Two Patients and a Caveat

The Use and Misuse of Molecular Methods in Mycobacterial Infections

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Patient #1: 13 y/o boy with IPEX syndrome; s/p BMT

- Constitutional symptoms
- Fever
- Lymph adenopathy

IPEX: Immune dysregulation syndrome, polyendocrinopathy, enteropathy, X-linked syndrome.
Faint blue macrophages in a fibrous stroma
H&E 200X

Macrophages with granular cytoplasm
H&E 400X

AFB Stain 400X

AFB Stain 400X
Patient #2: Otherwise Healthy Skin-Diver with a soft tissue infection of the arm following a coral scrape.

Superficial aspect of skin biopsy
H&E 40X magnification

Deeper Portion of Skin Biopsy

H&E, 40X

Fite, 1000x

ZN, 1000x
Who am I? Who should I be?

Patient 1 Patient 2

Cultures Negative with Prolonged Incubation — Next Steps

A. Re-biopsy/Re-culture
B. Submit for Broad-Range Mycobacterial PCR/DNA sequencing.
C. Treat for M. avium/M. intracellulare complex.
D. Treat for M. tuberculosis complex.
E. Treat for M. marinum.

Patient #1: 13 y/o boy with IPEX syndrome

- Broad-range Mycobacterial PCR / DNA sequencing disclosed:
  Mycobacterium genavense

Mycobacterium genavense

- First described in 1990.
- Slow-growing, fastidious mycobacterium
  - Recovered from tap water, various animals, human intestinal tract.
  - Causes infections in parrots and parakeets.
**Human Disease**

- Predominantly in patients with AIDS
  - One Swiss cohort, pre-HAART era
    - 12.8% of all NTMs, second to MAC
- Other immunocompromised patients
  - Solid organ transplants
  - Leukemia (AML)/Lymphoma (CLL)
  - Sarcoidosis
  - HyperIgE Syndrome

**Presentations**

- Usually Disseminated (similar to MAC)
  - Extensive GI involvement
  - Fever,
  - weight loss,
  - diarrhea,
  - abdominal pain,
  - lymphadenopathy,
  - anemia.
- Localized Disease
  - Cutaneous
  - Solitary cerebral mass
  - Genital tract

**Pathologic Findings**

- Variable granuloma production
  - As determined by the degree of immunosuppression.
- Large quantities of intracellular AFB
  - Pathologically indistinguishable from MAC.

**Culture**

- Fastidious NTM
- Requires
  - Liquid media,
  - Acid pH
  - Elevated Temperature (45 ºC)
  - Mycobactin J supplementation
  - 3 months incubation.
- Molecular methods usually needed for identification
Patient #2: Otherwise Healthy Skin-Diver with a soft tissue infection of the arm following a coral scrape.

- Broad-range Mycobacterial PCR / DNA sequencing disclosed:
  - *Mycobacterium haemophilum*

**Mycobacterium haemophilum**

- First Described by Sompolinsky et al in 1978; skin infection; immunocompromised host.
- Environmental mycobacteria; exact niche unknown.
- Immunocompromised:
  - AIDS, transplant recipients, SLE, Corticosteroid use, Diabetes Mellitus,
  - Disseminated disease limited to this population.
- Immunocompetent:
  - Children
  - Adults
- Animal Pathogen: Zebrafish (20% mortality in outbreaks); python pneumonia,
  and an intradural mass in a bison.

**References**

- GC content similar to *M. marinum* & *M. ulcerans*.
- Similar docosanoic acid (a fatty acid) content, a specific phenolic glycolipid, and major membrane protein 1 to *M. leprae*.
Diseases: Immunocompromised

- Skin and Soft Tissue
  - Presentation variable: nodules, plaques, abscesses & ulcers.
  - Pyomyositis: direct extension.
- Disseminated & Pulmonary
- Ophthalmologic: Conjunctivitis
- Osteomyelitis
- Less common
  - Central Catheter Related Infection
  - Epididymal Abscesses

Diseases: Immunocompetent

- Pediatric Cervicofacial Lymphadenitis
  - 2nd most common cause of NTM lymphadenitis in some locales (Israel and The Netherlands).
- Other Skin Lesions
  - Trauma
    - Coral scrapes

Diagnosis

- Histopathology: Necrotizing granulomas
- Acid fast staining: Short, slightly curved AFB
- Culture
  - Slow grower: Hold culture 8 weeks.
  - Incubate at lower temperatures: 30-32 °C
  - Iron supplementation required.
  - Hemin or ferric ammonium citrate.
The Caveat: Unregulated Broad-Range PCR and Sequencing for Mycobacteria

- 183 specimens / 18 months / $93,063 spend
- 2 patients with a mycobacterial infection.
  - Both with granulomas in tissue and AFB in tissue.
    - One smear positive / one smear negative
    - $93,063 / true positive detected
- 7/183 with granulomas (increased pre-test likelihood)
- No true "culture-negative/PCR-positive" infections detected
- One false-positive PCR and one false-positive culture.

Lesson: Molecular is not always better just because it is newer. Use these expensive tests judiciously.