



Importance of Hemogram Parameters for Predicting Primary Immunodeficiency

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Abstract

Early recognition of primary immunodeficiencies is life-saving. The complete blood count test contains important clues in the early diagnosis of immunodeficiencies. Lymphopenia, early onset frequent infections, neurological findings and low uric acid levels in infancy must suggest purine nucleoside phosphorylase deficiency. Compensatory monocytosis, eosinophilia and hypergammaglobulinemia may accompany severe congenital neutropenia. Herein, 2 patients with severe immunodeficiency disorder are presented to emphasize the importance of hemogram parameters in predicting PID.

Keywords: CBC; Lymphopenia; Neutropenia; Immunodeficiency

Introduction

Primary immunodeficiencies are a heterogeneous group of genetic disorders in which part of the immune system is missing or functions improperly, with increased susceptibility to infections, autoimmunity, lymphoproliferation, uncontrolled inflammation and malignancy. More than 350 distinct Primary Immunodeficiency Diseases (PID) have been described to date [1]. Although many of these disorders are rare, as a group it is estimated that between 1:2000 and 1:10 000 live births are affected by a PID [1-3]. All health care practitioners must be vigilant for PIDs as the timely precise diagnosis of these patients is critical for optimal treatment and improved outcome. PID patients often present with very common and/or nonspecific signs and symptoms, such as recurrent fever and upper respiratory tract infections, and also present in a subtle fashion that the diagnosis will be made only if the physician is aware of this group of disease [1,4,5]. The relative rarity of the individual PIDs also contributes to the diagnostic challenge. As a consequence, diagnostic delay is common.

There has been a rapid increase in the knowledge about the nature and pathogenesis of many inherited PIDs in recent years, with the advances in clinical immunology and molecular genetics technology, such as sophisticated functional tests and next generation sequencing techniques. Many of the new diagnostic methods remain in the realm of specific research laboratories. Besides detailed investigations, simple and accessible tests are very important for diagnosis and screening of PIDs. All family physicians must be aware of the clues in the basic tests for the assessment of immunity. Complete blood count (CBC) is the most laboratory ordered test and has very important parameters for foreseeing immunodeficiency. Herein, 2 patients with severe immunodeficiency disorder are presented to emphasize the importance of hemogram parameters in predicting PID.

Case Presentation

Case 1

Twelve month old girl was admitted to our hospital with complaints of recurrent respiratory tract infections since 3 months of age. Twin girls were born at 36th weeks of gestational age from third degree consanguineous healthy parents. Developmental milestones were late for age; she held her head up at 7 months and could not sit well unsupported yet. Her sister was healthy with normal development. Her weight was 7 kg (<3rd percentile), height was 67 cm (3rd percentile to 10th percentile) and head circumference was 42 cm (3rd percentile to 10th percentile). Pulmonary auscultation revealed multiple fine crackles on both lungs. Laboratory examinations showed leukocytosis with severe lymphopenia (WBC 27730/mm³, absolute neutrophil count 27340/mm³, absolute lymphocyte count 40/mm³) and panhypogammaglobulinemia (IgG 448 mg/dl, IgM <16 mg/dl, IgA <6 mg/dl, IgE 2.3 kU/l). Re-examination of previous hemogram results of other hospitals showed that the lymphopenia persisted from 3 months old. Additionally, serum

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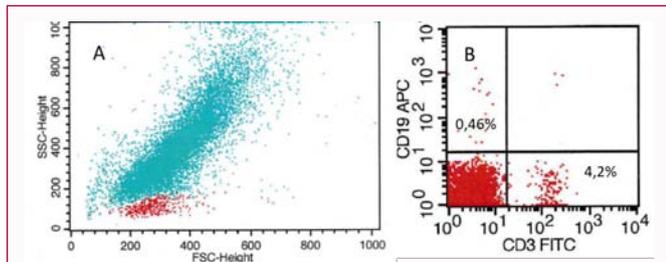


Figure 1: Flow cytometric dot blot representations of Case 1. A: Marked decrease in total lymphocytes, B: CD3⁺ T cell and CD19⁺ B cell lymphopenia.

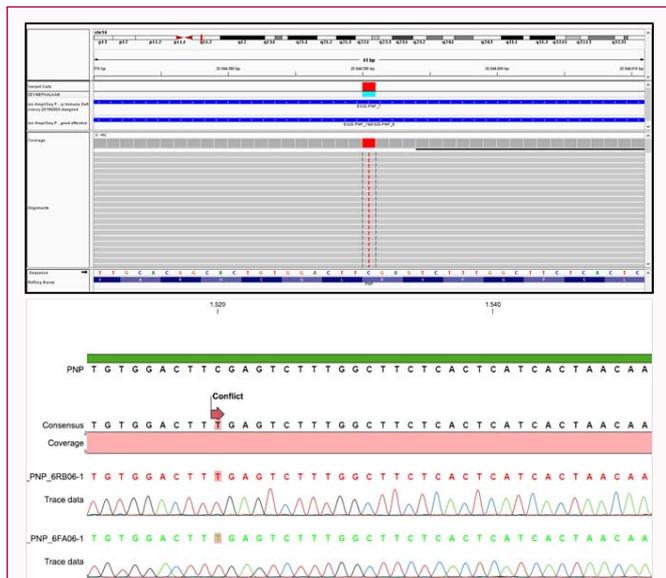


Figure 2: Genetic sequencing analysis showing a homozygous mutation in NP gene (c.700C>T, p.Arg234Ter (R24X) substitution in exon 2).

uric acid level was low (1.2 mg/dl, normal 2-5.5 mg/dl). Peripheral blood mononuclear cell phenotyping demonstrated CD3⁺ T cell and CD19⁺ B cell lymphopenia (CD3⁺ T cells 4.2%, CD19⁺ B cells 0.4%, CD3⁺ CD4⁺ T helper cells 2.2%, CD3⁺ CD8⁺ T cells 2.7%, CD3⁺ CD16/56⁺ natural killer cells 90%) (Figure 1). Genetic sequencing analysis revealed a homozygous mutation in NP gene (c.700C>T, p.Arg234Ter (R24X) substitution in exon 2) (Figure 2). Parainfluenza virus type 3, Coronavirus OC43 and Bocavirus were isolated from nasopharyngeal swab. The CMV DNA load was 3648 copies/ml in blood. Chest X-ray and thorax computed tomography showed bilateral infiltrations and parancymal nodules in both lungs (Figure 3). Despite robust treatment with intravenous immunoglobulin replacement, broad spectrum antibiotics, ganciclovir and antifungal voriconazole, the patient died from progressive lung infection and respiratory failure.

Case 2

Three year old girl was referred with the diagnosis of hyper IgE syndrome (HIES) from another university hospital. The clinicians suspected HIES with positive findings such as recurrent diaper dermatitis, otitis media, eosinophilia and elevated IgE level (2200 kU/l, normal <100 ku/l). She did not have any complaints on admission. Past medical history revealed multiple episodes of diaper dermatitis from infancy, recurrent otitis media since 2 years of age and sacral necrotising fasciitis 5 months ago. Pseudomonas aureginosa and Klebsiella pneumoniae had been isolated from debridement

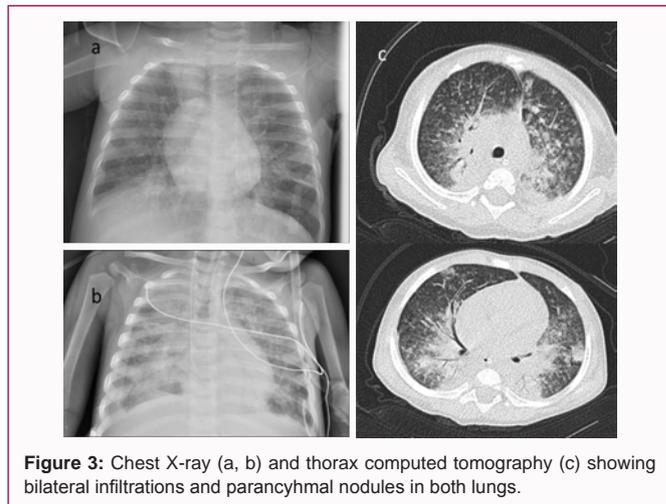


Figure 3: Chest X-ray (a, b) and thorax computed tomography (c) showing bilateral infiltrations and parancymal nodules in both lungs.

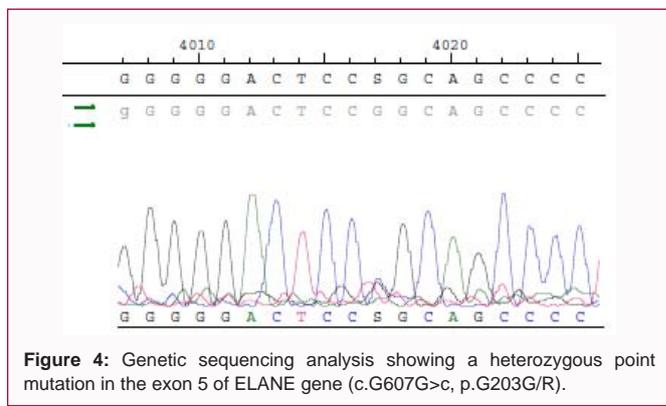


Figure 4: Genetic sequencing analysis showing a heterozygous point mutation in the exon 5 of ELANE gene (c.G607G>c, p.G203G/R).

material. She was born at term with a birth weight of 3600 gr from nonconsanguineous healthy parents. Developmental milestones were normal. Physical examination was unremarkable except aphthae on left cheek mucosa, multiple cervical <1 cm lymphadenopathies and the incision scar on sacral region. Her weight was 10 kg (10-25 percentile) and height was 86 cm (50-75 percentile). Laboratory investigations were as follows; WBC 6400/mm³, Hb 9.9 g/dl, hematocrit 30%, platelet 563000/mm³, 1% neutrophil (absolute neutrophil count 9/mm³), 44% lymphocyte, 44% monocyte and 6% eosinophil on peripheral blood smear, total protein 8 g/dl, albumin 3.3 g/dl (inverted albumin/globuline ratio) and hypergammaglobulinemia (IgG 2310 mg/dl, IgM 111 mg/dl, IgA 724 mg/dl, IgE 1140 ku/l). Lymphocyte subsets were in normal levels. Previous CBC results and repeated CBC tests in our hospital showed persistent neutropenia, absolute neutrophil counts were between 442-37/mm³. An infectious disease work up including evaluation for Parvovirus, CMV and EBV infections proved to be negative. Bone marrow aspiration smear and biopsy revealed normoplastic marrow with an apparent maturation arrest between promyelocytes and myelocytes, and no malignant transformation or myelodysplasia. A heterozygous point mutation in the exon 5 of ELANE gene (c.G607G>c, p.G203G/R) was identified, verifying severe congenital neutropenia (Figure 4).

Discussion

Analysis of a child with potential PID begins with the history and physical examination like in any other situation in pediatric practice. Historically considered rare, PIDs as a group are not rare. Since early diagnosis and appropriate therapy of most PID cases can save lives,

prevent morbidity, improve outcomes and patient quality of life, efforts to eliminate delay in diagnosis is essential. This early diagnosis is the task of the pediatrician who encounters the child for the first time. Potential PID should be suspected in time and appropriate diagnostic tests should be performed. The first step laboratory investigation is complete blood count with differential in patients presenting with recurrent sinopulmonary infections, broad infectious susceptibility, failure to thrive and recurrent abscess formation.

The complete blood count is more than a list of numbers. Understanding its strengths provides very useful information. When used in conjunction with careful review of medical history and physical examination, the CBC can be a more effective diagnostic and screening tool, allowing for proper direction toward making the diagnosis of ID.

Severe combined immunodeficiency (SCID) is a true medical emergency. Most newborns with SCID have no abnormalities at birth. Symptoms develop early in life and include frequent infections, failure to thrive, recurrent bronchiolitis, chronic diarrhea, moniliasis and life-threatening infections. All forms of SCID affect T cell development. As 70% of circulating lymphocytes are T cells in infants, lymphopenia is a key feature of most SCID. An absolute lymphocyte count below $2500/\text{mm}^3$ in the neonatal period should raise the suspicion of SCID [2,5,6]. The International Union of Immunological Societies Expert Committee for Primary Immunodeficiency updates biannually a detailed classification of molecularly defined PID [1]. Purine nucleoside phosphorylase (PNP) deficiency is in the combined immunodeficiencies with associated or syndromic features group. This group includes patients who have other characteristic syndromic features that might bring these patients to medical attention before the manifestation of immune defects [5]. PNP deficiency is a rare autosomal recessive combined PID accounting for approximately 5% of SCID [1,7]. Adenosine deaminase and PNP, expressed in practically all cell types, are consecutive enzymes important for purine degradation and salvage pathway [7,8]. PNP catalyzes the phosphorylation of inosine-deoxyinosine and of guanosine-deoxyguanosine to hypoxanthine and guanine, respectively. This metabolite undergoes detoxification to uric acid or salvage back to the nucleotide level. Loss of PNP function blocks production of xanthine, hypoxanthine and uric acid. In PNP deficiency patients, intracellular deoxyguanosine triphosphate accumulation is thought to be toxic to lymphoid cells [7,8]. Analysis of lymphocyte subpopulations in these patients often shows progressive T, B and NK cell lymphopenia. Patients usually have recurrent infections and may have failure to thrive, neurological impairment, autoimmunity and malignancy. Recurrent upper and lower respiratory tract infections due to common bacterial and viral pathogens or opportunistic organisms typically present in the first months of life [7,9,10]. Neurological abnormalities such as truncal hypotonia, spastic diplegia, ataxia, behavioral problems, mental retardation and developmental delay are observed in more than 50% of patients. Allogeneic hematopoietic stem cell transplantation (HSCT) is the curative treatment for PNP deficiency patients [11]. If lymphopenia had been taken into account at 3 months of age, Case 1 could have been diagnosed earlier and could have had a chance for HSCT. In addition to lymphopenia at the age of 12 months, neuromotor developmental delay and low uric acid levels facilitated the recognition of PNP deficiency.

Besides lymphocyte counts, numbers and ratios of neutrophils, monocytes and eosinophils also provide important clues for PIDs.

Inherited quantitative neutrophil disorders are termed congenital neutropenia (CN) classified by absolute neutrophil count in peripheral blood into mild ($1000/\text{mm}^3$ to $1500/\text{mm}^3$), moderate ($500/\text{mm}^3$ to $1000/\text{mm}^3$) or severe ($<500/\text{mm}^3$), which may be constant, intermittent or periodic. Estimated prevalence of severe CN is 1:300 000 [1,12]. Autosomal dominant heterozygous mutations of ELANE encoding neutrophil elastase are the most frequent cause of CN [12,14]. Patients have chronic profound neutropenia with a characteristic bone marrow maturation arrest at the promyelocyte stage. Neutropenia often presents with infections of mucous membranes, gingiva and skin. Common infections are oral ulcers, gingivitis, periodontitis, tooth loss, otitis media, sinusitis, pneumoniae, cellulitis, perirectal infections, deep tissue infections and sepsis. Treatment with Granulocyte Stimulating Factor (G-CSF) is standard treatment [15]. Invasive infection such as necrotizing fasciitis, eosinophilia and IgE elevation in the second patient, reminded HIES to the other physicians. Autosomal dominant HIES is characterized by eczematoid rashes, cold skin abscesses, recurrent sinopulmonary infections resulting in bronchiectasis and pneumatocele formation, recurrent boils, mucocutaneous candidiasis, coarse face, musculoskeletal abnormalities including hyperextensibility, scoliosis, minor trauma fractures, high arched palate and malignancy. Mutations in STAT3 were identified as the cause of autosomal dominant HIES in 2007 [16]. Case 2 did not have characteristic clinical and immunological features of AD-HIES. Moreover, she had chronic persistent neutropenia. Bone marrow myeloid arrest at promyelocyte stage with eosinophilia and monocytosis are well-known findings in patients with severe congenital neutropenia and ELANE mutation [12,14]. Compensatory hypergammaglobulinemia usually accompanies neutropenia. Genetic analysis confirmed the preliminary diagnosis of severe congenital neutropenia and G-CSF was started. G-CSF was well tolerated and considerably improved the patient's clinical situation.

Infections are the most common presentation of PIDs. Most PIDs can be readily diagnosed using standard laboratory tests. Early diagnosis before permanent organ damage or death has occurred is important, especially in those PIDs in which HSCT offers a curative treatment. The first steps in the evaluation of patients include a detailed medical history in search for anamnestic clues, careful and complete physical examination and initial workup with CBC and immunoglobulin levels. These 2 patients are reported to emphasize the importance of simple and basic tests in the diagnosis of severe diseases. Lymphopenia is the classic hallmark of severe combined immunodeficiency and it should be the definitely warning sign for SCID when observed in infancy. It must be kept in mind that permanent neutropenia, usually called severe congenital neutropenia, is associated with deep-seated bacterial infections, hypereosinophilia and compensatory hypergammaglobulinemia.

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