Coccidioidomycosis:
Diagnostic Issues and Considerations

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Now reported few cases from high desert in SCentral Washington

Few spots in Utah

CAP caused by Coccidioidomycosis

- Overall, in three published studies, the rate of CAP due to VF was 7-29%

56 patients with pneumonia recruited from 2 primary care sites and 1 urgent care clinic in Tucson
- 19 had positive antibody tests for valley fever (29%)
- 81% got antibiotics; 31% got > 1 course

Coccidioidomycosis as a Common Cause of Community-acquired Pneumonia

Lisa Valdivia,† David Nix,‖ Mark Wright,‖ Elizabeth Lindberg,‖ Timothy Fagan,§
Donald Lieberman,§ TPrien Stoffer,‖ Neil M. Ampel,‖† and John N. Galgiani†

Emerging Infectious Diseases Vol 12 No 6, June 2006
Slide from R. Sunenshine,2008, AZDHS
Issues with Coccidioidomycosis in Arizona

• Although, 40% are symptomatic
• < 1/3 are clinically evaluated,
• It is estimated that only 8-10% of total infections are serologically confirmed
• Only serologically confirmed are reported to public health

Sunenshine, R. 08. AZ Dept. Health Services
Coccidioidomycosis

- Incubation 7-28 days
- Primary Pulmonary (asymptomatic to mild to severe; erythema multiforme or nodosum usually good prognostic signs)
- Disseminating
  - Respiratory: pulmonary or extrapulmonary (pleural, chest wall)
  - Extrapulmonary: lymphatic, cutaneous, subcutaneous, skeletal, CNS, cardiac, endocrine, ophthalmic, urogenital
Coccidioidal Lesion

Big Hearted
Dissemination to skin

Lymph node
Coccidioidomycosis
Spectrum of Disease

100 Infections

60 No Symptoms

40 Symptoms

37 Recover

3-4 Recur

2-4 Progress Disseminate

Life-Long Immunity
Issues of Clinical and Laboratory Diagnosis of Coccidioidomycosis

- Fatigue: 84.4%
- Cough: 66.9%
- Fever: 58.8%
- Night sweats: 54.0%
- Chest pain: 51.9%
- Chills: 48.7%
- Joint pain: 47.7%
- Headache: 47.3%
- Muscle pain: 42.4%
- Wheezing: 41.0%
- Rash: 37.5%
- Stiff neck: 33.9%
- Sore throat: 29.4%
- Weight loss: 27.8%
- Cough blood: 24.8%
- Type of Symptoms: Percentage of Cases with Symptoms

- Average Days to Diagnosis: 147
- Median Days to Diagnosis: 39

Galgiani et. al.
Patients at Higher Risk of Severe Disease

- Patients with deficiencies in cellular immunity.
- Small number of patients, otherwise seemingly normal, identified with specific gene mutations that alter immunologic responses involving interferon-γ, interleukin 12 (IL-12), and other cellular immune pathways that appear to be responsible for their progressive coccidioidal infections.
  - In such patients, risk of disseminated infection can be as high as 75% rather than ordinarily approximately a 1% risk.
- Certain ethnic groups (e.g. Filipino or African ancestry) - modest
- Pregnant women (especially in third trimester)

Galgiani, et al. CID 2016:63
Case 1: Pulmonary Presentation

- A 52 y/o caucasian male presented moderately ill with pneumonia: chest X-ray showed a unilateral infiltrate
- Sputum Gram-stain showed many WBCs and light oropharyngeal contamination/without any PO associated with WBCs
- Sputum culture grew light growth of oropharyngeal flora
- The patient was treated with ceftriaxone and erythromycin for two days and sent home in stable condition on oral levofloxacin.
Case 1: Pulmonary Presentation

- After initial improvement the patient continued to have fevers and showed persistence of the infiltrate.
- He was seen by a pulmonologist for further workup and underwent a BAL.
- Routine, fungal and AFB cultures failed to determine the etiology after 4 weeks incubtion.
- Coccy serologies were negative at 1, 2 and 3 weeks after initial presentation.
- After the 4th week the IMDF IgM turned positive and a week later both the IMDF IgG and the CF titer turned positive (at only 1:2).
- At 6 weeks the CF titer peaked at 1:4
Case 1: Pulmonary Presentation

- The patient began to defervesce without specific antifungal therapy and was seemingly normal after a total of ten weeks.
- The patient did well for a period of 4 years without any specific symptoms (but he did complain of tiredness and some night sweats; he had no fever and now had negative IMDF and CF serologies.
- Significantly, his SED rate continued to be elevated.
- After 4 years, the patient suddenly complained of pain in his ankle.
Case 1: Pulmonary Presentation

- He presented without any fever but with increased WBC count and some eosinophilia.
- A scan of his ankle revealed a localized osteomyelitis.
- His Coccy CF serologies were now at 1:2 and became 1:4 two weeks later.
- He had no new pulmonary infiltrates.
- Therapy with high dose fluconazole ameliorated the pain and reduced the CF titer to 1:2 three weeks later and negative at six weeks. He was kept on fluconazole for six months and showed no symptoms other than a continued elevated SED rate.
Case 1: Pulmonary Presentation

- His fluconazole was stopped after 8 months; within three weeks his ankle pain returned and his CF titer became elevated at 1:2 (Davis Lab).
- He was placed on fluconazole and remained on fluconazole until his death 15 years later from other unrelated causes.
Case 2: Presentation: One Sick Kitty

- 27 y/o female veterinarian assistant (VA) worked on a cat brought in by a good samaritan who had found the cat lost and wandering
- The cat looked very ill with fever and overall failure to thrive
- The cat bit the VA on the hand, causing puncture wounds on the back of the hand
- The cat died four days later
Case Presentation 2 (slide 2)

- The VA did well for about 12 days when the bite wound began to suppurate and looked infected.
- She was seen by her clinician and placed on augmentin.
- The wound did not respond to the medication and now progressed along the lymph glands to the elbow, with swelling, but no open sores except at the initial wound mark.
Case Presentation 2 (slide 3)

- Serologies ordered and were negative.
- Biopsy performed on hand lesion; submitted for diagnostic cultures (routine, AFB, Fungus) and accompanying stained preps.
- Autopsy performed on cat (vet): had disseminated disease, including lesions on gums.
- *Coccidioides* spp. isolated from the hand and spherules found at autopsy on cat.
- Pt. treated with fluconazole and did well.
- Serologies at 3 months remained negative.
Biopsy of hand lesion: wet mount prep by fluorescence (Calcofluor KOH)
Laboratory Diagnosis (especially in CAP)

• Most beneficial for sicker patients (may benefit most from Rx)

• Other benefits of Dx may include:
  – Avoidance of use of bacterial antimicrobics
  – Avoidance of use of corticosteroids
  – Earlier identification of complications
  – Decreased need for added expensive Dx studies
  – Reduction in patient anxiety

Coccidioidomycosis: General Laboratory Diagnosis

- **Hematologic**
  - elevated erythrocyte sedimentation rate
  - eosinophilia

- **Meningitis (CSF)**
  - variable overall increased cell count
  - predominance of lymphocytes over PMNs
  - low to moderate elevation of protein
  - moderate decline in glucose
Coccidioidomycosis: Laboratory Diagnosis

- Serologic Skin Testing (recently FDA approved)
- Direct
  - Microscopy (spherules; endospores; mycelial forms)
  - *Coccidioides* antigen EIA - urine, Bronchoalveolar Lavage (BAL), serum, CSF (MiraVista Labs) CID 2008;47:e69
  - RT-PCR
- Culture (average time to recovery 4 days (2-16 days))
- Serologic evaluation (serum, CSF, other body fluids)
Coccidioidomycosis: Specimens

For Microscopy, Culture

Respiratory Secretions
- Sputum
- Bronchoscopy (wash, biopsy)
- Bronchoalveolar lavage (BAL)

Normally Sterile Body Fluids
- Pleural, Peritoneal, CSF, Blood, Abscess material, etc

Tissues
- FNA, Biopsies: Lung (open), Brain, Skin, other organs (abscesses-see above)

For Serology

Serum, CSF, Synovial and Pleural fluids
Coccidioidomycosis: Laboratory Diagnosis

- **Direct Microscopy (spherules; mycelial forms)**
  - KOH Wetmount
  - Calcofluor White Fluorescent Stain (with KOH)
    - At LSA Calcofluor stain sensitivity was 22% overall
  - Histopathologic Stains
    - Methenamine Silver stain (primary histopathologic)
    - Hematoxylin-Eosin (H&E), Periodic Acid Schiff (PAS), PAP Smear
Calcofluor White fluorescent stain X450

Gram stain with KOH x450

Spherules with endospores
Pulmonary tissue GMS stain - 1000 X Magnification

Low power magnification
Jim Dunn
Calcofluor of stool specimen
1000 X

Coccy spherules ??
Calcofluor of stool specimen
1000 X
Case Presentation from NY

- 39 y/o female with syncopy and 1 month history of decreased energy with occasional nausea and dyspnea
- Resided only in NY – rarely traveled
- Visited Israel 4 months before presentation: experienced self-limited fever, without pulmonary symptoms
- History of bipolar disorder but without any known immunocompromised
- X-ray and CT showed RUL cavitary lesion (2.0 x 2.0 cm)
- Negative PPD, Coccy, Histo, Crypto, Aspergillus, mycoplasma and Legionella serologies
- WBC count within normal limits
- Sputum grew *M. avium* complex and *M. kansasii*
- Patient placed on azithro, rifampin and ethambutol for 1 yr
Case continued

- CT scans were performed every six months as follow-up
- Over six months, lesion in RUL evolved onto thick-walled cavitary speculated abscess; CT at 1 yr shown below
- FNA of lesion was performed

Original thin-walled cavitary lesion in the right upper lobe of the lung

1 year later a partially solid and partially lucent, spiculated lesion in the right upper lobe of the lung

Diff-Quik of the fine-needle aspirate of the right upper lobe lesion in the lung, demonstrating poorly staining hyphal forms
GMS stain of FNA material

Note: Patient had no pulmonary symptoms, was not treated and was followed by chest CT
Coccidioidomycosis: Lab Diagnosis by Culture

Saubolle and Sussland, unpublished LSA data, 2009

– Not difficult to grow in lab
– Grows on almost all fungal and bacterial agar and broth media
– Incubation time (ambient air, 30°C) for 2-3 days to several weeks (at LSA/SQL lab average time to recovery 4 days, variation 2-16 days)

• Recovery by culture within specimen type (1998-2003: 55,788 total):
  – Respiratory specimens (10,372 total; 861 positive - 8.3%)
  – Other non-sterile body sites (25,628 total; 648 positive - 2.5%)
  – Other sterile body sites (11,566 total; 246 positive - 2.1%);
  – Bone marrow (267 total; 7 positive - 2.6%)
  – CNS (2,280 total; 20 positive - 0.9%);
  – Blood (5,026 total- 20 positive - 0.4%)
  – Urinary tract (649 total; 4 positive - 0.6%)

Overall: Recovered in Fungus culture – 71%; Bacterial culture: 29%
Coccidioides spp. – Colony Morphology

- Early growth – usually gray, membranous, flat, convex, or umbonate
- Mature colony – widely variable, but most are white, floccose, variable texture
- Other morphologies observed
Coccidioidomycosis: Lab ID

Identification

- Microscopic morphology of spherules (presumptive) in specimen
- Genetic Probe (Gen-Probe, San Diego) rRNA (identifies both species but will not differentiate between them)
Two Primary Ab Responses

- **Immunoglobulin M (IgM)** – also known as Tube Precipitin
  - polysaccharide is Ag
  - Tube precipitin because older form of testing included precipitin formation in a glass or plastic tube when Coecy Ag was mixed with positive sera
  - measurable earlier in acute phase usually between the first (50%) and third (90%) weeks of onset.
- **Immunoglobulin G (IgG)** – also know as CF antibody
  - because older method of detection included Complement Fixation (CF) testing alone
  - chitinase is Ag
  - becomes measurable between the 2nd and 28th week post onset. May remain for several months but is usually related to disease activity
Comparison of EIA, Immunodiffusion and CF Studies

<table>
<thead>
<tr>
<th>Study method</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA IgG</td>
<td>79%</td>
</tr>
<tr>
<td>EIA IgM</td>
<td>63%</td>
</tr>
<tr>
<td>EIA Combined</td>
<td>83%</td>
</tr>
<tr>
<td>ID</td>
<td>71%</td>
</tr>
<tr>
<td>CF</td>
<td>64%</td>
</tr>
</tbody>
</table>

Polage et. al. Abstract F-005, ASM Annual Meeting, 2006
EIA IgM vs IgG Results

Blair, JE and JT Currier, Mycopathologia 2008;16677-82

- Of 706 total EIAs
- 37 (5%) EIAs on 28 pts had only IgM + (i.e. IgG -)
- Of the 28 pts, there were no false + IgMs observed based on other laboratory data (other serologies, culture and histopathology)


- 17 patients with EIA IgM + but IgG – studied by reviewing medical records
- 5 pts were coded out at discharge as Coccy based solely on IgM+ (IgG -) and none were judged as Coccy by chart review.
- Of the pts with both IgM and IgG +, 12 (80%) were judged to have coccy infection based on chart review
Comparison of EIA IgM and IgG results from two manufacturers (Kit A and kit B) using sera from the same patients divided among three laboratories (two in Arizona & one in California) – Sunenshine, Khan, Saubolle, Lancaster, et. al.-2014

A. 150 sera from confirmed cocci cases were selected retrospectively and frozen (Mike Lancaster, Kern County Health Laboratory, CA):

- Laboratory confirmed with ID and/or Complement Fixation (CF).
- Independently reviewed for clinical evidence of cocci by an infectious disease physician.

B. 50 remnant sera from CDC employees from non-endemic area (controls).

C. Percent agreement:
## Results: Percent Agreement

### Percent Agreement Between All Laboratories

<table>
<thead>
<tr>
<th></th>
<th>Immy</th>
<th></th>
<th>Meridian</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>Combined</td>
<td>IgM</td>
</tr>
<tr>
<td>All Labs Agreed n (%</td>
<td>180 (90)</td>
<td>178 (89)</td>
<td>171 (85.5)</td>
<td>134 (67)</td>
</tr>
</tbody>
</table>
## Results: EIA Sensitivity and Specificity

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Combined IgM &amp; IgG</td>
<td>Combined IgM &amp; IgG</td>
</tr>
<tr>
<td><strong>EIA Immy Kit, Lab A</strong></td>
<td>65.8</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>EIA Immy Kit, Lab B</strong></td>
<td>62.7</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>EIA Immy Kit, Lab C</strong></td>
<td>77.0</td>
<td>98.0</td>
</tr>
<tr>
<td><strong>EIA Meridian Kit, Lab A</strong></td>
<td>87.3</td>
<td>74.0</td>
</tr>
<tr>
<td><strong>EIA Meridian Kit, Lab B</strong></td>
<td>57.3</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>EIA Meridian Kit, Lab C</strong></td>
<td>72.7</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>EIA Immy Kit, All Labs</strong></td>
<td>68.5</td>
<td>99.3</td>
</tr>
<tr>
<td><strong>EIA Meridian Kit, All Labs</strong></td>
<td>72.4</td>
<td>91.3</td>
</tr>
<tr>
<td><strong>EIA Both Kits, All Labs</strong></td>
<td>70.5</td>
<td>95.3</td>
</tr>
</tbody>
</table>

### Immunodiagnosis (Lab A)

<table>
<thead>
<tr>
<th>Combined IgM/IgG</th>
<th>True Negative</th>
<th>False Positive</th>
<th>Indeterminate</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA Immy Lab A</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>EIA Immy Lab B</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>EIA Immy lab C</td>
<td>48</td>
<td>1</td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td><strong>EIA Meridian Lab A</strong></td>
<td>37</td>
<td>13</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td>EIA Meridian Lab B</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>EIA Meridian Lab C</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>EIA Immy all labs</td>
<td>48</td>
<td>1</td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td>EIA Meridian all labs</td>
<td>37</td>
<td>13</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td>Immunodiagnosis (Lab A)</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>
Results Summary

• Percent Agreement
  – **Immy**: 86% of the time, all 3 labs obtained the same results using the Immy test kit for EIA IgG and IgM combined
  – **Meridian**: 71% of the time, all 3 labs obtained the same results using the Immy test kit EIA IgG and IgM combined

• Combined IgM and IgG Sensitivity
  – **Immy**: all labs combined 68.5%
  – **Meridian**: all labs combined 72.4%
  – Both kits, all labs 70.5%

• Combined IgM and IgG Specificity
  – **Immy**: all labs combined 99.3%
  – **Meridian**: all labs combined 91.3%
  – Both kits, all labs 95.3%
Specificity of Enzyme Immunoassays for Coccidioidomycosis in Sera from Endemic and Non-endemic Regions

<table>
<thead>
<tr>
<th></th>
<th>Puerto Rico (#534)</th>
<th></th>
<th>AZ (#1218)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pos (%)</td>
<td>Indet (%)</td>
<td>Pos (%)</td>
</tr>
<tr>
<td><strong>Meridian EIA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM-reactive</td>
<td>3.4</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>IgG-reactive</td>
<td>0.37</td>
<td>0.19</td>
<td>3.6</td>
</tr>
<tr>
<td>IgM+IgG-reactive</td>
<td>0</td>
<td>0</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3.7</td>
<td>2.6</td>
<td>6.6</td>
</tr>
<tr>
<td><strong>IMMY EIA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM-reactive</td>
<td>1.5</td>
<td>6.7</td>
<td>1.1</td>
</tr>
<tr>
<td>IgG-reactive</td>
<td>0.75</td>
<td>2.1</td>
<td>3.2</td>
</tr>
<tr>
<td>IgM+IgG-reactive</td>
<td>0</td>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2.2</td>
<td>8.8</td>
<td>4.6</td>
</tr>
</tbody>
</table>
Comparison of CF titers between 3 Laboratories

Coccy Complement Fixation

*Immunodiffusion was IgM/IgG positive for both SQL and UCD
  • Sample 7 – SQL results: 1:16 (8/2014); 1:8 (10/2014); 1:4 (12/2014); 1:2 (2/2015)
  • Sample 8: – SQL results: 1:2 (10/2014); 1:16 (11/2014)
The Interpretation of the CF Test

- Patients with disseminated disease have generally higher titers of CF antibodies than do those with infection confined to the lungs.
- However, this relationship does not hold for all patients with disseminated infection, and patients without disseminated infection, particularly those with pleural involvement, occasionally exhibit unexpectedly high CF antibody titers.
- Because of this variability, the diagnosis or lack of diagnosis of disseminated coccidioidal infection that is based solely on CF antibody titers is tenuous at best.
- CF results cannot be compared between laboratories (they may differ significantly).
- Do not follow individual (single) CF titers alone, but rather look for increasing or decreasing titers as indicators of disease progression or abeyance.

Galgiani, et al. CID 2016:63
Conclusions

• Variability between laboratories exists when performing serology using the same brand of EIA test kit on the same specimens
• Percent agreement is greater for the Immy test kit for both IgG, IgM and combined compared with the Meridian test kit
• Excluding this outlier, EIA IgG and IgM are very specific in our investigation ranging from 98% – 100%
• Both Immy and Meridian EIA IgG and IgM appear to be less sensitive than predicted ranging from 57% – 87%
Coccidioidomycosis: Serologic Dx

- Serologic studies are less sensitive than often thought, especially in self-limited clinical cases.
- Positive serologies are helpful, but negative ones cannot be relied on to rule out disease, especially early in disease process.
- False positive serologies can occur, especially with EIA IgM studies.
PCR Detection of *Coccidioides* spp.

- No commercially available kits at this time – not FDA approved
- Paucity of published studies on clinical value and outcomes
  - Respiratory (n=266): sens=100%; spec=98.4%
  - CSF poor
- Mitchell, et al. real-time PCR – BD MAX™ System (Becton Dickinson, Sparks, MD) (JCM. 2015 (poster #34 at this meeting)
- GeneSTAT *Coccidioides* Assay (DxNA LLC, St. George, UT) - TGen (Flagstaff, AZ) and NY Dept Health: July submission for FDA approval. Poster #58 at this meeting.
Reason for Evaluation of Program Outcomes

One of the great mistakes is to judge policies and programs by their intentions rather than their results
Milton Friedman (Nobel Prize 1976, US Economist)

Value of Smear and Culture of Needle Biopsy of Solitary Lung Nodules


<table>
<thead>
<tr>
<th>Dx by Smear</th>
<th># Patients</th>
<th>Fungal Culture</th>
<th>AFB Smear</th>
<th>AFB Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granuloma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spherules</td>
<td>49</td>
<td>1/33 (3%)</td>
<td>0/33</td>
<td>0/33</td>
</tr>
<tr>
<td>No Spherules</td>
<td>48</td>
<td>0/41</td>
<td>3/41 (7.3%)</td>
<td>1/41 (2.4%)</td>
</tr>
<tr>
<td>Nondiagnostic</td>
<td>94</td>
<td>2/55 (3.6%)</td>
<td>0/55</td>
<td>1/55 (1.8%)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>149</td>
<td>0/26</td>
<td>0/26</td>
<td>0/26</td>
</tr>
<tr>
<td>Benign</td>
<td>8</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Total</td>
<td>348</td>
<td>3/156 (1.9%)</td>
<td>3/156 (1.9%)</td>
<td>2/156 (1.3%)</td>
</tr>
</tbody>
</table>

• Only 3 cultures yielded new evidence (2 coccy, 1 AFB); Cost per Dx was $3,200
• Thoracotomy cost was > $6,000, so cultures cost-effective
• However, each of these 3 patients underwent a thoracotomy before cultures turned positive within 10 days
1. Effectiveness of PCR testing at Banner Medical Centers in the Phoenix area (2015)

166 consecutive pts with PCR ordered

(20 CSFs - 12%; 146 BALs – 88%)

- 162/166 (98%) negative by PCR
- 4/166 (2%) positive by PCR
- 0 CSF positive by PCR
- 4 BALs positive by PCR
  - 3 also positive by serology but 2-8 days sooner
  - 1 positive by PCR, negative by serology, but no follow up
- 8 BALs positive by sero, but negative by PCR

(Saubolle, LSA – unpublished data)
2. Review of Utilization *Coccidioides* PCR reference testing by one of Banner Medical Centers in 2016

Duration of study: Jan-Dec 2016; Total Patients tested: 101

PCR neg: 99 (98%)

PCR pos: 2 (2%)

PCR FNeg: 3 (3%)

A. The 2 positive patients had:

1. PCR collected on 12-6-16 and reported as positive on 12-8-16 (2 day TAT); Serologies: only CF was ordered and was anticomplementary; Cultures collected on 12-4-16 were reported growing a mould on 12-7-16 and finaled as *Coccidioides* spp on 12-9-16

Summary: Positive PCR did not contribute to patient care.

1. PCR positive on BAL 3-18-16; serologies and cultures all negative. Quantiferron psotive for tb; AFBV cultures negative; fungal cultures negative; patient responded to fluconazole.

Summary: possible coccy case – PCR may have been valid.

B. The two PCR negative patients had: positive serologies and one had positive cultures as well.
Sensitivity of Various VF Tests in Compromised and Normal Patients

- Immunocompromised patients may be unable to mount an adequate Antibody response causing Ab detecting studies less sensitive and thus less reliable

<table>
<thead>
<tr>
<th>Test</th>
<th>Compromised (N=62)</th>
<th>Healthy (N=298)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA</td>
<td>67%</td>
<td>87%</td>
</tr>
<tr>
<td>ID</td>
<td>53%</td>
<td>73%</td>
</tr>
<tr>
<td>CF</td>
<td>67%</td>
<td>75%</td>
</tr>
<tr>
<td>Any</td>
<td>84%</td>
<td>95%</td>
</tr>
</tbody>
</table>

Coccidioides Ag EIA
(MiraVista Diagnostics, Indiana; Joe Wheat)

• Rabbit anti-\textit{Coccidioides} galactomannan Ab in microplate wells / EIA
• Evaluated 22 pts with severe pneumonia and 2 pts with disseminated disease
• Antigenuria detected in 70.8% using \textit{Coccidioides} EIA
• Specificity : 99.4 (healthy individuals)
• X-reaction with other endemic mycoses: 10.7%

Durkin, et. al. CID 2008;47:e69-73
## Results of MV-Ag against other Dx in Six Fatal Cases of Coccidioidomycosis in Pts with Immunocompromise (IC)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cytology</th>
<th>Antigenuria</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>BAL neg</td>
<td>3.61 ng</td>
<td>EIA IgG pos</td>
</tr>
<tr>
<td>HIV</td>
<td>BAL, Spt neg</td>
<td>3.09 ng</td>
<td>EIA &amp; CF neg</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>Spt neg</td>
<td>0.65 ng</td>
<td>EIA IgM &amp; IgG pos, CF 1:32</td>
</tr>
<tr>
<td>Transplant</td>
<td>None</td>
<td>3.42 ng</td>
<td>EIA IgM &amp; IgG neg</td>
</tr>
<tr>
<td>Transplant</td>
<td>BM, BAL neg</td>
<td>3.88 ng</td>
<td>EIA IgM &amp; IgG neg, CF neg</td>
</tr>
<tr>
<td>HIV</td>
<td>None</td>
<td>Neg</td>
<td>CF 1:32</td>
</tr>
<tr>
<td>Positive/Total</td>
<td>0/6 (0%)</td>
<td>5/6 (83%)</td>
<td>3/6 (50%)</td>
</tr>
</tbody>
</table>

Durkin CID 2008
### MD-Ag Sensitivity

<table>
<thead>
<tr>
<th>Group</th>
<th>Urine AG</th>
<th>Serum AG</th>
<th>Either AG</th>
<th>CSF AG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate-Severe (N=24)</td>
<td>71%</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>Mild (N=24)</td>
<td>50%</td>
<td>62%</td>
<td>67%</td>
<td>Not done</td>
</tr>
<tr>
<td>Meningitis (N=52)</td>
<td>70% (n=28)</td>
<td>86% (n=22)</td>
<td>87% (n=31)</td>
<td>94% (n=52)</td>
</tr>
</tbody>
</table>

Comparative efficacy of MV-Ag and other Dx modalities in IC patients

<table>
<thead>
<tr>
<th>Test</th>
<th>Compromised (N=16)</th>
<th>Competent (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen</td>
<td>81%</td>
<td>50%</td>
</tr>
<tr>
<td>Pathology</td>
<td>50%</td>
<td>0%</td>
</tr>
<tr>
<td>Antibody</td>
<td>50%</td>
<td>100%</td>
</tr>
<tr>
<td>Ag, Ab, Path</td>
<td>94%</td>
<td>100%</td>
</tr>
<tr>
<td>Culture</td>
<td>100%</td>
<td>62%</td>
</tr>
</tbody>
</table>
Newer Tests: MiraVista Diagnostics for Coccidioides in CSF

- Thirty six patients with 42 episode of CM studied.
  - Sensitivity by Cocc Ag = 93%
  - Specificity by Cocc Ag = 100%
  - Cultures of CSF positive in 7%
  - Antibodies by ID positive in 67%
  - Antibodies by CF positive in 70%
  - IgM antibodies by EIA positive = 8%
  - IgG antibodies by EIA positive = 85%

**Conclusion.** Testing CSF for *Coccidioides* antigen is a useful addition to the diagnostic armamentarium in suspected CM, and complements testing by CSF antibodies and culture.

Anti-Coccidioides Ab by New MiraVista EIA in CSF

Wheat et. al. Poster 28: August 12th, 2017

<table>
<thead>
<tr>
<th></th>
<th>AB IgG+</th>
<th>AB IgG-</th>
<th>AB IgM+</th>
<th>AB IgM-</th>
<th>AG+</th>
<th>AG-</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF+ (n=8)</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
<td>83%</td>
<td>17%</td>
</tr>
<tr>
<td>CF- (n=41)</td>
<td>7%</td>
<td>93%</td>
<td>2%</td>
<td>98%</td>
<td>0%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Needs additional studies but looks promising

New Rapid Lateral Flow Products (IMMY, Norman OK)

- 'ValleyFeverDx' (CAB2003)- Poster #27, Stanford 2017
  - A two strip model intended as a qualitative replacement for immunodiffusion
  - Not submitted to FDA yet

- Sona Cocci Ab LFA (CTA2003)- Poster # 29, Stanford 2017
  - Purported to have a very high negative predictive value, used to screen out negative specimens – expect to be a waived test
Problems with Serologic Diagnosis

• Serologic procedures and reagents not standardized across the field
• Lab to lab Variability
• Can have reagent lot to lot variability
• Discrepancies between test systems
• Best run in parallel if following patient
• Clinicians should use clinical judgment for interpretation of serologic testing
Clinical Diagnosis of Coccidioidomycosis often difficult as presentation can be protean

Most presentations are of a respiratory nature but often can’t separate from other respiratory infections

At times patient does not realize that he/she has more than a virus until progression occurs

Must have high degree of suspicion and must understand laboratory studies (pros/cons; shortcomings)

Early serologies may be negative; repeat if suspicion high

False positives may occur, especially in IgMs (IMDF may help or repeating in 1-3 weeks may also clarify)

Do not compare CF results between labs (they may differ significantly) – do not follow individual (single) CF titers alone, but rather look for increasing or decreasing titers as indicators of disease progression or abeyance.
Coccidioidomycosis: Future Epidemiology

- Growing susceptible population
- Growing immunocompromised patient pool
- Expansion into desert
  - (record new home starts)
- Increasing travel and tourism
- Better education
- Possible expansion of geographic distribution in future

Conclusion: future of coccy seems assured
The END