positive for 4 days,	
Conjunctival culture	*Staphylococcus aureus MSSA*	
Swab of the external auditory canal	*Staphylococcus aureus MSSA, Escherichia coli* ESBL/-/	
PCR in whole blood for CMV, EBV, HSV-1, HSV-2, Enterovirus, Human paraechovirus, HHV-6, HHV-7, Parvovirus B19	negative	
Serum Galactomannan, mannan	negative	

CMV, Cytomegalovirus; EBV, Epstein-Barr virus; HHV-6, Human herpesvirus 6; HHV-7, Human herpesvirus 7; HSV-1, Herpes simplex virus type 1; HSV-2 – Herpes simplex virus type 2; IgA – immunoglobulin A; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; MSSA, methicillin-susceptible Staphylococcus aureus; PCR – polymerase chain reaction; PHA, phytohemagglutinin.

unrelated donor. At the age of 4 months, the girl was transplanted from a fully 10/10 HLA allele-matched unrelated donor using GEFA03 protocol, with intravenous busulfan at 0.5 mg/kg body weight (BW) given twice daily on days -4 and -3 (total dose 2 mg/kg), fludarabine i.v. at 30 mg/m2 daily from day -9 to day -4 (total dose 180 mg/m2), cyclophosphamide i.v. at 20 mg/kg daily on days -3 and -2 (total dose 40 mg/kg), and i.v. antithymocyte globulin (ATG)-Fresenius at 20 mg/kg BW daily from day -3 to day -1 (8).

Prophylaxis of a graft vs host disease (GVHD) was composed of cyclosporin A from -1 day and methotrexate on days +1, +3, +6. Peripheral blood progenitor cells in 14.67 x 10^6 CD34+ cells/kg BW dose were transplanted. Granulocytes recovered on +8 day after HSCT. From day +28, an isolated acute stage 2 skin GVHD in form of fine-grained rash on skin of the trunk and abdomen was diagnosed and treated with methylprednisolone. The patient developed mixed hematopoietic chimerism that stabilized at the level of 60-70% mononuclear cells of donor origin and more than 90% lymphocytes of donor origin (Supplemental Figure 1A). Immune reconstitution was regularly monitored (lymphocyte subset analysis results are shown on Supplemental Figure 1B), and the patient achieved stable CD4+ and CD8+ T-cell counts above 200 cells/mL after 2 months (Supplemental Figure 1C).

At the time of preparing of the manuscript, the girl has been followed-up for two years after HSCT. She is slightly delayed in physical and motor development. B-cell lymphopenia is observed permanently and the patient needs immunoglobulin therapy, as well as occasionally - red blood cells transfusions. The direct and indirect antiglobulin tests were negative, LDH

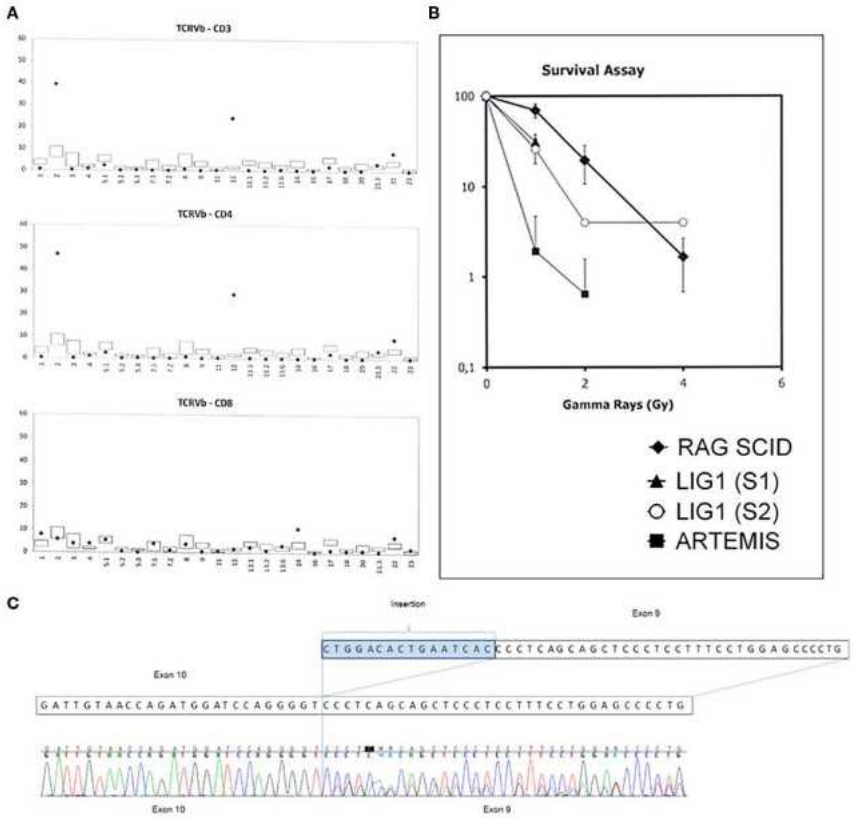


FIGURE 3
Diagnostic key-tests in the LIG1 deficiency patient. **(A)** Distribution of Vβ chain of the TCR receptor is oligoclonal with a significantly increased percentage of Vβ2 and Vβ12 chains among CD4 lymphocytes. **(B)** Clonogenic survival assay results. Fibroblasts cultured from skin biopsies of the patient and an Artemis-deficient SCID patient were exposed to increasing dose of irradiation and the percentage survival (axe Y) was determined after 8 days. S1- sample 1, S2- sample 2. RAG1 SCID patient sample was used as a control. **(C)** The cDNA sequencing results showing the splice site variant in the LIG1 which leads to an insertion of sixteen nucleotides in the exon 9 resulting in a shift of a reading frame and a premature protein termination at position 260.

activity, bilirubin direct and indirect results were within normal range. Due to anemia, the second transplantation has been considered, but the patient's parents did not consent to re-transplantation.

Discussion

The reported patient showed unique clinical features caused by presence of combined heterozygous mutation within the *LIG1* gene. The splice variant c.776+5G>T, p.Pro260* resulted in the insertion of 16 nucleotides in the intron 9 of *LIG1* leading to premature protein termination at position 260, and in consequence - to absence of functional domains of the protein. The missense variant (c. 2311G> T, p.Arg771Gly, R771G), which affected the structure of catalytic oligonucleotide/oligosaccharide binding-fold domain (OBD), was caused by defect located at the same position as reported in 4 other patients with *LIG1* deficiency (R771W), but associated with a different amino acid change (9, 10). However, both R771W and R641L variants decrease the activity of the DNA ligase (11, 12).

The biological function of ligase 1 in the replication and DNA repair is associated with sealing of Okazaki fragments during replication and catalyzing the ultimate ligation step of DNA repair (12). The cellular functions of LIG1 are mediated through its non-catalytic N-terminal domain (amino acids 1-261), that contains the nuclear localization signal and participates in protein–protein interactions (13).

DNA ligase I deficiency causes an extremely rare IEI with a diverse spectrum of clinical symptoms affecting not only immunity, but also growth, psychomotor development, and production of blood cells. The course of the disease in the

reported patient included severe anemia requiring multiple red blood cell transfusions since the first hours of life. It is unclear why mutations in the *LIG1* gene cause macrocytic anemia and other manifestations extending beyond immune deficiency, and whether any genotype-phenotype correlation exists. Macrocytic anemia probably results from an impaired DNA synthesis in hematopoietic precursor cells, yet in contrast to Fanconi anemia - it does not lead to bone marrow failure, but to hemolysis moderately compensated by reticulocyte production. Impaired DNA synthesis may also affect other rapidly dividing hematopoietic cells.

An IEI was for the first time suspected at the age of 8 weeks when absent T- and low B-lymphocyte counts were noted during work-up for constitutional hemolytic anemia. The diagnosis of T-B-NK+ SCID was similar to two other reported boys (3).

The presence of erythematous-exfoliating skin lesions suggestive of Omenn syndrome, due to uncontrolled proliferation of autologous T lymphocytes was a unique feature in the reported patient. The diagnosis was supported by clinical criteria of the ESID Registry, which state that probable diagnosis of Omenn's syndrome may be made when exfoliative erythroderma occurs in the first year of life and it is accompanied by at least one clinical symptom: lymphoproliferation, failure to thrive, chronic diarrhea, recurrent pneumonia, eosinophilia or elevated IgE with T-cell deficiency (low naive cells, reduced proliferation, oligoclonality), and with maternal engraftment and HIV infection excluded (14). Although there was no histopathological proof for such diagnosis, our patient was successfully treated as an Omenn syndrome with prednisone and cyclosporine: skin lesions, lymphadenopathy, and diarrhea disappeared, and the number of eosinophils normalized.

The essential consequence of *LIG1* defect is an impaired DNA repair mechanism. The reported patient's cells demonstrated an increased sensitivity to ionizing, UV radiation and DNA damaging agents *in vitro*, as in the first reported patient, or only to methyl methanosulphate, as in two SCID patients (4). Fibroblasts of our patient were sensitive to ionizing radiation and although sensitivity to other DNA damaging agents was not verified, it was considered as decisive information when HSCT procedure was planned. Based on the guidelines for HSCT in patients with radiosensitivity, and center experience - a reduced intensity conditioning protocol was administered. The peri-transplant period was relatively uneventful, mucosal toxicities were mild, and the patient was fed orally. However, the modified German Fanconi anemia protocol did not show sufficient myeloablative potential, as observed in ataxia-telangiectasia and Nijmegen breakage syndrome (15, 16).

Decreased pretransplant NK-cell counts can point out to exhaustion of common lymphoid progenitor compartment. At time of transplantation, the patient was profoundly lymphopenic and NK cell counts were not likely to affect the engraftment. It can not be ruled out, that suboptimal myelosuppression resulted in autologous recovery of NK cells and accelerated elimination of donor derived hematopoiesis. B- and NK-cell chimerism was not evaluated. The myeloid chimerism evaluation in CD15 positive cells was performed only once. All other chimerism measurements were carried up in mononuclear cells or in T-lymphocytes. It can be hypothesized, that vestigial donor hematopoiesis and myelopoesis (ca. 2% of all CD15 cells) are responsible for production of NK cells, and lack of competition from autologous lymphoid.

The reported experience with HSCT in *LIG1* deficiency suggested effectiveness of minimal intensity conditioning, but this was not confirmed in case of our patient. Both previously reported boys who underwent HSCT in Great Ormond Street Hospital (GOSH) from family donors,received minimal intensity conditioning: fludarabine 150 mg/m2, cyclophosphamide 1000 mg/m2, alemtuzumab 1 mg/kg and YTH 24/54 (anti-CD45 monoclonal antibodies) 800 µg/kg. Although the first patient received additionally YTH 24/54 anti-CD45 monoclonal antibodies in reduced dose due to adverse reaction during infusion, he achieved multilineage full donor chimerism. The second patient demonstrated low level B-cell engraftment, was transfusion-dependent, and remained on immunoglobulin replacement therapy. He also needed blood transfusions and chelation therapy (3).

Optimal conditioning protocol with sufficient myeloid engraftment is important in case of considered re-transplantation, but there is not enough data to determine the tolerance to chemotherapy regimens. In addition, despite limited long-term follow-up of patients with *LIG1* deficiency, an elevated risk of cancer incidence should be considered.

Conclusions

DNA ligase I deficiency should be considered in patients diagnosed with SCID associated with macrocytic anemia and features of Omenn syndrome. For the first time Omenn syndrome is described in a compound heterozygote carrying two novel variants p.Arg771Gly and p.Pro260* in the *LIG1* gene. HSCT is an option to cure LIG1 deficiency, however myeloid chimerism is required to control anemia and for sustained B-cell function, and an intensity of conditioning regimen remains questionable.

Data availability statement

The data presented in the study are deposited in the European Variation Archive (EVA) repository (https://www.ebi.ac.uk/eva/?eva-study=PRJEB56316), project number PRJEB56316, accession numbers ERZ14199911.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

ND-L, MP designed the concept of the manuscript. ND-L, AP, KB-P, BP, MU, MP wrote the manuscript with contribution from all co-authors. BP did the immunological studies AP, KB-P, MK did the genetic analysis. MB did the radiosensitivity of fibroblasts. ND-L, KB-S, EH-P, KG, KK, MU, MP contributed to clinical data collection and critical review. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.1033338/full#supplementary-material

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Case Report

Atypical Hemolytic Uremic Syndrome (aHUS) and Adenosine Deaminase (ADA)-Deficient Severe Combined Immunodeficiency (SCID)—Two Diseases That Exacerbate Each Other: Case Report

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Abstract: Hemolytic uremic syndrome (HUS) is defined by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury (AKI). Atypical HUS (aHUS), distinguished by its etiology, is caused by uncontrolled overactivation of the alternative complement pathway. The correct diagnosis of aHUS is complex and involves various gene mutations. Severe combined immunodeficiency (SCID), characterized by severe T-cell lymphocytopenia and a lack of antigen-specific T-cell and B-cell immune responses, is of seldom occurrence. In 10–15% of pediatric patients, SCID is caused by adenosine deaminase (ADA) deficiency. The authors describe the case of a boy who suffered from both aHUS and ADA-deficient SCID. At the age of 9 months, the patient presented acute kidney injury with anuria and coagulopathy. The diagnosis of aHUS was established on the basis of alternative complement pathway deregulation and disease-associated gene mutations. Further examination revealed immune system failure and, at the age of 13 months, the ADA deficiency was confirmed by genetic tests and the boy was diagnosed with ADA-SCID. ADA SCID has recently been described as a possible triggering factor of aHUS development and progression. However, more research is required in this field. Nevertheless, it is crucial in clinical practice to be aware of these two co-existing life-threatening diseases.

Keywords: atypical hemolytic uremic syndrome; thrombotic microangiopathy; severe combined immunodeficiency; adenosine deaminase deficiency; children

1. Introduction

Atypical hemolytic uremic syndrome (aHUS), a rare and severe disease, belongs to a family of thrombotic microangiopathies (TMAs). It is caused by uncontrolled, incessant activation of the alternative complement pathway, on account of defects in its regulation. Usually, aHUS is induced by the presence of genetic variants that diminish the function of complement regulating factors (e.g., factor H and I (CFH, CFI), and membrane cofactor protein (MCP = CD46)), as well as through activating mutations of C3 or complement factor B (CFB) or other related coagulation factors. Autoantibodies against CFH can provoke a clinical phenotype similar to aHUS, especially with the presence of CFH-related factor mutations. All children with HUS should also be tested for possible cobalamin C deficiency [1]. aHUS is characterized by the classic triad of symptoms: microangiopathic hemolytic anemia (MAHA), thrombocytopenia, and acute kidney injury. Renal manifestations of the disease are described as fibrin and platelet thrombi in capillaries and arterioles, endothelial cell swelling and detachment from the glomerular basement membrane, and the appearance of so-called double contours on the glomerular basement membrane [2].

Adenosine deaminase (ADA) deficiency, owing to *ADA* gene mutation, results in the purine metabolism disorder. The accumulation of toxic metabolites, such as deoxyadenosine triphosphate and deoxyadenosine, is the main pathogenic mechanism. Such a disruption affects various tissues, as ADA is ubiquitously expressed throughout the body; however, it profoundly impacts the immune system [3]. Consequently, the development and functioning of lymphocytes are impaired, finally causing severe combined immunodeficiency (SCID) [4]. A large population-based newborn screening ascertainment revealed that ADA deficiency occurs in 1 per 500,000 cases and accounts for 10–20% of human SCID. SCID is characterized by severe T-cell lymphocytopenia and a lack of antigen-specific T-cell and B-cell immune responses. This condition is uniformly fatal in the first two years of life unless immune reconstitution can be accomplished with allogeneic hematopoietic stem cell transplantation or gene therapy. ADA-SCID, being a non-lymphocyte-specific cause of immunodeficiency, is unique among the different genetic causes of SCID [5].

Aim

This manuscript presents the case of a boy who suffered from both aHUS and ADA SCID—two diseases that exacerbated each other.

2. Clinical Report

The boy was born to healthy Caucasian parents after the second pregnancy and the second delivery, at the 38th week of gestational age. The delivery was uncomplicated with a birth weight of 3150 g, body length of 54 cm, and an APGAR score of 9. Since infancy, the patient was presenting recurrent diarrhea, resulting in impaired weight gain—his weight remained at the third percentile for age and sex. Consequently, a retardation of psychomotor development was assumed.

At the age of three months, he developed varicella and, six months later, herpes zoster (Figure 1). The patient presented with anuric acute kidney injury, generalized edema, and respiratory failure, requiring immediate medical intervention. On admission to the intensive care unit, laboratory tests revealed various significant abnormalities (Table 1). An elevated reticulocytes count, hyperbilirubinemia, elevated serum creatinine, and increased lactate dehydrogenase (LDH) activity, together with proteinuria and hematuria, raised the suspicion of the diagnosis of HUS. Moreover, schistocytes were present in peripheral blood smear, accounting for 6% of RBC, and elevated aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), and c-reactive protein (CRP) values were noted. Owing to the severe clinical condition, the boy required peritoneal dialysis (PD) performed continuously, starting from hourly exchanges of dialysis fluid. In addition, owing to aHUS suspicion and coagulopathy (thrombocytopenia, prolonged activated partial thromboplastin time (aPTT) and prothrombin time (PT) along with reduced fibrinogen activity), blood product transfusions were performed. Shiga toxin-producing

Escherichia coli infection was excluded by fecal culture. Furthermore, assessment of complement components indicated slightly decreased C3 levels together with C4 levels within the normal range. ADAMTS13 (disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13) activity was 60%.



Figure 1. Severe course of Varicella Zoster in the described case.

Table 1. A,B. Laboratory test results. A—hospitalization at the age of 9 months, B—hospitalization at the age of 13 months.

Laboratory Tests	A	B	Reference Ranges
	Complete Blood Count		
	On admission On discharge		
Hematocrit [%]	32.5, 30.7	32.25	32–42
Red blood cell count [10^6/uL]	4.98, 3.52	4.05	3.8–5.4
Hemoglobin [g/dL]	11.3, 10.2	11.98	10.5–14
White cell count [10^3/uL]	3.79, 5.26	4.53	6–14
Granulocytes [10^3/uL]	1.35, 1.76	2.7	1.6–6
Lymphocytes [10^3/uL]	0.46, 0.93	0.4	1.0–3.3
Monocytes [10^3/uL]	1.56, 0.42	1.2	0.15–0.6
Mean corpuscular volume [fL]	65.3, 87.22	87.13	72–88
Mean corpuscular hemoglobin [pg]	22.7, 28.92	29.62	24–30
Mean corpuscular hemoglobin concentration [g/dL]	34.8, 33.16	34	32–36
Platelet count [10^3/uL]	50, 361	379	150–450
Reticulocyte count [10^9/L]	87.6	70.2	26–85

Table 1. *Cont.*

Laboratory Tests	A	B	Reference Ranges
	Serum laboratory tests		
Albumins [g/L]	29.84, 38.44	29.65	30–50
Aspartate aminotransferase [U/L]	660.6, 104.3	51.2	0.40
Alanin aminotransferase [U/L]	178.1, 49.2	33.2	0–40
Bilirubin (total) [umol/L]	22.8, 3.9	0.5	3.4–22
Complement component 3 [g/L]	0.84, 0.86	-	0.9–1.8
Complement component 4 [g/L]	0.1, 0.28	-	0.1–0.4
C-reactive protein [mg/L]	23.56, 2.63	37.95	0–5
Gamma glutamyltranspeptidase [U/L]	198, 127	360	5–65
Total protein [g/L]	41.1, 62.8	62.2	60–80
Creatinine [umol/L]	149, 61	55	21–53
Uric acid [umol/L]	-, 226	243	202.3–416.5
Urea [mmol/L]	23.1, 4.9	20.7	3.2–7.1
Lactate dehydrogenase [U/L]	3757, 591	-	180–435
Sodium [mmol/L]	121.1, 136	135	135–148
Potassium [mmol/L]	5.43, 5.84	3.74	3.5–5.0
Magnesium [mmol/L]	0.78, 0.87	1.05	0.7–1.1
Phosphate [mmol/L]	1.15, 1.74	1.02	0.81–1.45
Ionised calcium [mmol/L]	0.86, 1.19	1.11	1.1–1.35
Proteinuria [g/L]	26.0, 1.35	0.75	0–0.15
Hematuria [RBC/HPF]	Massive, 5–10	1–3	0–3
	Coagulation profile		
Activated partial thromboplastin time [s]	40.2, 38.1	27.8	28–40
Prothrombin time [s]	16.3, 13.1	12.5	11–16
International normalized ratio [INR]	1.31, 0.99	0.95	0.9–1.3
Fibrinogen [mg/dL]	178, 347	252	200–400
D-dimer [mg/mL]	3.1, 1.76	1.05	0–0.5
Antithrombin III [%]	90, 110	81	80–120

PD continued for 29 days (969 h), and blood plasma transfusions and pharmacological treatment did not provide a significant improvement in the patient's condition. Consequently, it was decided to conduct eight cycles of plasmapheresis, eventually resulting in a good clinical response (that time eculizumab treatment was unavailable) (Table 1). Three sessions of plasmapheresis were performed daily, and another five on alternating days. The amount of exchanged plasma was on average 600 mL/session (80 mL/kg/body weight). Additionally, the boy received combined hypotensive therapy, including amlodipine, bisoprolol, and ramipril. For the next month, the patient remained under outpatient nephrological care.

At the age of 13 months, the boy was admitted to the hospital because of high body temperature and severe dehydration accompanied by elevated AST, GGT, and CRP levels (Table 1). Further examination revealed an upper urinary tract infection (pharyngotonsillitis), followed by increased generalized edema, low urine output, and hypoalbuminemia in subsequent days. Consequently, amoxicillin with clavulanic acid, furosemide, and 20% albumin solution was administered. Unfortunately, the treatment was unsuccessful and, eventually, left-sided partial seizures, decorticate posturing, and opisthotonus occurred. Head computed tomography (CT) and cerebrospinal fluid analysis were performed, yet no abnormalities were found. Additionally, in the echocardiogram, a sinus venosus, an atrial septal defect, and a slight tricuspid valve regurgitation were present. In the following days, symptoms of pulmonary edema arose and the boy required PD and mechanical ventilation for 6 days. Further tests revealed a serum creatinine level of 37.7 µmol/L and urea concentration of 8.7 mmol/L. Platelet count was maintained in the range of 315–444 $10^3/\mu L$. A successful treatment was followed merely by several days of the patient's good condition. Pneumonia accompanied by severe anemia was disclosed within days. During blood transfusion, a sudden cardiac arrest occurred and the boy required intensive care once more. As soon as his condition improved, a renal biopsy was conducted, revealing mesangial cell

proliferation accompanied by hyaline degeneration, multiple hyaline casts in the glomerular lumen, and thickening within each layer of the arterial wall. Such a description strongly corresponded with membranoproliferative glomerulonephritis (MPGN) and vasculopathy (Figures 2 and 3). A part of the glomerular capillary wall with a widened sub-endothelial space and rich fluffy-like material beneath the endothelial cells was described in the performed electron microscopy examination (Figure 4). Bone marrow examination, conducted to broaden the diagnostic process, revealed 23% of lymphoblast-like cells, while precursor B cells in various development stages represented 13% of cells in immunophenotyping. No mature B cells were detected, neither in the bone marrow nor in peripheral blood.

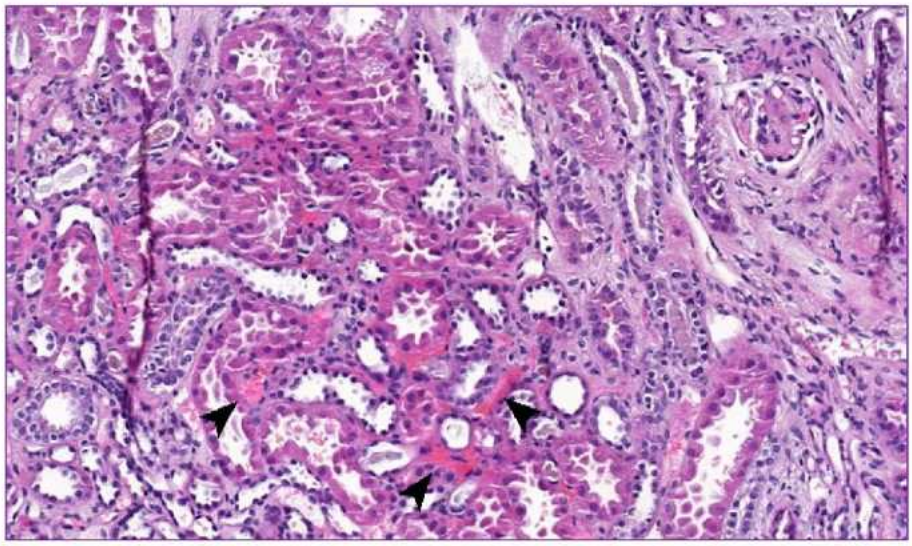


Figure 2. Small fibrin thrombi are visible in the interstitium (arrowheads).

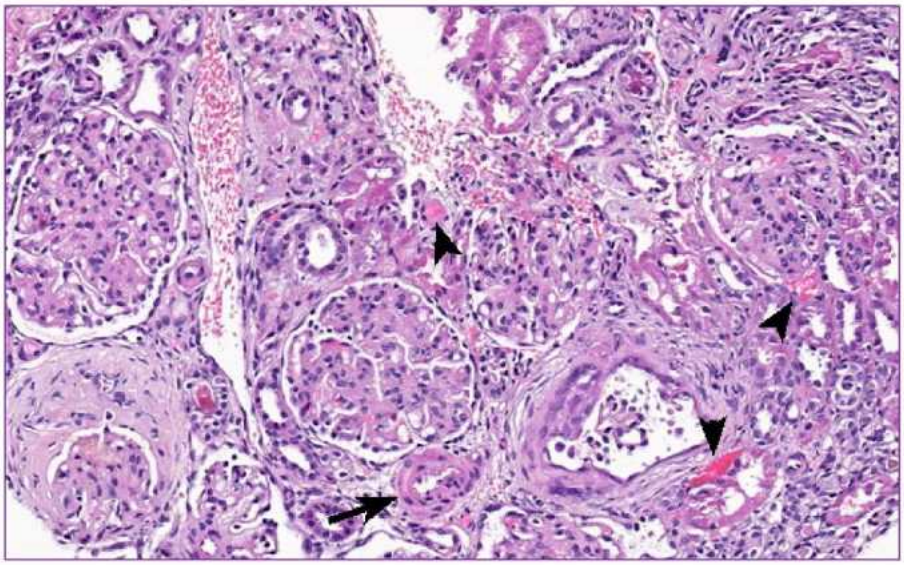


Figure 3. Increased cellularity of the glomeruli, accompanied by extracellular matrix expansion. Small thrombi located in the interstitium (arrowheads). Small arteriole with a thick wall (arrow).

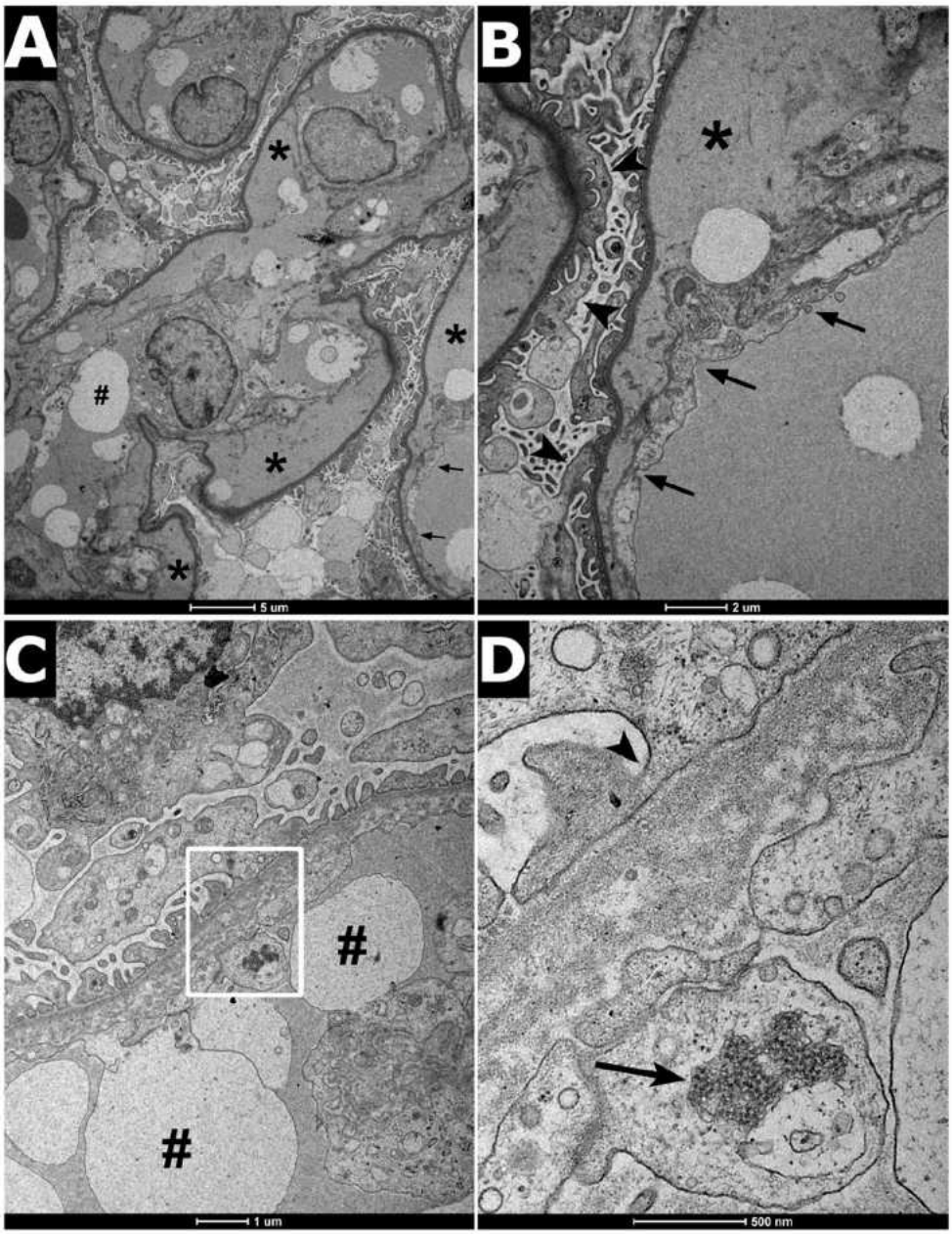


Figure 4. Electron microscopy findings in HUS. (**A**) Glomerular capillary separation of endothelial cells from the underlying glomerular capillary basement membrane (asterisks), markedly widened sub-endothelial space with fluffy-like material. Swelling of the capillary endothelial cells, electron lucent blebs are visible in the capilary lumen (#). Scale bar: 5 μm. (**B**) Higher magnification of a capillary wall; the arrows indicate the loss of endothelial fenestration, foot processes flattening, and microvilli on podocyte surface are also visible (arrowheads). Scale bar: 2 μm (**C**) Fragment of glomerular capillary wall with a widened sub-endothelial space and rich fluffy-like material under the endothelial cells. Capillary loop exhibits mild endothelial cell swelling with the absence of normal fenestra (#) Scale bar: 1 μm. (**D**) White box, magnification of (**C**) Endothelial cell contains a tubuloreticular inclusions (arrows). Moreover, flattening of podocyte foot processes also occurs (arrowhead). The presence of tubuloreticular inclusions (TRIs) in glomerular endothelial cells associated with viral infections (so-called interferon footprints) Scale bar: 500 nm.

Within a few days after renal biopsy, an appearance of blood in the urine was noticed and abdominal ultrasound revealed an arteriovenous fistula of the right kidney; therefore, the boy underwent successful embolization of the damaged artery.

Suspecting the presence of aHUS and considering its genetic basis, genetic testing was performed by Warsaw Genomics. *ADAMTS13; C3; C5; CD46; CFB; CFH; CFHR1; CFHR2; CFHR3; CFHR4; CFHR5; CFI; DGKE; MMACHC; PLG; THBD* genes were analyzed. Analysis of large deletions and rearrangements did not disclose any mutations in *CFHR1-5 gene*, as well as *DGKE; MMACHC; PLG; THBD, C3, C5, CFI genes*. Point mutation, included in the high-risk haplotype for aHUS and associated with a poor prognosis and greater risk of transplant rejection, was detected in *CFH* gene (TGTGT) [6]. Additionally three high-risk haplotypes were found in *CD46* gene (rs2796267, rs2796268, and rs859705) [7,8]. Both copies of *ADAMTS13 gene* were described with *the rs2301612* variant, previously detected in patients with aneurysms [9]. Furthermore, another variant, quite rare in the general population (MAF = 0.007 in the GnomAD database), was discovered in a single *CFB gene* copy (rs13194698; NM_001710.5:c.299-111C>T), yet its clinical manifestation is still unknown (Table 2 genes are listed in alphabetical order).

Table 2. Genetic testing.

Gene	Description	Inheritance	Additional Information
ADAMTS13	allele G rs2301612	mutations in both copies of gene	maternal and paternal
CD46	allele A rs2796267	mutations in both copies of gene	maternal and paternal
CD46	allele A rs2796268	mutations in both copies of gene	maternal and paternal
CD46	allele G rs859705	mutations in both copies of gene	maternal and paternal
CFB	allele T rs13194698	mutations in single copy of gene	maternal
CFH	allele T rs3753394	mutations in single copy of gene	paternal
CFH	allele G rs800292	mutations in both copies of gene	paternal
CFH	allele T rs1061170	mutations in single copy of gene	paternal
CFH	allele G rs3753396	mutations in single copy of gene	paternal
CFH	allele T rs1065489	mutations in single copy of gene	paternal
CFH	allele A rs2274700	mutations in single copy of gene	maternal and paternal

The patient demonstrated persistent lymphopenia with absolute lymphocyte numbers ranging from 0.17 to 0.8 $\times$ $10^3/\mu L$ (N: 1.0–4.4 $\times$ $10^3/\mu L$). The immunophenotyping of peripheral blood was performed with multicolor flow cytometry and the lymphocyte subsets were identified according to the EuroFlow standards [10]. This investigation showed decreased numbers of B-cells, T-cells, and NK-cells, which was suggestive of $T^-B^-NK^-$ SCID (Table 3) and prompted further investigation at the specialized institute. CD45+ cells unveiled RO surface antigen at 95.5%. The levels of all main subclasses of immunoglobulins were significantly below the normal range.

Concomitantly, genetic tests unraveled homozygous pathogenic mutation in *ADA gene* (c.302G > A, (p.Arg101Gln)). The clinical outcome and test results indicated adenosine deaminase (ADA)-deficient severe combined immune deficiency (SCID). Additionally, other immunodeficiency connected genes were analyzed and are presented in the Supplementary Material (Table S1). No further abnormalities were found.

At the age of 16 months, the boy was admitted to the district hospital because of a severe respiratory tract infection. Unfortunately, a progression of the infection with the severity of the underlying pathology led to multiorgan failure and the patient deceased.

Table 3. Immunological tests.

Lymphocyte Immunophenotyping			Immunoglobulins [g/L]	
Parameter	**Absolute Number [10^3/uL]**	**Percentage of Lymphocytes**		
T-cells	0.11 [N: 1.6–6.7]	79%	IgG	3.09 [N: 4.53–9.16]
B-cells	0.0056 [N: 0.6–2.7]	0.04%	IgA	0.11 [N: 0.19–1.46]
NK-cells	0.0287 [N; 0.2–1.2]	20.5%	IgM	0.1 [N: 0.2–1]
CD4 T-cells	0.00002 [N: 1.4–5.1]	1.2% of T-cells		
CD8 T-cells	0.10 [N: 0.6–2.2]	95.5% of T-cells		

The ranges of the presented parameters were adjusted to the child's age.

3. Discussion

3.1. Atypical Hemolytic Uremic Syndrome

Microangiopathic hemolysis (MAHA) was the initial diagnosis in the presented case. According to the current pathophysiological classification of TMAs, three main types are distinguished: primary (congenital and acquired), secondary, and infection induced. To exclude thrombotic thrombocytopenic purpura (TTP), ADAMTS13 activity was performed, which showed the level above 10%. HUS induced by Shiga toxin-producing bacteria (STPB, predominantly Shiga toxin-producing Escherichia coli (STEC); STEC HUS), classified as infection-induced TMA, is the most common type among young patients [2,11]. Thus, the main step in differential diagnosis of aHUS in children is the exclusion of STEC HUS. Therefore, fecal culture examination was performed in the described case, eliminating infection caused by verotoxin-producing bacterial strains. Furthermore, the combination of low C3 and normal C4 supported the diagnosis of aHUS. Kidney biopsy was also helpful in the diagnostic process. Description of membranoproliferative glomerulonephritis (MPGN) with the presence of thrombotic microangiopathy and fibrin deposits also supported aHUS-related pathology.

Although there has been substantial progress in this field of study, for about 40% of patients, the etiology of aHUS is still unknown [12]. Currently, aHUS diagnosis is still difficult to establish clearly, as no direct diagnostic test exists and available biomarkers are not completely reliable [2,11]. Moreover, the detection of complement gene variants is an obligatory element of the diagnostic process. Among aHUS-related gene disorders, *CFB, CFH,* and *MCP gene* mutations were found in the presented patient. Complement factor H haplotype TGTGT is a modest risk factor OR1.89 according to de Cordoba et al. [6]. Detected MCP gene mutations in our patient in accordance with previous findings of Fremeaux-Bacchi show that genetic variability in MCP is also associated with atypical HUS in that case [7].

In a differential diagnosis, we excluded the mutations in diacylglycerol kinase epsilon (DGKE) gene, whose presence could be responsible for the renal pathology of thrombotic microangiopathy or MPGN [13,14] and cobalamin C disease, which may also present as HUS with renal TMA during infancy [15]. Additionally, copies of ADAMTS13 gene were described with the rs2301612 variant defined before in patients with aneurysms, which is a benign variant that does not decrease ADAMTS13 activity [9,16]. In CT imaging the aneurysms were excluded in the presented patient. Such a variant of *ADAMTS13 gene* is frequently observed among patients with aHUS in our department.

Performing genetic tests should not delay the treatment of aHUS. Therefore, the described patient was given a suitable therapy in compliance with standards of care for the first presentation of aHUS, receiving plasma exchange therapy (PLEX), as eculizumab treatment was not yet available in Poland at that time [17].

In the described case, the incident of low urine output causing severe overhydration was defined as an exacerbation of chronic kidney disease during septic infection. Occurring reversible neurological complications with negative head CT scan, pulmonary edema, worsening anemia, and cardiac arrest were not related to aHUS.

3.2. Adenosine Deaminase (ADA)-Deficient Severe Combined Immune Deficiency (SCID)

In the presented case, genetic tests revealed homozygous pathogenic mutations in ADA gene that eventually led to purine metabolism disorder.

ADA deficiency is said to be the first acknowledged underlying pathology of SCID [4]. It is directly associated with a systemic purine metabolic disorder that is described to impact various organ systems, such as pulmonary, hematologic, gastrointestinal, neurologic, or skeletal [5]. Among the symptoms, hepatotoxicity (including hepatitis, hyperbilirubinemia, or even rapid hepatic failure) is frequently observed; likewise, it was described in the presented case. The differential diagnosis of increased activity of hepatic enzymes should be performed towards infectious, autoimmune, metabolic, and toxic causes, especially before establishing the diagnosis of ADA-SCID [18,19]. In the case of our patient, hepatotropic infections as well as autoimmune and common metabolic diseases were excluded. Pulmonary alveolar proteinosis of ADA-SCID should also be taken into consideration during differential diagnosis of pulmonary edema, especially when the *Pneumocystis jiroveci* infection has been excluded.

The lymphocyte functioning disorder due to ADA deficiency has already been deeply explored; however, other systemic manifestation mechanisms have not yet been fully understood. In the literature, a number of purine metabolism disorders were proved to indicate immunodeficiency [20]. ADA is a major enzyme in irreversible deamination of purine nucleosides, such as adenosine (Ado) into inosine and 2′-deoxyadenosine (d-Ado) into 2′-deoxyinosine (d-Ino) [21,22]. In the absence of this reaction in purine metabolism, d-Ado and Ado accumulate in fluids and cellular components of affected individuals, causing immune cell impairment, mainly affecting the development and function of T, B, and NK cells and, furthermore, systemic pathology [23,24]. The patient presented with profound lymphopenia from the beginning. Moreover, in conformity with the literature, he revealed an elevated percentage of memory CD45RO+ lymphocytes. It is worth mentioning that PLEX and repeated plasma transfusions, applied for aHUS treatment, might have provided the patient with passive humoral immunity.

Apart from immune system defect, Ado accumulation might be responsible for CNS complications. In our patient, a negative head CT scan documented only functional injury at the initial SCID presentation.

In genetic evaluation, we have presented the mutation in *ADA* gene and negative results in the analysis of genes connected with other congenital immunodeficiencies. Such a detailed evaluation has not been done previously.

Nowadays, early ADA restoration, by either enzyme replacement therapy, allogeneic hematopoietic stem cell transplantation (HSCT), or gene therapy, is available and prevents patients from severe immunodeficiency [3].

3.3. ADA-SCID and aHUS Interaction

According to the literature, which is limited to case reports, the mechanism of ADA-SCID influencing aHUS development might be connected with infection, deregulation of complement function, autoimmunity, or impaired metabolism; however, no certain connections were established [4,25]. However, these reports cannot be taken as evidence that the reported cases did have thrombotic microangiopathy due to defective regulation of the alternative complement pathway, because genetic variants were not demonstrated. We have tried to find the genetic variants confirming defective regulation of the alternative complement pathway in ADA-SCID child and TMA for the first time.

Constriction of afferent renal arterioles and subsequent glomerular filtration reduction are the consequences of signaling through the adenosine A1 receptor [26,27]. Therefore,

an elevated extracellular adenosine level within renal tissue in ADA-deficient patients is suspected to gradually induce nephrotic proteinuria, as ADA-SCID patients' autopsy reports disclosed mesangial sclerosis in the majority of cases and global glomerular sclerosis in up to 10% of glomeruli on death [28]. Such findings may impact the course of aHUS, accelerating the progress of renal failure and diminishing the clinical response to treatment. The findings of this case, together with the cases described in the cited references [4,25], suggest that ADA-SCID apart from aHUS may present with nephropathy during infancy, also owing to adenosine-mediated arterial and glomerular injury with concurrent hepatic injuries.

The obligatory combination of factors for aHUS to develop is believed to include infections, genetic disorders, and environmental susceptibility [29,30]. Hence, herpes zoster infection, presented in this case, was considered the triggering factor of aHUS development. Disseminated varicella caused by wild-type virus or following varicella vaccine in SCID patients, including ADA-SCID, have already been reported [31]. In our case, the mother's medical history concerning either VZ infection or vaccination remains unknown, which precludes the exact explanation of such an early infection. It is believed that ADA-SCID patients, owing to immune system abruption, are more likely to develop aHUS as a result of disease caused by a wider range of pathogens, or even an opportunistic infection.

As mentioned above, purine metabolism disorders caused by ADA deficiency might influence various tissues by currently unknown mechanisms. It is assumed that biologically active purine metabolites, accumulating in the organism, may trigger metabolic changes, which consequently might contribute to the predisposition to aHUS development, as was also mentioned in the study of Nikolajeva et al. [4].

4. Conclusions

In clinical practice, ADA-SCID patients should be carefully examined in terms of aHUS development possibilities. Furthermore, early treatment in this group of patients is essential, as both conditions, described as severe and life-threatening, are considered to exacerbate each other. However, currently, various therapies are available and should be induced as soon as possible.

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Mój wkład merytoryczny: koncepcja pracy, zbieranie danych, pisanie pracy

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3. Bernatowska Ewa, Skomska-Pawliszak Małgorzata, Wolska-Kuśnierz Beata, Pac Małgorzata, Heropolitańska-Pliszka Edyta, Pietrucha Barbara, Bernat-Sitarz Katarzyna, Dąbrowska-Leonik Nel, Bohynikowa Nadeżda, Piątosa Barbara, Lutyńska Anna, Augustynowicz Ewa, Augustynowicz-Kopeć Ewa, Korzeniewska-Koseła Maria, Krasińska Maria, Krzysztopa-Grzybowska Katarzyna, Wieteska-Klimczak Anna, Książyk Janusz, Jackowska Teresa, van der Burg Mirjam, van Dongen Jacques J.M., Casanova Jean-Laurent, Picard Capucine, Mikołuć Bożena. BCG Moreau vaccine safety profile and NK cells-double protection against disseminated BCG infection in retrospective study of BCG vaccination in 52 Polish children with severe combined immunodeficiency. Journal of Clinical Immunology: 2020: Vol. 40, Nr 1, s. 138-146. doi: 10.1007/s10875-019-00709-1. Epub 2019 Nov 20. PMID: 31749033; PMCID: PMC7082382.

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Mój wkład merytoryczny to zbieranie danych klinicznych

Jednocześnie wyrażam zgodę na przedłożenie w/w prac przez lek. Nel Dąbrowską-Leonik w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych

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- Mój wkład merytoryczny to zbieranie danych klinicznych

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1. Dąbrowska-Leonik Nel, Piątosa Barbara, Słomińska Ewa, Bohynikowa Nadeżda, Bernat-Sitarz Katarzyna, Bernatowska Ewa, Wolska-Kuśnierz Beata, Kałwak Krzysztof, Kołtan Sylwia, Dąbrowska Anna, Goździk Jolanta, Ussowicz Marek, Pac Małgorzata. National experience with adenosine deaminase deficiency related SCID in Polish children. Frontiers in Immunology: 2023: Vol. 14, January 2023, Article 1058623.

Mój wkład merytoryczny to: przygotowanie zbieranie danych, nanoszenie poprawek i uwag krytycznych

Jednocześnie wyrażam zgodę na przedłożenie w/w prac przez lek. Nel Dąbrowską-Leonik w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych

19-06-2026

Data i podpis

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1. Sharapova Svetlana O., Skomska-Pawliszak Małgorzata, Rodina Yulia A., Wolska-Kuśnierz Beata, Dąbrowska-Leonik Nel, Mikołuć Bożena, Pashchenko Olga E., Pasic Srdjan, Freiberger Tomas, Milota Tomas, Formankova Renata, Szaflarska Anna, Siedlar Maciej, Avcin Tadej, Markelj Gasper, Ciznar Peter, Kalwak Krzysztof, Kołtan Sylwia, Jackowska Teresa, Drabko Katarzyna, Gagro Alenka, Pac Małgorzata, Naumova Elissaveta, Kandilarova Snezhina, Babol-Pokora Katarzyna, Varabyou Dzmitry S., Barendregt Barbara H., Raykina Elena V., Varlamova Tatiana V., Pavlova Anna V., Grombirikova Hana, Debeljak Marusa, Mersiyanova Irina V., Bondarenko Anastasia V., Chernyshova Liudmyla I., Kostyuchenko Larysa V., Guseva Marina N., Rascon Jelena, Muleviciene Audrone, Preiksaitiene Egle, Geier Christoph B., Leiss-Piller Alexander, Yamazaki Yasuhiro, Kawai Tomoki, Walter Jolan E., Kondratenko Irina V., Sediva Anna, van der Burg Mirjam, Kuzmenko Natalia B., Notarangelo Luigi D., Bernatowska Ewa, Aleinikova Olga V. The clinical and genetic spectrum of 82 patients with RAG deficiency including a c.256_257delAA founder variant in Slavic countries. Frontiers in Immunology: 2020: Vol. 11, June 2020, s. e900.

Mój wkład merytoryczny to zbieranie danych pacjentów

2. Dąbrowska-Leonik Nel, Pastorczak Agata Karolina, Bąbol-Pokora Katarzyna, Bernat-Sitarz Katarzyna, Piątosa Barbara, Heropolitańska-Pliszka Edyta, Kacprzak Magdalena M., Kalwak Krzysztof, Gul Katarzyna, van der Burg Mirjam, Ussowicz Marek, Pac Małgorzata. Case report: severe combined immunodeficiency with ligase 1 deficiency and Omenn-like manifestation. Frontiers in Immunology: 2022: Vol. 13, October 2022, Article 033338.

Mój wkład merytoryczny to analiza genetyczna

Jednocześnie wyrażam zgodę na przedłożenie w/w prac przez lek. Nel Dąbrowską-Leonik w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych

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I declare that at work:

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My author conributions: designed the research, collected data, interpreted, analyzed the results and wrote the manuscript.

At the same time, I consent to the submission of the above-mentioned works by Dr. Nel Dąbrowska-Leonik in the proceedings for the award of a doctoral degree in the field of medical sciences.



18 of June 2026

.....

Date and signature

OŚWIADCZENIE WSPÓŁAUTORA

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Oświadczam, że w pracy:

1. Bogdał A, Badeński A, Pac M, Wójcicka A, Badeńska M, Didyk A, Trembecka-Dubel E, Dąbrowska-Leonik N, Walaszczyk M, Matysiak N, Morawiec-Knysak A, Szczepański T, Szczepańska M. Atypical Hemolytic Uremic Syndrome (aHUS) and Adenosine Deaminase (ADA)-Deficient Severe Combined Immunodeficiency (SCID)-Two Diseases That Exacerbate Each Other: Case Report. Int J Mol Sci. 2021 Aug 31;22(17):9479.

Mój wkład merytoryczny to: przygotowanie koncepcji merytorycznej, nanoszenie poprawek i uwag krytycznych

Jednocześnie wyrażam zgodę na przedłożenie w/w prac przez lek. Nel Dąbrowską-Leonik w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych

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1. Bogdał A, Badeński A, Pac M, Wójcicka A, Badeńska M, Didyk A, Trembecka-Dubel E, Dąbrowska-Leonik N, Walaszczyk M, Matysiak N, Morawiec-Knysak A, Szczepański T, Szczepańska M. Atypical Hemolytic Uremic Syndrome (aHUS) and Adenosine Deaminase (ADA)-Deficient Severe Combined Immunodeficiency (SCID)-Two Diseases That Exacerbate Each Other: Case Report. Int J Mol Sci. 2021 Aug 31;22(17):9479.

Mój wkład merytoryczny to: przygotowanie koncepcji merytorycznej, nanoszenie poprawek i uwag krytycznych

Jednocześnie wyrażam zgodę na przedłożenie w/w prac przez lek. Nel Dąbrowską-Leonik w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych

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1. Bogdał A, Badeński A, Pac M, Wójcicka A, Badeńska M, Didyk A, Trembecka-Dubel E, Dąbrowska-Leonik N, Walaszczyk M, Matysiak N, Morawiec-Knysak A, Szczepański T, Szczepańska M. Atypical Hemolytic Uremic Syndrome (aHUS) and Adenosine Deaminase (ADA)-Deficient Severe Combined Immunodeficiency (SCID)-Two Diseases That Exacerbate Each Other: Case Report. Int J Mol Sci. 2021 Aug 31;22(17):9479.

Mój wkład merytoryczny to: przygotowanie koncepcji merytorycznej, nanoszenie poprawek i uwag krytycznych

Jednocześnie wyrażam zgodę na przedłożenie w/w prac przez lek. Nel Dąbrowską-Leonik w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych

18.06.2026r. ..... A. Badeński

Data i podpis

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XV. Zgody Komisji Bioetycznej

KOMISJA BIOETYCZNA
przy Instytucie „Pomnik-Centrum Zdrowia Dziecka"
Al. Dzieci Polskich 20
04-730 Warszawa-Międzylesie
T. (22) 815-16-03

UCHWAŁA nr 30 /KBE/2023

OPINIA KOMISJI BIOETYCZNEJ
przy INSTYTUCIE „POMNIK-CENTRUM ZDROWIA DZIECKA"

Komisja Bioetyczna przy Instytucie Pomnik-Centrum Zdrowia Dziecka" **na posiedzeniu w dniu 20.06.2023** rozpatrzyła badanie pt: **Diagnostyka i leczenie pacjentów z ciężkim i złożonym niedoborem odporności -doświadczenia jednego ośrodka**

Gł. Badacz: lek med. Nel Dąbrowska-Leonik

Przedstawione streszczenie (w wersji poprawionej) planowanego badania retrospektywnego omawia cel pracy, planowany materiał badawczy i metody, a także spodziewane do osiągnięcia korzyści.
Na podstawie przedstawionej dokumentacji uważam, że badanie w planowanej formie nie budzi zastrzeżeń natury formalno-etycznej i wnoszę do Komisji Bioetycznej przy IP CZD w Warszawie o wydanie pozytywnej opinii

Przedłożone dokumenty:

1. Streszczenie projektu
2. Opinia eksperta

Na podstawie przedłożonej dokumentacji, dodatkowych wyjaśnień przedstawionych przez lek med. Nel Dąbrowską Leonik oraz dyskusji i wyniku tajnego głosowania Komisja Bioetyczna przy IPCZD wyraziła zgodę na przeprowadzenie badania. Skład i działanie Komisji zgodne z GCP oraz wymogami lokalnymi. Tekst uchwały został sporządzony w 2 jednobrzmiących egz. po jednym dla wnioskodawcy i Komisji Bioetycznej. Lista członków Komisji biorących udział w posiedzeniu stanowi załącznik do niniejszego dokumentu.

RZEWODNICZĄCA KOMISJI BIOETYCZNEJ

przy Instytucie „Pomnik-Centrum Zdrowia Dziecka"
prof. dr hab. n med. Katarzyna Kotulska-Jóźwiak

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posiedzenie w dniu 28.06.2023

LISTA OBECNOŚCI

Lp.	Imię, nazwisko	podpis
1.		
2.	**Mgr praw. Marcjanna Dębska Adwokat**	
3.		
4.	**Dr n med. farm Anna Cieślik**	
5.	**Dr n med. Ludmiła Bacewicz - IPCZD**	—
6.	**Prof. dr hab. n med. Katarzyna Kotulska-Jóźwiak- IPCZD**	
7.	**Prof. dr hab. n med. Wiesława Grajkowska- IPCZD**	
8.	**Dr hab. n med. Piotr Gutkowski - IPCZD**	

Potwierdzam udział w/w osób na spotkaniu Komisji Bioetycznej w dniu 28.06.2023

PRZEWODNICZĄCA KOMISJI BIOETYCZNEJ
przy Instytucie „Pomnik-Centrum Zdrowia Dziecka"

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UCHWAŁA nr 21/KBE/2022

OPINIA KOMISJI BIOETYCZNEJ
przy INSTYTUCIE „POMNIK-CENTRUM ZDROWIA DZIECKA"

Komisja Bioetyczna przy Instytucie Pomnik-Centrum Zdrowia Dziecka" **na posiedzeniu w dniu 18.05.2022r.** rozpatrzyła badanie pt: **Polskie doświadczenie w diagnostyce i leczeniu pacjentów z deficytem deaminazy adenozyny**

Gł. Badacz: lek med. Nel Dąbrowska –Leonik

Do Komisji Bioetycznej Instytutu Pomnik Centrum Zdrowia Dziecka wpłynął wniosek z dnia 27.04.2022r o wydanie opinii o projekcie eksperymentu medycznego „ Polskie doświadczenia w diagnostyce i leczeniu pacjentów z deficytem deaminazy adenozyny".

Badanie przeprowadzone będzie w Klinice Immunologii IPCZD, Katedrze i Klinice Transplantacji Szpiku, Onkologii Dziecięcej we Wrocławiu, Klinice Pediatrii, Hematologii i Onkologii w Toruniu, Ośrodku Transplantacji Uniwersyteckiego Szpitala w Krakowie, Pracowni Zgodności Tkankowej IPCZD.

Badacz główny- lek Nel Dąbrowska-Leonik

Badaniem objętych będzie 7 dzieci. Badanie ma charakter retrospektywny.

Cel Badania: retrospektywna ocena objawów klinicznych i wyników badań biochemicznych, immunologicznych i genetycznych prowadzących do postawienia diagnozy.

Do dokumentacji dołączone wymagane zgody Kierowników Klinik biorących udział ww badaniu. Zbierane będą rutynowe dane kliniczne, dane będą anonimizowane. Nie będą wykonywane żadne dodatkowe badania ani procedury medyczne związane z badaniem. Ochrona danych osobowych zgodna z wytycznymi RODO.

Złożona dokumentacja zawarta we wniosku o wydanie opinii do Komisji Bioetycznej przy IPCZD, w sprawie przeprowadzenia eksperymentu medycznego" Polskie doświadczenia w diagnostyce i leczeniu pacjentów z deficytem deaminazy adenozyny " pozwala uznać badanie za zasadne oraz stwierdzić, że sposób jego przeprowadzenia odbędzie się według zasad GCP.

Do zespołu badaczy należą:

Dr hab. n med. Małgorzata Pac
Lek med. Katarzyna Bernat-Sitarz
Lek med. Nadezda Bohynikova
Dr hab. n med. Barbara Piątosa

Przedłożone dokumenty:

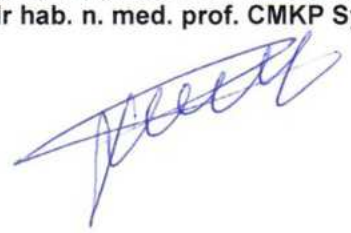
1. Wniosek o wydanie opinii o projekcie
2. Streszczenie projektu
3. Opinia Eksperta

Na podstawie przedłożonej dokumentacji, dodatkowych wyjaśnień przedstawionych przez lek med. Nel Dąbrowską – Leonik oraz dyskusji i wyniku tajnego głosowania Komisja Bioetyczna przy

IPCZD wyraziła zgodę na przeprowadzenie badania. Skład i działanie Komisji zgodne z GCP oraz wymogami lokalnymi. Tekst uchwały został sporządzony w 2 jednobrzmiących egz. po jednym dla wnioskodawcy i Komisji Bioetycznej. Lista członków Komisji biorących udział w posiedzeniu stanowi załącznik do niniejszego dokumentu.

PRZEWODNICZĄCY KOMISJI BIOETYCZNEJ

przy Instytucie „Pomnik-Centrum Zdrowia Dziecka"
dr hab. n. med. prof. CMKP Sylwester Prokurat



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posiedzenie w dniu 13 V 2022

LISTA OBECNOŚCI

Lp.	Imię, nazwisko, zawód /specjalność, funkcja	podpis
1.	**Prof. dr hab. n hum Paweł Łuków** Etyk	
2.	**Mgr praw. Marcjanna Dębska** Adwokat	-
3.	**Ks. Paweł Śmierzchalski** Teolog, Duszpasterstwo Służby Zdrowia	
4.	**Mgr Piel Ewa Szkiela** Pielęgniarka	
5.	**Dr n med.farm Anna Cieślik**	-
6.	**Dr n med. Ludmiła Bacewicz** Lekarz chirurg- IPCZD	
7.	**Prof. Dr hab. n med. Katarzyna Kotulska-Jóźwiak** Lekarz (neurolog, neurolog dziecięcy)- IPCZD	
8.	**Lek med. Jacek Rubik** IPCZD	
9.	**Dr n med. Małgorzata Łyszkowska** Lekarz (pediatra, transplantolog Kliniczny) - IPCZD	
10.	**Prof. nadzw dr hab. n med. Wiesława Grajkowska** IPCZD	
11.	**Dr hab. n med. Sylwester Prokurat, prof. nadzw** Lekarz (pediatra, nefrolog, transplantolog Kliniczny)- IPCZD	

Potwierdzam udział w w/w posiedzeniu Komisji Bioetycznej przy IPCZD w dniu 18.05.22