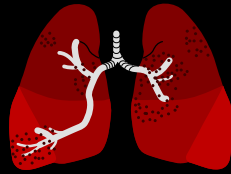


CHALLENGING MYCOBACTERIAL & FUNGAL INFECTIONS



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

*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. chelonae*
 - ✓ *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?

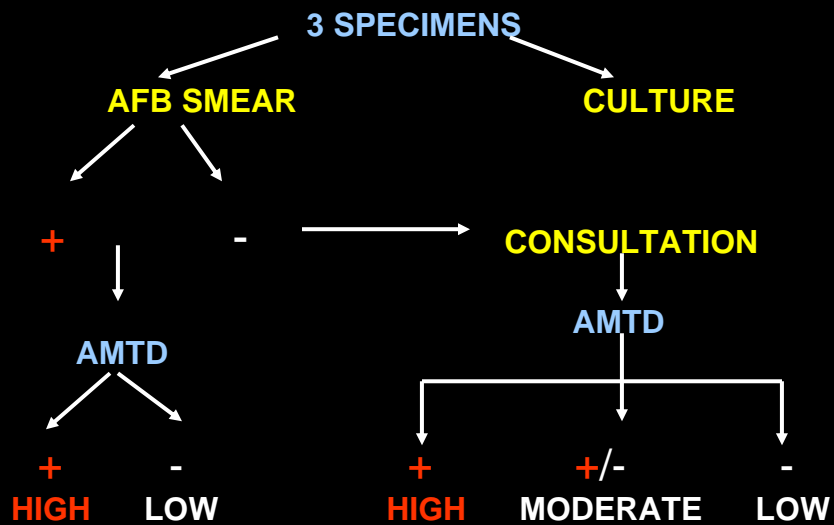
DX PEARLS

- **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

- **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 \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

- **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 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 & CLINICAL STAFF

CASE

- | PRESENTATION | SPECIMENS TO ORDER |
|---|---|
| <ul style="list-style-type: none">• 73 YR-OLD WOMAN• ACUTE MYELOID LEUKEMIA• FEVER & PANCYTOPENIA• BEGUN ON BROAD SPECTRUM ANTIBIOTICS AND ABL 5 MG/KG/DAY | <ul style="list-style-type: none">• BLOOD CULTURES<ul style="list-style-type: none">✓ BACTERIOLOGY✓ MYCOLOGY<ul style="list-style-type: none">✓ INEFFECTIVE, LOW YIELD✓ ISOLATOR TUBES• BIOPSIES<ul style="list-style-type: none">✓ 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 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

PATHOLOGY	MICROBIOLOGY
CONSISTENT WITH <i>CANDIDA</i>	<i>A. FUMIGATUS</i>
NON-SEPTATE HYPHAE	<i>A. FUMIGATUS</i>
FUNGAL HYPHAE, 45° ANGLE BRANCHING “Consistent with <i>Aspergillus</i> ”	<i>SYNCEPHALASTRUM</i> <i>FUSARIUM</i> <i>SCEDOSPORIUM</i>

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