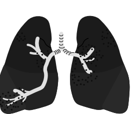
CHALLENGING MYCOBACTERIAL & FUNGAL INFECTIONS



Dr. Phyllis Della-Latta, 5-2929

OBJECTIVES

- Why is it important to "THINK TB" in your differential?
  - ✓Rapid Detection of TB
- Clinical relevance of nontuberculous mycobacteria
  - ✓Specimen Collection & Micro Tests
- Review clinical scenarios where fungal infections are suspect
  - ✓Micro Test Menu

TB FACTS

- 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
- Nearly 1% of the world's population is newly infected with TB each year

WHO Tuberculosis Fact Sheet

TB IN THE BIG APPLE

THEN- 1992

- ✓ Rate: 52 /100,000
- ✓ Mortality: 200
- ✓ Foreign Born: 18%
- ✓ MDR Cases: 441
- ✓ TB Labs: 5 day wk
- ✓ Rapid Molecular Tests for MTB Just Begun

Dr. Tom Frieden

- ✓ Director, Bureau of TB Control
- ✓ Directly Observed Therapy just initiated

NOW-2004

- ✓ Rate: 13 /100,000
- ✓ Mortality: 30
- ✓ Foreign Born: 68%
- ✓ MDR Cases: 18
- ✓ TB Labs: 7 day wk
- ✓ Rapid Tests for MTB Readily Available

Dr. Tom Frieden

- ✓ Commissioner, Dept of Health & Mental Hygiene
- ✓ DOT is routine

NYC DOH REQUIREMENTS

LAB REPORTING WITHIN 24 HRS

- AFB + smears
- Cultures + for *M. tuberculosis*
- Rapid NAAT for MTB
- Culture with solid & liquid media
- Pathology finding consistent with TB

NYC DOH

- 10 chest centers
- Contact investigations

SHOULD WE STILL THINK TB?
AT RISK GROUPS

- HIV infected
- Organ transplant, diabetes, CA
- Immunosuppressive therapy
- Radiographic evidence old/healed TB
- Immigrants from countries with high incidence TB (#1 China, #2 DR)
- Correctional facilities, homeless shelters, Nursing homes
- Injecting drug users
- PPD converters within past 2 yrs

MICRO TEST OVERVIEW BEFORE CASES

- COLLECT QUALITY SPECIMEN
- PERFORM AFB STAIN
- NUCLEIC ACID AMPLIFICATION TEST (NAAT)
- CULTURE & DNA PROBE IDENTIFICATION

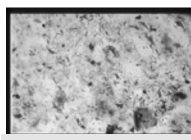
QUALITY SPECIMENS YIELD QUALITY RESULTS

- 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

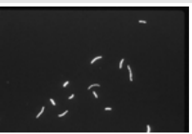
*DELLA-LATTA & WHITTIER. AM J CLIN PATH 110:301-310

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



KINYOUN STAIN



FLUORESCENT STAIN

MYCOBACTERIA CUMC MAIN PLAYERS

- MAJOR PATHOGENS
  - ✓ MTB complex (MTBC)
    - 30% OF CASES
    - GROWS 1-2 MTHS
  - ✓ *M. avium* complex (MAC)
    - 60% OF CASES
    - 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

DNA PROBE TESTS FROM CULTURE

PROBE ASSAYS

- Nucleic Acid Hybridization
- Target rRNA
- ss DNA probe complementary to rRNA
- DNA-RNA hybrid detected by chemiluminescence

1. *M. TUBERCULOSIS* COMPLEX (MTBC)
   - ✓ *M. tuberculosis*
   - ✓ *M. bovis*
     M. africanum
2. *M. AVIUM* COMPLEX
   - ✓ *M. avium*
   - ✓ *M. intracellulare*
   - ✓ X cluster (reactive with a third probe)
3. *M. KANSASII*
4. *M. GORDONAE*

NUCLEIC ACID AMPLIFICATION

- TEST DIRECTLY FROM SPECIMENS
- FASTER TIME TO RESULTS
  - ✓ 3 HR
- RAPID DX & START OF APPRO THERAPY
- 84% of all specimens AFB + & AMTD – are MAC
  - ✓ IMPACTS ON PT TX & MANAGEMENT DECISIONS
  - ✓ TOGETHER WITH CLINICAL IMPRESSION CAN RULE OUT TB
- 2004 NO FALSE + OR FALSE – PATIENTS
  - ✓ NO TEST IS 100%
  - ✓ TB OR NOT TB IS A CLINICAL CALL

NAAT MTBC

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

TB OR NOT TB? AMPLIFIED MTD TEST

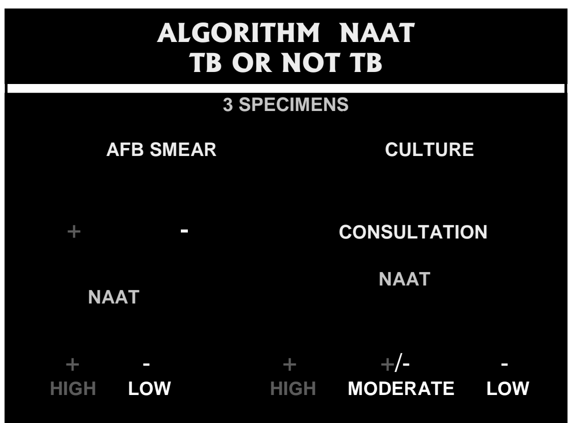
AFB SMEAR POSITIVE

- Specificity 100%
- Positive Predictive Value 95.5%

AFB SMEAR NEGATIVE

- Specificity* 97.6%
- Negative Predictive Value 96.4%

*Bloody specimens can give false positives



CASE PRESENTATION

HISTORY

- 51 YO MAN 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

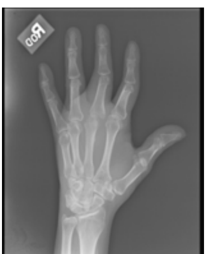
- DRAINAGE
  S. aureus

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 RESULT FROM HEMATOGENOUS DISSEMINATION OF PRIMARY TUBERCULOUS LESION MULTI-DRUG CHEMOTHERAPY SUCCESSFUL IN MOST PATIENTS
- 5-10% OF SKELETAL TB

"POT'S" DISEASE

CASE HISTORY

HISTORY:

- 5 YO BOY, ↑ SLEEPINESS, VOMITING
- PHONO/PHOTOPHOBIA
- 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)
NAAT + (4 hrs)
BRAIN BX: AFB smear -,
Culture MTB + (26 days)
NAAT + (4 hrs)

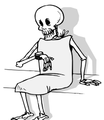
CT: 1st impression was arterial venous malformation
MRI: Tuberculoma in Lt cerebellar hemisphere (1st impression was metastatic tumor/acute hemorrhage)
PATHOLOGY BRAIN BX: Granuloma, inflammation, necrotic tissue, no AFB, lymphocytes

DX: TBM with TUBERCULOMA

CNS TB

QUESTIONS

- What questions should be asked of hx?
- What tests are most sensitive & rapid to r/o CNS TB DX?



DX PEARLS

- THINK TB! Thorough hx
- Symptoms nonspecific
- Consults critical
- Order CT/MRI
- RAPID TESTS FOR IDENTIFICATION, (NAAT)

NONTUBERCULOUS MYCOBACTERIA ARE THEY CLINICALLY SIGNIFICANT?

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

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

COMMON CLINICALLY IMPORTANT NTM

- RAPID GROWING MYCOBACTERIA (RGM)
  - ✓ M. ABSCESSUS
  - ✓ M. FORTUITUM
  - ✓ SKIN & SOFT TISSUE INFECTIONS
  - ✓ COSMETIC SURGERY (DR)
    - "LIPOTOURISM"
- MAC DISEASE
  - ✓ HOT TUB SYNDROME (SPAS)
  - ✓ COPD
  - ✓ CHRONIC BRONCHIECTASIS
  - ✓ DISSEMINATED DISEASE

M. ABSCESSUS NOSOCOMIAL INFECTIONS

- COSMETIC SURGERY
- CARDIAC SURGERY
  - ✓ STERNAL WOUND INFECTIONS, PROSTHETIC VALVE ENDOCARDITIS
- POSTINJECTION ABSCESSES
- DISSEMINATED INFECTIONS
- HEMODIALYSIS OUTBREAKS & PERITONEAL DIALYSIS
- CONTAMINATED BRONCHOSCOPES & ENDOSCOPES

TREATMENT *M. ABSCESSUS*

- CEFOXITIN AND CLARITHROMYCIN STARTED
- GENERAL SUSCEPTIBILITY
  - ✓ Clarithromycin, Amikacin, Imipenem, Cefoxitin
  - ✓ COMBINATION TX: cefoxitin/imipenem with clarithro/azithro
- TX DURATION
  - ✓ 6 TO 9 MTHS

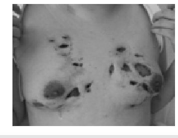
CASE PRESENTATION

- A 27 y.o. woman presented at CUMC with fevers, breast erythema and pain
- Pt reports that 1.5 years ago, in anticipation of breast implants, she started going to a nurse "Natasha" in Washington Heights/Queens, to receive silicone breast injections
- Two wks after the 6th injection, she developed acute onset swelling of both breasts, erythema and tenderness

MICROBIOLOGY TESTS

- BLOOD CULTURES ORDERED
  - ✓ NEGATIVE FOR BACTERIA, FUNGAL & AFB
- ACID FAST STAIN +
- AFB CULTURE FROM BREAST ABSCESS
  - ✓ GROWTH ON AGAR & BROTH MEDIA WITHIN 2 WKS
  - ✓ BIOCHEMICAL REACTIONS IDENTIFIED THE PATHOGEN AS RAPID GROWER
- FINAL IDENTIFICATION
  MYCOBACTERIUM ABSCESSUS

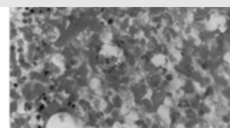
CASE PRESENTATION



M. ABSCESSUS

FOREIGN BODY GIANT CELL RESPONSE
- MANY CLEAR, VACANT SPACES C/W SILICONE INJECTION
- AFB IN VACUOLES
- GRANULATION TISSUE

PATHOLOGY BREAST ABSCESS



⚥

CDC AND CRIMINAL INVESTIGATION UPDATE

- NO IDENTIFIABLE SOURCE OF *M.ABSCESSUS*
- INSTRUMENTS AND MEDICATIONS CONFISCATED FROM THE APARTMENT OF THE ARRESTED COUPLE DID NOT GROW OUT *M.ABSCESSUS*

IN THE MEANTIME…

- July 17 2002 a couple from Venezuela was arrested in Queens on charges of assault, performing medical treatments without a license, scheming to fraud, grand larceny and possession of a weapon:

  THE HYPODERMIC NEEDLE

- They face 25 years in prison if convicted
- The practitioner who operated out of Washington Heights, identified only as a transsexual named Natasha, disappeared under police surveillance

INVESTIGATION CONTINUES…

- NURSE NATASHA REMAINS AT LARGE
- "SHE PROBABLY LEFT THE COUNTRY, WENT BACK TO DOMINICAN REPUBLIC" SAYS THE PATIENT
- PT DENIED ANY FURTHER KNOWLEDGE OF NURSE NATASHA'S WHEREABOUTS

THE FUNGAL JUNGLE *BEAUTIFUL BUT DEADLY*

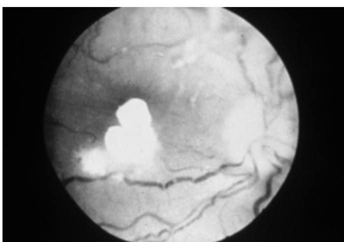
MICROBIOLOGY TESTS

- STAINS OF FLUIDS & TISSUE
  - ✓GRAM
  - ✓KOH
- CULTURE FLUIDS & TISSUE
  - ✓SELECTIVE MEDIA
- IDENTIFICATION BY BIOCHEMICAL TESTS

CLINICAL HISTORY

- 48 yr old woman presenting with uveitis of rt eye
  - ✓PMHx
    - 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

CANDIDA ENDOPHTHALMITIS



Lesions are often localized near the macula - ocular trauma, surgery, candidemia, indwelling catheters or drug abuse

SPECIMENS & TESTS

- PROCEDURES
  - ✓VITRECTOMY & LENSECTOMY
- SPECIMENS
  - ✓VITREOUS FLUID & LENSE
    - CULTURE FOR BACTERIA & FUNGUS
- MICROBIOLOGY CULTURE RESULTS
  - Fluid – *Candida albicans*
  - Tissue – *C. albicans*
  - Susceptible to all antifungals

INTERPRETATIONS

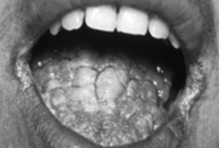
- SUSCEPTIBILE
  - ✓MOST OFTEN CORRELATES WITH SUCCESSFUL TREATMENT WITH THAT DRUG
- INTERMEDIATE
  - ✓SUSCEPTIBILITY IS UNCERTAIN & CANNOT BE CLEARLY CATEGORIZED AS S OR R
- RESISTANT
  - ✓MOST OFTEN CORRELATES WITH TREATMENT FAILURE WITH THAT DRUG
- *Candida krusei*
  - ✓ASSUMED TO BE INTRINSICALLY RESISTANT TO FLUCONAZONE

CANDIDA SUSCEPTIBILITY INTERPRETIVE GUIDELINES

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

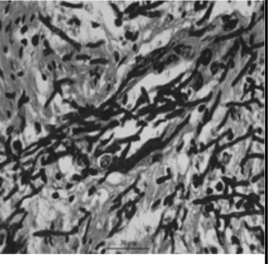
CANDIDIASIS CASE

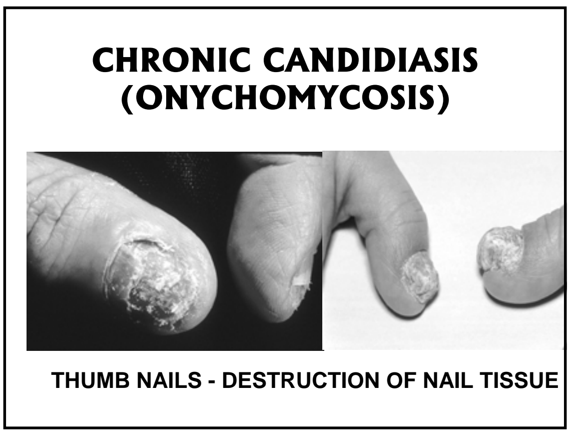
- Pt presents with Chronic oral candidiasis of the tongue in HIV clinic.
- Characteristic white pseudo membrane composed of cells & pseudohyphae of *C. albicans*.

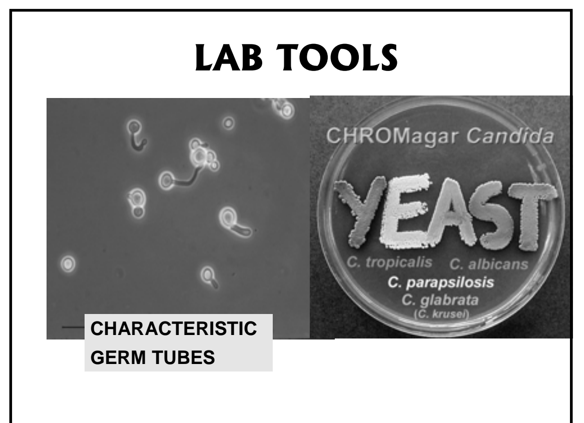


C. ALBICANS

- Periodic Acid-Schiff section of post-mortem esophagus shows invasion of blood vessels by *C. albicans*. Note blastoconidia & branched pseudohyphae.



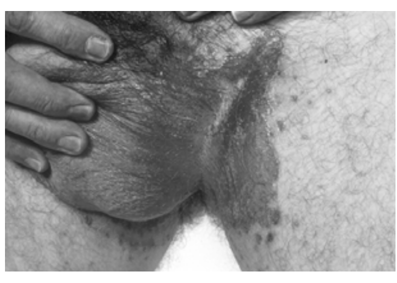


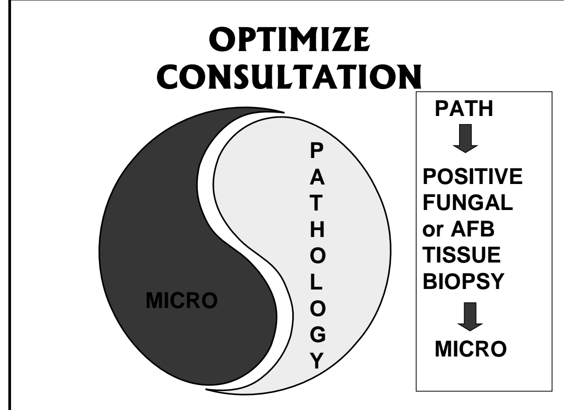


MYCOLOGY PITFALLS & SOLUTIONS

- 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

CANDIDIASIS OF THE GROIN MAY MIMIC TINEA CRURIS





7 LUNG BIOPSIES PATH & MICRO FUNGAL RESULTS

PATHOLOGY	MICROBIOLOGY
Consistent with *Candida*	*A. fumigatus*
Invasive *Aspergillus*	*A. flavus*
Aspergilloma	*A. fumigatus*
Fungal hyphae	*A. fumigatus*
Consistent with *Aspergillus*	No fungus isolated
Invasive *Aspergillus*	No fungus isolated
Suggestive of *Aspergillus*	No fungus isolated

CASE

PRESENTATION

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

SPECIMENS TO ORDER

- BLOOD CULTURES
  - ✓BACTERIOLOGY
  - ✓MYCOLOGY
    - ISOLATOR TUBE
- BIOPSIES
  - ✓MICROBIOLOGY & MYCOLOGY
  - ✓MYCOBACT
  - ✓PATHOLOGY
- BONE MARROW

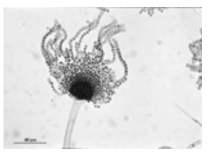
CASE

EXAM

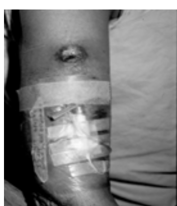
- 3-cm eschar appears on rt arm proximal to a PIC Line
- This occurred after 5 wks broad-spectrum antibiotics & ABLC
- Biopsy performed by the Dermatology consultant

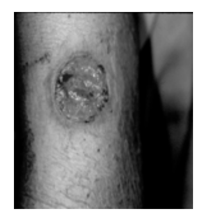
LAB RESULTS

- Septate mycelia, medusa head sporangium
- Culture grew *Aspergillus flavus*



ESCHAR





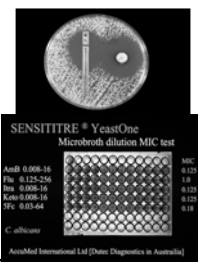
ESCHAR DETACHED & FELL OFF
after about three weeks
(patient remained pancytopenic)

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



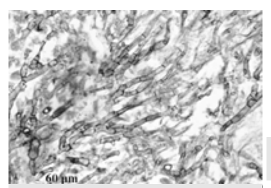
INVASIVE ASPERGILLOSIS

INCIDENCE

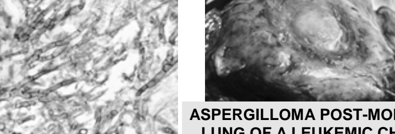
- LEUKEMIA (10%- 20%)
  - ✓ MORTALITY 50%
- BMT RECIPIENTS
  - ✓ INCIDENCE (5-13%)
  - ✓ MORTALITY 90%
- HEART LUNG TRANSPLANT (5-25%)
- RELAPSE COMMON, EVEN AFTER A "CURE"
- *A. FUMIGATUS* MOST PREVALENT (64%)
- *A. NIGER* (22%)

- SPECIMEN FROM STERILE BODY SITE IS BEST FOR CULTURE
  - ✓ CULTURE PROBLEMS: TISSUE BIOPSIES OR NEEDLE ASPIRATES 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

PHOTO GALLERY



DICHOTOMOUSLY BRANCHED, SEPTATE HYPHAE OF *A. FUMIGATUS*



ASPERGILLOMA POST-MORTEM LUNG OF A LEUKEMIC CHILD

NOTE CONIDIAL HEADS FORMING IN ALVEOLUS *A. FUMIGATUS*

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, corn flakes, rice, turkey, sausage) through damaged intestinal mucosa
  - ✓ Mould-derived antibiotics e.g. penicillin
- RECOMMENDED THAT THE TEST BE CONSIDERED TRUE POSITIVE ONLY WHEN >1 SAMPLE IS POSITIVE

MICROBIOLOGIC EVIDENCE SUGGESTIVE OF ASPERGILLOSIS

- POSITIVE CULTURE FROM BAL OR SPUTUM (>2 SPECIMENS)
- POSITIVE CULTURE & MICROSOPIC EXAM OF SINUS ASPIRATE
- POSITIVE GM TEST IN $\geq$ 2 BLOOD SPECIMENS

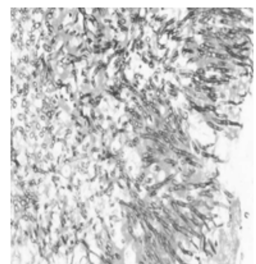
BRIEF CASE

- 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

PATHOLOGY RESULTS

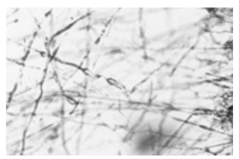
- Soft tissue & LVAD valve material examined
- PAS & silver stains positive
- Report Read

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



MICROBIOLOGY RESULTS

- 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
  - ✓ 2ND LINE DRUG FOR ASPERGILLOSIS
  - ✓ PT ISOLATE RESISTANT
- ✓ CASPOFUNGIN
  - ✓ SUSCEPTIBLE
  - ✓ NO RESISTANT MIC CUTOFF ESTABLISHED

PARTING THOUGHTS………..

PARTING THOUGHTS……

- EXPECT THE UNEXPECTED
- NTM ON THE RISE
- SEND BIOPSIES TO MICROBIOGOLY AS WELL AS PATHOLOGY