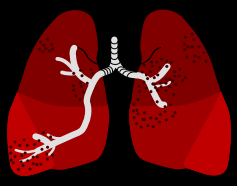
CHALLENGING MYCOBACTERIAL & FUNGAL INFECTIONS





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TB

- Between 2000 and 2020
  - ✓ One billion people will become newly infected
  - ✓ 200 million will get sick
  - ✓ 35 million will die
- Someone in the world is newly infected with TB every second
- 1% of the world's pop is newly infected each year

WHO Tuberculosis Fact Sheet, April 2000

THE BIG APPLE 2003

- 1140 CASES
- 14.2 CASES/100,000
- 3 X NATIONAL AVERAGE
- 5% CASE INCREASE SINCE 2002
- 67% IN FOREIGN BORN
- 43% IN HOMELESS

QUALITY SPECIMENS YIELD QUALITY RESULTS

- RESPIRATORY SPECIMEN COLLECTION
  - ✓ Double Container Reduces False Positives
- PATIENT WITH HIGH INDEX OF SUSPICION*
  - ✓ 75% Specimens Collected Were Culture Neg
  - ✓ 68% Normal Chest X-rays
- ADEQUATE NUMBER AND VOLUME
  - ✓ 3 Sputum Specimens
  - ✓ 5-10 ml/Specimen
- DIRECTLY SUPERVISED COLLECTION
  - ✓ Availability Of Sputum Induction

*Della-Latta & Whittier. Am J Clin Path 110:301-310

FROM SPECIMEN TO REPORTS

- SPECIMEN DIGESTION & DECONTAMINATION
  - ✓ NALC/NAOH Tx (3-4 HR)
- AFB STAINS – SAME DAY
  - ✓ FLUORESCENT STAIN - SPECIMENS
  - ✓ KINYOUN - CULTURE
  - ✓ STAINS MYCOLIC ACIDS
- NUCLEIC ACID AMPLIFICATION TESTS- 3H
  - ✓ ROUTINE ON ALL AFB SMEAR +, CONSULT FOR SMEAR NEG
- CULTURE GOLD STANDARD
  - ✓ TAT RESULTS 2-8 WEEKS
  - ✓ SOLID & LIQUID MEDIA
  - ✓ IDENTIFICATION
    - DNA PROBES & ROUTINE BIOCHEMICALS

MYCOBACTERIA CUMC MAIN PLAYERS

- SPECIES NUMBER
  - ✓ 30 species 25 yr ago
  - ✓ 100 species today
- MAJOR PATHOGENS
  - ✓ MTB complex (MTBC)
    - Grows 1-2 mths
  - ✓ *M. avium* complex (MAC)
    - Grows 2-4 wks
- SLOW GROWERS
  - ✓ *M. kansasii*
  - ✓ *M. xenopi*
- RAPID GROWERS
  - ✓ *M. abscessus*
    - 50% of rapid growers
  - ✓ *M. chelonei*
  - ✓ *M. marinum*
  - ✓ *M. fortuitum*
  - ✓ Grows 1-2 wks

4 DNA ACCUPROBE TEST FROM CULTURE

1. *M. tuberculosis* Complex (MTBC)
   - ✓ *M. tuberculosis*
   - ✓ *M. bovis*
     M. bovis BCG
   - ✓ *M. africanum*
     M. microti
     M. canetti
2. *M. avium* Complex
   - ✓ *M. avium*
   - ✓ *M. intracellulare*
   - ✓ X cluster (reactive with a third probe)
3. *M. kansasii*
4. *M. gordonae*

CASE PRESENTATION

HPI:

- 51 y.o. boy presented to ED after trauma to rt hand
- Worked as cook at Howard Johnson's where he hit hand on a large pot. No skin breakdown
- Pt noticed purulent drainage from palmer surface of the hand
- I&D of hand on admission

MICROBIOLOGY

- DRAINAGE
  - ✓ Meth Suscept *S. aureus*

TREATMENT

- IV Oxacillin for 4 wks

RADIOGRAPHY

- **Right hand:** Erosion of radial aspect of rt distal 3rd metacarpal, possibly involving proximal phalanx
- Diffuse dorsal soft tissue swelling compatible with osteomyelitis

ANY THOUGHTS?

MICRO & PATH

MICRO RESULTS

- All Smears were AFB negative
- Wound Specimen & Bone Specimen:
  M. tuberculosis + by NAAT
- Wound & Bone Culture: *M. tuberculosis* +

PATH RESULTS

- MICROSCOPIC DESCRIPTION
  Replacement of the bone marrow by a chronic, necrotizing granulomatous inflammatory infiltration containing poorly defined granulomata
- Special stains for bacterial (gram), fungal (GMS) and mycobacterial (AFB) organisms are negative
- DX: Necrotizing granulomatous osteomyelitis

THEN WE ASKED FOR A CT CHEST…

- Left lower lobe represents an old inactive granuloma
- LN enlargements, presumably reactive lymphadenitis
- Soft tissue swelling of the chest wall in association with focal lytic destruction of a rib
- Multifocal infection - *M. tuberculosis* highly probable

HOW WAS TB CONTRACTED?

- Two different types of lesions may be seen at different sites- tubercular osteomyelitis & arthritis
- SKELETAL TB may results from hematogenous dissemination of primary tuberculous lesion Multi-drug chemotherapy successful in most patients
- Rare, 5-10% of skeletal TB

CASE HISTORY

HPI: 5 yo boy, ↑ sleepiness, vomiting phono/photophobia,

PMHX: Family hx migraines

SISTER: PPD+, CXR (+1992, - 1997)

MOTHER: PPD+ (when 3 mths pregnant with pt) no TX,CXR -1997

FATHER: PPD+, CXR - 1997

PE: Febrile (102-103), neck supple, conjunctivitis

LAB RESULTS

MICROBIOLOGY:

CSF: AFB smear -

Culture MTB + (35 days)

NA Amp Test -/+ (4 hrs)

BRAIN BX: AFB smear -,

Culture MTB + (26 days)

NA Amp Test + (4 hrs)

DX: TBM with TUBERCULOMA

CT: 1st impression was arterial venous malformation

MRI: Tuberculoma in Lt cerebellar hemisphere (1st impression was metastatic tumor/acute hemorrhage)

PATHOLOGY BRAIN BX:

Granuloma, inflammation, necrotic tissue, no AFB, lymphocytes

CNS TB

QUESTIONS

- What questions should be asked of hx?
- What expertise is needed for CNS TB DX?
- What tests are most valuable?

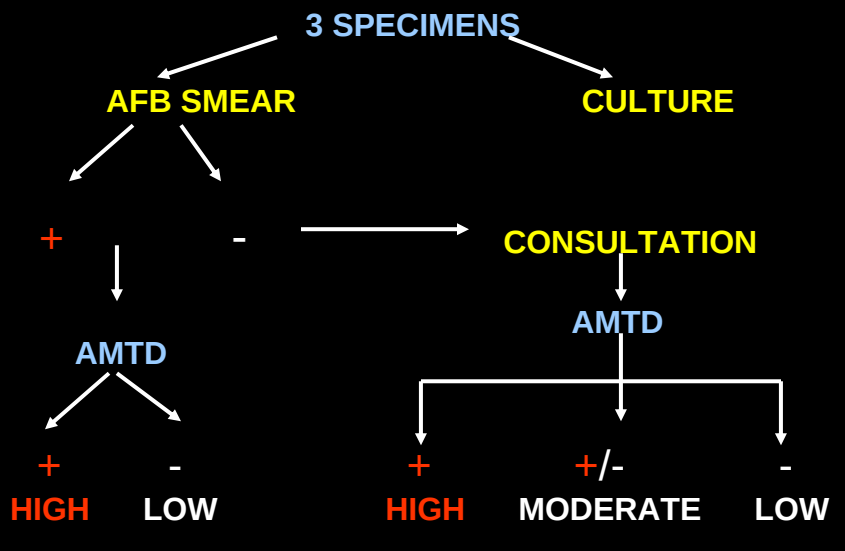
DX PEARLS

- **THINK TB!** Thorough hx
- Symptoms nonspecific
- Consults critical
- Order CT/MRI
- **Consider RAPID TESTS FOR IDENTIFICATION, (NA Amp tests)**

NA AMPLIFICATION MTBC

PARAMETERS	AMPLIFIED MTD
AMPLIFICATION METHOD	Transcription Mediated Amplification (NOT PCR)
TARGET	16S Ribosomal RNA
PROBE	DNA Acridinium ester labeled
DETECTION	Chemi-luminescence

ALGORITHM AMTD TB OR NOT TB INDEX OF SUSPICION



TB OR NOT TB?
AMPLIFIED MTD TEST

AFB SMEAR POSITIVE	AFB SMEAR NEGATIVE
• Specificity 100%	• Specificity* 97.6%
• Positive Predictive Value 95.5%	• Negative Predictive Value 96.4%

*Bloody specimens can give false positives

NONTUBERCULOUS MYCOBACTERIA ARE THEY CLINICALLY SIGNIFICANT?

- SKIN & SOFT-TISSUE INFECTIONS
  - ✓ Puncture Wounds Or Inoculations
  - ✓ Multiple Nodular Lesions
- PULMONARY INFECTION
  - ✓ Unilateral Noncavitary Lesion
- ENDOCARDITIS - 9% MORTALITY
- FOREIGN MATERIAL
  - ✓ Prosthetic Devices, Augmentation Mammoplasty
- POSTSURGICAL SITES e.g. sternal wounds

- NTM ARE NOT "ATYPICAL MYCOBACTERIA"!
- DISEASE, COLONIZATION, CONTAMINATION?
- ATS RECOMMENDATIONS FOR CLINCAL SIGNIFICANCE
  - ✓ 3 CULTURE +/AFB SMEAR - SPUTUM/BAL
  - ✓ 2 CULTURE +/1 AFB SMEAR +
  - ✓ 1 BAL CULTURE +/ AFB SMEAR $\geq$ 2+
  - ✓ ISOLATION FROM STERILE BODY SITE

CLINICAL HISTORY

- 48 yr old woman presenting with uveitis of rt eye
  - ✓ PMHx
    - Insulin-dependent diabetes
    - Recurrent pyelonephritis & UTI's
  - ✓ PSHx
    - Partial gastrectomy for obesity
- PE
  - ✓ Uveitis associated with sarcoid
  - ✓ Chest radiograph - Normal
  - ✓ Chest CT
    - No evidence of TB or sarcoid
  - ✓ Steroids initiated for treatment of sarcoid

CLINICAL COURSE

- NO IMPROVEMENT ON STEROIDS
- VITRECTOMY & LENSECTOMY
- PATHOLOGY – VITREOUS FLUID
  - Cytology – spores suggestive of *Candida*
- MICROBIOLOGY – VITREOUS TISSUE AND FLUID
  - Fluid – *Candida albicans*
  - Tissue – same as fluid
  - Susceptible to all antifungals

INTERPRETATIONS

- SUSCEPTIBILE
  - ✓ MOST OFTEN CORRELATES WITH SUCCESSFUL TREATMENT WITH THAT DRUG
- INTERMEDIATE
  - ✓ SUSCEPTIBILITY IS UNCERTAIN & CANNOT BE CLEARLY CATEGORIZED AS S OR R
- SUSCEPTIBLE DOSE DEPENDENT (SDD)
  - ✓ HIGHER DOSES MAY BE REQUIRED , e.g. FLUCONAZOLE >400 MG/DAY
- RESISTANT
  - ✓ MOST OFTEN CORRELATES WITH TREATMENT FAILURE WITH THAT DRUG
- *C. krusei*
  - ✓ ASSUMED TO BE INTRINSICALLY RESISTANT TO FLUCONAZONE

CLSI *CANDIDA* INTERPRETIVE GUIDELINES

AGENT	Suscep S	Suscep Dose-Depen SDD	Intermed I	Resistant R
AMPHO B	≤ 1	-	-	> 1
FLUCON	≤ 8	16- 32	-	≥ 64
ITRA	≤ 0.125	0.25- 0.5	-	≥ 1
5FC	≤ 4	-	8-16	≥ 32

MYCOLOGY PITFALLS & SOLUTIONS

- DO NOT RELY ONLY ON CLINICAL SYNDROMES
- SWABS ERRONEOUSLY SENT TO MICROBIOLOGY INSTEAD OF TISSUE
  - ✓ EDUCATION
  - ✓ REJECT SPECIMEN?
- LIMITATIONS WITH PATHOLOGY STAINS ONLY
  - ✓ HYPHAE ONLY SEEN
  - ✓ NO SPECIATION OR SUSCEPTIBILITY
- SOLUTIONS
  - ✓ SEND TISSUE TO BOTH PATHOLOGY & MICROBIOLOGY
  - ✓ COLLABORATION - PATHOLOGY & MICRO & CLINICIAL STAFF

CASE

PRESENTATION

- 73 YR-OLD WOMAN
- ACUTE MYELOID LEUKEMIA
- FEVER & PANCYTOPENIA
- BEGUN ON BROAD SPECTRUM ANTIBIOTICS AND ABLC 5 MG/KG/DAY

SPECIMENS TO ORDER

- BLOOD CULTURES
  - ✓ BACTERIOLOGY
  - ✓ MYCOLOGY
    - ✓ INEFFECTIVE, LOW YIELD
    - ✓ ISOLATOR TUBES
- BIOPSIES
  - ✓ MICROBIOLOGY & MYCOLOGY
  - ✓ MYCOBACTERIOLOGY
  - ✓ PATHOLOGY
- BONE MARROW

CASE PRESENTATION

EXAM

- 3-cm eschar appears on rt arm 4 cm proximal to a PIC Line
- This occurred after 5 wks broad-spectrum antibiotics and ABLC
- Biopsy performed by the Dermatology consultant

LAB RESULTS

- Narrow-caliber, septate mycelia, medusa head sporangium
- Culture grew ***Aspergillus flavus***

SUSCEPTIBILITY TESTS

- No change after one wk on ABLC & itraconazole
- In vitro susceptibility studies:
  - itraconazole-resistant
  - voriconazole-resistant
  - AMB- resistant
- Caspofungin acetate begun

PATIENT OUTCOME

- IMPROVED BUT DIED OF COMPLICATIONS ASSOCIATED WITH AML

INVASIVE ASPERGILLOSIS

INCIDENCE

- LEUKEMIA (10%- 20%)
  - ✓ MORTALITY 50%
- BMT RECIPIENTS
  - ✓ INCIDENCE (5-13%)
  - ✓ MORTALITY 90%
- HEART LUNG TRANSPLANT (5-25%)
- RELAPSE COMMON, EVEN AFTER A "CURE"
- *A. FUMIGATUS* MOST PREVALENT (64%)
- *A. NIGER* (22%)

- SPECIMEN FROM STERILE BODY SITE IS BEST FOR CULTURE
  - ✓ CULTURE PROBLEMS: TISSUE BIOPSIES OR NEEDLE ASPIRATES ARE OFTEN NOT SENT FOR MYCOLOGY, JUST PATH OR SENT ON SWABS
  - ✓ POSITIVES FROM NON STERILE SITE (SPUTUM) COULD BE CONTAMINANT
- CULTURE AS A STAND ALONE TEST HAS POOR SENSITIVITY
  - ✓ ISOLATION FROM BLOOD CULTURES NOT POSSIBLE USING CURRENT METHODS

GALACTOMANNAN TEST GREAT EXPECTATIONS

- GM TEST FOR *ASPERGILLUS* ANTIGEN DETECTION
  - ✓ PLATELIA (BIO-RAD)
  - ✓ FDA APPROVED MAY 2003
  - ✓ IMMUNOENZYMATIC SANDWICH EIA
  - ✓ EIA USING MONOCLONAL ANTIBODY TO GM POLYSACCHARIDE AG IN FUNGAL CELL WALL
  - ✓ 3 HR TEST
- SPECIMEN
  - ✓ SERUM

GALACTOMANNAN ASSAY

- FALSE POSITIVES (14%)
  - ✓*Paecilomyces, Penicillium & Rhodotorula*
  - ✓Translocation of GM antigen from food (e.g. bread, pasta, turkey, sausage) through damaged intestinal mucosa
  - ✓Mould-derived antibiotics e.g. penicillin
- POSITIVE CULTURE FROM BAL OR SPUTUM (>2 SPECIMENS)
- POSITIVE CULTURE & MICROSOPIC EXAM OF SINUS ASPIRATE
- POSITIVE GM TEST IN ≥ 2 BLOOD SPECIMENS

BRIEF CASE

- 56 yr old male, cardiomyopathy
- Transfer from another hospital
- Cardiac arrest during cardiac catherization
- LVAD implant, CABG x 2
- Sepsis
- LVAD dysfunction
- Cardiac arrest → Death → No autopsy

LAB RESULTS

PATHOLOGY

- Soft tissue & LVAD valve material examined
- PAS & silver stains positive
- Report Read

  "Fungal hyphae with 45^0 angle branching consistent with *Aspergillus*"

MICRO

- Blood cultures negative (3 sets)
- Tissue biopsy & sternal wound cultured
- No LVAD material sent for culture
- RESULT

  Syncephalastrum racemosum & NOT *Aspergillus*

DISCORDANT LUNG BIOPSIES

PATHOLOGY	MICROBIOLOGY
CONSISTENT WITH *CANDIDA*	*A. FUMIGATUS*
NON-SEPTATE HYPHAE	*A. FUMIGATUS*
FUNGAL HYPHAE, 45^0 ANGLE BRANCHING "Consistent with *Aspergillus*"	*SYNCEPHALASTRUM* *FUSARIUM* *SCEDOSPORIUM*

AND I CARE BECAUSE…? DRUG REGIMEN

- AMPHOTERICIN B
  - ✓STANDARD OF CARE FOR ASPERGILLOSIS
  - ✓PT ISOLATE RESISTANT
- ITRACONAZOLE
  - ✓2^{ND} LINE DRUG FOR ASPERGILLOSIS
  - ✓PT ISOLATE RESISTANT
- ✓ CASPOFUNGIN
  - ✓SUSCEPTIBLE
  - ✓NO RESISTANT MIC CUTOFF ESTABLISHED