Laboratory evaluation of primary immunodeficiencies

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More than 200 primary immunodeficiency diseases (PID) have been described.
Basic evaluation of the immune system

- Available at all the University Hospitals
- Can often be performed on day to day basis, if indicated

- Quantitative
  - Blood cell count (leukocytes)
  - S-immunoglobulin’s (Igs)
  - Isoagglutinins (anti-A and anti-B)
  - CH50 and AH50 (complement function)
  - Immunophenotyping (enumeration of leukocyte subpopulations)
  - Neutrophil oxidase function (intracellular killing)

- Qualitative
  - Specific antibody response (post immunization)
  - T-cell proliferation assays
Advanced evaluation of the immunosystem

- Second line test except for known familial cases or very suspicious clinical presentation
- Often only performed in a few labs in Denmark
- May require shipping of samples to labs outside DK
- Please ask your local immunologist who will be delighted to help you

Examples:

- Special B- and T-cell subpopulations
- NK-cell function
- Antibodies against neutrophil surface antigens
- Specific activation assays (e.g. TLR function)
- Immunophenotyping for specific intra or extracellular molecules (e.g. IFNGR1, BTK)
- Genetic testing (e.g. del22q11.2, TACI, B-cell somatic hypermutation, TNFRSF1)
Some (very) general aspects of laboratory testing

Normal range = 95% confidence interval
95% CI, 5/95 percentiles, prediction intervals

Never rely on lab values only
Always repeat an abnormal lab test that do not match the clinical presentation
Some general aspects of laboratory evaluation

- Age related differences are significant
- Always use age specific reference intervals
- Absolute count important
- Be aware of gender and ethnic variation

Comans-Bitter et al. 1997
Stoop et al. 1969
Peripheral blood sample → general immune evaluation

- Complement, Ig’s, antibodies
- DNA for genetic testing
- Whole blood for immunophenotyping
- Neutrophils for chemotaxis, chemiluminescence and MAIGA
- PMC for proliferation / stimulations

AALBORG UNIVERSITETSHOSPITAL
Complement function

- Defects in classical pathway: CH50 ~ 0%
- Defects in alternative pathway: AH50 ~ 0%

- Hemolytic assays:
  - Sample incubated with sensitized sheep erythrocytes (CH50) or non-sensitized rabbit erythrocytes (AH50).
  - Erythrocytes disintegrate
  - Lysis measured by decreased optical density
  - Deficient sera can be used to characterize discrete defects

Nilsson B, Ekdahl KN. 2012
Case - I

Twins, pneumococcal sepsis in first months of life
No familial history of immunodeficiency
Normal cell counts

Twin A: CH50: < 10%
Twin B: CH50: <10%

CH50 with deficient sera: C2 < 10%

<table>
<thead>
<tr>
<th>Patient</th>
<th>C2type 1 defekt</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>homozygot</td>
</tr>
<tr>
<td>B</td>
<td>homozygot</td>
</tr>
<tr>
<td>Mother</td>
<td>heterozygot</td>
</tr>
<tr>
<td>Father</td>
<td>heterozygot</td>
</tr>
</tbody>
</table>
Case - II

• 1 year old child
• Recurrent severe sinopulmonary infections
• Servere hypogamma
• Work up ?
Flowcytometric immunophenotyping

T-cells (CD3, CD4, CD8), B-cells (CD19, CD20), NK-cells (CD16, CD56)
  • Evaluation for a specific cell proteins (CD18, CD40/CD154, IFNγR, IL-12R)
  • Intracellular proteins: (BTK, FOXP3, STAT-1, STAT-4)

Assessment for biologic effect
  • Memory/naive T cells
  • Memory B cell - isotype switch (CD27, IgM, IgD)
  • Regulatory T- and B-cells
Flowcytometry

- "Advanced microscopy"
- FAST! – 10.000 cells/sec
- Multicolor cytometry allows for detailed characterization of cells
- Qualitative, semiquantitative, quantitative
Size and granularity
Subpopulations
Case - II

PATIENT

Controle

B-cells

T-cells
BTK staining

XLA patient
Characteristics of discrete subpopulations

• Class switched memory B-cells

• CVID
Classification of CVID – more data needed

A. Freiburg [6]
- CVID patient B-cell subsets
  - CD27⁺IgM⁺ IgD⁻ B cells <0.4% of PBL
  - CD27⁻IgM⁺ IgD⁺ B cells >0.4% of PBL

  - %CD21⁺ B cells >20%
  - %CD21⁻ B cells <20%

B. Paris [34]
- CVID patient B-cell subsets
  - %CD27⁺ B cells <11%
  - %CD27⁺IgM⁺IgD⁻ <8%

  - Neither MB0 nor MB1

C. EUROclass [6]
- CVID patient B-cell subsets
  - >1% B cells
    - >2% switched memory B cells
      - <10% CD21⁺ B cells
        - <10% CD21⁺ B cells
          - <1% CD4⁺CD45RA⁺CD62L⁻
          - 16–29% CD4⁺CD45RA⁺CD62L⁻
          - >30% CD4⁺CD45RA⁺CD62L⁻
        - >9% transitional B cells
          - <9% transitional B cells
          - 10% CD21⁺ B cells
          - <10% CD21⁺ B cells

  - <1% B cells

  - ≤2% switched memory B cells

D. Classification according to naive CD4⁺ T-cell numbers [56]
- CVID patient Naive CD4⁺ T-cell numbers
  - CD4⁺CD45RA⁺CD62L⁻ ≤15%
  - CD4⁺CD45RA⁺CD62L⁻ 16–29%
  - CD4⁺CD45RA⁺CD62L⁻ >30%


Bergbreiter et al. 2009
Case - III
Recurrent infections with atypical *mycobacteria*

Figure 11.16 The Immune System, 3ed. (© Garland Science 2009)
Case - III
Recurrent infections with atypical *mycobacteria*

Figure 1 Interferon-γ receptor expression on unstimulated lymphocytes (CD119). Blue and red curves, two normal controls. Green curve, patient.

Figure 2 STAT1γ production in interferon-γ (IFN-γ)-stimulated mononuclear cells (cytoometric bead assay). Blue, unstimulated control. Green, control stimulated with IFN-γ (10 IU/ml). Red, patient stimulated with IFN-γ (10 IU/ml).

Figure 3 Dominant interferon-γ receptor alpha-1 (IFNGR1) mutations in exon 6. Red box, a stop codon. *Deletion hotspot described by Jounguy et al. [6].
CASE - IV

- 10 months old girl
- Omphalitis at birth
- Conjunctivitis
- Nail infections
- Pneumonias
- Deep skin infections

- Normal Igs + complement
- Normal subpopulations
- Severe neutrocytosis
CASE - V

6 months old girl
Severe pneumonia
Neutropenia 0.11x10⁹

Autoimmune neutropenia
Flow + MAIGA: anti-HNA-1a

<table>
<thead>
<tr>
<th>Genotype</th>
<th>HNA-1a</th>
<th>HNA-1b</th>
<th>HNA-1c</th>
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</thead>
<tbody>
<tr>
<td>Pt.</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Mater</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
</tr>
</tbody>
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<tr>
<th>Commercial Luminex based HNA-antibody kit</th>
<th>Anti-HNA-1a</th>
<th>Anti-HNA-1b</th>
<th>Anti-HNA-1c</th>
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<tr>
<td>Pt.</td>
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Nielsen KR. et al. 2011, 2012
CASE - VI

- 4 week old boy
- Admitted with severe bacterial airway infection
- Neutropenia 0.3x10⁹/L
- Neutrophil count 1.1 at control 3 weeks after

- Neonatal alloimmune neutropenia

Granulocyte crossmatch

Kølbæk M, Nielsen KR. 2010
Evaluation of cell function

- Lymphocyte proliferation (mitogens, antigens, allogenic cells)
- NK-cell degranulation
- Granulocyte superoxide (Flowcytometry, chemiluminescens)
- Granulocyte chemotaxis
- Inflammatory mediator production (TLR signalling)
Case - VII

18 months old boy
Deep skin infections, sinopulmonary infections, salmonella osteomyelitis
Normal cell count, lgs, complement and immunophenotype

Chemilumiscens assay
Activation of neutrophils
Luminol (CGD and MPO)
Lucigenin (MPO sensitive)
X-linked CGD
CGD - variants
Lymphocyte proliferation (T-cell proliferation)

SCID ~ 0%
DiGeorge, CVID, others: Variate degress of impairment
NK-cell degranulation
TLR signalling

“My perfume? Why it’s activated complement proteins.
Do you like it?”
TLR signalling

Takeishi et al. 2009
Evaluating TLR Response

TLR specific ligand

PBMC

37°C
5% CO2

2.0 x 10^5 cells/well

Response

Supernatant

Multiplex assay

TNF ELISA

Von Bernuth, et al, Pediatrics, 2006
Inflammatory pathway function

Cytokine profile LPS stimulation

- IL-6
- IL-10
- TNFα

ng/ml

kontrol ustim
kontrol stim.
OBS stat-4 ustim.
OBS stat-4 stim.
CVID ustim.
CVID stim.
OBS NEMO ustim
Obs NEMO stim
Genetic testing

• Genetic testing is emerging as an important diagnostic test in resolving possible PID
  • Human genome 3.3x10^9 bases
  • Frederick Sanger developed DNA sequencing methods in 1977
  • Cost of Sanger sequencing : $2400 / 10^6 bases
  • Next generation sequencing 0.1-1$ / 10x6 bases

• Test of prognostic and diagnostic value often used:
  • TACI in CVID, 22q11.2 deletion, CGD : CYBB, CYBA, , NCF1, NCF2, Somatic hypermutation in B-cells
  • Mutations in SCID, Autoinflammatory syndroms – TNFRSF1A

• Genotype vs. phenotype
• Difficult to address the importance of a new mutation found
• All individuals have a SNPs at 1/1000 bases
• Multigene diseases and gene - gene interactions – epistasis,
Genetic testing – genetic screening?

SCID screening from PKU cards

Established f:1/60,000 newborn

False positives?

www.biomedcentral.com/.../1472-6750-3-18-1-l.jpg
Thank you!