Diagnosis of bone and joint infections: the point of view from the clinical metagenomist

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I have no financial disclosures
Goals of this presentation

• Review prosthetic joint infections (PJIs) and why they are good cases for metagenomic shotgun sequencing (MSS)

• Discuss metagenomic sequencing for PJIs in the context of ideal qualities of a test from the clinical metagenomist point of view

• Review data supporting why MSS may be useful, but not always necessary, for PJI
Case

- 68 yo female
- PMH: Crohn’s disease: adalimumab then vedolizumab

Bilateral TKA 8 yr ago → Bilateral Cx-negative PJI 2 yr ago → DAIR Vanc + cefep, Cefadroxil → Recurrent right PJI → First step of a 2-stage exchange

Necrotic tissue seen
Culture-negative
16S rDNA PCR neg
Targeted PCRs neg
Serologies neg

What do you go from here?
A little background

Prosthetic Joint Infection (PJI)

- Primarily bacterial, some fungal
- Acute or chronic
- Treatment is difficult
  - Surgery almost always required
  - Sometimes joint is removed for months (2-stage exchange)
  - Antibiotics from 6 weeks to lifelong

Metagenomic Shotgun Sequencing

- Nucleic acid (DNA and/or RNA) extracted directly from a clinical specimen
- No targeted amplification (e.g. 16S rRNA)
- Millions of short sequences obtained
- Sequences analyzed to detect microorganisms
What makes PJI an attractive target for MSS

• Cultures don’t always work
• Typically a sterile site
• “Wide” range of pathogens
• The diagnosis CAN wait a few days
• Long-term treatment implications
Goals of the clinical metagenomist

• To provide an accurate identification of pathogens to aid in the care of patients
  • We want to help patients
    • Tests must be effective, timely, and useful
  • We do not want to harm patients
    • Results must be accurate
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Does metagenomic sequencing work for PJI?

- To date, three larger studies
  - Two studies focused on sonicate fluid as a sample
  - One studied synovial fluid prior to surgery

Sonicate fluid:

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Molecular Diagnosis of Orthopedic-Device-Related Infection Directly from Sonication Fluid by Metagenomic Sequencing

Identification of Prosthetic Joint Infection Pathogens Using a Shotgun Metagenomics Approach

Direct Detection and Identification of Prosthetic Joint Infection Pathogens in Synovial Fluid by Metagenomic Shotgun Sequencing
Sonicate fluid MSS results

• Street, et al. results
  • 97 samples: 62 culture-positive, 35 culture-negative

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>New Identifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Versus sonicate fluid culture</td>
<td>88% (Genus level=93%)</td>
<td>88%</td>
<td>9 probable pathogens</td>
</tr>
<tr>
<td>Vs. sonicate fluid and PPT culture</td>
<td>68%</td>
<td>88%</td>
<td>6 probable pathogens</td>
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</table>

New Identifications:
Fusobacterium nucleatum, Veillonella parvula, Finegoldia magna, Parvimonas micra, Staphylococcus aureus, and Streptococcus dysgalactiae
Sonicate fluid MSS results

- Thoendel *et al.* results
  - 408 samples: 115 Culture-pos, 98 Cx-neg PJI, 195 aseptic failure

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<tr>
<td>Versus sonicate fluid culture</td>
<td>94.8% (115 Cx-pos PJI)</td>
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<td>11 from Cx-pos PJI (9.6%) 43 from Cx-Neg PJI (43.9%)</td>
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<tr>
<td>Vs. sonicate fluid and PPT culture</td>
<td>90.5% (137 Cx-pos PJI)</td>
<td>96.4% (Aseptic failures)</td>
<td>12 from Cx-pos PJI (8.8%) 27 from Cx-neg PJI (35.5)</td>
</tr>
<tr>
<td>Vs. sonicate fluid, PPT, and synovial fluid culture</td>
<td>89% (146 Cx-pos PJI)</td>
<td></td>
<td>12 from Cx-pos PJI (8.2%) 21 from Cx-neg PJI (31.3%)</td>
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Synovial Fluid MSS results

- Ivy, et al.
  - 168 samples: 82 Cx-pos PJI, 25 Cx-neg PJI, 61 aseptic failure

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<tr>
<td>Vs. synovial fluid culture</td>
<td>82.9%</td>
<td>93.4% (Aseptic failures)</td>
<td>3 from Cx-pos PJI (3.7%) 4 from Cx-neg PJI (16%)</td>
</tr>
</tbody>
</table>

New Identifications:
S. aureus, Salpingoea rosetta, Afipia broomeae, Bradyrhizobium japonicum, Enterococcus faecalis, Finegoldia magna, Anaerococcus vaginalis
Goals of the clinical metagenomist

• To provide an accurate identification of pathogens to aid in the care of patients
  • We want to help patients
    • Tests must be effective, *timely*, and useful
  • We do not want to harm patients
    • Results must be accurate
Nanopore-based sequencing for PJIs

- Successful in 7 out of 7 culture-positive PJIs
Goals of the clinical metagenomist

• To provide an accurate identification of pathogens to aid in the care of patients

  • We want to help patients
    • Tests must be effective, timely, and **useful**

  • We do not want to harm patients
    • Results must be accurate
How much difference could metagenomics make?

• Looked back at our study of 408 subjects
  • Now 2 to 8 years outcome data available

• Looked at treatment and outcomes of individuals where new potential pathogens were discovered by MSS
Study Design

• 39 subjects identified
  • 32 classified as PJI
  • 7 classified as aseptic failure

• Determined whether subsequent IV antibiotic therapy covered the identified microorganism
  • Also evaluated the reason the IV therapy was chosen

• Evaluated outcomes after surgery
Aseptic failure outcomes

Aseptic failures (7)

- Treatment covered potential pathogen (1)
  - Treatment successful (1)
  - Failed treatment (0)
- Pathogen not covered (6)
  - Treatment successful (6)
  - Failed treatment (0)

New organisms: *S. aureus* (3), *C. acnes* (2), *Streptococcus sanguinis* (2)
PJIs outcomes

Covered?

Outcome?

- Treatment covered potential pathogen (29)
  - Treatment successful (25)
    • Recurrent infection (C. albicans)
    • Would drainage requiring debridement (S. epidermidis)
    • Subsequent PJI with different organism (S. lugdunensis)
    • Death (S. dysgalactiae)
  - Failed treatment (4)
- Pathogen not covered (3)
  - Treatment successful (2) (S. epidermidis)
  - Failed treatment (1)
- Treatment successful (32)
  - Failed treatment (4)
  - Pathogen not covered (3)
    - Treatment successful (2)
    - Failed treatment (1)
- Recurrent infection (Mycoplasma salivarium)
How were we able to cover new pathogens in 29 of 32 cases?

Common pathogens?

Mainly Yes….
- *Corynebacterium pseudogenitalium*
- *Cutibacterium acnes* (4)
- *Staphylococcus aureus* (10)
- *Staphylococcus epidermidis* (7)
- *Staphylococcus haemolyticus*
- *Streptococcus agalactiae* (3)
- *Streptococcus dysgalactiae* (2)
- *Streptococcus sanguinis*
- *Enterococcus faecalis*

but also No…
- *Aerococcus urinae*
- *Candida albicans*
- *Clostridium perfringens*
- *Clostridium species*
- *Facklamia languida*
- *Finegoldia magna*
- *Peptoniphilus harei*
- *Peptoniphilus lacrimalis*
Reasons for choosing correct coverage in 29 cases

• 9 cases were empiric coverage
  • Daptomycin + ertapenem (2)
  • Vancomycin + ertapenem (2)
  • Vancomycin + cefepime
  • Ceftriaxone (2)
  • Cefepime
  • Cefazolin + rifampin

• 9 cases: past prior infection with detected pathogen
  • All underwent DAIR and were on suppression

• 8 cases: therapy directed at other culture-positive organisms

• 3 cases: Other positive cultures from acute episode

Conclusion: Metagenomics can help, but a good ID physician can go a long ways
Goals of the clinical metagenomist

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Why is accuracy difficult?

- How do you define prosthetic joint infection?
- Pathogen versus background?
  - Culture-negative PJI: often low burden of disease
  - Significant overlap between common reagent contaminants and reported PJI pathogens
  - Background varies
- New pathogens to discover?
Do analysis tools affect accuracy?
Does analysis tool choice matter?

- Evaluated three commercial analysis tools
- Hand-selected 24 “challenging” samples from PJI study
  - Uncommon pathogens, polymicrobial, culture-negative, etc.
- Submitted identical sequencing files to each company for analysis

- Determined whether there were differences in final interpretations based on the tool used
  - Culture-positive species detected?
  - New identifications?
    - If so, were the “corroborated” by other tools?
Does analysis tool choice matter?

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<tr>
<th></th>
<th>LMAT</th>
<th>CosmosID</th>
<th>One Codex</th>
<th>IDbyDNA</th>
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<tbody>
<tr>
<td>Culture- positive PJIs (16 samples)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species detected (24)</td>
<td>22</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Additional species detected:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corroborated</td>
<td>9</td>
<td>4</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Uncorroborated</td>
<td>0</td>
<td>1</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Culture-negative PJIs (4 samples)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Additional species detected:</td>
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<tr>
<td>Corroborated</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Uncorroborated</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>1</td>
</tr>
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Why is accuracy important?

• An accurate diagnosis can ideally lead to narrower and more effective therapy with better outcomes and fewer adverse effects.

• An inaccurate diagnosis can lead to harm:
  • Overtreatment if additional non-pathogens reported
  • Possible loss of treatment if only non-pathogen(s) reported

• A negative test will not create harm

• You cannot rely on physicians to sort out real versus not real
Summary

• Metagenomics has a role for PJI pathogen detection
• At this time metagenomic sequencing should be reserved for when conventional testing fails
• Accurate results will be key for clinical integration
• For PJI, specificity should trump sensitivity
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Pitfalls? How…?

- Interpreting the data is hard
- Tools were not designed to answer whether a pathogen is present
- As pathogen loads go down, reagent contaminant signals go up

<table>
<thead>
<tr>
<th>Sample #1 (with read #'s)</th>
<th>Sample #2</th>
<th>Sample #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus 334,354</td>
<td>Acinetobacter 4,915</td>
<td>Staphylococcus 666</td>
</tr>
<tr>
<td>Malassezia 8</td>
<td>Streptococcus 873</td>
<td>Cutibacterium 161</td>
</tr>
<tr>
<td>Corynebacterium 2</td>
<td>Prevotella 288</td>
<td>Streptococcus 141</td>
</tr>
<tr>
<td></td>
<td>Bradyrhizobium 326</td>
<td>Acinetobacter 133</td>
</tr>
<tr>
<td></td>
<td>Oribacterium 193</td>
<td>Malassezia 52</td>
</tr>
</tbody>
</table>

- *S. aureus* PJI
- Aseptic failure
- *S. epidermidis* PJI
Lessons learned from MSS analysis

• Simple read count or percentage cutoffs aren’t sufficient
  • Host DNA content and multiplexing influences these too much

• Subtracting negative control reads isn’t easy
  • Contains potential pathogens, changes over time

• Different tools can give different results

• A combination of metrics will likely be optimal
  • Signal strength, genome coverage, signal vs. internal controls

• A false positive result is much more dangerous than a negative result
Proposed role for MSS

• Currently: When all else fails
  • Cultures
  • Directed PCRs
  • 16S rRNA gene PCR
  • Serologic tests

• Best way to preserve samples?

• Future needs:
  • Faster and cheaper
  • Avoiding false positives

Alexander McAdam. J Clin Micro 2018, 56(8)
<table>
<thead>
<tr>
<th>Study</th>
<th>Samples</th>
<th>Microbial Enrichment</th>
<th>Extraction</th>
<th>Library + Sequencer</th>
<th>Analysis Tools</th>
<th>Comparison</th>
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<tr>
<td>Street, et al. J Clin Micro, 2017</td>
<td>Sonicate fluid (n=97)</td>
<td></td>
<td>Pathogen lysis tubes + EtOH precipitation</td>
<td>Nextera XT and MiSeq</td>
<td>Kraken</td>
<td>Sonicate fluid culture ± PPT</td>
</tr>
<tr>
<td>Thoendel, et al. CID, 2018</td>
<td>Sonicate fluid (n=408)</td>
<td>MolYsis</td>
<td>MoBio Bacteremia DNA kit</td>
<td>NEBNext Ultra and HiSeq 2500</td>
<td>LMAT + Metaphlan2</td>
<td>Sonicate fluid culture ± PPT ± synovial fluid culture</td>
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LMAT = Livermore Metagenomics Analysis Toolkit
PPT = Periprosthetic tissue (intraoperative)