Case Presentation

- 67 year-old man with known long-standing mitral valve regurgitation
- Recent decrease in effort tolerance with shortness of breath.
- Recent dental work, with extraction of 14 teeth
  - 1 week of amoxicillin therapy.
- Transthoracic echocardiogram:
  - Myxomatous changes of the anterior mitral valve leaflet.
  - Appearance and motion of ruptured cords leading to a partial flail leaflet and severe mitral valve regurgitation.
- Underwent elective mitral valve repair
  - Tissue was sent to pathology. Cultures not performed.
Impression so far?
A. No organisms present (calcifications seen)
B. Bacteria present, defer to additional stains
C. Gram positive cocci present

What other stain could help us?

Additional testing
- Culture was not performed
- Broad range (16s rRNA gene) sequencing for bacteria detected Streptococcus mitis DNA

Basic Principles for Diagnosing Infective Endocarditis (IE)

Basic Principle #1: Role of Histology
- Primary features of infective endocarditis: vegetations, valvular inflammation, fibrosis, destruction
- Inflammatory infiltrate
  - Can be seen with non-infectious etiologies (e.g. most degenerative diseases)
  - Composition may vary with the type and duration of infection
- Different approaches for native valve infective endocarditis (IE) and prosthetic valve IE (bioprosthetic vs. mechanical)
Basic principle #2

• Bacteria may be visible on H&E
  • Colonies of embedded organisms may be seen
  • Bacteria often stain basophilic
    • Does not indicate that they are Gram positive!
    • Gram stain is needed to determine Gram classification

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Basic principle #3

• Gram stain works the same as in the microbiology lab...with a few caveats
Streptococcus pneumoniae, Gram stain, 1000x total magnification

Haemophilus parainfluenzae, Gram stain, 1000x total magnification

Caveat: decolorization

Caveat: thick regions
Caveat: treatment effect

Caveat: non-viable organisms

Staphylococcus sp., H&E, 400x total magnification

Caveat: non-viable organisms

Staphylococcus sp., H&E, 1000x total magnification

Caveat: non-viable organisms

Caveat: non-viable organisms

Basic Principle #4

- Role for GMS and PAS as supplemental stains:
  - Dead organisms
  - Fungi
  - T. whipplei (PAS-D)
- Caveats:
  - High background
  - Staining of non-infectious substances

Caveat: other organisms not reliably seen with Gram stain

- *Tropheryma whippelii*
  - See infiltrations of foamy macrophages
- *Coxiella burnetii*
  - See numerous giant cells with lymphocytes
- *Bartonella* spp.
  - Non-specific fibrosis, granulation tissue, endothelial proliferation, variable inflammation
- Mycobacteria (may be Gram positive or Gram ‘invisible’)
Propionibacterium, GMS stain, 400x total magnification

Propionibacterium, GMS stain, 1000x total magnification

Caveat: artifactual staining

Propionibacterium sp., PAS stain, 400x total magnification

Propionibacterium sp., PAS stain, 1000x total magnification
Whipple’s disease – A special consideration for PAS

Role of molecular testing
Primary molecular testing options

- Pathogen-specific PCR
  - E.g. Tropheryma whipperi, Coxiella burnetii, Bartonella henselae
  - Especially helpful for confirming the histologic impression in the correct clinical context
  - Requires knowing what you are looking for
  - Can get expensive if multiple assays are ordered
- Broad-range amplification and sequencing assays
  - 16S ribosomal RNA gene (rDNA) for bacteria (including mycobacteria)
  - If PCR positive, amplicon sequencing is performed for identification
  - Other targets for mycobacteria (rpoB), fungi (28S rDNA, internal transcribed spacer [ITS] 1 and 2)
  - No broad-range assays for viruses (usually pathogen-specific PCR, IHC or ISH).

Role of 16S rDNA sequencing

- Broad-range
  - Advantage: not specific to a particular bacterium
  - Disadvantage: May not be as sensitive as pathogen-specific PCR assays
  - Useful in the setting of blood-culture negative endocarditis
  - General impression: most useful when bacteria are seen by Gram or GMS

Supporting studies

- Many studies available dating back to early 2000s
- Limitations:
  - Early studies used older blood culture and PCR/sequencing methods and PCR contamination was a big issue
  - Most recent studies do not compare full histologic examination to culture and 16S rDNA sequencing
  - In general, all show improved sensitivity of 16S rDNA sequencing over blood or valve culture for detection of bacterial pathogens

Select recent studies

- Shrestha et al. Ann Thorac Surg 2015;99:33-7 (Cleveland Clinic)
  - Retrospective review of 174 valves from 174 patients who underwent surgery for active infective endocarditis (2010-2013; Modified Duke criteria)
  - 80 with native valve endocarditis, 94 with prosthetic valve endocarditis
  - Compared results of blood culture, valve culture, and valve sequencing (bacterial, fungal and mycobacterial – FRESH tissue)
  - Study only included patients with acute inflammation or microorganisms on special staining (no further information given)
  - Definitive microbial cause of endocarditis – defined a same pathogen in at least 2 of 3 methods.

Pathogens detected by sequencing

- Viridans group streptococci (36)
- S. aureus (33)
- Coagulase-negative staphylococci (27)
- Enterococcus (21)
- Pyogenic (beta-hemolytic) streptococci (7)
- HACEK organisms (6)
- "nutritionally variant" streptococci (5)
- Bartonella (5)
- Other pathogen (12): Erysipelothrix rhusiopathiae, Mycoplasma hominis, Tropheryma whippelite
- Fungi (all negative by fungal PCR/sequencing)

Shrestha et al. - Findings

<table>
<thead>
<tr>
<th>Test</th>
<th># positive</th>
<th># tested</th>
<th>True positives</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture</td>
<td>140</td>
<td>163</td>
<td>128</td>
<td>79%</td>
</tr>
<tr>
<td>Valve culture</td>
<td>74</td>
<td>163</td>
<td>51</td>
<td>31%</td>
</tr>
<tr>
<td>16S sequencing*</td>
<td>152</td>
<td>166</td>
<td>150**</td>
<td>90%</td>
</tr>
</tbody>
</table>

* Fungal and mycobacterial sequencing was non-contributory
** 25 probable pathogens were detected only by sequencing
Shrestha et al. – Conclusions

• Valve sequencing rather than culture should be the primary test for detection of bacteria. Is this a new paradigm?

• Limitations:
  • Retrospective study; not all valves sent for sequencing (sampling bias)?
  • Only specimens with histopathologic evidence of infection were included in this study
  • Test was not performed in house (delayed TAT)
  • Economics of testing not covered

• An alternate approach: target the cases that will most benefit from 16S rDNA sequencing

Our Data – Concordant Negative Cases

<table>
<thead>
<tr>
<th>Type of Tissue</th>
<th>Specimen</th>
<th>16S rDNA PCR/Sequencing</th>
<th>Microbiology Gram stain/Culture Result</th>
<th>Final path result</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFPE Aortic valve</td>
<td>negative</td>
<td>negative</td>
<td>no organs seen</td>
<td></td>
</tr>
<tr>
<td>FFPE Heart valve, nos</td>
<td>negative</td>
<td>negative</td>
<td>no organs seen</td>
<td></td>
</tr>
<tr>
<td>FFPE mitral valve sewing ring</td>
<td>negative</td>
<td>negative</td>
<td>no organs seen</td>
<td></td>
</tr>
<tr>
<td>FFPE Cardiac tissue</td>
<td>negative</td>
<td>negative</td>
<td>no organs seen</td>
<td></td>
</tr>
<tr>
<td>FFPE Aortic homograft wall</td>
<td>negative</td>
<td>negative</td>
<td>no organs seen</td>
<td></td>
</tr>
<tr>
<td>FFPE Aortic root abscesses</td>
<td>negative</td>
<td>No cultures ordered</td>
<td>no organs seen</td>
<td></td>
</tr>
<tr>
<td>FFPE Graft tissue</td>
<td>negative</td>
<td>negative</td>
<td>no organs seen</td>
<td></td>
</tr>
<tr>
<td>fresh Peri-aortic root tissue</td>
<td>negative</td>
<td>negative</td>
<td>no organs seen</td>
<td></td>
</tr>
<tr>
<td>fresh Heart valve, nos</td>
<td>negative</td>
<td>negative</td>
<td>no organs seen</td>
<td></td>
</tr>
<tr>
<td>fresh Tricuspid aortic valve</td>
<td>negative</td>
<td>negative</td>
<td>no organs seen</td>
<td></td>
</tr>
</tbody>
</table>

Our Data – Concordant Positive Cases

<table>
<thead>
<tr>
<th>Type of Tissue</th>
<th>Specimen</th>
<th>16S rDNA PCR/Sequencing</th>
<th>Microbiology Gram stain/Culture Result</th>
<th>Final path result</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFPE Mitral valve</td>
<td>Streptococcus mitis</td>
<td>No Culture ordered</td>
<td>Paucimyelocytic; Gram variable</td>
<td></td>
</tr>
<tr>
<td>FFPE Aortic valve</td>
<td>Cardiobacterium hominis</td>
<td>No Culture ordered</td>
<td>Focal bacilli</td>
<td></td>
</tr>
<tr>
<td>FFPE prosthetic Mitral valve</td>
<td>Streptococcus anginosus</td>
<td>No Culture ordered</td>
<td>Macrophages</td>
<td></td>
</tr>
<tr>
<td>FFPE Cardiac valve</td>
<td>Propionibacterium acnes</td>
<td>No Culture ordered</td>
<td>GPCs in chains</td>
<td></td>
</tr>
<tr>
<td>fresh Aortic Tissue</td>
<td>Propionibacterium acnes</td>
<td>No Culture ordered</td>
<td>Gram positive bacilli</td>
<td></td>
</tr>
<tr>
<td>fresh Aortic Valve</td>
<td>Streptococcus dysgalactiae</td>
<td>rare cocci</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFPE Aortic valve</td>
<td>Streptococcus gigas (Streptococcus mitis group)</td>
<td>no organs seen</td>
<td>Bacilli present</td>
<td></td>
</tr>
</tbody>
</table>

Our Data – False Negatives

<table>
<thead>
<tr>
<th>Type of Tissue</th>
<th>Specimen</th>
<th>16S rDNA PCR/Sequencing</th>
<th>Microbiology Gram stain/Culture Result</th>
<th>Final path result</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFPE Mitral valve</td>
<td>prosthetic</td>
<td>negative</td>
<td>negative</td>
<td>Bacilli; somewhat striped diplococci</td>
</tr>
<tr>
<td>FFPE Abdominal aorta</td>
<td>negative</td>
<td>No Culture ordered</td>
<td>negative</td>
<td>GPCs</td>
</tr>
<tr>
<td>fresh Aortic valve</td>
<td>negative</td>
<td>No Culture ordered</td>
<td>negative</td>
<td>Bacilli present</td>
</tr>
</tbody>
</table>

Our Data – Additional Positive

<table>
<thead>
<tr>
<th>Type of Tissue</th>
<th>Specimen</th>
<th>16S rDNA PCR/Sequencing</th>
<th>Microbiology Gram stain/Culture Result</th>
<th>Final path result</th>
</tr>
</thead>
<tbody>
<tr>
<td>fresh Heart Valve</td>
<td>Staphylococcus epidermidis</td>
<td>Negative**</td>
<td>no organs seen; acute inflammation present</td>
<td></td>
</tr>
</tbody>
</table>

Summary thoughts

• Continued role for histopathology
• 16S rDNA sequencing offers increased sensitivity for pathogen detection over CULTURE
  • May not be useful in every case
  • Unlikely to be positive if organisms are NOT seen by histopathology
  • Culture is still required for antimicrobial susceptibility testing
• Important considerations:
  • Sequencing is not yet incorporated into the modified Duke criteria
  • Slow turn around time if not performed in-house
  • May not be cost-effective
THANK YOU