Step by Step Diagnosis of Patients with Primary Immunodeficiency Diseases: Case Scenarios

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Improving Access to Care for Patients with Primary Immunodeficiency

22nd - 29th April 2016
Test - Diagnose - Treat
www.worldpiweek.org

Raising awareness for diagnosis and treatment of PI together
Case 1: Recurrent infections and cardiac and urogenital malformations

- A girl from consanguineous parents
  - History of recurrent pneumonia
  - History of recurrent diarrhea
  - History of oral candidiasis

- Colostomy due to rectovaginal fistula

- Cardiac surgery due to ASD

**Which laboratory test should be requested at first step?**
A. Immunoglobulin levels  
B. Nitroblue-Tetrazolium (NBT) test  
C. Complete blood cell count  
D. C3, C4, Complement hemolytic 50 (CH50) assays
**Laboratory test**

*CBC*

<table>
<thead>
<tr>
<th></th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Test 4</th>
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<tbody>
<tr>
<td><strong>WBC (*/mm³)</strong></td>
<td>2200</td>
<td>1500</td>
<td>3700</td>
<td>2300</td>
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<tr>
<td><strong>PMN (%)</strong></td>
<td>10</td>
<td>55</td>
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<td><strong>Lymph (%)</strong></td>
<td>80</td>
<td>42</td>
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<td><strong>ANC (*/mm³)</strong></td>
<td>220</td>
<td>825</td>
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*Severe persistent neutropenia*

What is the probable diagnosis?
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<tr>
<th>CN variant</th>
<th>Congenital neutropenia</th>
<th>Osteopenia</th>
<th>Skeletal system (Growth)</th>
<th>Skin/Hair</th>
<th>Neurological system</th>
<th>Cardiovascular system</th>
<th>Urogenital system</th>
<th>Gastrointestinal system</th>
<th>Endocrine system</th>
<th>Adaptive immune system</th>
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<td>Mitochondrial DNA</td>
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</table>
Genetic defect

**Diagnosis: G6PC3 deficiency**

P22- G6PC3 mutation G → A

An amino acid exchange Arg253 to His.


Case 2

- A boy from consanguineous parents
  * History of recurrent upper and lower respiratory infections, diarrhea and short stature
  * Mental retardation and ADHD

What is your next action?
A. CBC
B. Chest X-ray
C. Physical examination
D. Antibiotic therapy
Physical Examination

Physical examination was remarkable for height and weight less than fifth percentile for age and a significant chest deformity (Bell-Shaped Thorax)

WBC 3,600/mm³
- 93% lymphocytes
- 4% segments/bands
- 1% monocytes
- 2% undifferentiated cells

Hemoglobin 11.5 g/dL
Platelet 48,000/mm³

Abdominal sonography showed typical “white” pancreas (arrows) due to lipomatosis
Laboratory tests and Imaging

Radiological examination demonstrated metaphyseal dysplasia and pectum carinatum, with significant bilateral lung atelectasis.

- Evaluation for failure to thrive was significant for low levels of pancreatic enzymes (elastase)

- A sweat test was done to screen for cystic fibrosis and was reported normal

What is your favorite diagnosis?
A. Severe congenital neutropenia (SCN)
B. Chediak-Higashi syndrome (CHS)
C. Shwachman-Diamond syndrome (SDS)
D. Chronic granulomatous disease (CGD)
Shwachman-Diamond syndrome

- The differential blood count is compatible with SCN. However, thrombocytopenia is not a typical finding in SCN.

- SDS is associated with exocrine pancreatic insufficiency particularly in the first years of life.

- Molecular analysis showed the homozygous mutations in the SBDS gene, as expected in this autosomal recessive disease.
Case 3

A boy was referred to our center with lymphadenopathy and omphalitis.

He had history of deep-seated abscesses due to *Staphylococcus aureus* and *Aspergillus*.

His family history was relevant for a maternal uncle with recurrent bacterial skin and liver infections who died at age 14 years due to *Aspergillus nidulans* pneumonia.
Laboratory data: WBC=15,600/uL (PMN=76%, Lymph=20%) 
Hb=11.5% 
Plt=427,000 
ESR=98mm/hr 
IgG=1230 mg/dL, IgA=32 mg/dL, IgM=78 mg/dL, IgE=56 IU/mL.

HIV Elisa was negative

Which of the following tests could most probably give you the underlying diagnosis in this patient? 
A. FACS study 
B. DHR assay 
C. DTH study 
D. CH50 assay
Laboratory data

*Defective DHR test

What is the most likely diagnosis?
A: Leukocyte adhesion deficiency (LAD)
B: Chronic granulomatous disease (CGD)
C: Common variable immunodeficiency (CVID)
D: Severe combined immunodeficiency (SCID)
Diagnosis

Chronic granulomatous disease (CGD)

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<tr>
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<td>CYBA</td>
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<td>AR</td>
<td>NCF1</td>
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<td>p67phox deficiency</td>
<td>AR</td>
<td>NCF2</td>
</tr>
<tr>
<td>p40phox deficiency</td>
<td>AR</td>
<td>NCF4</td>
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</table>

Which of the following is the appropriate next step follow-up recommendation?

A. Avoid all live-virus vaccines and initiate antiviral prophylaxis
B. Initiate antibacterial, antifungal, and IFN-γ prophylaxis
C. Avoid any prophylactic medication to reduce the risk of selecting flora
D. Haploidentical HSCT
Case 4

A 10-year old boy was referred with fever and chest pain for 6 days, and was admitted to the hospital. He had history of recurrent sinopulmonary infections with encapsulated bacteria.

He was well until the age of 6 months, when he developed pneumonia, requiring hospitalization. The patient had experienced recurrent episodes of acute otitis media since the age of 1 year. He also experienced vaccine-related poliomyelitis.

Physical examination shows perforated left tympanic membrane and absent tonsils with no adenopathy.

Chest X-ray showed a lobar pneumonia at right lung.
Laboratory data

WBC: 22,500/ml (PMN: 88%, lymphocytes: 7%)
Hemoglobin: 12.2 g/dl
Platelets: 290,000/ml
C-reactive protein (CRP) +++
Serum immunoglobulins levels:
  IgG=90 (256–1067) mg/dL
  IgA=5 (12–103) mg/dL
  IgM=10 (47–173) mg/dL

Which of the following approach could make a diagnosis?
A. Lymphocyte subset enumeration
B. Nitroblue-Tetrazolium (NBT) test
C. Delayed cutaneous hypersensitivity
D. C3, C4, Complement hemolytic 50 (CH50) assays
The patient was diagnosed to have bacterial pneumonia because of a consolidation in the chest X-ray, hyperleukocytosis, and an abnormal titer of CRP.

He was suspected to have an antibody deficiency or phagocytic disorder, and the presence of panhypogammaglobulinemia indicates that he may have an antibody deficiency.

Flow cytometry to determine lymphocyte subsets: CD3= 82%, CD4= 50%, CD8= 32% and CD19= 1%

**What is the most likely diagnosis?**
A. Common variable immunodeficiency (CVID)
B. X-linked hyper IgM syndrome (HIGM)
C. X-linked agammaglobulinemia (XLA)
D. Severe combined immunodeficiency (SCID)
Diagnosis

- Recurrent pneumonia and sinopulmonary infections with encapsulated bacteria since early infancy
- Absence of tonsils
- Significant decreased serum levels of IgG, IgM and IgA
- Low number of B-cells, normal number of T-cells

X-linked agammaglobulinemia
(Btk deficiency)

- SCID and HIGM are combined immunodeficiency, and the patients sometimes present with interstitial pneumonia caused by *Pneumocystic jiroveci* or cytomegalovirus infection.

- The patient showed bacterial pneumonia and hypogammaglobulinemia, which was therefore suggestive of antibody deficiency. CVID and XLA are the major forms of antibody deficiency. The case showed the absence of tonsils, which is a typical sign for XLA.
Case 5

An 11-year-old boy presented with high-grade fever, cough, and intermittent hematuria lasting for 3 days. His medical history included a recurrent eczematoid rash before the age of 1 year, as well as repeated skin infections. The patient had presented recurrent otitis media and sinusitis, some isolated episodes of bloody diarrhea, and 2 episodes of pneumonia. At 10 months of age, he presented recurrent thrombocytopenia.

What is the most likely diagnosis?
A. Ataxia-Telangiectasia
B. Hyper-IgE syndrome
C. Di George syndrome
D. Wiskott Aldrich syndrome
Diagnosis

WBC: 12,700/mm$^3$ (PMN: 70%, Lymph: 14%)
Platelets, 51,400/mm$^3$
IgG, 928 mg/dl, IgA, 896 mg/dl; IgM, 13 mg/dl

Chronic idiopathic thrombocytopenia

Wiskott Aldrich syndrome
Case 6: Oculocutaneous hypopigmentation

- A 6-month boy, the second child of consanguineous parents

- CC:
  * Prolonged fever

- PE:
  * Silvery hair, eyelashes, eyebrows
  * Hepatosplenomegaly

Laboratory test?
Laboratory findings

White blood cell count: 3400 cells/mm³
Polymophonuclears <1%
Lymphocytes: 98%
Monocytes; 2%
Hb: 9 gr/dl
Platelets count: 38×10⁹/L

ALT 51 IU/L (normal range: 10-40)
AST 41 IU/L (normal range: 10-40 IU/L)
LDH 360 IU/L (normal range: up to 450 IU/L)
Triglyceride was 392 mg/dl (normal range: 35-200 mg/dL)
Ferritin was 4660 ng/ml (normal range: 6-140 ng/mL)

Next step?
Further laboratory findings

* Peripheral blood smear: No giant cytoplasmic granules in leukocytes

* Microscopic examination of the hair: Irregular agglomerations of pigment in hair shafts

* Bone marrow aspiration biopsy: Hemophagocytosis without evidence of infiltrative or malignant process

Diagnosis?
What gene defect?
Diagnosis, Genetic defect

- Diagnosis: Griscelli syndrome type 2

  * Results of sequencing the RAB27A gene:

    A novel homozygosis mutation in exon 5, a single base substitution (g.42996 A>G, EMBL: AF443871), which leads to an amino acid change (S115G) from Serine (AGC) to Glycine (GGC)

# Characteristics of the immunodeficiency syndromes with hypopigmentation

<table>
<thead>
<tr>
<th></th>
<th>Chédiak-Higashi syndrome</th>
<th>Griscelli syndrome, type 2</th>
<th>Hermansky-Pudlak syndrome, type 2</th>
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<tr>
<td><strong>Hypopigmentation</strong></td>
<td>Variable</td>
<td>Variable</td>
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<td>Prominent</td>
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<tr>
<td><strong>Hair shaft findings</strong></td>
<td>Distributed regular melanin granules</td>
<td>Large irregular melanin granules</td>
<td>Normal or distributed small clumps of pigment</td>
<td>-</td>
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<tr>
<td><strong>Prominent facial features</strong></td>
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<td>+</td>
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<td><strong>Giant intracellular granules</strong></td>
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<td><strong>Short stature</strong></td>
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Light-microscopic hair shaft analysis of CHS and GS2 vs. control
Case 7: Recurrent infections and oral lesions

* A 3 year-old boy

* Recurrent pneumonia
* Oral and anal ulcers
* Necrotic oral lesions
* Periodontitis

Laboratory test?
Laboratory findings

White Blood Cells = 6600 cells/mm³
Polymorphonuclear = 7%
Lymphocytes = 92%
Monocytes = 1%

Absolute neutrophils count (ANC) of 462 cells/mm³

* Severe consistent neutropenia

Preliminary Diagnosis:
Severe Congenital Neutropenia (SCN)
Genetic studies of SCN

*Granulocyte Colony-Stimulating Factor (GCSF) was started for the patient in addition to antibiotics.

*ANC increased to 2697 cells/mm³.

*The patient was discharged from the hospital with a good condition and was advised to continue GCSF therapy.

*Although clinical and laboratory findings of the patient were compatible with a diagnosis of SCN, molecular studies revealed that ELA2 and HAX1 genes were intact.

*He experienced acute diarrhea, superficial abscesses, and necrotic cutaneous lesions at 20 months old

Further studies?
Further studies

*Immunological studies:
IgG = 120 (normal range: 656-1350) mg/dl
IgA = 35 (normal range: 86-320) mg/dl
IgM = 270 (normal range: 120-320) mg/dl
IgE = 2.1 (normal range: 0-46) IU

*Incidental finding in another *in vitro* project:
Defects in cytokine production, after stimulation of T-cells with PHA

IL-2: 235 pg/ml before and 238.6 pg/ml after stimulation
(normal control: 159 and 5,694 pg/ml before and after stimulation, respectively)

IL-4: 24.7 pg/ml before and 46.5 pg/ml after stimulation
(normal control: 44.6 and 288.8 pg/ml before and after stimulation, respectively).

**Diagnosis?**
**What gene defect?**
Diagnosis, Genetic defect

* Results of sequencing the **CD40L** gene:

A 17 base pair deletion in exon 2, resulting in a frameshift after aa E61 and a premature stop codon in the extracellular domain.

➢ Definite Diagnosis: CD40L deficiency


Reference

*Rezaei N, Aghamohammadi A, Notarangelo LD. Primary immunodeficiency diseases: definition, diagnosis and management. © Springer
8th International Conference on Primary Immunodeficiency Diseases
April 23rd - 24th, 2016
Children's Medical Center, Tehran, Iran
RCID is pleased to announce the following speakers for the 8th conference
2016 Keynote Lecturers

Professor Hans D. Ochs, MD
Professor Reinhold Schmidt, MD
Professor Ralf S. Gella, MD
Eleonora Gambineri, MD
Andrew Gennery, MD

A preliminary agenda is available. Register now!

Join us for this momentous occasion that promises to be one of the best conferences on primary immunodeficiency.

We look forward to seeing you in Tehran!

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