II. Streszczenie w języku angielskim

Severe combined immunodeficiency (SCID) is an inborn error of immunity resulting from impaired maturation and differentiation of T lymphocytes from hematopoietic stem cells in the bone marrow. In some genotypes, the impaired maturation also affects B lymphocytes and/or NK cells. The incidence of the disease, determined based on newborn screening tests, varies between countries and is: in Germany 1:54,000, in the USA 1:58,000-1:66,000, in Israel 1:29,000. In countries with a high percentage of consanguineous marriages, the incidence is higher, e.g., in Saudi Arabia 1:2,906, and in Oman 1:22,000 [1-6].

SCID is diagnosed based on immunological tests (quantitative assessment of T, B, NK lymphocyte subpopulations using flow cytometry, functional lymphoproliferative response) and molecular with the assessment of genetic variants. Depending on the clinical symptoms and test results, the disease can be classified as typical, atypical (usually with a milder course), or Omenn syndrome [7]. Genes with pathogenic variants most commonly causing SCID include *IL2RG*, *RAG1/2*, *ADA*, *IL7R*, *DCLRE1C*, and *JAK3*; these constitute over 80% of SCID genotypes in the USA [7] and over 70% in Germany [2].

Untreated, the disease leads to death in the first years of life, primarily due to infections, including opportunistic ones. The primary treatment option is hematopoietic stem cell transplantation (HSCT). Gene therapies are available in Europe to address pathogenic changes in certain genes: *ADA*, *IL2RG*, *RAG1*, and *DCLRE1C*. However, only Strimvelis has received a positive opinion from the European Medicines Agency for the treatment of ADA-SCID. Enzyme replacement therapy (ERT) is available for patients with adenosine deaminase deficiency (ADA-SCID). In Omenn syndrome, immunosuppressive therapy is used prior to targeted therapy.

Patients with ADA-SCID are a special group. The disease is an inherited disorder of purine metabolism, and symptoms result from the accumulation of toxic deoxyadenosine, a substrate of the missing ADA enzyme, in cells. They affect not only the bone marrow, leading to severe combined immunodeficiency, but also other organs: the brain, lungs, liver, and skeletal system. ADA-SCID has a prevalence of 1 in 200,000–1 in 1 million births and accounts for approximately 10–20% of all SCID patients. Treatment includes hematopoietic stem cell transplantation, enzyme replacement therapy, and gene therapy [8].

Early diagnosis of SCID increases the chances of successful treatment. The survival rate for infants undergoing HSCT before the age of 3.5 months and before infectious complications occurs is 94%, while in older infants with concomitant infections, this rate decreased to 17- 82% [9-10]. In some countries, newborn screening helps to detect patients with SCID before infections develop. In Poland newborn screening for SCID is available from September 2025. Previously diagnosis could only be made in siblings of children diagnosed with SCID. It is important to increase awareness of the symptoms of SCID and to identify patients early.

The primary aim of this work is to characterize the clinical and laboratory factors, including immunogenetic characteristics, of patients with various types of SCID diagnosed at the Immunology Department of the Children's Memorial Health Institute (CMHI). The dissertation

comprises a series of six publications, four original articles and two case reports, which address the specific objectives outlined below.

The first publication, titled "What a pediatrician should know about different types of severe combined immunodeficiency – a single-center experience," aims to characterize the clinical symptoms and laboratory results, including immunogenetic, of patients diagnosed with typical, atypical SCID, and Omenn syndrome at the Immunology Department CMHI.

In the second publication, entitled "National experience with adenosine deaminase deficiency related SCID in Polish children", the aim was to characterize clinical, laboratory and immunogenetic characteristics of patients diagnosed with ADA-SCID and to evaluate the effects of ERT treatment of children with ADA-SCID at the Immunology Department CMHI.

In the third publication, titled “BCG Moreau vaccine safety profile and NK cells—double protection against disseminated BCG infection in a retrospective study of BCG vaccination in 52 Polish children with severe combined immunodeficiency,” the aim was to assess the incidence of adverse reactions following administration of the live BCG Moreau vaccine in patients with SCID in Poland.

The aim of the fourth publication, "The clinical and genetic spectrum of 82 patients with RAG deficiency, including a c.256_257delAA founder variant in Slavic countries," was to estimate the prevalence, present clinical outcome, and genetic variability of patients diagnosed with SCID caused by variants in *RAG* genes in populations living in Slavic countries.

The aim of the fifth publication, "Case report: severe combined immunodeficiency with ligase 1 deficiency and Omenn-like manifestation," and the sixth publication, "Atypical Hemolytic Uremic Syndrome (aHUS) and Adenosine Deaminase (ADA)-Deficient Severe Combined Immunodeficiency (SCID)-Two Diseases That Exacerbate Each Other: Case Report," was to present the unique disease courses of two patients diagnosed with SCID.

Results

In the publication "What pediatricians should know about different types of severe combined immunodeficiency – a single center experience," the median age at diagnosis of SCID was 4.4 months, higher in AS (8 months, $p<0.05$). The median diagnostic delay (DD) was 2.5 months (4.7 months in children with AS). The disease was suspected by pediatricians (in 44.4% of all children), hematologists (19.4% of all children, 26.3% of children with AS, DD - 20 months), or dermatologists. The initial symptoms were infections (64%), rash (22.2%), chronic diarrhea (5.6%), anemia (5.6%), and lymphopenia (2.8%). Lymphopenia was present in 79.5% of patients at diagnosis. Median recent thymic emigrants (RTE) were 0.2%, 2.1%, and 0.3% in TS, AS, and OS, respectively. The most common 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%). Survival was 69% in all children.

In the publication "National experience with adenosine deaminase deficiency related SCID in Polish children," the diagnosis of ADA-SCID was made between 7 weeks and 27 months of age, in

the first three patients between 16 and 27 months of age, and in the last four between 7 weeks and 6 months of age. At the time of suspected SCID diagnosis, all patients had leukopenia with neutropenia and lymphopenia. Extramedullary manifestations included: elevated transaminase activity (5 patients), skeletal dysplasia (2 patients), atrophy in the central nervous system (1 patient), and atypical hemolytic-uremic syndrome (1 patient). In all patients, the counts of T lymphocytes (both CD4+ and CD8+), B lymphocytes, and NK cells were below age-appropriate reference values. The concentration of immunoglobulin G was decreased in two patients, and IgM in three patients. The diagnosis of ADA-SCID was made based on ADA activity in red blood cells (4 patients) and/or genetic testing (pathogenic variants were identified in 6 patients). Six patients underwent HSCT; all are alive; one patient died before qualifying for HSCT. Four patients in the Immunology Department CMHI received ERT prior to HSCT, and normalization of lymphocyte counts and transaminase activity was observed. None of the patients underwent gene therapy.

The publication "BCG Moreau vaccine safety profile and NK cells—double protection against disseminated BCG infection in a retrospective study of BCG vaccination in 52 Polish children with severe combined immunodeficiency" demonstrated that disseminated BCG infection occurs in 19% of children with SCID vaccinated in Poland, a rate lower than in other global populations (34% in Marciano's study (p=0.0028) [11]. Disseminated BCG infection was observed only in patients with low NK cell counts (p=0.0161). NK cell counts did not influence the occurrence of local adverse reactions to the vaccine.

In the publication “The clinical and genetic spectrum of 82 patients with RAG deficiency including a c.256_257delAA founder variant in Slavic countries,” a group of 82 patients with RAG deficiency demonstrated typical (n = 20), atypical (n = 21), Omenn syndrome (n = 37) SCID phenotypes and CID (n=4). Sixty-seven (81.7%) patients were *RAG1* carriers, and 15 patients (18.3%) had biallelic *RAG2* variants. We estimate that the minimum annual incidence of RAG deficiency in Slavic countries ranges from 1 in 180,000 to 1 in 300,000 live births. In the cohort of patients with *RAG1* variants, 70% (n = 47) carried the c.256_257delAA allele in a homozygous (n = 18, 27%) or compound heterozygous (n = 29, 43%). The majority (77%) of patients with the homozygous *RAG1* c.256_257delAA variant originated from the Vistula River basin in central-eastern Poland, and cases of compound heterozygote occurred in all Slavic countries except Bulgaria. The clinical and immunological course of patients with the homozygous c.256_257delAA variant of the *RAG1* gene was very diverse (typical SCID, atypical SCID, Omenn syndrome).

The publication "Case report: severe combined immunodeficiency with ligase 1 deficiency and Omenn-like manifestation" presents a child with Omenn-like SCID and macrocytic anemia, who was diagnosed with ligase DNA I deficiency. Severe macrocytic anemia and lymphopenia in peripheral blood and bone marrow prompted rapid diagnostic workup for inborn errors of immunity. At 8 weeks of age, SCID of the Omenn syndrome type was diagnosed. Genetic testing revealed the presence of two new variants in the *LIG1* gene, and exposure of fibroblasts to gamma radiation revealed increased radiosensitivity. At 4 months of age, HSCT was performed from a matched unrelated donor according to the protocol for radiosensitive patients. The publication "Atypical Hemolytic Uremic Syndrome (aHUS) and Adenosine Deaminase (ADA)-Deficient Severe Combined Immunodeficiency (SCID)-Two Diseases That Exacerbate Each Other: Case Report"

describes a patient with an atypical course of ADA-SCID characterized by predominant renal symptoms. Within the first few months, atypical hemolytic uremic syndrome and renal failure were diagnosed. ADA-SCID was suspected only after end-stage multiple organ failure and confirmed *post mortem*.

Summary

The topic of this dissertation is the diagnosis and treatment of patients with severe combined immunodeficiency diagnosed in the Immunology Department CMHI, and these issues are presented in all papers. Paper 2 also included two Polish patients from outside the IPCZD, and Paper 4 also included patients from other Slavic countries.

In most patients, the symptoms causing the suspicion of SCID and referral for immunological diagnostics were infections – severe, life-threatening, recurrent, opportunistic, or disseminated BCG infection. Most patients (80%) had lymphopenia at that time, which should have been a reason for prior investigation of SCID. The diagnosis of SCID was made significantly later in cases of AS than in TS and OS. The type of first symptoms does not differ between the different types of SCID.

Publications No. 1 and No. 2 found that the diagnosis of SCID and ADA-SCID is being made in increasingly younger patients. Early diagnosis in ADA-SCID patients may result from the presence of leukopenia (with neutropenia and lymphopenia), which alerted pediatricians and allowed for the suspicion of immunodeficiency. Isolated lymphopenia without leukopenia and neutropenia in patients with other forms of SCID can be overlooked. The longest delay in diagnosis was seen in patients with atypical SCID, all of whom had lymphopenia. Therefore, evaluation for SCID is recommended in infants with lymphopenia, especially if chest radiography shows thymic aplasia/hypoplasia or dysplastic bone changes that may indicate ADA-SCID (acute scapular angle).

A serious complication of SCID can be disseminated BCG infection, which affects nearly 20% of SCID patients vaccinated in Poland, as presented in publication no. 3. The Moreau BCG substrain produced in Poland by Biomed is derived from the parent *Mycobacterium bovis BCG Moreau Rio de Janeiro* substrain, but it is safer than in the Brazilian population (48% of BCG-vaccinated SCID patients had disseminated infection) or in other global populations (34%).

Genetic diagnostics showed that the most common SCID genotypes in patients diagnosed by the Immunology Department CMHI between 2010 and 2023 were *IL2RG*, *RAG 1/2*, *JAK3*, and *ADA*.

In the *RAG1* gene, our patients have the dominant homozygous variant c.256_257delAA. The high prevalence of this variant among children from Eastern Poland and the presence of this variant in all Slavic peoples may indicate that it is a founder variant originating from the Vistula River basin in Poland, as presented in publication no. 4.

The causal treatment for SCID patients is HSCT, but some patients with severe infections or multi-organ failure die at diagnosis or in the peri-transplant period. The survival rate of SCID patients diagnosed by the Immunology Department CMHI in the years 2010-2023 was above 80% in

patients with a positive family history and diagnosed in the first days and treated with HSCT in the first months of life, and in patients diagnosed after the onset of symptoms it was 68% (26/38), including 78% (26/34) who underwent HSCT (4 patients died before HSCT) – publication no. 1.

ERT was effective in all four treated patients with ADA-SCID. None of the patients underwent gene therapy, due to limited access to the procedure in a few centers in Europe and the longer duration of treatment and immune reconstitution with gene therapy compared to HSCT.

SCID is an ultra-rare disease in the European population, and some patients have unique symptom manifestations, as described in two case reports. Publication No. 5 describes a child with SCID of the Omenn syndrome type, accompanied by severe congenital macrocytic anemia. Molecular diagnostics revealed that the cause of the symptoms was ligase 1 deficiency. This is an extremely rare disease; to date, seven patients with ligase 1 deficiency have been described worldwide, with a diverse spectrum of immunological manifestations. Three of these patients (including our patient) underwent HSCT. Early diagnosis in this patient was due to the presence of severe macrocytic anemia, requiring numerous red blood cell transfusions and referral to a hematologist, who noted the accompanying lymphopenia. Publication No. 6 describes a patient with an atypical course of ADA-SCID in the form of dominant nephrological symptoms: atypical hemolytic uremic syndrome and renal failure were diagnosed in the first months, and ADA-SCID was suspected at the end-stage multiple organ failure.

The characteristics of clinical symptoms and patient test results presented in the above-mentioned works may allow for improving the diagnosis of SCID before organ complications occur and thus increasing the survival rate of these patients.