Too many Respiratory and other infections:
When to suspect
How to screen
Confirm
And what can be done about it

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Infections – when to be concerned?

• Too many?
• Localized or systemic, what organ is affected?
• How serious?
• Response to therapy?
• What are the microorganisms?
• Related to live vaccines?
• Other, non-infectious findings, syndromic?
• Family history?
4 Compartments of the Immune System

**innate Immunity**

- Complement
  - Recurrent disseminated Neisserial infections.
  - Pyogenic bacterial infections.
  - Angioedema of face, hands, feet, or GI tract.
  - Autoimmune symptoms (Lupus).
  - History suggestive of autosomal dominant inheritance.

- Phagocytic
  - Soft tissue abscesses or lymphadenitis.
  - Infection with catalase(+) organisms (Staph aureus, Serratia, E. coli, Aspergillus).
  - Poor wound healing.
  - Delayed separation of the umbilical cord.
  - Chronic gingivitis and periodontal disease.
  - Mucosal ulcerations.

**adaptive Immunity**

- B Cell
  - Recurrent bacterial sino-pulmonary infections or sepsis, particularly with encapsulated organisms.
  - Chronic or recurrent gastroenteritis (Giardia and Enterovirus common).
  - Chronic enteroviral meningoencephalitis.
  - Arthritis
  - Unexplained bronchiectasis.

- T Cell
  - *Pneumocystis carinii* pneumonia.
  - Fungal infections.
  - GVHD (rash, abnormal LFT’s, and chronic diarrhea).
  - Recurrent, severe, or unusual viral infections.
  - Failure to thrive.

**Combined**
3 year old boy with recurrent pyogenic bacterial infections, systemic lupus erythematosus (SLE), nephritis, CH50 = 0

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**Complement**
- Recurrent disseminated Neisserial infections.
- Pyogenic bacterial infections.
- Angioedema of face, hands, feet, or GI tract.
- Autoimmune symptoms (Lupus).
- History suggestive of autosomal dominant or recessive inheritance.

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- Soft tissue abscesses or lymphadenitis.
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**LABS**
- CH50 - tests entire complement cascade (U$ 83.‐)
- CBC/Differential ($40)
- Neutrophil oxydative burst assay - AKA NBT assay ($86)
- CBC/Differential ($40)
- Quantitative IgG, IgM, IgA, and IgE ($127)
- Anti-tetanus titers ($57)
- Anti-pneumococcal titers pre- & 4 wks post-Immunization
- CBC/Differential ($40)
- T & B cell subset analysis ($202)
- DTH reaction to Candida & Tetanus
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You have decided to investigate

The 2 Goals of all Immunologic Testing

• NUMBERS
• FUNCTION
Testing - Complement

- **Number:**
  - Individual complement protein levels (C3, C4, etc.)

- **Function:**
  - CH50 test
C-Deficiencies
what can be done?

• Hyper-immunization (PPS, neisseria)
• Antibiotic prophylaxis (+/-)
• IVIG prophylaxis (+/-)
• Replacement/prophylaxis C1-esterase inhibitor
• Check family members
Phagocytes

- Soft tissue abscesses or lymphadenitis.
- Catalase(+) organisms (Staph aureus, Serratia marcesans, Aspergillus) -> CGD
- Poor wound healing.
- Delayed separation of the umbilical cord.
- Chronic gingivitis and periodontal disease -> LAD (1/2/3)
- -> severe neutropenia

*Delves and Roitt, NEJM 343:37-49*
**Immunodeficiency Algorithm**

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**Combined**
- CH50 - tests entire complement cascade ($83)
- Neutrophil oxidative burst assay (Rhodamine) ($86)
- NBT (< 1 US$)
- CBC/Differential ($40)
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- Anti-tetanus titers ($57)
- Anti-pneumococcal titers pre- & 4 wks post-Immunization
- CBC/Differential ($40)
- DTH reaction to Candida & Tetanus

9 month old male with purulent cervical lymphadenitis and a history of pneumonia at age 6 months. A maternal grand-uncle died of acute lung infection at age 2 years in the 1950ies.
Testing - Phagocytes

• Number:
  – CBC/differential (neutropenia)

• Function:
  – Neutrophil oxidative burst (CGD)
  – CD18 Expression (LAD1)
Neutrophil Defects
what can be done?

• G-CSF, antibiotics, BMT for severe (congenital) Neutropenia
• Antibiotic/antifungal prophylaxis (CGD)
  Interferon gamma
  steroids to reduce inflammation
  BMT
• White cell transfusions, antibiotics, BMT (LAD)
B Cells

- Unexplained bronchiectasis.
- Recurrent bacterial sinopulmonary infections.
- Chronic or recurrent gastroenteritis (Giardia, Cryptosporidium, etc.).
- Chronic enteroviral menigoencephalitis.
- Arthritis -> XLA
Antibody Deficiencies (AD)
When to suspect?

• Recurrent
• respiratory
• Mild or severe: “colds or pneumonia
• Chronic: sinusitis, bronchiectasis
• Sepsis
• Meningitis
• Not: lymphadenitis, candida, failure to thrive, recurrent viral infections
Example: XLA

- Gene: Btk
- Function: allows B cell development
- Diagnosis: low Ig, low # B, mutation of Btk
- Effective screening test by flow
- At least 5 other genes (a.r.)
- Improved Ig tx: self infusion at home SC
- Unsolved problems: chron. Lung disease
- Progressive neurodegeneration of unknown cause
18 month old boy admitted with acute pneumonia and sepsis, due to Strep pneumoniae. He started to have upper respiratory infections at age 11 month. On examination, he had no tonsillar tissue. A maternal uncle died of paralytic polio after oral polio vaccine.

**DEFECT TYPE**

- Complement
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**Immunodeficiency Algorithm**
Testing – B cells & Antibodies

• Number:
  – CBC/differential (lymphopenia)
  – Lymphocyte subsets (T/B/NK)
  – Quantitative IgG, IgM, IgA, IgE

• Function:
  – Vaccine titers (protein & carbohydrate antigens)
Flow-Based Assays

- **Basic Subset Analysis – T/B/NK**
- **Detailed Immunophenotyping**
- **Disease-specific Testing**
- **Functional Testing**
Tagging Cells for Flow Cytometry

Macrophages  B Cells  T Cells
Flow Cytometry - Mechanism

Cells

Laser

Detector

Counter

Fluorescence (T Cells)
Disease-Specific Flow Tests

**WASp (WAS)**

**BTK (XLA)**

**SAP (XLP1)**

**XIAP (XLP2)**

**FOXP3 (IPEX)**

**Normal**

**Patient**
Antibody Deficiencies
what can be done?

• IVIG, subcutaneous IG self infusion
• Prophylactic antibiotics (+/-) prevent chronic disease (bronchiectasis)
• Recognize: autoimmune, malignant complications
T Cells

Generals

- *Pneumocystis jirovecii* pneumonia
- Other fungal infections
- GVHD/Autoimmunity (rash, diarrhea, ↑LFT’s, etc.)
- Recurrent, severe, or unusual viral infections (CMV, Adenovirus, EBV, Metapneumovirus, etc.)
- Failure to thrive
SCID: Symptoms
(Severe Combined Immune Deficiency)

Lymphopenia – often, but not always!
Low TREC
Small thymus
Early onset (1-3 months)
Bacterial, viral, fungal, parasitic and systemic infections
Diarrhea and failure to thrive (70%)
Rash
Early Death
18 month old boy admitted with acute pneumonia and sepsis, due to Strep pneumoniae. He started to have upper respiratory infections at age 11 month. On examination, he had no tonsillar tissue. A maternal uncle died of paralytic polio after oral polio vaccine.

**Defect Type**

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**Laboratory Tests**

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- Anti-tetanus titers ($57)
- Anti-pneumococcal titers pre- & 4 wks post-Immunization
- DTH reaction to Candida & Tetanus
Testing – T cells

- Number:
  - CBC/Differential (lymphopenia)
  - Lymphocyte subsets (T/B/NK)

- Function:
  - T cell proliferation to mitogens and antigens
  - Vaccine titers
T cell/(sever)combined Immunodeficiency, a medical Emergency! what can be done?

- Early diagnosis is essential
- IVIG, subcutaneous IG
- Prophylactic antibiotics/antiviral
  - PCP prophylaxis
- No live vaccines
- **Hematopoietic stem cell transplantation**
- Gene therapy (+/-) and, in the future, gene correction
Evaluation of SCID

Sequencing

IL2RG
JAK3
ADA
IL7Rα
Artemis
RAG1/RAG2
Etc.

Figure 4 Relative frequencies of the different genetic types of SCID among 170 patients seen consecutively by the author over 3.5 decades.


Function
STAT5B Phosphorylation

STAT5B Phosphorylation

Normal

X-SCID (E110X)

IL-2 (2000 U/ml)
IL-2 (500 U/ml)

X-SCID (E110X)

IL-2 (2000 U/ml)
IL-2 (500 U/ml)
Syndromic PID

**X-linked inheritance
**Thrombocytopenia, small platelets
*Eczema

Bloody diarrhea
Recurrent infections due to Immunodeficiency (bacterial, viral)
Autoimmune hemolytic anemia
Wiskott-Aldrich Syndrome / XLT

**X-linked inheritance

**Thrombocytopenia, small platelets

*Eczema

Bloody diarrhea

Recurrent infections due to Immunedef.

Autoimmunitiy, Malignacies

Tx: symptomatic; stem cell transplantation

(gene therapy)
3 year old boy
mutation c.C290T in the WASP Gen
thrombocytopenia (10.000-25.000/µl)
very mild eczema
no immunodeficiency

10 month old boy
mutation c.C290T
thrombocytopenia (20.000-40.000/µl)
no eczema
no immunodeficiency
Disease-Specific Flow Tests

WASp (WAS)

BTK (XLA)

SAP (XLP1)

XIAP (XLP2)

FOXP3 (IPEX)

Normal

Patient

FL1-H: WASP-FITC

FL1-H: FL1-FITC

FL1-H: FL1-FITC

FL1-H: FL1-FITC

FL1-H: FL1-FITC

% of Max

% of Max

% of Max

% of Max

% of Max

normal

Normal Control (Father)
Type of Infections vs. Genetic Defect

- Bacterial, gram+ -> antibody deficiency
- Encapsulated bacterial sepsis -> C-deficiency
- Bacterial (staph, klebsiella), fungal -> CGD
- Bacterial, fungal -> neutropenia
- Staph without pus -> LAD
- Bacterial, fungal, opportunistic -> SCID
- Atypical mycobacteria (BCG) -> IFNg/IL-12 auto-antibody to IFNg
- Assoc. with autoimmunity: IPEX, IPEX-like
PID-Evaluation by the Expert

- Flow studies: lymphocyte subsets
  T and B cell maturation
  protein analysis (membrane, cytoplasm, nuclear
  functional eval: pSTAT1; CD40L (CD40Ig-binding)
- Chromosome analysis (FISH, DNA breaks)
- T cell excision circles (TRECs) thymic function
- Gene sequencing: Sanger, WES, WGS, multiplex
  platforms (panel for SCID, neutropenia, HLH)
The Evolution of PID Knowledge

• ADA Deficiency first PIDD molecularly defined in 1972 (Giblett et al.)

• Approximately 300 molecularly defined disorders of the immune system in 2016
  – SCID – 21 genes
### WAS Scoring System:

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