214 Diagnosis of Infectious Diseases in Surgical and Cytopathology with Microbiology Recommendations

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AMERICAN SOCIETY FOR CLINICAL PATHOLOGY
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214 Diagnosis of Infectious Diseases in Surgical and Cytopathology with Microbiology Recommendations

This session will cover the identification of viral, bacterial, fungal and parasitic infectious disease agents in surgical pathology and cytopathology specimens with recommendations for microbiological studies. This session will address commonly encountered infectious diseases as well as emerging and re-emerging infectious disease agents. Special attention to common infectious agents that might be encountered from global traveling will also be addressed. Participants will be encouraged to share their experiences and diagnostic practice.

- Participants will be able to evaluate surgical and cytology specimens for the identification of infectious disease agents.
- Participants will be able to decide on microbiological studies important for the identification of infectious disease agents and/or communicating such studies to clinicians.
- Participants with establish a working knowledge of emerging infectious diseases and infectious diseases common to travel abroad.

FACULTY:

Sherri Yong MD
Eva Wojcik MD

Entire Pathology Team
Global Pathology
Global Pathology
2.0 CME/CMLE Credits

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**Case 1**

- 50 year old male was found to have a lung nodule.
- History of smoking cigarettes
- Immunocompetent
- Travel significant for vacationing on Vancouver Island in British Columbia
What to do?

- Aspirate material
  - Minimum 2-3 drops in a sterile container
  - Flush the needle with sterile saline and send fluid
  - Fungal culture and direct smear (calcofluor)
- Cell block
  - Special stains
    - Fungal: GMS, PAS, Mucicarmine and Fontana-Masson
  - Molecular studies if necessary

Cell block

PAP stain  Mucicarmine

FNA of Lung Mass
Smaller sized yeasts involving lung

- Cryptococcosis
- Sporotrichosis
- *Penicillium marneffei*
- *Pneumocystis jiroveci*
- *Candida glabrata*
- *Histoplasma capsulatum*

Smaller yeast variants involving lung

- Blastomycosis
  - Microforms
- Coccidiomycosis
  - Endospores
- Paracoccidiomycosis
  - Blastoconidia

Case 1

- Capsular yeast 2-10 microns
- Mucicarmine positive
- Cultures: *Cryptococcus neoformans*
Cryptococcus

- Typically an opportunistic infection in immunocompromised hosts
- Can infect immunocompetent hosts
- Most common cause of fungal meningitis
- Clinically presents
  - Meningitis or meningoencephalitis
  - Pulmonary disease
  - Other; cutaneous, mucocutaneous, osseous and visceral

Cryptococcosis

- Cryptococcus neoformans
  - Global distribution
- Cryptococcus gattii
  - New emerging pathogen in immunocompetent hosts
  - Typically tropical and subtropical
  - Since 1999: Vancouver Island, Canada
  - NOW: Pacific Northwest and recently in southeastern US
- Rare
  - Cryptococcus laurantii
  - Cryptococcus albidus

Epidemiology

- Soil contaminated with pigeon, chicken or turkey droppings
  - C. neoformans
- Eucalyptus trees and decaying hallows in living trees; British Columbia
  Douglas-fir and Alder trees
  - C. gattii
Cryptococcus

- Inhalation of infected aerosolized particles
  - Lung
- Hematogenous dissemination
  - Brain
  - Skin, bone, joints, lymph nodes, spleen, liver, genitourinary tract, eyes, myocardium
- Direct inoculation of skin

Cryptococcus and Imaging Studies

- Pulmonary
  - Pulmonary nodules or masses; range 1-10
    - 6.0 mm to >3.0 cm
    - Homogenous on CT with low attenuation, calcification and rarely cavitation
  - Segmental or lobar consolidation
  - Reticulonodular (diffuse interstitial)
  - Mediastinal or hilar adenopathy
  - Pleural effusion
- Bone
  - Osteolytic lesions (long bones, pelvis, vertebrae and ribs)
Cryptococcus

- Pleomorphic yeast 2-20 microns
- Mucopolysaccharide capsule
- Capsule deficient variants
  - Associated with false negative Cryptococcal Antigen tests
- Narrow based budding
- Rare
  - Pseudohyphae
  - Branched septated hyphae
  - "germ tube" formation
- Cryptococcus neoformans and C. gattii are histologically identical
Microbiology

- Monomorphic yeast
- Most bacterial and fungal culture media (Sabourauds and 5% sheep blood agar) 2-7 days
- Isolates confirms by demonstrating the production of caffeic acid
- Canavanine-glycine bromothymol blue agar (CGB)
  - C. gattii triggers a blue reaction
MUCICARMINE

Rhinosporidiosis
mucicarmine positive
Blastomyces reported to be occasionally weakly mucicarmine positive

Prototheca mucicarmine positive
Capsular deficient variant

Mucicarmine stain

Electron microscopy of Cryptococcus

Electron microscopy of Cryptococcus
Dematiaceous fungi stain positive for Fontana Masson
Cryptococcosis

- Identification important
  - C. neoformans and C. gattii are always a pathogen
  - Mucicarmine positive
  - Fontana Masson positive and will stain capsular deficient variant
- Culture positive in 2-7 days, average
- Emerging pathogen;
  - Cryptococcus gattii in immunocompetent hosts
  - Reported to be more aggressive
  - Predilection to form cryptococcomas

Emerging pathogen and travel

Differential diagnosis Summary

- Cryptococcus
  - Fontanna Masson and mucicarmine positive
- Sporotrichosis
  - Pleomorphic with oval and spherical
- Penicillium marneffei
  - Southeast or Far East Asia, has a single transverse septum
- Pneumocystis jiroveci
  - PAS negative
- Candida glabrata
  - Difficult to differentiate from H. capsulatum, but are amphiphilic on H&E and strongly gram +
- Histoplasma capsulatum
  - Mucicarmine negative
Summary

• *Cryptococcus gattii*
  – Emerging pathogen with an expanding geographic area and environmental niche
  – More common in immunocompetent patients that *C. neoformans*
  – Mostly isolated pulmonary disease
    • Frequently mistaken as a malignancy
  – Histologically identical to *C. neoformans*
  – Differentiated by culture or sequencing
  – Treatment: similar to *C. neoformans*
    • May have disparate susceptibilities to certain antifungal agents
    • May require longer antifungal treatment or surgical removal of cryptococcoma

CASE 2

• 35 year old male from Chicago and avid baseball fan traveled to Phoenix, Arizona in July 2011 to watch the Homerun Derby and the Baseball All Star game

July 5th Phoenix Arizona
Arizona Dust Storm “Haboob”
Chase Stadium in Arizona

Arizona's Chase Field
Home of the Arizona Diamondbacks and 2011 Baseball All Star game

US Presswire, Chicago Tribune
After returning home… like the rest of the 47,994 attendees

- Several weeks later he had had a “flu” like illness
- He continued to feel tired and sought medical attention
- Chest xray showed a few pulmonary nodules
What to do?

- Aspirate material
  - Minimum 2-3 drops in a sterile container
  - Flush the needle with sterile saline and send fluid
  - Fungal culture and direct smear (calcofluor)
  - WARN Clinical microbiology lab of a possible coccidioidomycosis
- Cell block
  - Special stains
    - Fungal: GMS, PAS, Mucicarmine and Fontana-Masson
    - Molecular studies if necessary

Intermediate to Larger yeast structures

- Coccidiomycosis
- Paracoccidiomycosis
- Blastomycosis
- Cryptococcus
- *Histoplasmosis duboisii*
Differential Diagnosis of Intermediate to large Fungal Yeast / Spherules

- Chicago’s Disease
  - Blastomyces dermatitidis
- Valley Fever
  - Coccidioides immitis / posadasii
- South American Blastomycosis
  - Paracoccidioides brasiliensis
- Cryptococcosis
  - Cryptococcus neoformans / gattii
- African Histoplasmosis
  - Histoplasma duboisii

Blastomycosis

- Blastomyces dermatitidis
- Upper Great Lakes, lower Mississippi Valley, Southeast United States
- Canada, India, Israel, Saudi Arabia, and Africa
- Inhalation of spores aerosolized from the soil or rotting wood
- Rare: primary cutaneous infection
  - Accidental inoculation of spore (microbiologists and pathologists)
  - Following dog bites

Blastomycosis

- Asymptomatic spontaneously healing pneumonia
- Localized in lung
  - Severe pneumonitis (ARDS)
  - Pulmonary nodules “coin lesion”
- Disseminate
  - Skin
  - Bone, genitourinary, larynx, joints, CNS, lymph nodes, heart, adrenal
Coccidioidomycosis

- United States
- Argentina, Brazil, Colombia, Guatemala, Honduras, Mexico, Nicaragua, Paraguay and Venezuela

Coccidioidomycosis

- *Coccidioides immitis*
- *Coccidioides posadasii*
- Inhalation of arthroconidia from desert soil and dust
  - Same form that is grown in clinical microbiology lab (Biosafety level 3)
- Infections often follow earthquakes and dust storms and activities that disturb the ground
- **Only fungi on the “Select Agent Rule” enacted in the United States to track potentially hazardous agents**

Coccidioidomycosis: At risk population

- Exposure
  - 47,994 baseball fans at the All Star Game
  - Construction, landscaping, mining, agriculture
  - Archaeological excavation
  - Military maneuvers
  - Recreational activities; dirt biking
  - Donor-related transmission
  - Exposure to dirt/dust from endemic areas
- Dissemination
  - Immunosuppressed patients: HIV, transplants, tx with corticosteroids or tumor necrosis factor
  - African Americans, Filipinos, Pregnant women 3rd trimester
Coccidioidomycosis

- Pulmonary
  - Asymptomatic
  - Acute primary pulmonary coccidioidomycosis
  - Residual
    - Fibrocaseous nodules (Coccidiomomas)
    - Fibrocavitary nodules
- Disseminated
  - CNS, Bone, joints, soft tissues, rare peritoneum and GI
- Skin
  - Mostly likely disseminated disease
  - Rare primary cutaneous disease
Life cycle of Coccidioides

Paracoccidioidomycosis

- Latin America countries and some Caribbean islands
- *Paracoccidioides brasiliensis*
- Inhalation of conidia
- Pulmonary
  - Acute/subacute and chronic
- Skin
- Disseminated
  - Oropharyngeal mucosa, larynx, spleen, liver, adrenal glands, lymph nodes, intestinal tract, kidneys and bones
Paracoccidioidomycosis

• Acute pulmonary
  – Seen in children
  – Bronchopneumonia
  – Associated lymph node complex

• Chronic pulmonary
  – Interstitial fibrosis, fibrocaseous and necrotic granulomas
  – Solitary residual nodules “coin lesions” from acute disease
**Histoplasma duboisii**

- Endemic in Central and West Africa
  - Three cases reported from France in HIV+ patients from Democratic Republic of Congo
- Ecology and pathogenesis is poorly understood
- Co-exists with *Histoplasma capsulatum* in some African areas

Louengue, P et al, Literature Review and Case Histories of Histoplasma capsulatum var. duboisii Infections in HIV-infected Patients, Emerging Infectious Diseases, Vol 12, No 11, Nov 2007

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**Histoplasma duboisii**

Chandler F et al, Pathologic Diagnosis of Fungal Infections, ASCP Press 1987
**Histoplasma duboisii**

- Pulmonary lesions uncommon
- Chronic localized infections
  - Skin most common, papules or nodules that ulcerate or form abscesses
  - Bone; any bone osteolysis and osteomyelitis
- Rapid progressive and disseminated form
  - Fever, anemia, weight loss
  - Hepatosplenomegaly, and lymphadenopathy
  - Liver, spleen, intestine

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**Histoplasma duboisii**

- Uncommon
- In culture; indistinguishable from *H. capsulatum*
- Very rare, but can be confused with *Blastomyces dermatitidis* in tissue
- Treatment is extrapolated from the treatment used for *Histoplasma capsulatum*

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**Intermediate to Larger yeast structures**

- Coccidioidomycosis
  - Serologic studies
  - Culture is a laboratory hazard
- Paracoccidioidomycosis
  - History of travel to South America
- Blastomyces
  - Urinary antigen
- Cryptococcus
  - Serum antigen studies
- *Histoplasmosis duboisii*
  - Usually bone,
  - History of travel to Africa
  - Uninucleate
Why does this matter?

<table>
<thead>
<tr>
<th>Organism</th>
<th>Treatment</th>
<th>Alternate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coccidioidomycosis</td>
<td>Itraconazole</td>
<td>Fluconazole</td>
</tr>
<tr>
<td>Paracoccidioidomycosis</td>
<td>Trimethoprim/sulfmethoxazole</td>
<td>Itraconazole</td>
</tr>
<tr>
<td>Blastomycesis</td>
<td>Itraconazole</td>
<td></td>
</tr>
<tr>
<td>Cryptococcus</td>
<td>Fluconazole</td>
<td>Ampho B</td>
</tr>
<tr>
<td>Histoplasmosis duboissii</td>
<td>Ampho B</td>
<td>Itraconazole</td>
</tr>
</tbody>
</table>

Treatment for uncomplicated pulmonary disease at LUMC per ID physicians.
Case 3

- 50 year old male presents with respiratory distress to the ER
- Recently treated for community acquired pneumonia
- History of travel to a pathology conference in Las Vegas
- Chest X-ray shows bilateral interstitial infiltrates
- BAL performed

BAL of lung
Viral Inclusions

- Identification
- Immunohistochemical stains
  - Cytomegalovirus
  - HSV 1 and 2
  - VZV
  - Adenovirus
  - Respiratory syncytial virus

Does identifying the virus matter?

- Most care is supportive
  - Chicken soup
  - Sick leave
- Not many antiviral drugs available anyway
Antiviral treatment of severe viral pneumonia

<table>
<thead>
<tr>
<th>Viral Infection</th>
<th>Antiviral Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A and B</td>
<td>Oseltamivir (oral), zanamivir (inhalation, IV); peramivir (IV)</td>
</tr>
<tr>
<td>Influenza A</td>
<td>Amantadine (oral) rimantadine (oral)</td>
</tr>
<tr>
<td>RSV</td>
<td>Ribavirin (inhalation, IV)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Cidofovir (IV)</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>Pleconaril* (compassionate cases)</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>Pleconaril* (compassionate cases)</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>Ribivirin (IV)</td>
</tr>
<tr>
<td>Hantavirus</td>
<td>Ribivirin (IV)</td>
</tr>
<tr>
<td>VZV</td>
<td>Aciclovir (IV)</td>
</tr>
</tbody>
</table>

Pulmonary Viral infections

Viral Cytopathic Changes

- Clinical Microbiology
  - Viral induced damage to the monolayer of cells in a viral culture
    - Rounding, swollen, shrunken, granular, glassy, multinucleated/syncytial cells
- Surgical and Cytopathology
  - Multinucleated cells
  - Inclusions
    - Nuclear, cytoplasmic or both
Pulmonary Viral Infections

- Community acquired infections
  - Pediatric and adult populations
  - Immunocompromised / transplant population

- Global Travel
  - Unusual Viral Infections

Viral Community Acquired Pneumonia

- Respiratory syncytial virus
- Human metapneumovirus
- Parainfluenza
- Adenovirus
- Varicella-zoster
- Herpes simplex
- Cytomegalovirus
- Measles
- Rhinovirus
- Influenza A and B
- Human bocavirus
- Coronavirus
- Enterovirus
- Hantavirus
- Parechovirus
- Epstein-Barr virus
- Human herpesvirus 6 and 7
- Mimivirus

Global Travel Viral Pneumonias

- Severe Acute Respiratory Syndrome (SARS) coronavirus
- Influenza A (H5N1, H1N1)
- Hantavirus pulmonary Syndrome
- Hemorrhagic fever viruses
Viral Pneumonias

**Viral Inclusions**

- Respiratory syncytial virus
- Human metapneumovirus
- Parainfluenza
- Adenovirus
- Varicella-zoster
- Herpes simplex
- Cytomegalovirus
- Measles

**No Viral Inclusions**

- Rhinovirus
- Influenza A and B
- Human bocavirus
- SARS / Coronavirus
- Enterovirus
- Hantavirus
- Parechovirus
- Epstein-Barr virus
- Human herpesvirus 6 & 7
- Mimivirus
- Viral hemorrhagic fevers

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**Viral Inclusions**

- Single Nucleated cells
- Multinucleated cells

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**Inclusions**

- Respiratory syncytial virus
- Human metapneumovirus
- Parainfluenza
- Adenovirus
- HSV / VZ
- CMV
- Measles

**Single Nucleated cells**

- Adenovirus
- CMV

**Multinucleated cells**

- Respiratory syncytial virus
- Human metapneumovirus
- Parainfluenza
- HSV / VZ
- CMV (rarely)
- Measles
INCLUSIONS IN SINGLE NUCLEATED CELLS

- Adenovirus
  - Nuclear
- CMV
  - Cytoplasmic
- CMV
  - Both
Adenovirus

Adenovirus IHC

INCLUSIONS IN SINGLE NUCLEATED CELLS

- Adenovirus
- CMV

Nuclear
Cytoplasmic
Both
Inclusions in Multinucleated Cells

- HSV/VZV
- RSV
- Parainfluenza
- CMV
- Measles
- Human metapneumovirus

Nuclear  Cytoplasmic  Both
Inclusions in Multinucleated cells

- HSV
- VZV
- RSV
- Parainfluenza
- CMV
- Measles
- Human metapneumovirus

- Nuclear
- Cytoplasmic
- Both

**RSV case**

- Four month old infant premie
- Nasopharyngeal aspirates were negative for RSV x 2
- Viral cultures of lungs done at autopsy grew RSV
Inclusions in Multinucleated cells

- HSV
- VZV
- RSV
- Parainfluenza
- CMV
- Measles
- Human metapneumovirus

Nuclear
Cytoplasmic
Both

Measles
The figure above shows the cumulative number of measles cases reported, by month of rash onset, in the United States during 2001-2011. During January 1-May 20, 2011, a total of 118 cases were reported, the highest number reported for the same period since 1996.

CDC MMWR May 27, 2011/60(20);666-668

The figure above shows the distribution and origin of reported measles cases (N = 118) in the United States during January 1-May 20, 2011.

CDC MMWR May 27, 2011/60(20);666-668
Human Metapneumovirus

- Humans only known natural host
- Presumably spread respiratory droplets
- Worldwide; North and South America, Europe, Asia, Africa and Australia
- Seasonal, simultaneous with or slightly later than RSV
- 5-10% of the lower respiratory tract infection in infants
- Peak age 5-22 months, can infect young health adults and older adults in long term health facilities

Human metapneumovirus

- Lower respiratory tract infection
  - Bronchiolitis, pneumonia, asthma exacerbation, croup
  - Exacerbation of COPD in adults
  - Severe disease in immunocompromised
- Upper respiratory tract infection
  - Fever, coryza, cough, rhinitis, pharyngitis, otalgia and abnormal tympanic membranes

Human metapneumovirus diagnosis

- Viral respiratory shell vial cultures
  - Typically: Influenza A and B, RSV, Parainfluenza 1,2,3 and adenovirus
  - Monoclonal antibody specific for hMPV

- Immunofluorescent staining of respiratory secretions

- Real time RT-PCR FDA approved
  - Higher sensitivity than culture and IFA
  - Nasopharyngeal aspirates and BAL specimens
  - xTAG Respiratory Viral Panel
    - Luminex, Austin, TX USA
    - proHMPV+ assay
    - Gen-Probe Prodesse, Waukesha, WI, USA

Cytomegalovirus

Double CMV
CMV pneumonia in Immunocompetent hosts

- First described in 1968
- Uncommon, but recognized more frequently
- Severe viral CAP in immunocompetent hosts
  - Influenza (human, avian and swine)
  - Adenovirus
  - CMV
- Culture; may take up to 21 days
- Serologic tests; IgM and IgG
- CMV antigen assays
- CMV PCR


Viral Community Acquired Pneumonia

- Respiratory syncytial virus
- Human metapneumovirus
- Parainfluenza
- Adenovirus
- Varicella-zoster
- Herpes simplex
- Cytomegalovirus
- Measles
- Rhinovirus
- Influenza A and B
- Human bocavirus
- Coronavirus
- Enterovirus
- Hantavirus
- Parechovirus
- Epstein-Barr virus
- Human herpesvirus 6 and 7
- Mimivirus


A near “cat” tastrophie
Case 4
Clinical History

- 36 year-old woman with no prior history of liver disease presents with fever and abdominal pain for five days.
- MRI showed a 2 cm lesion, thought to be an abscess.
- FNA of lesion performed.

FNA of liver lesion

FNA of liver lesion
Recommendations for limited sample

- Culture
  - Bacterial aerobic and anaerobic
  - Fungal
  - Mycobacterial
- Smears

Chocolate agar  Anaerobic blood  Non-select fungal media  Mycobacterial broth media

Clinical course

- Treated with a course of antibiotics
- Two months later, presents with worsening right upper quadrant pain and fever.
- Imaging studies showed a 4.8 cm mass, an abscess favored

Surgery: Right hepatectomy
Symptoms recur after surgery

- Fever, pain and nausea and vomiting
- Extensive infectious disease workup begun
- Pancultures negative
- 3.3 cm lesion at surgical site
- New lesions in right and left lobe, largest 4.1 cm

<table>
<thead>
<tr>
<th>Test</th>
<th>Reference Range</th>
<th>Result Admission 1</th>
<th>Result Admission 2</th>
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</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>150-200 mg/dL</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Platelets</td>
<td>150-450 x 10^9/L</td>
<td>Negative</td>
<td>Negative</td>
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<tr>
<td>Prothrombin Time</td>
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<tr>
<td>Extensive Infectious Disease Workup Begun</td>
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<td>Pancultures Negative</td>
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</tbody>
</table>

Granulomatous Hepatitis...get out
“The Big List”

- Infectious Diseases
  - Bacterial
  - Mycobacterial
  - Rickettsial
  - Chlamydial
  - Fungal
  - Viral
  - Parasitic
- Hypersensitivity
- Immunologic Diseases
- Foreign Materials
- Neoplasms
- Miscellaneous

Granulomatous hepatitis: Infectious Diseases

**Bacterial**
- Actinomycosis
- Borrelia
- Botryomycosis
- Brucellosis
- Cat-scratch disease
- Granuloma inguinale
- Yersinia

**Mycobacterial**
- Tuberculosis
- Atypical mycobacteria
- BCG immunization and tox
- Leptospirosis

**Rickettsial**
- Boutonneuse
- Q fever
- Rochalimaea conorii

**Chlamydial**
- Lymphopathia venerum
- Psittacosis


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Granulomatous Hepatitis: Infectious Disease

**Fungal**
- Aspergillosis
- Blastomycosis
- Candidiasis
- Coccidioidomycosis
- Cryptococcus

**Parasitic**
- Amoebiasis
- Ancylostomiasis
- Capillariasis
- Enterobius vermicularis infection
- Fascioliasis
- Giardiasis
- Linguatula serrata
- Paragonimiasis
- Opisthorchiasis
- Schistosomiasis
- Toxocariasis
- Visceral leishmaniasis


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<table>
<thead>
<tr>
<th>Infectious</th>
<th>Bacterial</th>
<th>Fungal</th>
<th>Parasitic</th>
<th>Viral</th>
<th>Fatty liver disease</th>
<th>Primary biliary cirrhosis</th>
<th>Autoimmune liver disease</th>
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<tr>
<td>Actinomycosis</td>
<td>Brucellosis</td>
<td>Candidiasis</td>
<td>Amoebiasis</td>
<td>CMV infection</td>
<td>Autoimmune</td>
<td>Hepatitis A, B, C</td>
<td>Cytomegalovirus</td>
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<td>Botryomycosis</td>
<td>Coccidioidomycosis</td>
<td>Ancylostomiasis</td>
<td>EBV-infected</td>
<td>Drug reaction</td>
<td>Ebola</td>
<td>Epstein-Barr virus</td>
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<td>Botryomycosis</td>
<td>Brucellosis</td>
<td>Cryptococcus</td>
<td>Capillariasis</td>
<td>EBV-infectious mono</td>
<td>Foreign body reaction</td>
<td>Ebola hemorrhagic fever</td>
<td>Erythema infectiosum</td>
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<td>Tuberculosis</td>
<td>Fascioliasis</td>
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<td>Yersinia</td>
<td>Tuberculosis</td>
<td>Tuberculosis</td>
<td>Paragonimiasis</td>
<td>Enterobius vermicularis</td>
<td>Foreign body reaction</td>
<td>Ebola hemorrhagic fever</td>
<td>Erythema infectiosum</td>
</tr>
<tr>
<td>Yersinia</td>
<td>Tuberculosis</td>
<td>Tuberculosis</td>
<td>Opisthorchiasis</td>
<td>Enterobius vermicularis</td>
<td>Foreign body reaction</td>
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<tr>
<td>Yersinia</td>
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<td>Tuberculosis</td>
<td>Schistosomiasis</td>
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<td>Foreign body reaction</td>
<td>Ebola hemorrhagic fever</td>
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<tr>
<td>Yersinia</td>
<td>Tuberculosis</td>
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<td>Toxocariasis</td>
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<td>Foreign body reaction</td>
<td>Ebola hemorrhagic fever</td>
<td>Erythema infectiosum</td>
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<td>Yersinia</td>
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<td>Tuberculosis</td>
<td>Visceral leishmaniasis</td>
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<td>Foreign body reaction</td>
<td>Ebola hemorrhagic fever</td>
<td>Erythema infectiosum</td>
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<tr>
<td>Yersinia</td>
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<td>Tuberculosis</td>
<td>Autoimmune liver disease</td>
<td>Enterobius vermicularis</td>
<td>Foreign body reaction</td>
<td>Ebola hemorrhagic fever</td>
<td>Erythema infectiosum</td>
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<tr>
<td>Yersinia</td>
<td>Tuberculosis</td>
<td>Tuberculosis</td>
<td>Other</td>
<td>Enterobius vermicularis</td>
<td>Foreign body reaction</td>
<td>Ebola hemorrhagic fever</td>
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</tr>
<tr>
<td>Yersinia</td>
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<td>Enterobius vermicularis</td>
<td>Foreign body reaction</td>
<td>Ebola hemorrhagic fever</td>
<td>Erythema infectiosum</td>
</tr>
</tbody>
</table>
Molecular Studies

- **PCR positive for** *Bartonella henselae*

- **Remember....her serologic studies were negative** were negative for *Bartonella* and culture studies were negative

Classical Cat Scratch Disease

- Cat scratch disease (CSD) caused by *Bartonella henselae*, a fastidious, aerobic, Gram negative bacterium.
  - *Aflia felis* and *Bartonella quintana* rare causes
- Transmitted bite or scratch of a cat.
- Typical symptoms include fevers, malaise, weight loss, chills, headaches and lymphadenopathy.
- Hepatic bartonellosis has been reported in 0.3-2% of CSD cases
  - Typically multiple lesions and abdominal lymphadenopathy
**Diagnosis**

- Currently there is no "gold standard" for the diagnosis of bartonellosis
- Traditional diagnostic criteria:
  1. Contact with a cat and history of a scratch or other inoculation even
  2. Positive cat scratch skin test reaction
  3. Regional lymphadenopathy with no other apparent etiology
  4. Characteristic histopathologic features on biopsy


**Pathological Diagnosis**

- Stellate microabscesses with granulomatous inflammation are the histologic hallmark of Bartonella hepatitis.
- Peliosis hepatitis in HIV and immunocompromised patients
- Activated macrophages, with a surrounding rim of lymphocytes and fibroblasts.
- Single and clumps of pleomorphic bacilli seen on silver stain

**Diagnosis: Silver Stains**

- Variably detected
- Silver stains
  - Warthin Starry
  - Steiner
  - Dieterle
Silver impregnation stains

- Deposition of silver salts on cell walls
- In theory, all eubacteria and mycobacteria +
- Some controversy, but in general yield comparable results
- Weak gram reactive or non-gram reactive bacteria
- *Treponema, Borrelia, Bartonella, Leptospira and Calymmatobacterium spp*
- *Leigonella, Burkholderia, Francisella and Helicobacter spp*

Silver stain: Steiner

Silver stain: Dieterle
Silver stain on Gram stain control

Immunohistochemical

- Immunohistochemistry (IHC) monoclonal antibody reacts with a 43-kDa epitope.


Diagnosis: PCR

- PCR on paraffin embedded tissue
Comparison of PCR, IHC and silver stains

- 24 formalin-fixed paraffin embedded (FFPE) cases of lymphadenitis with histologic and/or clinical suspicion of CSD.

Control cases included 14 cases of lymphadenopathy other than CSD.


Comparison of Silver stain, IHC and PCR on paraffin embedded block

<table>
<thead>
<tr>
<th>Immunochemical Result</th>
<th>PCR-SSS-F (p&lt;0.01)</th>
<th>PCR-SSS-IF (p&lt;0.01)</th>
<th>PCR-SSS-IF (p&lt;0.01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (F)</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Negative (F)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Values indicate percentage of cases where Bartonella henselae was detected.


IHC vs SSS vs PCR

- Positive cases were as follows:
  - Silver stain: 11 (46%)
  - PCR: 9 (38%)
  - IHC: 6 (25%).

- Only 2 cases (8%) were positive for all 3 studies.

- Diagnostic sensitivity of these 3 tests is low for CSD.

- Silver stain seems to be the most sensitive test but is the least specific.

Diagnosis: Culture

- Conventional bacterial cultures are rarely diagnostic, as organism is fastidious, slow-growing, and difficult to culture.

- Culture methods include Chocolate or heart-infusion supplemented with 5% rabbit or horse blood.
  - Inoculated plates should be incubated in 5% CO2 at 35°C for at least three weeks.
  - Colonies are rough, cauliflower-like, and deeply embedded into agar.

- LUMC we do not culture for Bartonella, we send the specimens out for PCR studies

Serologic testing

- Studies show a 50% of bacteremic patients do not mount a measurable antibody titers.

- Causes:
  - Technical limitations
  - Sample handling
  - Subjective interpretation
  - Variations in seroprevalence
  - Genetic heterogeneity

- Regardless of the mechanism, our finding of repeatedly negative antibody testing for B. henselae and B. quintana in our patient illustrates the limitations of serologic testing.


Treatment

- Multiple courses of single-agent antimicrobial therapy, including
  - clarithromycin (9 weeks)
  - azithromycin (2 wks)
  - ciprofloxacin (6wks).

- Prednisone has been suggested as adjunctive therapy

- Surgical treatment maybe required in some cases.
Back to this patient

- Continues to have fevers, treating with antibiotics: azithromycin, and clindamycin.
- Repeat liver biopsy show small lipogranulomas.
- PCR was positive on liver bx and blood for B. henselae.

Summary

- This case illustrates the challenges of establishing the diagnosis of B. henselae-induced granulomatous hepatitis.
- Silver stains may/may not reveal the organism.
- Serologic tests may be negative.
- Conventional bacterial cultures are unlikely to identify this fastidious organism.
- PCR studies can aid in making the diagnosis for hepatic bartonellosis.

CASE 5

- 63 year old male s/p right lower lung lobectomy for large cell carcinoma, size 2.3 cm, pT1 N0
- One year later LUL mass identified
  - FNA positive for tumor
  - Treated with stereotactic radiotherapy
Clinical course

- Mass continues to grow despite radiotherapy
- Work up shows a cluster of PET positive nodules in the left upper lobe
- No other PET positive lesions
- Patient undergoes resection of left upper lobe
Fused PET and CT scan

PET scan

- Positron emission tomography
- Short lived radioactive tracer
- \(^{18}F\) fluorodeoxyglucose
- Maximum standardized uptake value \([\text{SUV}_{\text{max}}]\) = extent of radiotracer uptake
  - SUV of 2.0 commonly used to differentiate between benign and malignant tumors
- PET used for treatment decisions
  - 36.5% change in treatment
  - 38.0% change in treatment (30% from nontreatment to treatment)

False positive PET scans with elevated SUV

Tumors
- Hibernomas
- Fibrous dysplasia
- Neurofibroma
- Schwannoma
- Desmoid
- Giant cell tumor of tendon sheath
- Villonodular synovitis

Non tumorous lesions
- Sarcoïdosis
- Mycobacterial
- Fungal
- Bacterial
- Radiation pneumonitis
- Post operative surgical procedures
- Pneumoconiosis
- Other granulomatous diseases

False negative PET scans

- Tumors with low metabolic activity
  – Bronchiolalveolar carcinoma
  – Carcinoid tumors
  – Low grade lymphomas
  – adenomas

Case 5 clinical course

- Left upper lobectomy
- Frozen section biopsies of mass and bronchial margin
- N4, N7, N9, N10 and N11 lymph node resection
**Acid fast positive organisms in processed specimens**

**Acid fast**
- all *Mycobacterium*, except *M. lepra*
- *Nocardi a sp* (occasionally)
- Hooklets of *Echinococcus*

**Modified acid fast**
- *Mycobacterium lepra*
- *Nocardia sp*
- *Rodococcus*
- *Legionella micdadei*
- Shell and spine of *Schistosoma mansoni*

*Peanut oil and/or weaker acid alcohol*

**Carbolfuchsin Acid Fast Stains**

- **Acid Fast stain**
  - Ziehl-Neelsen
  - Kinyoun
    - HCL acid alcohol
- **Modified acid fast stains**
  - Fite
    - Peanut oil
    - HCL acid alcohol
  - Coates Fite
    - Peanut oil
    - H$_2$SO$_4$ acid alcohol

*Laboratory Methods in Histotechnology, AFIP, 1992, editor Prophet, 3 et al.*
Back to our patient

• Granulomatous lung mass associated with many acid fast positive bacillary organisms
• Patient placed in isolation
• Cryostat decontaminated
• Infection control contacted for possible exposure testing
• Patient: QuantiFERON gold test - negative
Pulmonary Mycobacterial Infections - Past

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td>• Tuberculin skin test (TST)</td>
</tr>
<tr>
<td>Non tuberculosis</td>
<td>• Smear</td>
</tr>
<tr>
<td>• MAI</td>
<td>• Auramine-rhodamine</td>
</tr>
<tr>
<td>• Rapid growers</td>
<td>• AFB stain (Kinyon or Ziehl-Neelsen)</td>
</tr>
<tr>
<td>• Miscellaneous</td>
<td>• Culture</td>
</tr>
<tr>
<td>• Contaminants</td>
<td>• Lowenstein-Jensen slants</td>
</tr>
<tr>
<td></td>
<td>• Broth culture</td>
</tr>
<tr>
<td></td>
<td>• Biochemicals test for identification</td>
</tr>
</tbody>
</table>

Pulmonary Mycobacterial Infections - Present

<table>
<thead>
<tr>
<th>Organism</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. tuberculosis complex</td>
<td>• Interferon- gamma release assays</td>
</tr>
<tr>
<td>M. avium-intracellular complex</td>
<td>• Tuberculin skin test (TST)</td>
</tr>
<tr>
<td>M. kansasii</td>
<td>• AFB smear</td>
</tr>
<tr>
<td>Rapid growers: M. abscessus, M. chelonae, M. fortuitum</td>
<td>• Direct specimen testing</td>
</tr>
<tr>
<td>M. xenopi</td>
<td>• Culture and probe ID</td>
</tr>
<tr>
<td>M. szulgai</td>
<td>• PCR on paraffin embedded tissue block</td>
</tr>
<tr>
<td>M. simiae</td>
<td>• Antimicrobial Sensitivity testing</td>
</tr>
<tr>
<td>others</td>
<td></td>
</tr>
</tbody>
</table>

Interferon gamma release assay

• QuantiFERON Gold In-tube test (QFT-GIT)
• T-SPOT. TB test
  • Blood is incubated with Mtb antigens, with mitogen (control) and with no antigen
  • Mtb antigens; ESAT-6, CFP10 (and TB7.7 in QFT-GIT)
  • T-lymphocytes in individuals with M. tuberculosis will be stimulated to produce cytokine gamma interferon
  • ELISA test measures interferon gamma produced (QFT-GIT)
  • T-SPOT counts the number of activated T lymphocytes that secrete interferon γ. Enzyme linked immunospot assay
**Tuberculin skin test vs Interferon gamma release assays**

<table>
<thead>
<tr>
<th>TEST</th>
<th>SENSITIVITY (pooled Head to Head studies)</th>
<th>SPECIFICITY (pooled studies)</th>
<th>SPECIFICITY (pooled studies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculin skin test</td>
<td>95%</td>
<td>85%</td>
<td>86%</td>
</tr>
<tr>
<td>T-SPOT.TB test</td>
<td>91%</td>
<td>88%</td>
<td></td>
</tr>
<tr>
<td>QuantiFERON-TB Gold In-tube</td>
<td>84%</td>
<td>99%</td>
<td></td>
</tr>
</tbody>
</table>


**Interferon Gamma Release Assays**

- An IGRA may be used in place of a tuberculin skin test in all situations in which CDC recommend tuberculin skin testing as an aid in diagnosing *M. tuberculosis* infection with some preferences and special considerations.

- IGRA preferred
  - Patients with a history of low rates of returning to have TSTs read (ie; homeless and drug users)
  - Patients who have received BCG (vaccine or cancer therapy)

- TST preferred
  - Testing children < 5 years of age


**Identification**

- Tissue block sent for PCR analysis (6 days)
  - *Mycobacterium xenopi* DNA detected
  - NO *M. tuberculosis complex or M. avium complex* DNA detected

- Culture studies: (+MAC DNA probe 20 days, culture 31 days)
  - Lung tissue: *Mycobacterium avium intracellular complex*
  - Sputum: *Mycobacterium avium intracellular complex*
What happened?

• Mixed infections
  – PCR identified only *M. xenopi*
  – Culture grew only *M. avium intracelluar complex*

• PCR
  – Formalin fixation degrades DNA

• Clinical microbiology
  – *M. xenopi* can be missed in nonradiometric systems if bacterial count is low

Summary

• Not all PET positive lesions are malignant, even in patients with known malignancies
• Different acid fast stains
• Interferon gamma studies are slowly replacing the tuberculin skin tests
• Non tuberculosis mycobacterium can cause significant pulmonary disease
• PCR studies are available, culture is still important

CASE 6

• 40 year old male and former member of Army special forces presents with cutaneous lesions and was found to have splenomegaly
• FNA of spleen performed
FNA of Spleen

FNA of spleen

- Most reports from outside United States
- Safety and risks are controversial
- Indications
  - Metastatic diseases
  - Hematologic malignancies
  - Infectious diseases
    - Leishmaniasis
    - Tuberculosis
    - Abscess organisms
    - Other
Recent Military Conflicts

- Afghanistan (Operation Enduring Freedom)
  - October 7, 2001
- Iraq (Operation Iraqi Freedom)
  - March 20, 2003
- Military deployment
  - Multiple rotations to the area
- Others
  - Civilian contractors employed by US government

Emerging Infectious Diseases – U.S. Service Members in Afghanistan and Iraq

- Brucellosis
- Crimean Congo hemorrhagic fever
- Dengue fever
- Hepatitis E
- Leishmaniasis (cutaneous and visceral)
- Leptospirosis
- Malaria (Plasmodium vivax and P. falciparum)
- Pertussis
- Plague
- Q fever
- Sandfly fever
- Multidrug resistant tuberculosis
- Typhoid fever
- Murine typhus
- Wound infections by multidrug resistant gram negative bacteria

Gaydos J, et al, A Roundtable Discussion on Emerging Infectious Disease – Risks to U.S. Service Members in Afghanistan and Iraq, Military Medicine, Vol 175, Dec 2010
Patient

- In addition, a skin punch biopsy of lesion was performed with a clinical history....
  R/O Leishmaniasis
Leishmaniasis

- Bite from an infected Sandfly
  - Rare reports via blood transfusion, transplacental, contaminated needles and organ transplant
- Promastigotes travel to phagocytic cells of the reticuloendothelial system and transform into the amastigote form
- Clinical spectrum

Leishmaniasis: Protozoan with two separate lives

- Promastigote stage
  - Sandfly
  - Tissue culture
- Amastigote stage
  - In humans or animal reservoir
  - Aflagellar form

Cutaneous Leishmaniasis

New World
- *Leishmania amazonensis*
- *Leishmania mexicana*
- *Leishmania venezuelensis*

Old World
- *Leishmania major*
- *Leishmania tropica*
- *Leishmania aethiopica*

*Image library CDC*
Cutaneous Leishmaniasis

- **Acute**
  - Nodules that ulceration
  - Flat plaques hyperkeratotic and wart like lesions

- **Chronic**
  - Single or multiple, raised non-ulcerated plaques

- **Recidivous**
  - Papules in proximity to scar of healed lesions
  - *L. tropica*

- **Disseminated**
  - Macules and nodules
  - Anergic individual

- **HIV**
  - Wide variation of lesions
  - Immune reconstitution inflammatory syndrome

Cutaneous Pathology

- **Acute**
  - Hyperkeratosis, acanthosis, or atrophy
  - Progressive pseudoepltheliomatous hyperplasia
  - Plasma cells, lymphocytes, parasitized macrophages, eosinophils
  - Amastigotes

- **Chronic**
  - Tuberculoid, noncaseating granulomas
  - Rare amastigotes

- **Recidivous**
  - Resembles lupus vulgaris with tubercles surrounded by histiocytes
  - Amastigotes are hard to find

- **Disseminated**
  - Sheets of amastigotes macrophages
Mucocutaneous

http://pathmicro.med.sc.edu/parasitology/bloodproto.htm
http://www.uq.edu.edu/depts/id/espundia.jpg

Mucocutaneous pathology

- Mucocutaneous
  - Ulceration and adjacent pseudoepitheliomatous hyperplasia and granulation tissue reaction
  - Granulomatous inflammation with lymphocytes, plasma cells and histiocytes
  - Few parasitized macrophages

Visceral Leishmaniasis / Post kala-azar dermal leishmaniasis

Old World
- Leishmaniasis donovani
- Leishmaniasis infantum

New World
- Leishmaniasis chagasi
Leishmaniasis

• **Visceral – kala-azar**
  - 2-8 month incubation (rarely up to 2 years)
  - Fever, fatigue, weakness, loss of weight, anemia
  - Enlarged lymph nodes, spleen and liver
  - Other: diarrhea, cough, bleeding
  - Chronic disease evolving over several month to years

• **Post kala-azar dermal leishmaniasis**
  - Papular then nodular skin lesions beginning on face then whole body
  - Contain numerous parasites
  - Important role in sandfly transmission and dissemination of parasite
  - Follows visceral disease

Pathology

• **Visceral**
  - Parasites in macrophages in
    - Bone marrow, spleen, liver, lymph nodes
    - Intestine and lung
  - Immunosuppressed / advanced cases
  - all organs can be involved

Pathology: Leishmaniasis amastigote
Differential diagnosis: Histoplasmosis

Leishmaniasis

Histoplasmosis

Differential diagnosis: Toxoplasmosis
Differential diagnosis: Trypanosomiasis
Immunohistochemical stain for Leishmaniasis

Richard L. Kradin, MD, Diagnostic Pathology of Infectious Disease, Figure 19-110. Cutaneous leishmaniasis (immunoperoxidase stain): Another example of the so-called marquee sign. (Courtesy of Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

CDC Leishmaniasis services “gratis”

- Parasitic Disease Branch Frank Steurer 404-718-4175
- Examination of slides (biopsy specimens, impression smear and dermal scrapings)
- Leishmanial culture medium
- Culturing (with isoenzyme analysis) & PCR for diagnosis of leishmaniasis and species identification (cultures held for 4 weeks)
- Serologic testing for anti-leishmaniasis antibodies (visceral, HIV negative and some cases of mucocutaneous)

CDC Leishmaniasis algorithm

http://www.cdc.gov/parasites/leishmaniasis/health_professionals/index.html
Types of specimens

• Skin biopsy: youngest, most active and least superinfected skin lesion
  – Fresh
    • Leishmanial culture medium (culture and PCR)
    • Bacterial, fungal and mycobacterial culture
    • Impression smears
  – Formalin fixed
    • H&E
    • Giemsa
    • AFB and GMS

• Aspirate:
  – Place in leishmanial culture medium or sterile tube

• Aspirate: Spleen, lymph node, bone marrow
  – Contact CDC
    • Frank Steurer 404-718-4175
    • Parasite Diseases Branch

• Dermal scraping
  – Make thin smear, fix in methanol, stain with giemsa

Types of specimen

• Aspirate: skin
  – Place in leishmanial culture medium or sterile tube

• Aspirate: Spleen, lymph node, bone marrow
  – Contact CDC
    • Frank Steurer 404-718-4175
    • Parasite Diseases Branch

• Dermal scraping
  – Make thin smear, fix in methanol, stain with giemsa

Treatment

• Individualized according to country of acquisition and species
  – Pentavalent antimonial
    • Meglumine antimoniate
    • Sodium stibogluconate
  – Amphotericin B liposomal
  – Pentamidine
Leishmaniasis

- Increasing cases
  - Travel to endemic areas (even if it occurred several months to years earlier)
  - Adventure travel to endemic forest areas
  - Military personnel
  - Researchers
  - Refugees
  - Multinational contractors
  - Transplant or immunosuppressed patients with previous history leishmaniasis

Summary

- Pathologic examination and identification of Leishmaniasis amastigote
  - Aspirations of
    - Bone marrow
    - Spleen (most sensitive)
    - Lymph node
    - Infiltrative edge of skin lesion
  - Biopsy of skin or mucosal lesions
  - Scraping of involved skin
  - Unusual sites parasite found
    - CSF, normal skin, GI mucosa, BAL, pleural fluid

Summary

- Culture
  - Novy-MacNeal-Nicolle (NNN media), 24-26 degrees C, grows into promastigote stage 1-3 weeks
  - Scheider-Drosophila medium 1 week
  - Allow for species identification and drug sensitivity studies
  - Some organisms difficult to culture
Summary

• Molecular studies; PCR
  – Skin specimens
  – Culture specimens
  – Aspirate specimens

• Serologic tests
  – Diagnosis of visceral and some mucocutaneous diseases
  – HIV negative