CHALLENGING MYCOBACTERIAL & FUNGAL INFECTIONS

Dr. Phyllis Della-Latta, 5-2929

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

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
- 3X NATIONAL AVERAGE
- 5% CASE INCREASE SINCE 2002
- 67% IN FOREIGN BORN
- 43% IN HOMELESS

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

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


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

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**RADIOGRAPHY**

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

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**CASE HISTORY**

<table>
<thead>
<tr>
<th>HPI</th>
<th>SISTER</th>
<th>MOTHER</th>
<th>FATHER</th>
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</thead>
<tbody>
<tr>
<td>PE</td>
<td>PHON</td>
<td>PHOT</td>
<td></td>
</tr>
<tr>
<td>Febrile (102-103), neck supp, conjunctivitis</td>
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**MICRO & PATH**

<table>
<thead>
<tr>
<th>MICRO RESULTS</th>
<th>PATH RESULTS</th>
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</thead>
<tbody>
<tr>
<td>All Smears were AFB negative</td>
<td>MICROSCOPIC DESCRIPTION</td>
</tr>
<tr>
<td>Wound Specimen &amp; Bone Specimen: M. tuberculosis + by NAAT</td>
<td>Replacement of the bone marrow by a chronic, necrotizing granulomatous inflammatory infiltration containing poorly defined granulomata</td>
</tr>
<tr>
<td>Wound &amp; Bone Culture: M. tuberculosis +</td>
<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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**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

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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 results from hematogenous dissemination of primary tuberculous lesion Multi-drug chemotherapy successful in most patients
- Rare, 5-10% of skeletal TB

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**ANY THOUGHTS?**
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

ALGORITHM AMTD TB OR NOT TB INDEX OF SUSPICION

3 SPECIMENS

- AFB SMEAR
- CULTURE

CONSULTATION

AMTD

HIGH LOW

HIGH LOW

HIGH MODERATE LOW

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
  Consulate critical
  Order CT/MRI
  Consider RAPID TESTS FOR IDENTIFICATION, (NA Amp tests)

TB OR NOT TB? AMPLIFIED MTD TEST

AFB SMEAR

- POSITIVE
  - Specificity 100%
  - Positive Predictive Value 95.5%

- NEGATIVE
  - Specificity* 97.6%
  - Negative Predictive Value 96.4%

*Nontuberculous Mycobacteria are clinically significant? sidered

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>
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NON Tuberculous 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 Mammaplasty
  - POSTSURGICAL SITES e.g. sternal wounds

- Nontuberculous Mycobacteria are not “atypical mycobacteria!”
- DISEASE, COLONIZATION, CONTAMINATION?
- ATS RECOMMENDATIONS FOR CLINICAL SIGNIFICANCE
  - 2 CULTURE +/- AFB SMEAR - SPUTUM/BAL
  - 2 CULTURE +/- 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

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 & CLINICAL 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

CLSI CANDIDA INTERPRETIVE GUIDELINES

<table>
<thead>
<tr>
<th>AGENT</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
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<tbody>
<tr>
<td>AMPHOB</td>
<td>≤ 1</td>
<td>-</td>
<td>&gt; 1</td>
</tr>
<tr>
<td>FLUCON</td>
<td>≤ 8</td>
<td>16 - 32</td>
<td>&gt; 64</td>
</tr>
<tr>
<td>ITRA</td>
<td>≤ 0.125</td>
<td>0.25 - 0.5</td>
<td>&gt; 1</td>
</tr>
<tr>
<td>5FC</td>
<td>≤ 4</td>
<td>8 - 16</td>
<td>≥ 32</td>
</tr>
</tbody>
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CLSI CANDIDA INTERPRETIVE GUIDELINES

- 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

INTERPRETATIONS

- SUSCEPTIBLE
- INTERMEDIATE
- SUSCEPTIBLE DOSE DEPENDENT (SDD)
- RESISTANT
- C. krusei
- ASSUMED TO BE INTRINSICALLY RESISTANT TO FLUCONAZOLE
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

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
- SPECIMEN
  - SERUM

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 BIOSPIES 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

BRIEF CASE
- 56 yr old male, cardiomyopathy
- Transfer from another hospital
- Cardiac arrest during cardiac catheterization
- 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° 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>A. FUMIGATUS</td>
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<tr>
<td>NON-SEPTATE HYPHAE</td>
<td>A. FUMIGATUS</td>
</tr>
<tr>
<td>FUNGAL HYPHAE, 45° ANGLE BRANCHING</td>
<td>SYNCEPHALASTRUM FUSARIUM</td>
</tr>
<tr>
<td>“Consistent with Aspergillus”</td>
<td>SCEDOSPORIUM</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