CHALLENGING MYCOBACTERIAL & FUNGAL INFECTIONS

Dr. Phyllis Della-Latta, 5-2929

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


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?
<table>
<thead>
<tr>
<th>MICRO &amp; PATH</th>
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<tbody>
<tr>
<td><strong>MICRO RESULTS</strong></td>
</tr>
<tr>
<td>• All Smears were AFB negative</td>
</tr>
<tr>
<td>• Wound Specimen &amp; Bone Specimen: <em>M. tuberculosis</em> + by NAAT</td>
</tr>
<tr>
<td>• Wound &amp; Bone Culture: <em>M. tuberculosis</em> +</td>
</tr>
<tr>
<td><strong>PATH RESULTS</strong></td>
</tr>
<tr>
<td>• <strong>MICROSCOPIC DESCRIPTION</strong></td>
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<tr>
<td>Replacement of the bone marrow by a chronic, necrotizing granulomatous inflammatory infiltration containing poorly defined granulomata</td>
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<tr>
<td>• Special stains for bacterial (gram), fungal (GMS) and mycobacterial (AFB) organisms are negative</td>
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<tr>
<td>• DX: Necrotizing granulomatous osteomyelitis</td>
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<table>
<thead>
<tr>
<th>THEN WE ASKED FOR A CT CHEST...</th>
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<tr>
<td>• Left lower lobe represents an old inactive granuloma</td>
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<tr>
<td>• LN enlargements, presumably reactive lymphadenitis</td>
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<td>• Soft tissue swelling of the chest wall in association with focal lytic destruction of a rib</td>
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<tr>
<td>• Multifocal infection - <em>M. tuberculosis</em> highly probable</td>
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HOW WAS TB CONTRACTED?

- Two different types of lesions may be seen at different sites - tubercular osteomyelitis & arthritis
- **SKELETAL TB** may result 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)

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

DX: TBM with TUBERCULOMA

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

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>AMPLIFIED MTD</th>
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<tbody>
<tr>
<td>AMPLIFICATION METHOD</td>
<td>Transcription Mediated Amplification (NOT PCR)</td>
</tr>
<tr>
<td>TARGET</td>
<td>16S Ribosomal RNA</td>
</tr>
<tr>
<td>PROBE</td>
<td>DNA Acridinium ester labeled</td>
</tr>
<tr>
<td>DETECTION</td>
<td>Chemi-luminescence</td>
</tr>
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ALGORITHM AMTD TB OR NOT TB INDEX OF SUSPICION

3 SPECIMENS

AFB SMEAR

+  -

AMTD

+  -

HIGH LOW

CULTURE

CONSULTATION

AMTD

+  +/-  -

HIGH MODERATE LOW
TB OR NOT TB?
AMPLIFIED MTD TEST

AFB SMEAR POSITIVE
• Specificity 100%
• Positive Predictive Value 95.5%

AFB SMEAR NEGATIVE
• Specificity* 97.6%
• 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 CLINICAL SIGNIFICANCE
  ✓ 3 CULTURE +/AFB SMEAR - SPUTUM/BAL
  ✓ 2 CULTURE +/1 AFB SMEAR +
  ✓ 1 BAL CULTURE +/ AFB SMEAR ≥ 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

• SUSCEPTIBLE
  ✓ 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 FLUCONAZOLE

CLSI CANDIDA INTERPRETIVE GUIDELINES

<table>
<thead>
<tr>
<th>AGENT</th>
<th>Suscep S</th>
<th>Suscep Dose-Depen SDD</th>
<th>Intermed I</th>
<th>Resistant R</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPHOB</td>
<td>&lt; 1</td>
<td>-</td>
<td>-</td>
<td>&gt; 1</td>
</tr>
<tr>
<td>FLUCON</td>
<td>&lt; 8</td>
<td>16-32</td>
<td>-</td>
<td>≥ 64</td>
</tr>
<tr>
<td>ITRA</td>
<td>&lt; 0.125</td>
<td>0.25-0.5</td>
<td>-</td>
<td>&gt; 1</td>
</tr>
<tr>
<td>5FC</td>
<td>&lt; 4</td>
<td>-</td>
<td>8-16</td>
<td>≥ 32</td>
</tr>
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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 & MICROSCOPIC 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

<table>
<thead>
<tr>
<th>PATHOLOGY</th>
<th>MICROBIOLOGY</th>
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<tbody>
<tr>
<td>CONSISTENT WITH CANDIDA</td>
<td><em>A. Fumigatus</em></td>
</tr>
<tr>
<td>NON-SEPTATE HYPHAE</td>
<td><em>A. Fumigatus</em></td>
</tr>
<tr>
<td>FUNGAL HYPHAE, 45(^0) ANGLE BRANCHING</td>
<td><em>Syncephalastrum</em></td>
</tr>
<tr>
<td>“Consistent with <em>Aspergillus</em>”</td>
<td><em>Fusarium</em></td>
</tr>
<tr>
<td></td>
<td><em>Scedosporium</em></td>
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AND I CARE BECAUSE...?
DRUG REGIMEN

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