Chapter

Primary Immunodeficiency

Renfen Chen

Abstract

Primary immunodeficiency (PID) is a large group of rare diseases present with chronic, serious, or life-threatening infections and other immune complications caused by defects or dysfunction of human immune system. Unlike secondary immunodeficiency acquired from an environmental factor or other medical conditions, PIDs are initiated by genetic defects. PIDs are divided into innate/adaptive immunodeficiencies, phagocytic deficiencies, complement deficiencies, and immune dysregulation. Due to the heterogeneous nature of the clinical presentations, diagnosis of PIDs can be of significant challenge. Review of clinical history and physical examination is important for raising initial suspicion of PIDs, whereas laboratory testing is essential to establish a diagnosis. Laboratory investigation includes the assessment of antibody and cellular response, as well as evaluation of the phagocytic and complement system. Flow cytometry and genetic assays are generally served as confirmation tools to validate a diagnosis. The recent exponential increase of genetic analysis has facilitated the identification of known and novel mutations. The advances in understanding of the immune system, development of novel cellular and molecular methodologies, and increased clinical awareness have led to significant improvement of disease management and clinical outcome for these diseases.

Keywords: primary immunodeficiency, innate immunity, adaptive immunity, phenotype, gene defect, infection, autoimmune disorder, classification, clinical awareness, laboratory diagnosis, flow cytometry, genetic testing, treatment, prognosis

1. Introduction

Primary immunodeficiency (PID) is a large group of rare diseases attributed to inborn genetic errors that impair different components of adaptive and innate immune system, resulting in chronic, serious infections, or other complications. The diseases are often accompanied by a predisposition to autoimmune disorders, autoinflammation, atopy, and malignancy [1–4]. Unlike secondary immunodeficiency acquired from other diseases or conditions such as malnutrition, immunosuppression, or HIV infections, PIDs are triggered by genetic defects. Based on the abnormality of one or more components of human immunity, PIDs can be divided into antibody deficiencies, combined T- and B-cell deficiencies, deficiencies in the phagocytic or complement system, and immune dysregulation [1]. Diagnosis of these disorders requires good clinical awareness and specialized laboratory testing. Flow cytometry and genetic testing are essential to identify the phenotypic and genetic defects of the diseases and to confirm the diagnosis. Accurate diagnosis and efficient management are important for reducing morbidity and mortality in

patients with PID [2]. The chapter provides an overview of the classification and manifestation as well as the diagnosis and management of these disorders.

2. Prevalence

Individual type of PIDs is considered to be rare in the population; however, recent studies have shown that PIDs may be more common than previously estimated 1% of the population when all varieties are combined [5]. The prevalence of PIDs varies depending on the type of immunodeficiencies and is difficult to be precisely calculated as the number of diagnosed cases is rapidly increasing. A 2018 global survey from the Jeffrey Modell Centers Network (JMCN) reported the case of PID patients followed in the JMCN increased by 35.4% to 102,097, while the case of patients identified with a specific gene defect increased 21.8% to 67,308 during the same period [5]. Up to 2018, 354 distinct disorders with 344 different gene defects were recognized [6]. Of note, most of the cases reported are from developed countries. It is estimated that 70–90% of individuals living with a PID are undiagnosed [7], particularly in the area with poor medical condition and lacking laboratory resources. With the extensive application of exome or whole genome sequencing, it was predicted that the associated PID genetic defects would reach 1000 under current trend in next decade [5]. **Table 1** listed the reported number of 18 most common PID defects among 354 inborn errors of immunity [5]. As shown, antibody deficiencies have much higher occurrence rate against other types of the disorders. Studies also showed that the selective IgA deficiency has the highest prevalence worldwide with a range from 1 in

Rank	PID defects	Global number
1	Predominantly antibody deficiencies, including selective IgA deficiency, unspecified hypogammaglobulinemia, and hyper-IgM syndrome	13,333
2	Common variable immunodeficiency (CVID), AR	11996
3	Chrom 22q11.2 deletion syndrome (de novo HS or AD)	5215
4	IgG subclass deficiency, isolated, AR	4612
5	Specific antibody deficiency (normal Ig and B cells), AR	4072
6	Hypogammaglobulinemia of infancy, transient (normal B cells), AR	4028
7	MEFV deficiency (familial Mediterranean fever (FMF), AD/AR)	2835
8	ATM deficiency (ataxia-telangiectasia (AT)), AR	2514
9	C1QA deficiency (C1 inhibitor), AD	2420
10	BTK deficiency, XL	2486
11	TACI deficiency	2239
12	Immunodeficiencies affecting cellular and humoral immunity, including SCID	2178
13	Autoinflammatory disorders, including PFAPA	1983
14	Complement deficiencies	1629
15	Congenital defects of phagocyte number, function, or both	1563
16	IgA with IgG subclass deficiency, AR	1562
17	CGD, XL (gp91phox deficiency)	1385
18	WAS deficiency (Wiskott-Aldrich syndrome), XL	1258
	Total	67308

AR: autosomal recessive transmission; AD: autosomal dominant transmission; Ig: immunoglobulins; SCID: severe combined immunodeficiency; CGD: Chronic Granulomatous Disease; XL: X-linked transmission; PFAPA: periodic fever with aphthous stomatitis, pharyngitis and adenitis.

Table 1.
Global prevalence of PIDs reported by Jeffrey Modell Centers Network [5].

223 to 1 in 1000 depending on ethnic background [8], while severe combined immunodeficiency (SCID), although fatal, is much rarer (1 in 100,000) [9, 10].

3. Classification

The classification of PIDs is generally based on the defects of the major components of human immunity, such as innate/adaptive immunodeficiencies, phagocytic deficiencies, complement deficiencies, and immune dysregulation. The classification has evolved over time with more phenotypic and genetic defects identified [4, 11].

Major category	Subcategory
1. Immunodeficiencies affecting cellular and humoral immunity	Severe combined immunodeficiencies, defined by CD3 T cell lymphopenia Combined immunodeficiencies generally less profound than severe combined immunodeficiency
2. Combined immunodeficiencies with associated or syndromic features	Immunodeficiency with congenital thrombocytopenia DNA repair defects other than those listed in major category 1 Thymic defects with additional congenital anomalies Immuno-osseous dysplasias Hyper IgE syndromes Dyskeratosis congenita, myelodysplasia, short telomeres Defects of vitamin B12 and folate metabolism Anhidrotic ectodermal dysplasia with immunodeficiency Calcium channel defects Others
3. Predominantly antibody deficiencies	Hypogammaglobulinemia (Severe reduction in all serum immunoglobulin isotypes with profoundly decreased or absent B Cells) Other antibody deficiencies • Severe reduction in at least 2 serum immunoglobulin isotypes with normal or low number of B Cells, CVID phenotype • Severe reduction in serum IgG and IgA with normal/elevated IgM and normal numbers of B cells, hyper IgM • Isotype, light chain, or functional deficiencies with generally normal numbers of B Cells
4. Diseases of immune dysregulation	Hemophagocytic lymphohistocytosis (HLH) & EBV susceptibility Syndromes with autoimmunity and others
5. Congenital defects of phagocyte number or function or both	Congenital neutropenias Functional defects • Defects of respiratory burst • Other non-lymphoid defects
6. Defects in intrinsic and innate immunity	Bacterial and parasitic infections Mendelian susceptibility to mycobacterial disease and viral infection
7. Autoinflammatory disorders	Recurrent inflammation Systemic inflammation on with urticaria rash Sterile inflammation (skin / bone / joints) Type 1 interferonopathies Others
8. Complement deficiencies	Susceptibility to infections (high) • Disseminated neisserial infections • Recurrent pyogenic infections Susceptibility to infections (low) • SLE-like syndrome • Atypical hemolytic uremic syndrome • Others
9. Phenocopies of PID	Associated with somatic mutations Associated with autoantibodies

Table 2.
The 2017 IUIS phenotypic categorization of PIDs [6, 11].

The International Union of Immunological Societies (IUIS) expert committee, currently named as Inborn Errors of Immunity Committee, has been responsible for issuing the classification of PIDs every other year from 1970. The complete catalog of classification has now been widely used as a reference by clinicians and researchers. From 2013, IUIS published more user-friendly phenotypic classification in two formats: one is a pdf file, namely clinically oriented phenotype categorization in the Journal of Clinical Immunology, and the other is a csv file containing a comprehensive list of various disorders that can be downloaded from http://www.iuisonline.org [6, 11]. The phenotypic categorization published in the journal has been well designed for clinical use, while the online list contains the most updated information demonstrated in a digital friendly excel format that can be sorted by phenotypic and genetic features, which are very useful for designing sequencing panels, disease code lists, and diagnostic algorithms.

The major category and subcategory of PIDs from the revised 2017 IUIS phenotypic classification are summarized in **Table 2**.

4. Clinical presentations

Patients with PID present highly heterogeneous clinical symptoms with increased susceptibility to infections and other immune complications [12, 13]. Recurrent infection is the hallmark of the PIDs although a variety of other clinical manifestations may appear before the infection [13, 14]. In fact, noninfectious manifestations, such as gastrointestinal disorders, hematological diseases, autoimmune/autoinflammatory conditions, atopy or malignancy, can be the predominant clinical presentations in some patients with underlying immunodeficiency [3, 15]. Furthermore, patients with PID also demonstrate overlapping symptoms and share similarities with many "routine" diseases.

4.1 Infections

Majority of patients with PID suffer mild to severe or life-threatening infections. The unique clinical characteristics of infections in PIDs are recurring, chronic, and can appear in multiple anatomic sites. Recurrent infections in both the sinuses and the respiratory tract, such as sinusitis, bronchitis, otitis, and pneumonia, are the most frequent symptoms observed in patients with PID [16], while recurrent systemic infections (e.g., meningitis and bacteremia) are also not rare [17, 18]. Patients with SCID may suffer from unusual or opportunistic infections leading to unexpected complications or death [19].

4.2 Autoimmune and autoinflammatory disorders

Autoimmune and autoinflammatory disorders are more frequently seen in some categories of the PIDs than in other diseases [20]. The associated conditions in PID individuals may present in a single tissue or organ, such as autoimmune hemolytic anemia, thrombocytopenia, and autoimmune thyroiditis, or affect multiple organs, exemplified by an related vasculitis, or resemble rheumatic symptoms such as (e.g., dermatomyositis, rheumatoid arthritis, and systemic lupus erythematosus) [3, 20, 21]. To note, family members that carry the same gene mutation may present different types of autoimmune/autoinflammatory symptoms, or without such disorders [22]. In comparison with other types of defects, the autoimmune presentations are relatively common in PIDs with antibody deficiencies (e.g., CVID, selective IgA deficiency), and absence of initial components (C1–C4) of the classical complement system [23, 24].

4.3 Gastrointestinal and hematological disorders

Patients with PID, particularly infants and young children, may manifest chronic diarrhea, malnutrition, and malabsorption. Some individuals may undergo infections in gastrointestinal tract, such as chronic giardiasis and rotavirus [25], while others may experience a variety of autoimmune or autoinflammatory disorders including inflammatory bowel disease, atrophic gastritis with pernicious anemia, or gluten-sensitive enteropathy [20].

Hematological disorders, such as autoimmune hemolytic anemia, and/or neutropenia and/or thrombocytopenia, are also frequently seen in patients with CVID or selective IgA deficiency [26]. Patients with the Wiskott-Aldrich syndrome, a disease characterized by variable defects in B- and T-lymphocyte function, can present with reduced platelet volume and significant thrombocytopenia [27].

4.4 Immunodeficiency syndromes

PID patients may also present with a syndrome complex. For example, recurrent bacterial/fungal infections and chronic inflammation of the gastrointestinal and respiratory tract often present in patients with chronic granulomatous disease, while an individual suffering from Wiskott-Aldrich syndrome may have manifestations of eczema, recurrent bacterial infections, autoimmune disorders, and thrombocytopenia [27]. In addition, congenital heart disease and hypocalcemic tetany frequently appear in a newborn baby with the DiGeorge syndrome [28, 29]. In fact, the investigation of patients with a syndrome derived from immunodeficiency may trigger an early diagnosis of PID before the typical immunodeficiency symptoms appear [4].

4.5 Malignancy

Compared to individuals with a healthy immune system, patients with PID are expected to have higher prevalence and/or broader spectrum of malignancies [30]. A study showed that lymphoma, the most common malignancy seen in PID patients, has increased 10-folds in male and 8.34-folds in female compared to age-matched controls [31]. Other types of cancer with higher frequency in PIDs are leukemia, digestive tract cancers, and virus-induced cancers [30]. Interestingly, the four most common cancers routinely occurred in men and women (lung, colon, breast, and prostate cancers) do not have significant elevation in subjects diagnosed with PID [31]. Evidence also demonstrated that patients with specific forms of immunodeficiency caused by highly penetrant gene defects have higher risk of developing cancer [32].

5. Clinical investigation

Early diagnosis of PID is critical for reducing morbidity or mortality and improving treatment outcomes. Review of clinical and family history and physical examination are the first steps in evaluating the need for further laboratory investigation.

The differentiation of PIDs from other medical conditions can be complicated as the symptoms of infection (e.g., sinusitis, bronchitis, pneumonia, gastroenteritis, meningitis, or sepsis) and other manifestations often present in patients with non-PID. Hence, it is important to delineate the infectious organisms, the pattern of infections, and clinical pictures for guiding the clinical judgment, prior to focusing on laboratory testing.

	Warning signs in children	Warning signs in adults
1	Equal or more than four new ear infections within one year	Equal or more than two new ear infections within one year
2	Equal or more than two serious sinus infections within one year	Equal or more than two new sinus infections within one year without allergy
3	Equal or more than two months on antibiotics with little effect	One pneumonia per year for more than one year
4	Equal or more than two pneumonias within one year	Chronic diarrhea with weight loss
5	Failure of an infant to gain weight or grow normally	Recurrent viral infections (colds, herpes, warts, condyloma)
6	Recurrent, deep skin or organ abscesses	Recurrent need for IV antibiotics to clear infections
7	Persistent thrush in mouth or fungal infection on skin	Recurrent, deep abscesses of the skin or internal organs
8	Need for intravenous antibiotics to clear infections	Persistent thrush or fungal infection on skin or elsewhere
9	Equal or more than two deep-seated infections including septicemia	Infection with normally harmless tuberculosis-like bacteria
10	A family history of PID	A family history of PID

Adapted from Jeffrey Modell Foundation http://www.info4pi.org/library/educational-materials/10-warning-signs. Accessed on 09/Oct/2019.

Table 3.
Clinical warning signs of PIDs.

Due to the highly variable clinical presentations and low frequency of the PIDs, the diagnosis of patients is often delayed for years. To raise clinical awareness, JMCN has promoted 10 warning signs for children and adults (**Table 3**). Patients presenting with two or more of the clinical warning signs should be prompted for further investigation for the possible underlying immunodeficiencies and referred to immunologists for proper disease management.

6. Laboratory diagnosis

The laboratory testing is essential to diagnose and delineate the immunologic defects of PIDs. Patients with clinical suspicion should be further investigated for the response of innate immunity and adaptive immunity [33, 34]. **Table 4** listed the most common tests used for initial screening of PIDs.

6.1 Evaluation of humoral immunity

Measurement of serum immunoglobulins is the first-line test for evaluating B-lymphocyte functions. Quantitative measurements of IgG, IgA, IgM, and IgE will identify either hypogammaglobulinemia or deficiency of an individual class of immunoglobulins. Evaluation of IgG subclasses may be required when a patient has strong implication of humoral immunodeficiency but the total IgG is normal. To be mindful, the results of immunoglobulin quantitation must be interpreted with appropriate age-specific ranges. Assessment of antibody responses to immunization with protein antigens (e.g., tetanus or diphtheria toxoids) and polysaccharide antigens (e.g., pneumococcal capsular) is another way to evaluate humoral immunity,

Suspected immunodeficiency	Laboratory tests
Humoral immunity	Quantitation of immunoglobulins (IgG, IgA, IgM), IgE, IgG subclass Antibody response to immunization (Tetanus/diphtheria toxoids, pneumococcal) B cell (CD19) and subtype enumeration
Cell-mediated immunity	Lymphocyte enumeration T cell and subtype enumeration (CD3,CD4, CD8) T cell functions • Delayed type hypersensitivity skin tests • Vaccine-specific T cell responses • In vitro proliferation to the stimulation of mitogen (e.g. PHA, PMA or specific antigen(candida, tetanus toxoid) • Cytotoxicity • Cytokines in response to stimulation
Complement system	Complement activity for classical pathway (CH50) Complement activity for alternate pathway (AH50) C1 inhibitor (quantitation and function) Individual complement level
Phagocytosis system	Neutrophil enumeration Nitroblue tetrazolium (NBT) dye test Oxidative burst by Dihydrorhodamine 123
NK cells	NK cell enumeration NK cell functions • ^{51}Chromium release assay • CD107 expression • Perforin / granzyme expression

Table 4.
Initial laboratory tests for PIDs.

although note is to be taken that live viral vaccines must be restricted to a patient with underlying immunodeficiency [35].

6.2 Evaluation of cellular immunity

Delayed-type hypersensitivity (DTH) skin test is commonly used to screen whether the patient has intact cell-mediated immune response. A positive DTH skin test generally rules out the possible defect of cellular immunity [36]. Nevertheless, the test requires that individuals must have sufficient prior exposure and sensitization to the testing antigen; therefore, it may not be suitable for infants and young children. Quantitation of T-lymphocytes (CD3, CD4, CD8) in peripheral blood is able to indirectly reflect the aberrant cellular immunity and can be easily performed by flow cytometry. More specialized T-cell function tests would provide in-depth information in immune system, which include the assessment of lymphocyte proliferation in response to stimulus such as mitogens (e.g., phytohemagglutinin, ConA, and PMA), or specific antigens (e.g., candida). Furthermore, in vitro measurements of intra- and/or extracellular cytokine responses (e.g., interleukin 2, interferon-gamma, BAFF, and TNF) are informative for the investigation of T- and B-lymphocyte regulation [37].

6.3 Evaluation of phagocytic function

Leukocyte count and differential can assess the phagocytic disorders such as congenital agranulocytosis or cyclic neutropenia. Phagocytic function can be indirectly

assessed by traditional nitroblue tetrazolium (NBT) assay, which measures phagocytic cells' killing capability in response to an oxidative burst. More recently, a simpler dihydrorhodamine 123 (DHR) assay based on flow cytometry has replaced NBT test for assisting the diagnosis of chronic granulomatous disease (CGD), Rac2 deficiency, and complete myeloperoxidase deficiency [38, 39]. Other complicated in-vitro functional methods such as the measurement of directed cell movement (chemotaxis), ingestion (phagocytosis), and intracellular killing (bactericidal activity) are available in some specialized laboratories [40].

6.4 Evaluation of NK function

The importance of evaluating NK cells in human immunity has been previously underscored, and this is supported by two evidences: significantly increased number of patients with reduced NK cells and/or functions, and over 40 genetically defined congenital immunodeficiencies present with impaired NK cell functions [41]. There are several methods utilized for the examination of NK cell functions including ^{51}chromium release assay, flow cytometry-based perforin/granzyme expression and CD107a degranulation. These assays are particularly valuable for the patients suspected of primary hemophagocytic lymphohistiocytosis [42, 43].

6.5 Evaluation of the complement system

The complement system can be evaluated by measuring the level or function of complement proteins that are involved in the classical and alternative activation pathways. C3 and C4 are the complements routinely tested. Quantitative and functional assay of C1 esterase inhibitor is essential for the diagnosis of hereditary angioedema. Assays of CH50 and AH50 are, respectively, used to measure the overall complement activity in the classical or alternative pathway. Combining the results of CH50 and AH50 is indicative for further investigation of individual complement proteins that initiate the classical or alternative pathway or common terminal pathway [44].

6.6 Flow cytometry

As our understanding of the defect or dysfunction of immune system increases, immunophenotypic and functional assays based on flow cytometry have been extensively used in identifying the abnormality of various cell types and their functions associated with certain diseases, including PIDs. Furthermore, flow cytometry is also a favorable technique for the measurement of intra- and extracellular cytokine production (e.g., IL12, IFN, TNF, and TH17), cell surface protein expression (e.g., Foxp3, CTLA-4, and BTK), and cellular signaling pathways (e.g., phosphor-STAT) [45]. The information gained from flow cytometry analysis can assist not only in the diagnosis, monitoring, and treatment of the diseases but also in understanding the influence of immune system associated with genetic defects that are newly identified. **Table 5** lists the flow cytometry assays used for common PID disorders. Most of the tests listed are required to be undertaken in a specialized laboratory, with the exception of TBNK cell populations, memory B cells, and some function assays that can be performed in a routine diagnostic laboratory.

Proper instrument setting, standardized operating procedures, and good quality controls must be exercised when performing flow cytometric analysis, as flow cytometry is susceptible to assay variation,. The reported data must include both percentage and absolute number of specific cell population. Moreover, appropriate age-matched reference ranges should also be provided in the final report [47, 48].

Ideally, each laboratory should establish their own normal ranges, but this is often not feasible; alternatively, published reference ranges may be used if a proper validation has been undertaken.

To date, flow cytometry has also been widely used for evaluating cell functions. Traditionally, lymphocyte functions were tested by radioactive methods, such as cytotoxicity of T and NK cells (chromium release) or proliferation of T cells

Defect of PIDs	Flow cytometric assessment
Combined immunodeficiencies	
SCID	Absent T cells and variable number of B and NK cells, reduced or absent $CD31^+$ recent thymus emigrant, disruptions in naïve and memory T cells[a]
X-SCID	Reduced or absent of CD132 on lymphocytes [a], reduced phosphorylation of STAT5 in response to cytokine stimulation [c]
Omenn Syndrome and leaky SCID	Reduced memory T cells, TCR analysis for oligoclonality[a]
BLS Type 1	Reduced or absent MHC Class I, decreased CD8 T cells [a]
BLS Type 2	Reduced or absent MHC Class II, decreased CD4 T cells[a]
X-linked HIGM	Reduced expression or function of CD40L on activated T cells[c]
Autosomal recessive HIGM	Absent CD40 expression on B cells[a]
Jak-3 mutations	Reduced phosphorylation of STAT5 in response to cytokine stimulation [c]
ZAP-70 deficiency	Decreased CD8 T cells[a], absent ZAP70 in T cells[b]
DOCK8 deficiency	Absent DOCK8 expression in lymphocytes[b]
Combined immunodeficiencies with syndromic or associated features	
Wiskott-Aldrich syndrome (WAS)	Reduced WAS protein in major mature B cell subsets and regulatory B cells [b]
Hyper-IgE Syndrome	Disruptions in T and B cell populations [a], reduced TH17 cells [b,c]
Antibody deficiencies	
X-linked Agammaglobulinemia	Reduced/Absent B cells[a], reduced/absent BTK protein in monocytes[b]
CVID	Absent CD19 and BAFF-R on B cells[a] , disruptions in switched, non-switched, transitional B cells [a], reduced inducible costimulator on activated T cells [c]
Autosomal recessive HIGM (uracil N glycosylase, AICDA)	Reduced memory B cells, disruptions in switched and non-switched memory B cells [a]
Intrinsic and innate immune defects	
Mendelian susceptibility to mycobacterial disease	Reduced IFNγR1 expression on monocyte and IL12Rβ1 expression on activated T cells [a], reduced phosphorylation of STAT1, or STAT4 in monocytes[b,c]
STAT1 GOF	Delayed dephosphorylation of STAT1 in response to IFNγ or IFNα in monocytes[b,c]
IL17 RA deficiency	Reduced IL17 RA expression on lymphocytes and monocytes [a]

IRAK-4 and MyD88 deficiency	Reduced intracellular TNF-α in monocytes in response to LPS[b,c]
Neutrophil defects	
CGD- X-linked	Reduced surface expression of gp91[a], reduced or absent oxidative burst in DHR123 assay[c]
CGD - autosomal recessive	Reduced surface expression of gp22[a], reduced intracellular expression of gp47 or gp67[b], reduced oxidative burst in DHR123 assay[c]
Leukocyte adhesion defect I	Reduced expression of CD18, CD11a, CD11b, and CD11c[a]
Leukocyte adhesion defect II	Reduced CD15s expression[a]
Complement defects	
Atypical hemolytic uremic syndrome	Reduced CD46 (MCP) expression in neutrophils[a]
Immune dysregulation	
Autoimmune lymphoproliferative syndrome	Elevated $CD3^{+}TCR\alpha\beta^{+}$ CD4-CD8- T cells [a]
X-linked lymphoproliferative disease type 1	Significantly reduced iNKT cells[a], decreased intracellular SAP in CD8 + T cells[b] and NK cells
X-linked lymphoproliferative disease type 2	Reduced intracellular XIAP in lymphocytes[b]
Immune dysregulation, polyendocrinopathy, X-linked syndrome	Reduced $FoxP3^{+}$ Treg cells[b]
CTLA-4 haploinsufficiency	Reduced CTLA-4 expression in Treg cells[b]
LRBA deficiency	Reduced intracellular LRBA in stimulated PBMC[b, c]and decreased CTLA-4 in Tregs[b]
STAT3-GOF	Delayed dephosphorylation of STAT3 following stimulation with IL-6[b,c]
Familial HLH3, 4 and 5, Chediak-Higashi, Griscelli or Hermansky-Pudlak Syndromes	Reduced CD107a expression on NK cells in response to target cells[c]
Familial HLH2 (PRF1 mutations)	Reduced intracellular perforin in CD8 T cells and NK cells[b]
IL-10R deficiency	Reduced STAT3 phosphorylation in lymphocytes in response to IL-10[c]
Infantile-onset multisystem autoimmune disease 1 (heterozygous GOF mutation in STAT 3)	Enhanced STAT3 phosphorylation[c]
Phenocopies of PIDs	
Autoantibodies to IFNγ	Inhibition of IFNγ-induced phosphorylation of STAT1 with patient's serum [b,c]
Autoantibodies to GMCSF	Inhibition of GMCSF-induced phosphorylation of STAT5 with patient's serum [b,c]

SCID: severe combined immunodeficiency; STAT: signal transducer and activator of transcription; BLS: bare lymphocyte syndrome; HIGM: hyper-IgM syndrome; BTK: Bruton's tyrosine kinase; CVID: common variable immunodeficiency; CGD: chronic granulomatous disease; DHR: dihydrorhodamine; HLH: hemophagocytic lymphohistiocytosis; CTLA-4: cytotoxic T-lymphocyte associated protein-4; LRBA: LPS responsive Beige-like anchor protein; GOF: gain-of-function; GMCSF: granulocyte macrophage colony stimulating factor.

[a] Detection of surface molecules. [b] Detection of intracellular or intranuclear molecules. [c] Detection of cellular function.

Table 5.
Phenotypic and functional assessment for PIDs by flow cytometry [45, 46].

(tritiated thymidine uptake). These approaches are still recognized as gold standard by some clinicians. However, radioactive methods have the following intrinsic limitations: involvement of radioactivity, labor intensive, high expertise required, and poor result reproducibility. Additionally, seeking for a consistent healthy

Method	Advantages	Limitations	Recommendations by Immunology Society
Chromosomal microarray	Detection of CNVs and majority of structural variations Greater analytical sensitivity than conventional cytogenetics Fast result TAT	Unable to detect SNVs and very small deletions/duplications (<100kb) or chromosomal rearrangements that do not affect the nucleotide copy number	Use as initial screening test to narrow the list of genetic candidates in an undetermined phenotype Complement each other with WES
Sanger sequencing	Detection of noncoding variants Lowest overall cost Fast result TAT High accuracy	Unable to detect CNVs and structural variations Inability to detect portions not included in the assay	Use for the detection of monogenetic PID for patient and family members Technical confirmation of genetic mutations detected by other approaches
Gene panel by massively parallel sequencing	High throughput Increased depth of sequencing coverage in target genes with fewer regions missed Higher accuracy and sensitivity versus NGS Detection of mosaicism Less expensive than WES or GS	Unable to detect non-targeted variants Newly defined variants/genes need to be constantly updated in the testing panel Inability to detect novel disease-causing genes Only very small gene (1-25 nucleotide) insertions and deletions can reliably be detected	Use as rapid testing for limited gene candidates
Whole exome sequencing (WES)	Covers all known coding regions of all genes. High diagnostic yield with the detection of the majority of important pathogenic variants in only 1% of the genome Capable of discovering new pathogenic genes Identification of defects in multiple genes	Poor coverage of noncoding, novel exons or poorly understood regions Unreliable detection of copy number, structural variations and insertions or deletions more than 25 nucleotides More expensive than Sanger and panel sequencing Detection of variants of unclear significance Baits cannot be optimized for all target genes	Use in individuals with complicated manifestations or phenotypes with significant locus heterogeneity Use in conjunction with chromosomal microarray when a genetic diagnosis cannot be secure after Sanger or gene panel testing
Genome sequencing (GS)	More consistent coverage across all genes Identification of variants in coding, noncoding, regulatory regions and duplications and deletions not covered by WES. Detect CNVs and structural variations and large deletions Discover new pathogenic genes	Highest cost Slowest result TAT Detection of variants of unclear significance Labor and knowledge -intensive in the interpretation of large volume of data (around 3 billion base pairs of DNA)	Use when other genomic methods can not discover the genetic variations or secure the genetic diagnosis

CNVs: copy-number variations; SNV: single-nucleotide variant; TAT: turn around time; NGS: Next generation sequencing.

Table 6.
The advantages, limitations and recommendations of genetic technologies [50, 53].

fresh blood as assay normal control and obtaining a proper reference range can be challenging in routine laboratory practice. Therefore, they have been gradually replaced by other methodologies, such as bioluminescence-based assay or flow cytometry–based assay, which use specific dye for the detection of cell proliferation (e.g., CFSE, PKH-2, or PKH-26) or cell death (e.g., 7AAD and Annexing V) [49]. Many assays based on flow cytometry have been increasingly popular as they are easier to perform, have quicker turnaround time, are nonradioactive, are capable of using whole blood, and are more robust compared to the traditional radioactive assays.

6.7 Genetic testing

Genetic testing plays a critical role in patients with PID in confirming diagnosis, predicting the prognosis, assessing the influences of genotype-phenotype associations, and family planning [50, 51]. Besides, early and accurate molecular diagnosis is vital for guiding the selection of appropriate treatment including genetic therapy. Several molecular tests are available in identifying the genetic defects of PIDs, such as chromosomal analysis, fluorescence in situ hybridization, chromosomal microarray, single gene by Sanger sequencing, gene panels by massively parallel, whole exome, and genome by next-generation sequencing [52]. The selection of these assays should consider their inherent advantages and limitations [50, 53]. The summary of these tests is shown in **Table 6**. Recent emerged simple molecular assays for measuring circular DNA segments namely T-cell receptor excision circles and kappa-deleting recombination excision circles, based on quantitative PCR amplification of DNA extracted from dried blood spots, enable for a quick screening of newborn SCID [54].

The choice of specific gene(s) for examination is suggested by the patient's clinical history and phenotypical and functional results. Clinicians are required to have a basic understanding of the utility, accessibility of different genetic approaches. The selection criteria of molecular methodology should be based on the greatest odds of achieving the diagnosis within an acceptable time frame with the most cost-effective test. There is no specific algorithm for genetic testing in patients with PID as individual's genetic mutation is often unique, the technology, cost, and the assay turnaround time are constantly changing, and each molecular method has inherent advantages and limitations. Practically, two or more approaches are often used together to achieve an optimal diagnosis [50]. For example, single gene Sanger sequencing is considered to be not only a simple and reliable assay for testing patients with known monogenic mutations of PID or their family members, but it can also serve as a tool for confirming the genetic variants detected by whole exome sequencing. When assessing large numbers of mutations, gene panels or whole genome/exome approach may be more cost-effective and faster than single gene analysis. Since genetic testing in primary immunodeficiency is highly personalized, and a specific genetic mutation does not always translate into a disease, test results must be interpreted with caution by genetic consultants and immunologists.

The recent advances of sequencing technologies have facilitated the genomic assays to become the standard of care in some hospitals although these techniques may face the challenges of cost, accessibility, and interpretation issues. The exponential growth of genetic analysis by next-generation sequencing and other novel molecular technologies has enabled quick identification of known and novel mutations, which contributed to a dramatic expansion of the number and types of PIDs [16, 53, 55].

7. Treatment

Treatments for PIDs involve preventing and controlling recurrent infections, treating symptoms, strengthening the immunity, and treating the underlying cause of the immune defects. Illness associated with PIDs such as autoimmune disorders or malignancies should also be managed [1, 13].

More aggressive and/or longer course of antibiotics than "normal infections" is usually prescribed in patients with PID, in order to control the infections caused by bacteria or fungi. Some patients may require prolonged antibiotic therapy to prevent infections and permanent damage to organs [13]. Routine immunizations can also provide protective immunity to those at risk of infections, but the attenuated

vaccines such as oral polio and measles-mumps-rubella might not be suitable for children with PIDs. For viral infection, interferon-gamma therapy may be of choice besides other antiviral drugs routinely used (e.g., amantadine and acyclovir) [13]. In patients with chronic granulomatous disease, using granulocyte colony–stimulating factor, a glycoprotein that is able to stimulate the proliferation/differentiation and improve the functions of neutrophil, can help increase the levels of immune-strengthening leukocytes to control the infections [56].

Immunoglobulin replacement has been the pillar of therapy for recurrent infections of PIDs, since around 60% of PID cases have impaired antibody production [57]. In fact, most of these patients will require life-time immunoglobulin replacement therapy. Immunoglobulin can be delivered either intravenously (abbreviated IVIG) or subcutaneously (abbreviated SCIG). The choice of which route depends on the circumstance although both of them have been demonstrated to be effective. Because higher IgG levels can be obtained through intravenous administration, IVIG has been routinely used for preventing serious/recurrent infections [58]; however, SCIG has recently emerged as a popular route for delivery due to its fewer side effects and greater flexibility [57, 59]. Future research direction is focusing on more precise IgG replacement in PIDs, such as the development of IgG subclass-specific enriched preparation and microbe-specific IgG [58].

Defects	Supportive treatment	Definitive treatment
CIDs/SCID	Ig replacement (IV or SC) Enzyme replacement Antibiotic prophylaxis Antifungal prophylaxis Aggressive prevention and management of infections Immunosuppressants for autoinflammation	Thymus transplantation [66] Stem cell transplant Gene therapy
Antibody deficiencies	Ig replacement therapy (IV or SC) Antibiotic prophylaxis Antifungal prophylaxis Biological agents or immunosuppressants for autoinflammation	Stem cell transplant Gene therapy
Innate immunodeficiencies	Antibiotic prophylaxis Antifungal prophylaxis Cytokine replacement Granulocyte colony stimulating factor Immunizations Ig replacement if indicated	Stem cell transplant Gene therapy
Autoimmune/autoinflammatory disorders	Corticosteroids Other immunosuppressants Biological agents	Stem cell transplant Gene therapy
Immune dysregulation disorders	Antibiotic prophylaxis Antifungal prophylaxis Immunizations Immunosuppressants Biological agents	Stem cell transplant Gene therapy

Ig: immunoglobulin; IV: intravenous; SC: subcutaneous; CID: combined immunodeficiency; SCID: severe combined immunodeficiency.

Table 7.
Current strategies for the treatment of PIDs [1, 13, 66].

Apart from controlling infections, the considerable morbidity and mortality caused by noninfectious complications of PIDs can also be troublesome to clinicians. To standardize clinical practices and improve treatment outcome, British Society of Immunology has recently published the first set of recommendations for monitoring and managing the noninfectious complications of CVID [60].

Bone marrow transplantation (BMT) and hematopoietic stem cell transplantation (HSCT) are feasible options for a permanent cure for several types of life-threatening immunodeficiency, with SCID in particular [61, 62]. Immune system can reconstitute when stem cells harvested from bone marrow or cord blood are transferred to the patients with PID. However, the successful rate of biological match, possibility of life-threatening graft-versus-host-disease, and the risk of uncontrolled infections following the destruction of the patient's own immune system prior to the transplant should be well evaluated.

The technical advances of genetic engineering provide another hope to cure PIDs. Substantial progress has been made in the past decade in treating several types of PIDs (e.g., adenosine deaminase-SCID, SCID-X1, chronic granulomatous disorder, and Wiskott-Aldrich syndrome) with gene therapy [63–65]. Current treatment scenario is mostly based on ex-vivo deliver of therapeutic transgene through viral vectors to autologous stem cells, followed by transplantation back to the same patient. Although the overall outcome from all the clinical trials targeting different PIDs has been extremely promising, however, serious adverse events (e.g., vector-mediated oncogenesis) and high cost may be a hindrance to clinical trials and promotion of gene therapy [63, 65]. A summary of current strategies for treatment and management of PIDs is shown in **Table 7**.

8. Prognosis

The prognosis of patients with PID is extremely variable depending on the type of immune defects. Infants with SCID will die in the first 2 years of life without HSCT/BMT or gene therapy. Individuals who obtained stem cell transplantation in early childhood (before 3.5 months) have better prognosis [67]. Many PID patients who received proper medical care and treatments are able to live healthy and independent life for a long term. With the enhancement in managing infections and other complications and growing application of definitive therapies, the outcomes and long-term survival of PIDs have improved dramatically since the 1970s [13].

9. Conclusion

The investigation of PIDs has provided valuable insights to understand the specific gene defect that impairs the immune system. Flow cytometry and genetic testing enable to identify existing and novel phenotypes and genotypes as well as their impact on PIDs. The applications of flow cytometry and genetic technologies have expanded dramatically with more types of PID is defined, and the use of mass sequencing technologies has accelerated the identification of novel disorders. To efficiently use these complex assays, clinicians should have a good understanding of these methods and know how to interpret the results for diagnosis and disease management [33].

The management of patients with PID is based on three aspects of diagnosis: suspicious clinical manifestations, aberrant results of immune response, and the underlying genetic defect [4]. However, the diagnosis of PIDs may confront significant challenges: there are large numbers of variable types of PIDs to be recognized

and most of them have alike clinical presentations with common diseases; immunodeficiencies derived from multiple gene defects can share similar symptoms, and a defect in the same gene may have various clinical manifestations [1]. While severe forms of PIDs are relatively easier to be recognized, milder immunodeficiencies may not raise alertness until typical presentation occurs [68, 69]. Additionally, the criteria for constituting a PID diagnosis are subjective, for example, the degree of frequency and the severity of the infections for establishing the diagnosis are unclear, the association of PIDs with autoimmune disorder or malignancy is ambiguous, and some individuals may not have noticeable symptoms apart from laboratory findings. Furthermore, advanced laboratory examination such as specialized flow cytometric and genetic analysis is not always easy to access. All these factors may contribute to delayed or missed diagnosis of the diseases.

To combat the challenges, clinical warning signs of PIDs should be disseminated to all clinicians for raising earlier recognition of the diseases, and an immunologist must be consulted for proper diagnosis and management. Due to the complexity of clinical presentations and large number of disease types, the use of scoring system based on the codes of the international classification of PIDs [69] assisted by artificial intelligence may be beneficial for clinicians to differentiate these disorders from other diseases and raise initial recognition. The recent advances in understanding the human immune system, development of novel cellular and molecular assays, and collaborations from the international/national organizations have led to significant increase of clinical awareness and cases diagnosed and improvement of disease management and treatment outcomes for PIDs.

Acknowledgements

The author thanks Dr. Alexander Richardson from the Royal College of Pathologists of Australasia for assisting the references work and Mrs. Xiumei Wei from the Sutherland Centre of Immunology in NSW, Australia, for valuable opinions.

Conflict of interest

The author declares that there is no conflict of interest.

Author details

Renfen Chen
Central Sydney Immunology Laboratory, Royal Prince Alfred Hospital, NSW Health Pathology, Camperdown, NSW, Australia

*Address all correspondence to: ren.chen@health.nsw.gov.au

References

[1] Raje N, Dinakar C. Overview of immunodeficiency disorders. Immunology and Allergy Clinics of North America. 2015;**35**(4):599-623. DOI: 10.1016/j.iac.2015.07.001

[2] Condino-Neto A, Espinosa-Rosales FJ. Changing the lives of people with primary immunodeficiencies (PI) with early testing and diagnosis. Frontiers in Immunology. 2018;**9**:1439. DOI: 10.3389/fimmu.2018.01439

[3] Amaya-Uribe L et al. Primary immunodeficiency and autoimmunity: A comprehensive review. Journal of Autoimmunity. 2019;**99**:52-72. DOI: 10.1016/j.jaut.2019.01.011

[4] Mahlaoui N et al. Advances in the care of primary immunodeficiencies (PIDs): From birth to adulthood. Journal of Clinical Immunology. 2017;**37**(5):452-460. DOI: 10.1007/s10875-017-0401-y

[5] Modell V et al. Global report on primary immunodeficiencies: 2018 update from the Jeffrey Modell Centers Network on disease classification, regional trends, treatment modalities, and physician reported outcomes. Immunologic Research. 2018;**66**(3): 367-380. DOI: 10.1007/s12026-018-8996-5

[6] Picard C et al. International Union of Immunological Societies: 2017 primary immunodeficiency diseases committee report on inborn errors of immunity. Journal of Clinical Immunology. 2018;**38**(1):96-128. DOI: 10.1007/s10875-017-0464-9

[7] Primary Immunodeficiencies (PID). Driving Diagnosis for Optimal Care in Europe, European Reference Paper. Available from: http://worldpiweek.org/sites/default/files/basic_page_documents/PI_European_Reference_Paper.pdf

[8] Yel L. Selective IgA deficiency. Journal of Clinical Immunology. 2010;**30**(1):10-16. DOI: 10.1007/s10875-009-9357-x

[9] Kwan A et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. JAMA. 2014;**312**(7):729-738. DOI: 10.1001/jama.2014.9132

[10] Rivers L, Gaspar HB. Severe combined immunodeficiency: Recent developments and guidance on clinical management. Archives of Disease in Childhood. 2015;**100**(7):667-672. DOI: 10.1136/archdischild-2014-306425

[11] Bousfiha A et al. The 2017 IUIS phenotypic classification for primary immunodeficiencies. Journal of Clinical Immunology. 2018;**38**(1):129-143. DOI: 10.1007/s10875-017-0465-8

[12] Carneiro-Sampaio M, Coutinho A. Immunity to microbes: Lessons from primary immunodeficiencies. Infection and Immunity. 2007;**75**(4):1545-1555. DOI: 10.1128/IAI.00787-06

[13] McCusker C, Upton J, Warrington R. Primary immunodeficiency. Allergy, Asthma and Clinical Immunology. 2018;**14**(Suppl 2):61. DOI: 10.1186/s13223-018-0290-5

[14] Bazregari S et al. Evaluation of infectious and non-infectious complications in patients with primary immunodeficiency. Central European Journal of Immunology. 2017;**42**(4): 336-341. DOI: 10.5114/ceji.2017.72825

[15] Guffroy A et al. Primary immunodeficiency and autoimmunity. La Revue de Médecine Interne. 2017;**38**(6):383-392. DOI: 10.1016/j.revmed.2016.10.388

[16] Jesenak M et al. Pulmonary manifestations of primary

immunodeficiency disorders in children. Frontiers in Pediatrics. 2014;**2**:77. DOI: 10.3389/fped.2014.00077

[17] Ruffner MA, Sullivan KE, Henrickson SE. Recurrent and sustained viral infections in primary immunodeficiencies. Frontiers in Immunology. 2017;**8**:665. DOI: 10.3389/fimmu.2017.00665

[18] Sanges S et al. Diagnosis of primary antibody and complement deficiencies in young adults after a first invasive bacterial infection. Clinical Microbiology and Infection. 2017;**23**(8):576-576. DOI: 10.1016/j.cmi.2017.02.005

[19] Burg M, Gennery AR. Educational paper. The expanding clinical and immunological spectrum of severe combined immunodeficiency. European Journal of Pediatrics. 2011;**170**(5):561-571. DOI: 10.1007/s00431-011-1452-3

[20] Fischer A et al. Autoimmune and inflammatory manifestations occur frequently in patients with primary immunodeficiencies. The Journal of Allergy and Clinical Immunology. 2017;**140**(5):1388-1393. DOI: 10.1016/j.jaci.2016.12.978

[21] Arakelyan A et al. Autoimmunity and autoinflammation: A systems view on signaling pathway dysregulation profiles. PLoS One. 2017;**12**(11):e0187572. DOI: 10.1371/journal.pone.0187572

[22] Martorana D et al. Monogenic autoinflammatory diseases with Mendelian inheritance: Genes, mutations, and genotype/phenotype correlations. Frontiers in Immunology. 2017;**8**:344. DOI: 10.3389/fimmu.2017.00344

[23] Sarmiento E et al. Autoimmune disease in primary antibody deficiencies. Allergologia et Immunopathologia. 2005;**33**(2):69-73

[24] Sparchez M et al. Primary complement and antibody deficiencies in autoimmune rheumatologic diseases with juvenile onset: A prospective study at two centers. Pediatric Rheumatology Online Journal. 2015;**13**:51. DOI: 10.1186/s12969-015-0050-8

[25] Agarwal S, Cunningham-Rundles C. Gastrointestinal manifestations and complications of primary immunodeficiency disorders. Immunology and Allergy Clinics of North America. 2019;**39**(1):81-94. DOI: 10.1016/j.iac.2018.08.006

[26] Azizi G et al. Autoimmunity in primary T-cell immunodeficiencies. Expert Review of Clinical Immunology. 2016;**12**(9):989-1006. DOI: 10.1080/1744666X.2016.1177458

[27] Park JY et al. Early deficit of lymphocytes in Wiskott-Aldrich syndrome: Possible role of WASP in human lymphocyte maturation. Clinical and Experimental Immunology. 2004;**136**(1):104-110. DOI: 10.1111/j.1365-2249.2004.02409.x

[28] Grassi MS et al. Congenital heart disease as a warning sign for the diagnosis of the 22q11.2 deletion. Arquivos Brasileiros de Cardiologia. 2014;**103**(5):382-390. DOI: 10.5935/abc.20140145

[29] Rayannavar A et al. Association of hypocalcemia with congenital heart disease in 22q11.2 deletion syndrome. American Journal of Medical Genetics. Part A. 2018;**176**(10):2099-2103. DOI: 10.1002/ajmg.a.40495

[30] Satge D. A tumor profile in primary immune deficiencies challenges the cancer immune surveillance concept. Frontiers in Immunology. 2018;**9**:1149. DOI: 10.3389/fimmu.2018.01149

[31] Mayor PC et al. Cancer in primary immunodeficiency diseases: Cancer incidence in the United States immune

deficiency network registry. The Journal of Allergy and Clinical Immunology. 2018;**141**(3):1028-1035. DOI: 10.1016/j.jaci.2017.05.024

[32] Haas OA. Primary immunodeficiency and cancer predisposition revisited: Embedding two closely related concepts into an integrative conceptual framework. Frontiers in Immunology. 2018;**9**:3136. DOI: 10.3389/fimmu.2018.03136

[33] Locke BA, Dasu T, Verbsky JW. Laboratory diagnosis of primary immunodeficiencies. Clinical Reviews in Allergy and Immunology. 2014;**46**(2):154-168. DOI: 10.1007/s12016-014-8412-4

[34] Nagy G. Laboratory evaluation of immunological dysfunctions in primary immunodeficiency diseases. Orvosi Hetilap. 2018;**159**(49):2087-2094. DOI: 10.1556/650.2018.31261

[35] Eibl MM, Wolf HM. Vaccination in patients with primary immune deficiency, secondary immune deficiency and autoimmunity with immune regulatory abnormalities. Immunotherapy. 2015;**7**(12):1273-1292. DOI: 10.2217/IMT.15.74

[36] Folds JD, Schmitz JL. 24. Clinical and laboratory assessment of immunity. The Journal of Allergy and Clinical Immunology. 2003;**111**(2 Suppl):S702-S711. DOI: 10.1067/mai.2003.122

[37] Varzaneh FN et al. Cytokines in common variable immunodeficiency as signs of immune dysregulation and potential therapeutic targets: A review of the current knowledge. Journal of Clinical Immunology. 2014;**34**(5):524-543. DOI: 10.1007/s10875-014-0053-0

[38] Abraham RS, Aubert G. Flow cytometry, a versatile tool for diagnosis and monitoring of primary immunodeficiencies. Clinical and Vaccine Immunology. 2016;**23**(4):254-271. DOI: 10.1128/CVI.00001-16

[39] Milligan KL et al. Complete myeloperoxidase deficiency: Beware the "false-positive" dihydrorhodamine oxidation. The Journal of Pediatrics. 2016;**176**:204-206. DOI: 10.1016/j.jpeds.2016.05.047

[40] Dinauer MC. Disorders of neutrophil function: An overview. Methods in Molecular Biology. 2007;**412**:489-504. DOI: 10.1007/978-1-59745-467-4_30

[41] Orange JS. Natural killer cell deficiency. The Journal of Allergy and Clinical Immunology. 2013;**132**(3):515-525. DOI: 10.1016/j.jaci.2013.07.020

[42] Rubin TS et al. Perforin and CD107a testing is superior to NK cell function testing for screening patients for genetic HLH. Blood. 2017;**129**(22):2993-2999. DOI: 10.1182/blood-2016-12-753830

[43] Chiang SCC, Bleesing JJ, Marsh RA. Current flow cytometric assays for the screening and diagnosis of primary HLH. Frontiers in Immunology. 2019;**10**:1740. DOI: 10.3389/fimmu.2019.01740

[44] Nilsson B, Ekdahl KN. Complement diagnostics: Concepts, indications, and practical guidelines. Clinical & Developmental Immunology. 2012;**2012**:962702. DOI: 10.1155/2012/962702

[45] Kanegane H, et al. Flow cytometry-based diagnosis of primary immunodeficiency diseases. Allergology International. 2018;**67**(1):43-54. DOI: 10.1016/j.alit.2017.06.003

[46] Knight V. The utility of flow cytometry for the diagnosis of primary immunodeficiencies. International Journal of Laboratory Hematology. 2019;**41**(Suppl 1):63-72. DOI: 10.1111/ijlh.13010

[47] Piatosa B et al. B cell subsets in healthy children: Reference values for evaluation of B cell maturation process in peripheral blood. Cytometry. Part B, Clinical Cytometry. 2010;**78**(6):372-381. DOI: 10.1002/cyto.b.20536

[48] Schatorje EJ et al. Age-matched reference values for B-lymphocyte subpopulations and CVID classifications in children. Scandinavian Journal of Immunology. 2011;**74**(5):502-510. DOI: 10.1111/j.1365-3083.2011.02609.x

[49] Karimi MA et al. Measuring cytotoxicity by bioluminescence imaging outperforms the standard chromium-51 release assay. PLoS One. 2014;**9**(2):e89357. DOI: 10.1371/journal.pone.0089357

[50] Heimall JR et al. Use of genetic testing for primary immunodeficiency patients. Journal of Clinical Immunology. 2018;**38**(3):320-329. DOI: 10.1007/s10875-018-0489-8

[51] Ameratunga R et al. The clinical utility of molecular diagnostic testing for primary immune deficiency disorders: A case based review. Allergy, Asthma and Clinical Immunology. 2010;**6**(1):12. DOI: 10.1186/1710-1492-6-12

[52] Zheng X et al. The effects of cryoprotectants on sperm motility of the Chinese pearl oyster, *Pinctada fucata* martensii. Cryobiology. 2018;**82**:64-69. DOI: 10.1016/j.cryobiol.2018.04.007

[53] Chinn IK, Bostwick BL. The role of genomic approaches in diagnosis and management of primary immunodeficiency. Current Opinion in Pediatrics. 2018;**30**(6):791-797. DOI: 10.1097/MOP.0000000000000695

[54] Barbaro M et al. Newborn screening for severe primary immunodeficiency diseases in Sweden-a 2-year pilot TREC and KREC screening study. Journal of Clinical Immunology. 2017;**37**(1):51-60. DOI: 10.1007/s10875-016-0347-5

[55] Raje N et al. Utility of next generation sequencing in clinical primary immunodeficiencies. Current Allergy and Asthma Reports. 2014;**14**(10):468. DOI: 10.1007/s11882-014-0468-y

[56] Dinauer MC. Primary immune deficiencies with defects in neutrophil function. Hematology. American Society of Hematology. Education Program. 2016;**2016**(1):43-50. DOI: 10.1182/asheducation-2016.1.43

[57] Krivan G et al. New insights in the use of immunoglobulins for the management of immune deficiency (PID) patients. American Journal of Clinical and Experimental Immunology. 2017;**6**(5):76-83

[58] Bonagura VR. Using intravenous immunoglobulin (IVIG) to treat patients with primary immune deficiency disease. Journal of Clinical Immunology. 2013;**33**(Suppl 2):S90-S94. DOI: 10.1007/s10875-012-9838-1

[59] Shapiro RS et al. Emerging paradigm of primary immunodeficiency disease: Individualizing immunoglobulin dose and delivery to enhance outcomes. Journal of Clinical Immunology. 2017;**37**(2):190-196. DOI: 10.1007/s10875-014-9990-x

[60] Bethune C et al. British Society for Immunology/United Kingdom Primary Immunodeficiency Network consensus statement on managing non-infectious complications of common variable immunodeficiency disorders. Clinical and Experimental Immunology. 2019;**196**(3):328-335. DOI: 10.1111/cei.13272

[61] Heimall J et al. Current knowledge and priorities for future research in late effects after hematopoietic stem cell transplantation (HCT) for severe

combined immunodeficiency patients: A consensus statement from the second pediatric blood and marrow transplant consortium international conference on late effects after pediatric HCT. Biology of Blood and Marrow Transplantation. 2017;**23**(3):379-387. DOI: 10.1016/j.bbmt.2016.12.619

[62] Castagnoli R et al. Hematopoietic stem cell transplantation in primary immunodeficiency diseases: Current status and future perspectives. Frontiers in Pediatrics. 2019;**7**:295. DOI: 10.3389/fped.2019.00295

[63] Chinen J, Puck JM. Successes and risks of gene therapy in primary immunodeficiencies. The Journal of Allergy and Clinical Immunology. 2004;**113**(4):595-603. DOI: 10.1016/j.jaci.2004.01.765

[64] Booth C et al. Gene therapy for primary immunodeficiency. Human Molecular Genetics. 2019. DOI: 10.1093/hmg/ddz170. [Epub ahead of print]

[65] Mukherjee S, Thrasher AJ. Gene therapy for PIDs: Progress, pitfalls and prospects. Gene. 2013;**525**(2):174-181. DOI: 10.1016/j.gene.2013.03.098

[66] Markert ML, Devlin BH, McCarthy EA. Thymus transplantation. Clinical Immunology. 2010;**135**(2):236-246. DOI: 10.1016/j.clim.2010.02.007

[67] Wahlstrom JT, Dvorak CC, Cowan MJ. Hematopoietic stem cell transplantation for severe combined immunodeficiency. Current Pediatrics Reports. 2015;**3**(1):1-10. DOI: 10.1007/s40124-014-0071-7

[68] Starr SP. Immunology update: Primary immunodeficiency diseases. FP Essentials. 2016;**450**:35-53

[69] Yarmohammadi H et al. Recognizing primary immune deficiency in clinical practice. Clinical and Vaccine Immunology. 2006;**13**(3):329-332. DOI: 10.1128/CVI.13.3.329-332.2006