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STATE-OF-THE-ART

Primary immunodeficiencies: *up-to-date*

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ABSTRACT

Primary immunodeficiencies (PID) represent a large group of genetic diseases with the common characteristic of a malfunctioning immune system. In Mauno Vihinen'classification there are 79 entities. These diseases are diagnosed in the first years of life. The frequent clues to this diagnosis are recurrent, protracted, chronic and/or opportunistic infections and/or infections resistant to first line antibiotics. The main pathogenic methods of treatment in PID include bone marrow transplant (BMT), stem cells transplant, enzyme replacement, immunoglobulin replacement therapy, cytokines substitution therapy. The gene therapy is still the therapeutic perspective.

Key words: primary immunodeficiency, diagnosis, therapy

The main goal of immunity is to preserve one's integrity. More and more complex ways of completing this task have been developed throughout our phylogeny. No matter the action site these mechanisms are specialized and unspecialized, specific and unspecific.

Immunodeficiencies represent a group of diseases characterized by a malfunction of one component of the defense system. They may be primary or acquired (secondary to other diseases), with partial defects (specific/nonspecific or cellular/humoral immunity involved) or with entire system impairment.

Primary immunodeficiencies represent a particular subject among pediatricians and are related to the immune system development in fetus and child. Some of them appear only in childhood. Several classifications are accepted. One of these is the Mauno Vihinen'classification (1), with 79 entities. ❑

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CLASSIFICATION OF PRIMARY IMMUNODEFICIENCIES (1)

1. Specific immunity disorders

A. Humoral immunity disorders

1. X-linked agammaglobulinemia (Bruton's disease)
2. Non X-linked hyper IgM syndrome
3. Immunoglobulin (Ig) heavy-chain gene deletions
4. Kappa chain deficiency
5. Selective deficiencies of IgG or IgA subclasses or IgE class:
   1. gamma1 (IGHG1); gamma2 (IGHG2); partial gamma3 (IGHG3); gamma4(IGHG4)
   2. alfa1 (IGHA1); alfa2 (IGHA2)
   3. epsilon (IGHE)
6. Antibody deficiency with normal Igs
7. Common variable immunodeficiency
8. IgA deficiency
9. Transient hypogammaglobulinemia of infancy
10. Autosomal recessive agammaglobulinemia

B. Cellular immunity disorders:

1. Thymus dysplasia/aplasia with normal Ig (Nezelof Syndrome)
2. Purine nucleoside phosphorylase (PNP) deficiency
3. CD3 gamma deficiency
4. CD3 epsilon deficiency
5. ZAP-70 deficiency

C. Combined immunodeficiencies (CIDs):

1. Severe combined immunodeficiencies (SCIDs)

A. T-B+ SCIDs
   A. X-linked gamma chain deficiency
   B. Autosomal recessive Jak3 deficiency

B. T-B- SCIDs
   A. RAG 1 deficiency
   B. RAG 2 deficiency
   C. Adenosine deaminase (ADA) deficiency
   D. Reticular dysgenesis

C. Other SCIDs
   A. X-linked hyper IgM syndrome
   B. CIITA, MHCII transactivating protein deficiency
   C. RFX-5, MHCII promoter X box regulatory factor 5 deficiency
   D. RFXAP, Regulatory factor X-associated protein deficiency
   E. TAP-2 deficiency

2. Other CIDs

A. Wiskott-Aldrich (WA) syndrome
B. Ataxia-telangiectasia
C. DiGeorge syndrome

2. Nonspecific immunity disorders

A. Humoral immunity disorders

1. C1q deficiency
2. C1r deficiency
3. C1s deficiency
4. C4 deficiency
5. C2 deficiency
6. C3 deficiency
7. C5 deficiency
8. C6 deficiency
9. C7 deficiency
10. C8 alpha deficiency
11. C8 beta deficiency
12. C9 deficiency
13. C1 inhibitor deficiency
14. Factor I deficiency
15. Factor H deficiency
16. Factor D deficiency
17. Properdin deficiency

B. Cellular immunity disorders:

1. Severe congenital neutropenia
2. Cyclic neutropenia
3. Leukocyte adhesion defect 1 (deficiency of beta chain (CD18) of LFA-1)
4. Leukocyte adhesion defect 2 (failure to convert GDP mannose to fucose)
5. Chediak-Higashi syndrome
6. Specific granule deficiency
7. Schwachman syndrome
8. X-linked chronic granulomatous disease (CGD) (cyt b 91kD)
9. Autosomal recessive CGD deficiency of p22 phox
10. Autosomal recessive CGD deficiency of p47 phox
11. Autosomal recessive CGD deficiency of p67 phox
12. Neutrophil G6PD deficiency
13. Myeloperoxidase deficiency
14. IFN-gamma receptor deficiency

3. Combined immunodeficiency

– Congenital aleukia

4. Primary immunodeficiencies associated with some congenital diseases:

1. Down syndrome
2. 18th chromosome breakage
3. Turner syndrome
4. chromosome instability syndrome
5. DNA defective repair
6. Fanconi anemia
7. Bloom syndrome
8. Xeroderma pigmentosum
9. ICF syndrome
10. Nijmegen breakage syndrome
11. metabolic defects
12. skeletal malformations
13. growth retardation
14. chronic mucocutaneous candidiasis
15. Job syndrome

PRIMARY IMMUNODEFICIENCIES (PID) are the best-known immunity diseases. They represent true "experiments of the nature". Most of them are inherited, all of them having genetic causes. The immune system has many components and it is the target of many mutations. Some of them cannot be compensated by back-ups and this leads to phenotypic expression, with a variety of diseases and symptoms.

Formerly they were thought to be rare (as only the most severe were recognized). Today, PID are more frequently encountered, but as they can present in mild/moderate forms, the diagnosis can be delayed as late as to adolescence or even adulthood, while others can be clinically not manifested.

Severe PID are evident from birth, but most of them are recognized within the first year of life. ❑

Epidemiology

Estimations (in USA) are around 400 births / year. 25,000 to 50,000 people in USA have a primary immunodeficiency (2). As science progresses, PID cases are recognized and more PID classes are described. Although WHO gives over 70 types of PID (2), a Spanish study (3) shows that PID with known causes are over 100. SCIDs are still rarely encountered. DiGeorge syndrome is more frequent diagnosed now. The selective IgA deficiency is rather high: 1/300 (a "healthy" blood donor's evaluation). ❑

Pathogeny

PID are results of genetic defects whereas acquired immunodeficiencies are the result of viral/chemical/physical agents' action on the immune system. In PID the defect is either *de novo* or inherited from one or both parents. The mutated gene acts through modified protein expression.

If there is no normal allele, the disease is clinically manifested (autosomal recessive diseases). The same clinical signs may be seen in other family members. ❑

Clinical features are characterized by:

1. infections
   - recurrent, protracted, chronic infections
   - sinusal, bronchial or multiple systems involvement
   - severe, bacterial, admission-requiring infections (pneumonia, meningitis, osteomielitis, sepsis, deep tissues abscess)
   - opportunistic infections: P. carinii, T. gondii
   - resistant to first line antibiotics (AB)
2. immunologic disorders
   - immune hemolytic anemia
   - diabetes mellitus
   - systemic lupus erythematosus
3. somatic, neurological and psychical retarded development
4. associated features (parts of complex syndromes)
   1. DiGeorge: thyme dysplasia, lack of T cells, parotid and facial malformations
   2. Wiskott-Aldrich: infections, hemorrhagic syndrome, rash.

PID diagnosis is established on anamnesis (patient's history, familial antecedents), clinical features and biologic tests. Sometimes the diagnostic is not so evident. Severe infections are not always associated to PID, in many cases (50%) they occur in normal children. European Society for Immunodeficiencies (ESID) has established the criteria necessary for definitive, probable or possible diagnosis and also exclusion criteria for some of these diseases (4).

- *Patient's history* is of recurrent, protracted, chronic infections.
- *Familial antecedents* are present in PID cases, with infant deaths and other elements suggestive for X-linkage (only boys affected).
- *Clinical exam* can evidence the child nutrition state, growth and development assessment, respiratory disease signs, mucous membranes lesions (oral aphtae), splenomegalia, arthritis, lymphatic system assessment (ganglia, tonsils).

Diagnostic can become easier if some of these ten signs of PID (2) are present:

1. otic infections $\geq$ 8/year
2. severe sinusitis $\geq$ 2/year
3. antibiotic requirements > 2 months/year (and with poor results)
4. pneumonias $\geq$ 2/year
5. unsatisfactory weight curve evolution in infants
6. recurrent deep abscesses
7. persistent aphtous oral lesion after 1 year
8. intravenous (i.v.) antibiotic needed for intercurrent infections

9. ≥ 2 severe infections (meningitis, osteomielitis, sepsis, cellulites)
10. familial history of PID

Some data about the signs and diagnostic in PID are summarized in the following tables (1,2,3)

Differentials are made with malnutrition, severe accidents, burns, drugs (corticoids), severe diseases (leukemia), some infections (Epstein Barr virus – EBV, measles, varicella, HIV).

Always present	Frequent	Occasional
Recurrent infections	Weight and stature retard	Prolonged fever
Otitis	Opportunistic infections	Delayed umbilical detachment
Sinusitis	Chronic diarrhea	Arthralgia
Pneumonia	Malabsorptive syndromes	Periodontitis
Unusual bacterial infections	Abscesses	Stomatitis
Inappropriate response to AB treatment	Autoimmune disorders	Adenopathies
		Hepatosplenomegaly
		Lymphoid neoplasia
Familial history of PID		

TABLE 1. Suggestive signs for PID (3)

	Humoral defects	Phagocyitic defects	Combined severe PID
Onset	5-6 mo	Any age	Birth
Infections	Respiratory	Respiratory	Respiratory
	Digestive	Skin	Digestive
		Deep tissues	Sepsis
Germs	Bacteria	Bacteria	Bacteria
	Enteroviruses	Fungi	Viruses
			Mycobacteria.
			Opportunistic (cytomegalovirus, Candida, P.carinii)
Lymphoid tissue	Normal/absent	Normal	Absent

TABLE 2. Characteristic clinical features (3)

Clinical picture	Possible PID	Differentials
Recurrent enteritis or lower airways infections	Antibody or complement deficiency	Cystic fibrosis Ciliary dyskinesia Allergic asthma Adenoid hypertrophy
Weight and stature retard Protracted diarrhea Opportunistic infections	Severe combined PID	Malabsorption Chronic bowel diseases Chemotherapy Immunosuppression Acquired immunodeficiencies (HIV, cytomegalovirus, congenital rubella)
Superficial or systemic infections with pyogenic germs or fungi	Phagocytic disorders Intracellular bactericidal defects Hyper IgE syndrome	Local factors Hematogenous dissemination Eczema
Recurrent infections with Neisseria spp., atypic Mycobact., viruses	Complement defects IFN gamma/IL12 defects	Local factors Intrafamilial infections
Associated specific clinical features (ataxia-telangiectasia, cardiopathy, hypocalcaemia, eczema, thrombocytopenia)	DiGeorge syndrome Wiskott-Aldrich syndrome	
Delayed umbilical detachment	Adhesion molecule defect	
Autoimmune disorders	Common variable PID	

TABLE 3. Clinical features and diagnostic (3)

Immunologic evaluation

A "global" screening of immunity in all children with recurrent infections is not feasible. Thus, clinical picture and PID's "key" features should be the starting point. The diagnostic procedure should be progressive ("first things first"): a complete blood count (CBC) and formula, quantitative immunoglobulins, levels of antibodies to current vaccines, complement components and CH50.

Advanced tests like antibodies level for antipneumococcic and anti HiB vaccines, lymphocytes types and subtypes, cytokines and their receptors' levels, intracellular oxidation tests (NBT test, fluorescence), granulocytic adhesion molecules levels, mycobacterium killing test, function of complement components, genetic tests for C1-inhibitor deficiency and other genetic tests are the following.

There are many diseases from the immunodeficiencies group where the genetic tests are part of the definitive diagnosis (X linked agammaglobulinemia, ataxia telangiectasia, Wiskott – Aldrich syndrome, severe combined immunodeficiency, X-linked hyper IgM, X-linked severe combined immunodeficiency, MHC – class II deficiency, DiGeorge syndrome, chronic granulomatous disease, leukocyte adhesion defects, X-linked lymphoproliferative syndrome) (4). In one immunodeficiencies database – IDbases (http://bioinf.uta.fi/IDbases) there have been identified more than 100 affected genes. Mutations described at DNA, mRNA, and protein levels can be inherited in an X-linked, an autosomal recessive, or an autosomal dominant way (5).

The absence of the isohemagglutinins or the poor response to vaccine are important criteria in the diagnosis of some immunodeficiencies.

Infections work-up like germ culture, antibiogramme can be performed.

The antenatal diagnosis is recommended in families with PID cases. The fetal cells are harvested by amniocentesis (from week 14th), chorionic villus sampling (from week 9-10th), and fetal blood (from week 18th). These cells are tested for cell abnormalities or enzymes deficiencies. Knowing the diagnosis before birth means to be prepared to treat, to consider early bone marrow/stem cells transplant.

Treatment

PID treatment has as main objective the infections clearance. This is realized using first line antibiotics (oral), second line antibiotics, if needed intravenous (IV) and adjuvants like non steroidal anti-inflammatory drugs and physical therapy for respiratory infections.

Infection prophylaxis follows a strict hygiene, adequate nutrition, crowded place and sick people avoidance, chronic antibiotherapy (continuous long-term low-dose antibiotics) with trimethoprim – sulfonamide combination (TMP-SMD) to prevent Pneumocystis carinii pneumonia in kids with low T cells, vaccinations with killed (inactivated) germs.

The pathogenic methods of treatment in PID include bone marrow transplant (BMT), enzyme replacement, immunoglobulin replacement therapy, cytokines substitution therapy, stem cells transplant.

BMT was first done in 1968 and there are almost 1000 children treated this way so far. This offers the chance of a radical, complete and permanent treatment for some PID. It was performed in SCID, WA syndrome, leucocytes adhesion deficit. The results are good, offering antinfectious protection, weight gain, and an almost normal life. The limits consists in some compatibility problems, (identical, or nearly identical HLA antigens between the donor's tissues and the recipient's tissues), age (better to be performed at small age), suitable only for *some* PID, no infections at the moment of BMT.

Enzyme replacement can be recommended in PID characterized by unique enzyme defect, especially in children with SCID that lack compatible donor and ADA (adenosine deaminase) deficiency (15% of SCID). It consists in the periodic administration of the enzyme, linked with a macromolecule (PEG) which prevents enzyme's rapid decay.

Ig replacement therapy at variable intervals (every 3-4 weeks, generally monthly) is based on the half-life of the principal Ig component (IgG). It is dependent on the immunologic "experience" of the donors. The minimum dosage is 300-400 mg/kg. This method has good results. It must respect a certain administration technique. The way of administration can be IV or subcutaneous. There are studies that suggest that the subcutaneous IgG replacement therapy at home is effective and safe in children and adults with primary immunodeficiencies and high IgG levels were easily maintained (6).

Cytokines substitution therapy is a good method of treatment for chronic granulomatous disease (CGD) – using gamma-interferon (IFN). Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) and Granulocyte Colony

Stimulating Factor (G-CSF) are also used in neutropenia. These are two glycoproteins of the CSF family. **GM-CSF** is secreted by macrophages and stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophiles) and macrophages. It is used as a medication (trademarked Leukine®), to stimulate the production of white blood cells, especially granulocytes and macrophages, following chemotherapy. **G-CSF** regulates haematopoietic cell proliferation and differentiation (7). It is produced by endothelium, macrophages, and other immune cells. There are two forms of the natural G-CSF: one of a 174 and other of a 180 amino-acid-long protein. The more-abundant and more-active form is the first one. This form is used in the development of pharmaceutical products by recombinant DNA (rDNA) technology. Australia was the country where G-CSF was first recognized and purified in 1983 (mouse G-CSF), but the human G-CSF gene was cloned, for the first time, in Japan and United States three years later. There are two forms of recombinant human G-CSF. One is synthesized in an *E. coli* expression system, and it is called filgrastim. There are slight differences between the filgrastim and the natural glycoprotein. Filgrastim (Neupogen®) and PEG-filgrastim (Neulasta®) are two commercially available forms of rhG-CSF (recombinant human G-CSF). PEG means polyethylene glycol and it confers a much longer half-life, reducing the necessity of daily injections. The other form of recombinant human G-CSF, called lenograstim, is synthesized in Chinese Hamster Ovary cells (CHO cells). Lenograstim is indistinguishable from the 174-amino acid natural human G-CSF. No clinical or therapeutic consequences linked to the differences between filgrastim and lenograstim have yet been identified, but there are no formal comparative studies.

Stem cells transplant (SCT) is an easier technique than the BMT with a longer life-span of transplanted cells. Stem cells are easier to harvest (from umbilical cord) (8) even from unrelated umbilical donor cord blood (UCB) when a suitable HLA-matched donor is not available. It was used in children with Wiskott-Aldrich syndrome and other patients with malignant and nonmalignant disorders treated with myeloablative therapy (9). There are UCB banks in many countries in the world for allogeneic unrelated and related SCT. UCB banks report available units to national and international donor registries. There is a second model of UCB, the family banking, where UCB is stored for the benefit of the donor or of their family members. In many studies, the UCB transplants, related and unrelated, seems to be equivalent to or superior to bone marrow transplants, especially in children (9). There has been reported the allogeneic haematopoietic stem cell transplantation even in utero in cases of severe immunodeficiencies (10).

Important cautions in PID

The avoidance of live attenuated vaccines (oral antipolio – OAP, measles, and varicella) in patients with ATS, Bruton disease or T cell defects must be respected in order to prevent the disseminated infections with these attenuated pathogens. But, now, in United States of America OAP was replaced with inactivated polio vaccine. In certain immunocompromised children, the Advisory Committee on Immunization Practices and American Academy of Pediatrics recommends the administration of the live viral vaccines (11). It is recommended that the persons with primary B-cell immunodeficiencies (not persons with T-cell immunodeficiencies) must avoid OAP being at risk to disseminated infections with attenuated pathogens (12). In DiGeorge syndrome, MMR (measles, mumps, rubella) vaccine was administrated without incidence regarding side effects in patients who had no evidence of severe immunocompromise (13).

Also, blood products need not only to be virus-free (hepatitis type B or C, cytomegalovirus) but should also be irradiated, in order to prevent the T-cell – mediated graft-versus-host-disease (GVHD) in the recipient.

Therapeutic perspectives

Genic therapy represents the "insertion" of the normal gene in the patient's DNA using a vector. For the first time this type of therapy was tried in 1990, in two girls with SCID (ADA deficit) with good results, although the two are still receiving PEG-ADA. T lymphocytes (TL) were harvested and were "infected" with a vector carrying the desired DNA sequence (retrovirus). The TL were then perfused back into the patient. There are vectors (MFG-S-hIL2RG) that provide the best opportunity for in vivo selection and development of B and T lymphocytes for human XSCID gene therapy (14). There were reported cases of leukemia proliferations (15,16). But, trials shown that gene therapy for patients with X-linked severe combined immun-

odeficiency (XSCID) (eight cases from the group of ten) (17) and adenosine deaminase-deficient severe combined immunodeficiency have restored immune competence (18). In patients with X-SCID, the first curative effects for gene therapy were obtained after approximately 10 years (19). This kind of treatment is now studied in other immunodeficiency disorders, including chronic granulomatous disease and Wiskott-Aldrich syndrome (18). There are studies on mouse model that show that integration sites are near or within established protooncogenes and that T cell transformation is dependent on the insertion vector (20). This therapy is still an "edge" therapy. ❑

Conclusion

1. Recent medicine progresses have changed the diagnosis and treatment of primary immunodeficiencies. Not only these patients do survive, but they can live almost normal lives.
2. Early diagnosis is a must, in order to modify these patients' evolution, by preventing irreversible lesions and life endangering.
3. Infections that are more than "simple infections" must be recognized because diagnosis procedures and treatment should immediately follow.
4. Intravenous immunoglobulins, bone marrow transplant and enzyme replacement are efficient treatment methods and they improve continuously.
5. Collaboration is required and also is the identification of the centers with the technique equipment and expertise required in exploring the immune system.

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