Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica

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ORIGINAL ARTICLE

OPEN ACCESS

Diagnosis of primary immunodeficiency diseases in pediatric patients hospitalized for recurrent, severe, or unusual infections

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Abstract

Background: Primary immunodeficiency diseases (PID) usually presents itself with recurrent, severe, and unusual infections, along with autoimmunity and various other malignancies. But, the diversity of PID often makes the diagnosis of patients difficult for physicians other than clinical immunologists. This study aimed to describe the characteristics of patients diagnosed with PIDs during the inpatient treatment for infectious diseases, and to highlight the cases in which a PID diagnosis should be considered.

Methods: The clinical, immunological, and molecular features of 81 pediatric patients treated for infectious diseases, who were diagnosed with a PID during hospitalization was retrospectively analyzed. The diagnosis was based on the PID criteria of the International Union of Immunological Societies.

Results: The five main PID sub-types were identified. Predominantly, antibody deficiencies were the most common (61.7%) group. The average delay in diagnosis was 34.6 months, and the positive family history rate was 24.7%, while the consanguineous marriage rate was 45.7%. Around thirty-five (43%) patients were found to have mutated PID-related genes. While lower respiratory tract infections were the most common symptom, a fever of unknown origin was another remarkable diagnosis. Eight (9.9%) patients underwent allogeneic hematopoietic stem cell transplantation.

Conclusions: Clinicians should consider a PID diagnosis, especially in the cases of recurrent, severe, or atypical infections. Increased knowledge of the alarm features of PID can promote early diagnosis.

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Introduction

Primary immunodeficiency diseases (PID) are a heterogeneous group of inherited immune system disorders that predispose the patients to recurrent infections, allergies, autoimmunity, and other malignancies. PIDs comprise of more than 400 disorders, which the International Union of Immunological Societies (IUIS) expert committee has categorized into 10 groups based on the patients' genetic and molecular characteristics.[1]

PIDs are common, and their prevalence may be considerably higher than generally thought. It is estimated that 1 in 1,200 people worldwide lives with a PID.[2] In Turkey, the estimated prevalence of PIDs among the general population has been estimated to be 30.5/100,000 people.[3] The prevalence of PIDs has increased significantly over the years.[1,3-5] Higher number of patients are diagnosed with PID every year, and more immunodeficiency syndromes are described every year.[2]

The diagnosis of PID was based on the updated criteria of the IUIS (2019)[1], according to which the PIDs are classified into 10 groups: immunodeficiencies affecting cellular and humoral immunity, combined immunodeficiency (CID) with associated or syndromic features, predominantly antibody deficiencies, diseases of immune dysregulation, congenital defects of phagocyte number, function, or both, defects in intrinsic and innate immunity, auto-inflammatory disorders, complement deficiencies, bone marrow failure, and phenocopies of PID.[1]

To reduce PID-associated morbidity and mortality rates, early diagnosis is the most important step. This can be achieved by increasing the physicians' awareness of warning signs. This study is aimed to describe the clinical features of PID patients, and highlight the cases in which a PID diagnosis should be considered.

Materials and Methods

The clinical and laboratory features of PID patients (n=81) who received inpatient treatment at the Division of Pediatric Allergy and Immunology of Istanbul Cerrahpaşa Medical School between 2007 and 2017 was retrospectively analyzed. The patients who were hospitalized for any infectious disease and diagnosed with PID were included in the study.

The data on the demographic characteristics, parental consanguinity status, family history for PID, clinical manifestations (age at onset of the disease, age at diagnosis, and infection history), laboratory investigations (immunological and hematological parameters), treatment modality, and follow-up were retrieved from patient files and electronic records. Time to diagnosis (diagnostic delay) in PID diagnosis was defined as the time between the initial presentation and the time of diagnosis.

Complete blood count analyses (white blood cell, lymphocyte, and neutrophil counts) were performed using a Beckman Coulter LH 780 device. CD3+, CD4+, and CD8+ T cell, CD19+ B cell, and CD16/56+ natural killer (NK) cell counts were performed with a Beckton Dickinson FACSCalibur flow cytometer using monoclonal antibodies. Serum immunoglobulin (IgG, IgA, IgM, and IgE) measurements were also performed. The serum immunoglobulin

levels and leukocyte counts were evaluated by comparing it with the local age-related normal range values.[6,7] Nitro blue tetrazolium dye tests and assessments of the expression of CD18/CD11 on neutrophils were performed using flow cytometry. The chromosome 22q11.2 deletion in DiGeorge syndrome patients was detected using the fluorescence in situ hybridization method. The genetic diagnoses of all patients except severe combined immunodeficiency (SCID) patients were performed by whole exome sequencing (WES) in outer research laboratories. A previously designed custom-made targeted NGS panel which contains 18 SCID related genes was performed on SCID patients.[8]

The study was approved by the Ethics Committee of the Faculty of Medicine of Istanbul Cerrahpaşa University (decision number 1791; February 2018). Informed consent was obtained from all participants' parents or legal guardians.

Results

General features of the patients

Eighty-one patients hospitalized for infectious diseases and diagnosed with PID were included in the study. Of those, 35 (43.2%) were female and 46 (56.8%) were male. The patients were categorized into five groups according to the IUIS classification.[1] Predominantly antibody deficiency (PAD) patients were the most common group in our cohort, including 22 (27.2%) patients with common variable immune deficiency (CVID), 26 (32.1%) patients with IgA deficiency, and two (2.5%) patients with hyper-IgM syndrome (Group 1). The second common group was the one with congenital defects of phagocyte number or function (Group 2), which included 11 (13.6%) chronic granulomatous disease (CGD) and two (2.5%) leukocyte adhesion deficiency type 3 (LAD3) patients. The immunodeficiencies affecting cellular and humoral immunity group, the most severe form of PID, comprised of 9 (11.1%) SCID patients (Group 3). About five (6.2%) patients with DiGeorge syndrome and 1 (1.2%) patient with the hyper-IgE syndrome were grouped as combined immune deficiency (CID) with associated or syndromic features group (Group 4). Lastly, three patients were included in the defects in the intrinsic and innate immunity group (Group 5).

The median age at the onset of symptoms was eight months (range: 1–192 months), and the median age at diagnosis was 48 months (range: 1–216 months). The mean time to diagnosis was 34.6 months. The longest mean time to diagnosis was observed in the PAD group (51.7 months), whereas the shortest was observed in DiGeorge syndrome and SCID patients. The average delay between onset of symptoms and diagnosis differed between the groups: 48.7 months in Group 1, 22 months in Group 2, 3.6 months in Group 3, 3.5 months in Group 4, and 11 months in Group 5. The clinical and immunological features of the patients according to their classification is shown in Table 1.

Genetic diagnosis in PID patients

Whole exome or targeted panel sequencing was performed on our cohort, and the genetic diagnoses were archived in 35 (43%) patients (Figure 1). Whole exome sequencing

Table 1. General features of patients according to PID sub-type

Diagnosis	Patient number	Gender (F/M)	Age at onset (month, median range)	Age at diagnosis (month, median, range)	Time to diagnosis (month) average	Mortality n (%)	Consanguinity rate (%)	Family history of PID (%)	HSCT (%)
Group 1									
CVID	22	10/12	12 (1.5-192)	49.5 (48-216)	51.7	0	36.4	31.8	0
IgA deficiency	26	12/14	27 (6-156)	96 (12-216)	48.7	0	42.3	3.8	0
Hyper-IgM syndrome	2	0/2	7.5 (7-8)	16 (14-18)	8.5	0	0	0	0
Group 2									
CGD	11	1/10	4 (1-60)	24 (3-168)	24.9	1 (9.1)	36.4	45.5	9.1
LAD3	2	1/1	1	6.5 (1-12)	5.5	1 (50)	100	100	100
Group 3									
SCID	9	4/5	2 (1-6)	5 (1.5-12)	3.6	4 (44.4)	88.9	44.4	55.6
Group 4									
DiGeorge syndrome	5	5/0	4 (2-9)	4 (2-16)	3	0	20	0	0
Hyper-IgE syndrome	1	1/0	4	10	6	0	100	0	0
Group 5									
IRAK4 deficiency	1	0/1	9	22	13	0	100	100	0
MyD88 deficiency	1	1/0	2	14	12	0	0	0	0
IFN-gammaR1 deficiency	1	0/1	6	14	8	0	100	0	0
Total	81	35/46	8 (1-192)	48 (1-216)	34.6	6 (7.4)	45.7	24.7	9.9
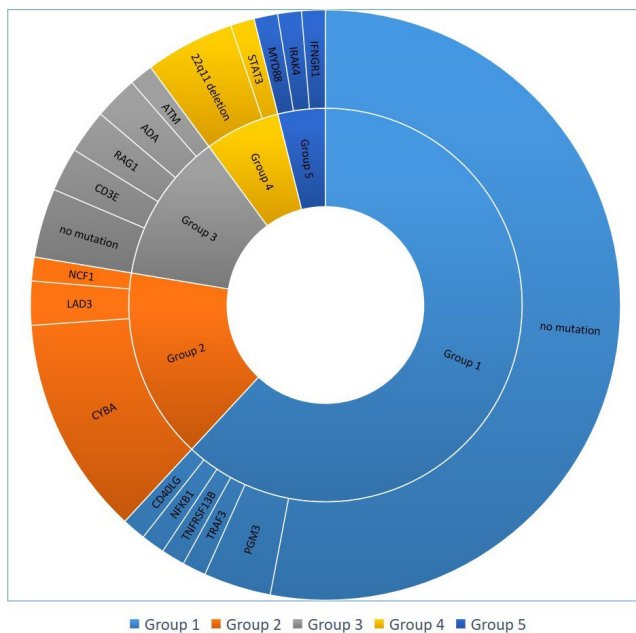
Figure 1 Mutation rates in primary immunodeficiency patients Group1: Predominantly antibody deficiency PAD; Group 2: The congenital defects of phagocyte number or function; Group 3: Immunodeficiencies affecting cellular and humoral immunity; Group 4: Combined immune deficiency (CID) with associated or syndromic features; Group 5: The defects in intrinsic and innate immunity.

was performed in all the groups except SCID, and the disease-causing variants were detected in all the members of groups 2, 4, and 5.

In the congenital defects of phagocyte number or function (Group 2) group, 11 patients were diagnosed as CGD, 10 patients were diagnosed with *CYBA* and 1 patient had an *NCF1* variant. In the intrinsic and innate immunity group (Group 5), all the patients were diagnosed with *IFN-gammaR1, IRAK4,* or *MYD88* deficiency. In the CID group (Group 4), there were four patients with DiGeorge syndrome and one Hyper IgE patient with a *STAT3* gene variant. In the PAD group (group 1), seven patients carried *CD40LG, PGM3, TNFRSF13B,* and *NFKB1* variants.

For the SCID group (group 3), a targeted-NGS panel was previously created, and *ADA, CD3E, RAG1,* and *ATM* gene variants were identified in out seven of 10 patients.

Clinical presentations and outcomes

The most common reason for hospitalization was found to be lower respiratory tract infection (LRTI), which was observed in five (60%) of our cohort, followed by fever of unknown origin (FUO) in 9.9%, sepsis in 4%, gastroenteritis in 3.7%, arthritis in 3.7%, meningitis in 3.7%, lymphadenitis in 3.7%, liver abscess in 3.7%, 1 osteomyelitis, 1 myocarditis, 1 omphalitis, and 1 perianal abscess in our cohort. The leucocyte counts, hemoglobin values, platelet counts, and serum immunoglobulin levels in each group is shown in Table 2, and the reasons for hospitalization that eventually led to PID diagnosis is shown in Table 3.

In the SCID cohort, 5 patients were hospitalized with an LRTI diagnosis, three had sepsis, and one had gastroenteritis. The blood culture of one of the patients diagnosed with

Table 2 Leucocyte counts, hemoglobin values, platelet counts, serum immunoglobulin levels, and peripheral blood lymphocyte subsets.

Laboratory	Group 1	Group 2	Group 3	Group 4	Group 5
Leucocytes (/mm³)	6,850 (1,169-22,700)	22,000 (8,400-49,300)	2,400 (1400-6,300)	12,250 (7,300-18,700)	22,400 (12,800-26,700)
Hgb (g/dL)	11.7 (8.1-15.2)	9.4 (7.3-11.8)	10 (9-11.1)	10.25 (9.5-11.7)	10.5 (10.2-10.8)
Platelets (× 10³)	254 (52-476)	495 (31-780)	286 (209-430)	285 (260-380)	380 (360-440)
Neutrophils (/mm³)	3,300 (500-13,900)	14,000 (3,000-43,398)	1,150 (600-4,200)	3,658 (2,000-8,800)	17,600 (8,200-22,700)
Lymphocytes (/mm³)	2,200 (600-7,100)	5,600 (2,800-11,000)	800 (300-2,200)	6500 (2,555-9,400)	3,800 (3,200-7,300)
Eosinophils (/mm³)	100 (100-1,100)	400 (100-2,200)	740 (150-1,100)	300 (100-1,100)	130 (100-150)
IgG (gr/dL)	254 (60-557)	1,090 (535-2,132)	190 (73-340)	390 (314-530)	740 (560-820)
IgA (gr/dL)	8.65 (0-125)	68 (14.6-462)	0 (0-16)	32.4 (10.7-70)	110 (66-110)
IgM (gr/dL)	27 (2.9-206)	164 (31.2-431)	24 (19-63)	58 (19.3-120)	160 (110-180)
IgE (kU/L)	39 (2-5,100)	18 (16-5,100)	1,100 (17-1,600)	17 (17-2,300)	17 (17-17)
CD3 (/mm³)	1,803 (510-6,106)	3,180 (1,792-6,882)	308 (0-1,122)	2,557 (1,200-6,670)	2,280 (2,272-4,453)
CD4 (/mm³)	736 (122-2,440)	1,978 (882-9,108)	120 (0-319)	1,815 (264-3,535)	1,232 (1,056-2,200)
CD8 (/mm³)	706.5 (91-3,840)	1,008 (444-1,848)	77 (0-297)	767 (108-1,555)	704 (616-1,100)
CD19 (/mm³)	175 (20-1,540)	616 (230-1,554)	95 (49-576)	2154 (300-3,618)	380 (352-730)
CD16-56 (/mm³)	107 (25-848)	256 (112-555)	24 (12-660)	348 (248-540)	228 (192-365)

All values are medians (ranges). Hgb: haemoglobin; Ig: immunoglobulin.

Table 3 Causes of hospitalization.

Diagnosis	n	%
LRTI	49	60.5
FUO	8	9.9
Sepsis	6	7.4
Gastroenteritis	3	3.7
Arthritis	3	3.7
Meningitis	3	3.7
Lymphadenitis	3	3.7
Liver abscess	2	2.5
Osteomyelitis	1	1.2
Myocarditis	1	1.2
Omphalitis	1	1.2
Perianal abscess	1	1.2
Total	**81**	**100**

FUO: fever of unknown origin; LRTI: lower respiratory tract infection; n: number

sepsis showed *Klebsiella pneumoniae* growth. About eight of the nine patients had cytomegalovirus viremia (CMV).

In DiGeorge syndrome patients, three were hospitalized with an LRTI diagnosis, one had sepsis, and one had FUO. The blood culture of the patient with sepsis showed *Pseudomonas aeruginosa* growth.

In CVID patients, the most common symptom was LRTI (81.8%), followed by sepsis, meningitis, and FUO. One patient was diagnosed with pulmonary tuberculosis in a follow-up examination. The patients with IgA deficiency were mostly hospitalized with an LRTI diagnosis (61.5%). It was found that three patients were presented with arthritis.

Three patients were admitted with an LRTI diagnosis, two had a liver abscess, two were diagnosed with lymphadenitis, one had a perianal abscess, one had sepsis, one had myocarditis, and one had osteomyelitis in CGD patients. *Salmonella* growth was observed in the blood and stool cultures of the patient with sepsis. Methicillin-resistant *Staphylococcus aureus* was isolated from four patients with liver and perianal abscesses and lymphadenitis. *Aspergillus fumigatus* was detected in the sputum sample of one patient. One patient with a LAD3 variant was admitted with an LTRI. The other LAD3 deficiency patient was diagnosed with omphalitis.

The patients with IFN-gammaR1 deficiency was hospitalized for tuberculosis lymphadenitis. The patients diagnosed with *IRAK4* and *MyD88* deficiency were followed up with a diagnosis of streptococcal pneumococcal meningitis.

Alarm features suggesting PID

Recurrent upper and lower respiratory tract infection histories were found in 63% of the patients, while recurrent acute gastroenteritis was found in 11.1% of the patients. These findings were seen mostly in Group 1. Oral candidiasis was observed in nearly all patients (88.9%) in Group 3. Bronchiectasis was found only in Group 1 (n = 21, 25.9%). The failure to thrive and growth retardation were observed in 50 (61.7%) patients. Bacillus Calmette-Guérin (BCG) vaccine complications (BCGitis) were found in nine (11.1%)

Table 4 Warning signs of PID.

Sign	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Group 4 n (%)	Group 5 n (%)	Total n (%)
Recurrent URTI	42 (84)	4 (30.8)	2 (22.2)	1 (16.7)	2 (66.7)	51 (63)
Recurrent LRTI	34 (68)	7 (53.8)	5 (55.6)	3 (50)	2 (66.7)	51 (63)
Recurrent AGE	6 (12)	2 (15.4)	1 (11.1)	0 (0)	0 (0)	9 (11.1)
Oral candidiasis	0 (0)	0 (0)	8 (88.9)	0 (0)	0 (0)	8 (9.9)
Bronchiectasis	21 (42)	0 (0)	0 (0)	0 (0)	0 (0)	21 (25.9)
Growth retardation	30 (60)	6 (46.2)	7 (77.8)	5 (83.3)	2 (66.7)	50 (61.7)
BCGitis	0 (0)	4 (30.8)	3 (33.3)	1 (16.7)	1 (33.3)	9 (11.1)
Eczema	6 (12)	2 (15.4)	5 (55.6)	1 (16.7)	0 (0)	14 (17.3)
Hypereosinophilia	6 (12)	6 (46.2)	8 (88.9)	2 (33.3)	0 (0)	22 (27.2)
Hyper-IgE	19 (38)	4 (30.8)	8 (88.9)	1 (16.7)	0 (0)	32 (39.5)
Recurrent hospitalization	18 (36)	10 (76.9)	7 (77.8)	2 (33.3)	3 (100)	40 (49.4)
Family history of PID	8 (16)	7 (53.8)	4 (44.4)	0 (0)	1 (33.3)	20 (24.7)
Hepatomegaly	9 (18)	6 (46.2)	5 (55.6)	1 (16.7)	2 (66.7)	23 (28.4)
Splenomegaly	18 (36)	6 (46.2)	5 (55.6)	1 (16.7)	2 (66.7)	32 (39.5)
Allergic disease	18 (36)	2 (15.4)	5 (55.6)	1 (16.7)	0 (0)	26 (32.1)

AGE: acute gastroenteritis; BCG: Bacillus Calmette-Guérin; IgE: immunoglobulin E; LRTI: lower respiratory tract infection; PID: primary immunodeficiency disease; URTI: upper respiratory tract infection.

patients. Recurrent hospitalizations were seen in nearly half (49.4%) of the patients. The alarm features of the patients are shown in Table 4.

The parental consanguinity rate was 45.7% overall and was highest in Group 3. The rate of family history of PID was 24.7% overall and was highest in Groups 2 and 3.

Treatment and follow-up

Prophylactic antibiotic and intravenous immunoglobulin replacement (IVIG) therapies were the most administered therapies (n = 55, 67.9%, and n = 33, 40.7%, respectively). Antifungal prophylaxis was administered to 20 (24.7%) patients. CGD patients received IFN-gamma and antifungal agents.

Eight patients (9.9%) underwent allogeneic hematopoietic stem cell transplantation (HSCT). Among the eight, five had SCID, two had LAD3, and one had CGD. After HSCT, two patients (one with LAD3 and one with SCID) died. The mortality rate was 7.4% overall, and was the highest in Group 1 (44.4%).

Discussion

Primary immunodeficiencies are a heterogeneous group of inborn errors of immunity characterized by recurrent, severe, or atypical infections, autoimmunity, developmental delays, allergic diseases, and malignancies.[9] In the present study, more than half of the patients were diagnosed with PID. This finding is consistent with previous studies.[5,10,11] Moreover, the number of male patients was higher than females. Similar ratios which suggest an X-linked inheritance of some PIDs have been reported by other studies[1,3,4,11-13]

In PID, the rate of consanguineous marriages is higher than in the general population.[1,11] Nevertheless, it varies

widely between geographic regions, ranging from 15% to 80%.[4,5,11,13,14] A family history of PID is another important warning sign that should be kept in mind. Previous studies have reported rates between 23% and 43%.[10,12-14] In our study, the positive family history rate was found 24.7%, and the consanguineous marriage rate was found to be 45.7%.

Diagnostic delay in PID is a common event that causes high morbidity and mortality in patients. Diagnostic delay is considerably shorter in combined T and B cell deficiency.[3] In our cohort, the average time between the onset of clinical symptoms and PID diagnosis was 34.6 months, but was only approximately four months in Groups 1 and 4. In the literature, diagnostic delay varies between 19 and 60 months. The differences may be due to different cohort sizes.[3,12-15]

The patients diagnosed with PID may present with different clinical findings. Recurrent infections are the most common manifestation.[4,12,13] LRTI is observed in nearly half of all patients.[4,14] In the present study, nearly half of the patients had a history of previous hospitalizations for infections. Therefore, especially serious infection cases requiring hospitalization should be screened for a possible PID. FUO is another diagnosis that should be carefully considered. In our study, nine patients hospitalized for FUO were diagnosed with a PID. It can sometimes be the first manifestation of PID.[12] A predisposition to specific infectious diseases has been identified in some PID sub-types.[16] In the present study, two patients with streptococcal pneumococcal meningitis were diagnosed with IRAK4 and MyD88 deficiencies.

Early diagnosis is important for reducing morbidity and mortality rates, and the choice of treatment options.[17,18] Therefore, clinicians should carefully evaluate the warning signs of PID. The Jeffrey Modell Foundation has created the 10 Warning Signs of Primary Immunodeficiency in children. These mainly include frequent, recurrent, severe, or atypical infections, growth, and developmental retardation, and family history of PID.[19] It has been suggested that these

warning signs should be refined considering our increasing knowledge of PID, and classified according to different medical disciplines such as intensive care, rheumatology, hematology, oncology, gastroenterology, or neurology.[20] In the present study, recurrent upper and lower respiratory tract infections, growth retardation, and recurrent hospitalization histories were observed in almost half of the patients. Findings such as BCGitis, oral candidiasis, allergic diseases, and eczema demonstrate the complexity of immune dysregulation in PID, and can alert clinicians.[10,12]

Many infectious and non-infectious skin lesions are seen in PID patients.[21] Eczematous dermatitis is a non-specific cutaneous lesion most seen after infectious lesions.[22,23] It usually begins in infancy or early childhood, and its severity may be related to disease activity.[20] It is seen in 13-19% of PID patients, and is mostly associated with combined immunodeficiencies.[24] In the present study, eczema was seen in 17.3% of the patients, most of whom were diagnosed with SCID. The presence of skin manifestations prior to immunological diagnosis suggests that clinicians should consider PID in the differential diagnosis of skin lesions.

Immunoglobulin replacement therapy is one of the cornerstones of treatment. In our study, around 40% of the patients were treated with immunoglobulins. This rate is consistent with the literature.[13] HSCT is the only curative option for a wide range of PIDs.[14,25,26] Early diagnosis, appropriate human leukocyte antigen-matched donors, and improvement of post-transplant supportive therapies increase the success of HSCT in patients.[25-27] In this study, eight patients underwent allogeneic HSCT, six of whom are alive.

Conclusion

Despite the increasing number of diagnoses each year, there are still significant delays in PID diagnosis. Early diagnosis is of critical importance for patients' clinical course and prognosis. Especially in cases of recurrent, severe, or atypical infections, clinicians should consider a PID diagnosis. Raising awareness of PID and PID-related symptoms can improve the rates of diagnosis and successful treatment.

Acknowledgments

Conflict of Interest

The authors declare that they have no conflicts of interest.

References

1. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, et al. Human Inborn Errors of Immunity: 2019 Update of the IUIS Phenotypical Classification. J Clin Immunol. 2020;40(1):66-81. https://doi.org/10.1007/s10875-020-00758-x

2. Bousfiha AA, Jeddane L, Ailal F, Benhsaien I, Mahlaoui N, Casanova JL, et al. Primary immunodeficiency diseases worldwide: more common than generally thought. J Clin Immunol. 2013;33(1):1-7. https://doi.org/10.1007/s10875-012-9751-7

3. Kilic SS, Ozel M, Hafizoglu D, Karaca NE, Aksu G, Kutukculer N. The prevalences [correction] and patient characteristics of primary immunodeficiency diseases in Turkey-two centers study. J Clin Immunol. 2013;33(1):74-83. https://doi.org/10.1007/s10875-012-9763-3

4. Mellouli F, Mustapha IB, Khaled MB, Besbes H, Ouederni M, Mekki N, et al. Report of the Tunisian Registry of Primary Immunodeficiencies: 25-Years of Experience (1988-2012). J Clin Immunol. 2015;35(8):745-53. https://doi.org/10.1007/s10875-015-0206-9

5. Abolhassani H, Kiaee F, Tavakol M, Chavoshzadeh Z, Mahdaviani SA, Momen T, et al. Fourth Update on the Iranian National Registry of Primary Immunodeficiencies: Integration of Molecular Diagnosis. J Clin Immunol. 2018;38(7):816-32. https://doi.org/10.1007/s10875-018-0556-1

6. Bayram, R. O., Özdemir, H., Emsen, A., Türk Dağı, H., & Artaç, H. Reference ranges for serum immunoglobulin (IgG, IgA, and IgM) and IgG subclass levels in healthy children. Turkish journal of medical sciences.2019;49(2), 497–505. https://doi.org/10.3906/sag-1807-282

7. Ikinciogullari A, Kendirli T, Dogu F, Egin Y, Reisli I, Cin S, et al. Peripheral blood lymphocyte subsets in healthy Turkish children. Turk J Pediatr. 2004;46(2):125-30. https://doi.org/10.1046/j.1442-200x.2004.01856.x

8. Firtina S, Yin Ng Y, Hatirnaz Ng O, Kiykim A, Aydiner E, Nepesov S, et al. Mutational landscape of severe combined immunodeficiency patients from Turkey. Int J Immunogenet. 2020;47(6):529-38. https://doi.org/10.1111/iji.12496

9. de Vries E, European Society for Immunodeficiencies m. Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update. Clin Exp Immunol. 2012;167(1):108-19. https://doi.org/10.1111/j.1365-2249.2011.04461.x

10. Kirkpatrick P, Riminton S. Primary immunodeficiency diseases in Australia and New Zealand. J Clin Immunol. 2007;27(5):517-24. https://doi.org/10.1007/s10875-007-9105-z

11. group CTFPs. The French national registry of primary immunodeficiency diseases. Clin Immunol. 2010;135(2):264-72. https://doi.org/10.1016/j.clim.2010.02.021

12. Rhim JW, Kim KH, Kim DS, Kim BS, Kim JS, Kim CH, et al. Prevalence of primary immunodeficiency in Korea. J Korean Med Sci. 2012;27(7):788-93. https://doi.org/10.3346/jkms.2012.27.7.788

13. Al-Herz W. Primary immunodeficiency disorders in Kuwait: first report from Kuwait National Primary Immunodeficiency Registry (2004--2006). J Clin Immunol. 2008;28(2):186-93. https://doi.org/10.1007/s10875-007-9144-5

14. Gupta D, Thakral D, Kumar P, Kabra SK, Lodha R, Kumari R, et al. Primary Immunodeficiency Disorders Among North Indian Children. Indian J Pediatr. 2019;86(10):885-91. https://doi.org/10.1007/s12098-019-02971-y

15. Linares NA, Bouchard M, Gutierrez NS, Colmenares M, Cantor-Garcia A, Gabaldon-Figueira JC, et al. Immunological features in pediatric patients with recurrent and severe infection: Identification of Primary Immunodeficiency Diseases in Merida, Venezuela. Allergol Immunopathol (Madr). 2019;47(5):437-48. https://doi.org/10.1016/j.aller.2019.02.002

16. Bousfiha A, Picard C, Boisson-Dupuis S, Zhang SY, Bustamante J, Puel A, et al. Primary immunodeficiencies of protective immunity to primary infections. Clin Immunol. 2010;135(2):204-9. https://doi.org/10.1016/j.clim.2010.02.001

17. Quartier P, Debre M, De Blic J, de Sauverzac R, Sayegh N, Jabado N, et al. Early and prolonged intravenous immunoglobulin replacement therapy in childhood agammaglobulinemia: a

retrospective survey of 31 patients. J Pediatr. 1999;134(5):589-96. https://doi.org/10.1016/S0022-3476(99)70246-5

18. Busse PJ, Razvi S, Cunningham-Rundles C. Efficacy of intravenous immunoglobulin in the prevention of pneumonia in patients with common variable immunodeficiency. J Allergy Clin Immunol. 2002;109(6):1001-4. https://doi.org/10.1067/mai.2002.124999

19. Foundation JM. 10 Warning Signs of Primary Immunodeficiency.

20. O'Sullivan MD, Cant AJ. The 10 warning signs: a time for a change? Curr Opin Allergy Clin Immunol. 2012;12(6):588-94. https://doi.org/10.1097/ACI.0b013e3283591534

21. Relan M, Lehman HK. Common dermatologic manifestations of primary immune deficiencies. Curr Allergy Asthma Rep. 2014;14(12):480. https://doi.org/10.1007/s11882-014-0480-2

22. Lehman H, Gordon C. The Skin as a Window into Primary Immune Deficiency Diseases: Atopic Dermatitis and Chronic Mucocutaneous Candidiasis. J Allergy Clin Immunol Pract. 2019;7(3):788-98. https://doi.org/10.1016/j.jaip.2018.11.026

23. Al-Herz W, Nanda A. Skin manifestations in primary immunodeficient children. Pediatr Dermatol. 2011;28(5):494-501. https://doi.org/10.1111/j.1525-1470.2011.01409.x

24. Hoskins S, Skoda-Smith S, Torgerson TR, Boos MD. Eczematous dermatitis in primary immunodeficiencies: A review of cutaneous clues to diagnosis. Clin Immunol. 2020;211:108330. https://doi.org/10.1016/j.clim.2019.108330

25. Slatter MA, Cant AJ. Hematopoietic stem cell transplantation for primary immunodeficiency diseases. Ann N Y Acad Sci. 2011;1238:122-31. https://doi.org/10.1111/j.1749-6632.2011.06243.x

26. Ikinciogullari A, Cagdas D, Dogu F, Tugrul T, Karasu G, Haskologlu S, et al. Clinical Features and HSCT Outcome for SCID in Turkey. J Clin Immunol. 2019;39(3):316-23. https://doi.org/10.1007/s10875-019-00610-x

27. Gennery AR, Albert MH, Slatter MA, Lankester A. Hematopoietic Stem Cell Transplantation for Primary Immunodeficiencies. Front Pediatr. 2019;7:445. https://doi.org/10.3389/fped.2019.00445