Renal Pathology Specialty Conference

Case 1
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Clinical History
- A 56 y.o. male with ESLD due to idiopathic pulmonary fibrosis underwent a left lung transplant in 2009
  - Immunosuppressive regimen
    - Anti-thymocyte globulin followed by Tacrolimus, MMF and Prednisone
    - Prophylaxis
      - Valganciclovir and TMP-SMX
  - Social History
    - Works as a cattle rancher with herding dogs x 30 years
- 1 week post-transplant, he developed ACR with humoral component treated with steroids and rituximab
  - Lung biopsies x 2 GMS and AFB stains negative for organisms
  - Rejection resolved over 6 weeks

Post Transplant Period
- 4 months
  - Episodic fevers and persistent dry cough
  - Lung biopsy: GMS & AFB stains negative
- 5.5 months
  - Admitted for fever, cough, altered mental status, diarrhea
    - Chest CT: small ground glass infiltrates LLL
    - Brain MRI: unrevealing (CSF w/u negative for bacteria, AFB, fungi, virus)
  - Colonoscopy and stool cultures: negative
  - Blood, urine, sputum cultures: negative for bacteria, AFB and fungi
  - Serum studies negative: Hepatitis B and C, HIV, CMV, EBV, HSV, coccidiomycosis antibody, ANA and ANCA
  - Empiric therapy: Vancomycin, Cefepime, Acyclovir
  - Renal function worsened: Creatinine 1.2-1.6 mg/dl

Post Transplant Period
- 6.5 months
  - Admitted with fever, cough, confusion, acute renal failure (peak Cr 4.3 mg/dl)
    - Diarrhea resolved.
    - Blood, urine, stool, sputum, CSF cultures: negative
    - Tacrolimus blood levels 1 (14-23 ng/ml)
    - Renal function transiently improved with ↓ Tacrolimus dose
    - BK and JC virus PCR: negative
    - Urinalysis: sterile pyuria, rod-shaped organisms and tubular epithelial cells with inclusions
  - 7 months
    - Renal Biopsy performed

Allograft Biopsy Results
- Acute granulomatous interstitial nephritis
- Nekomocellular glomeruli
- Severe interstitial fibrosis and tubular atrophy

Interstitial
- Intestinal granulomatous infiltrate
- Poorly formed granulomas admixed with lymphs, monocytes and a few neutrophils
- Occasional well-formed granulomas
Tubules
- Few tubules with tubulitis
- TEC with enlarged hyperchromatic nuclei
- Partial to complete tubular necrosis

Vessels
- Granulomatous inflammation surrounds vessels with fibrinoid necrosis of wall
- Tacrolimus effect: arteriolar hyalinization with beaded hyalinization

Organisms
- Small purplish pleomorphic rod-shaped organisms in tubular lumens and epithelial cells
- Granulomas with organisms in histiocytes

Special Stains
- Gram stain (Brown&Brenn): irregularly stained gram positive pleomorphic rod-shaped organisms
- PAS stain - dot pattern
- AFB stain - negative
- GMS stain - negative
- Urine re-examine

Electron Microscopy - Diagnostic!
- Encephalitozoon cuniculi
- Organisms develop in parasitophorous vacuole in host cell
- Vacuole has all stages of development:
  - Meront
  - Sporont
  - Spores

Special Stains
Renal Biopsy
- GMS stain - negative
- AFB stain - negative
- PAS stain - dot pattern

Organisms show single pink dot that corresponds to "posterior vacuole"
Renal Biopsy Diagnosis:

- Acute granulomatous interstitial nephritis due to *Encephalitozoon cuniculi*
- Infection-related arterial and arteriolar focal necrosis
- Interstitial fibrosis and tubular atrophy, severe, due to ongoing interstitial nephritis
- Arteriolar hyalinosis, beaded, Tacrolimus-related

Pathology of *E. cuniculi*

Spectrum of pathologic changes in the kidney:

- HIV negative kidney transplants
  - Mohindra AR et al., 2002: Tubulointerstitial infiltrate and coccoid organisms in renal tubules
  - Gamboa-Dominguez A et al., 2003: Abscesses with tubular necrosis
- Mahmood MN et al., 2003: Tubules with round structures and surrounding neutrophilic infiltrates
- HIV positive native kidney cases
  - Tosina A et al., 2002: Tubulointerstitial nephritis and granulomatous reaction; spores in necrotic tubules
  - Other reports: No inflammatory response in tissues
- Animals
  - Kunzel F et al., 2010: Granulomatous interstitial nephritis, necrosis
- Vasculitis with fibrinoid necrosis in various tissues

Disorders Associated With GIN

- 3 retrospective studies evaluated the cause of GIN
- GIN is uncommon in native kidneys and organ transplant recipients

Retrospective studies of GIN (mainly native kidneys)

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Aug %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug-induced</td>
<td>33.6%</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>21.0%</td>
</tr>
<tr>
<td>Wegener’s</td>
<td>12.7%</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>3.1%</td>
</tr>
<tr>
<td>MSA</td>
<td>8.3%</td>
</tr>
<tr>
<td>Foreign body GMR</td>
<td>4.2%</td>
</tr>
<tr>
<td>BCG therapy</td>
<td>0.9%</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>0.9%</td>
</tr>
<tr>
<td>Missed</td>
<td>14.5%</td>
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</tbody>
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**Differential Diagnosis #1**

- **Rhodococcus**
  - Clinical: Acquired by animal contact (horses, cows) or inhalation of Rhodococcus in soil
  - 1 case in allograft kidney: lung infection and sepsis, disseminated to kidney
  - Culture: blood, sputum, urine positive for Rhodococcus equi

- **Pathology**
  - Multiple noncaseating granulomas with giant cells
  - Macrophages contain gram positive 2-3 μm pleomorphic coccobacilli

**Epidemiology**

- World wide distribution
- Most commonly occurs in immunosuppressed patients:
  - Immunocompromised patients with AIDS (15%)
  - Immunocompromised patients without AIDS – Organ transplant recipients, patients with malignancies, diabetics
- Increasingly reported in immunocompetent patients:
  - Children and Elderly
  - Travelers
  - Subset of asymptomatic humans may harbor latent infection
- Mode of transmission:
  - Fecal-oral
  - Ingestion of contaminated water/flood
  - Inhalation of contaminated aerosols
  - Person-to-person
  - Contact with animal (Zoonotic infection)

**Phylum Microsporidia**

- Obligate intracellular, spore-forming parasites, related to fungi; identified in 1857 as cause of silkworm disease
- Infect vertebrate and invertebrate hosts: rodents, insects, birds, fish, mammals including cattle and dogs (domestic and wild)
- Responsible for infectious disease problems and economic losses in the fishing, honeybee and silkworm industries
- 1959 first human case of microsporidiosis; 1987 emerged as cause of opportunistic infections in HIV patients
- Phylum contains 3,200 species with 14 known to infect humans: most common:
  - Enterocytozoon bieneusi (diarrhea in AIDS patients and travelers)
  - Encephalitozoon sp. (E. intestinalis, E. cuniculi, E. hellem)

**Classification & Clinical Features**

- 14 species are known to infect humans
- Clinical presentation varies depending on site of infection

<table>
<thead>
<tr>
<th>Species</th>
<th>Sites of Infection</th>
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<tbody>
<tr>
<td>E. bieneusi</td>
<td>Intestinal, biliary, respiratory, urinary tract</td>
</tr>
<tr>
<td>E. intestinalis</td>
<td>Systemic, intestine, respiratory/luminal tract</td>
</tr>
<tr>
<td>E. cuniculi</td>
<td>Systemic, eye, respiratory/luminal tract</td>
</tr>
<tr>
<td>E. hellem</td>
<td>Systemic, eye, respiratory/luminal tract</td>
</tr>
<tr>
<td>N. caninum</td>
<td>Systemic</td>
</tr>
<tr>
<td>T. arthroplasty</td>
<td>Systemic, eye, brain</td>
</tr>
<tr>
<td>T. hominis</td>
<td>Systemic, eye, skeletal muscle</td>
</tr>
<tr>
<td>B. algemar</td>
<td>Eye, skeletal muscle, skin</td>
</tr>
<tr>
<td>M. oseledetsii</td>
<td>Eye</td>
</tr>
<tr>
<td>M. gilchristi</td>
<td>Eye</td>
</tr>
<tr>
<td>N. ocularis</td>
<td>Eye</td>
</tr>
<tr>
<td>V. corneae</td>
<td>Eye, urinary tract</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Clinical Presentation</th>
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<tbody>
<tr>
<td>Enteral/Extrahepatic</td>
</tr>
<tr>
<td>Cholestatic/Hepatitis</td>
</tr>
<tr>
<td>Bronchitis/Pneumonia</td>
</tr>
<tr>
<td>Cystitis/Nephritis</td>
</tr>
<tr>
<td>Paranasal sinusitis</td>
</tr>
<tr>
<td>Myositis</td>
</tr>
<tr>
<td>Encephalitis</td>
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**Morphology and Cellular Infection**

- E. cuniculi/spore: Oval 2-3 μm diameter
  - Electron-dense exosporum (Ex) (glycoprotein)
  - Electron-luculent endosporum (En) (chitin)
- Polar tubule infects cell
- Cytoplasm: Nucleus (N), Posterior vacuole (PV), Polar tubule (PT) (5-7 cells)

**Comparative Morphology**

- Distinct features of E. cuniculi by EM

| E. cuniculi vs. E. hellem |

**Mechanisms of Host Defense**

Cell- and Humoral-mediated immunity contribute to host defense against infection:

- Human studies: 1. severity of disease in AIDS patients with declining CD4+ T cells
  - Mortality of mice infected with E. cuniculi and depleted of T cells
  - Importance of CD8+ T cells is shown to reduce mice orally infected with E. cuniculi
  - Survival improved by adoptive transfer of CD8+ T cells from WT mice
  - Survival of mice reconstituted with CD8+ T cells was further prolonged by administration of anti-E. cuniculi antibody

Macrophage function is also important for host defense against infection...
Microsporidiosis in HIV Negative Transplant Recipients

### Diagnostic Tests

- **Histopathological Methods**
  1. Clinical Fluids (detect spores in stool, urine, sputum, tissue imprint):
    - Chemofluorescent agents: calcofluor white
    - Modified trichrome (chromotrope-based) stains (pink spores/belt stripe)
    - Hematoxylin and Eosin (H&E) stain (bacterial and fungal)
    - Immunofluorescent stain: species-specific antibodies
  2. Tissue Paraffin Sections
    - Gram stain (blue spores with belt stripe)
    - PAS-fungal stain (capsule vacuole as pink dot)
    - Silver stain (Warthin-Starry) (solid black rods)
    - Immunohistochemistry using species-specific antibodies

- **Serology** (detect carrier; not routine in immunocompromised patients)

### Treatment of E. cuniculi

- CDC recommends Albendazole for E. cuniculi infection
  - Leads to clinical improvement/clearance of parasites in cases of *Encephalitozoon* infection. Fumagillin is effective for *E. bieneusi*.
  - Side effects are rare: hypersensitivity, neutropenia, thrombocytopenia
  - Also: lower or transiently discontinue immunosuppressive therapy

- Randomized, double-blind, placebo controlled study:
  - Albendazole (400 mg bid x 3 weeks) vs Rx and prophylaxis of *E. intestinales* in AIDS patients (J Infect Dis 177:1373, 1998)

- **Results**
  - Clearance of parasite and clinical benefit:
    - Albendazole: 4/4
    - Placebo: 0/4, P = 0.01
    - All cases cleared microsporidia with albendazole
  - Prevention of relapse: All 8 patients randomized to Albendazole (400 mg bid) x 12 months vs No Rx
    - Albendazole: 0/2 recurred (8-9 mo) No Rx: 3/5 recurred

### Clinical Course

- **Albendazole 400 mg bid x 4 wks**
  - Immunosuppression was reduced
- **Urine and sputum were monitored for spores using the Calcofluor white stain**
- **After 5 days: pt responded to therapy, afebrile, reduction in E. cuniculi spores 1 wk later: Aspergillus pneumonia and expired**
- **8 months autopsy: disseminated E. cuniculi in lung, kidney, brain, liver, spleen**
Summary:
- 56 y.o. lung transplant recipient: heavy immunosuppressive therapy and history of direct contact with cattle/dogs
- At risk for microsporidia
- 4 months post-transplant: fever, cough, lung infiltrate, + lung biopsy (in retrospect) for *E. cuniculi*
- Probably acquired zoonotic infection
- 5.5 to 7 months post-transplant: persistent fever, cough, confusion, renal failure. Cultures/serology negative
- R/O Tacrolimus vs ATIN
- *E. cuniculi* spread to kidney
- Granulomatous Interstitial Nephritis due to *E. cuniculi*
- Albendazole, ↓Immunosuppression
- 8 months autopsy: Aspergillus pneumonia and disseminated *E. cuniculi*

Conclusions:
- Our case is the first report of disseminated *E. cuniculi* in an HIV negative lung transplant recipient
- HIV patients and immunocompromised non-HIV transplant recipients should be considered risk groups for microsporidia
- Microsporidia should be considered in cases of FUO and/or multiorgan infection, especially with renal failure, after other causes are excluded
- Examine urine carefully; if organisms are identified but cultures are negative, screen for microsporidia (calcofluor white/modified trichrome)
- In certain cases, serotesting of donors may be useful to detect carriers of microsporidiosis to prevent donor-related infection
- Further studies to elucidate the cellular mechanisms by which microsporidia infect tissues may lead to new therapeutic strategies

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