Which « procedures » for an optimal Microbiological Diagnosis of Osteoarticular Infections?

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Microbiological Diagnosis of Osteoarticular Infections

- Microbiological procedures
  - conventional culture based
  - PCR based techniques
- Sampling procedures
  - superficial swab sampling
  - invasive surgical specimens
Microbiological Diagnosis of Osteoarticular Infections

Guided by epidemiological data that may differ

• Adult or pediatric population
• Primary arthritis or bone infections versus prosthetic device related infections
• Diabetic foot related osteomyelitis
Culture of Etiologic Agents in Cases of Septic Arthritis-Osteomyelitis

- Bacterial etiology of suspected septic arthritis remains unproven in one-third of cases:
  - patients received antimicrobial therapy
  - arthritis was of reactive nature
  - microorganisms are not detected by currently available techniques
  - concentrations of bacteria in synovial fluid may be of low magnitude
  - purulent exudates exert an inhibitory effect upon bacterial growth
Investigations in Pediatric Bone and Joint Infections

- White cell count: poor indicator of osteomyelitis elevated in only 35-40% of cases
- Erythrocyte sedimentation rate > 20 in 70-92% of cases
- CRP: most sensitive test: ↑ in up to 98% at admission
- Blood cultures: positive in 30-50% of patients
- Aspirations of affected bone: detection rate ↑ to 75-80%

Kingella kingae: An Emerging Pathogen of Acute Osteoarticular Infections in Children

- Review of data of suspected osteoarticular infections in 406 children hospitalized during a 3.5-year period
- 74 bacteriologically proven cases: 38 septic arthritis, 36 bone infections
  - *Staphylococcus aureus* 44% most frequent ≥ 36m
    (including PVL+ CA-MRSA: 62% of all *S. aureus*, ↑ ERS and CRP)
  - *K. kingae* 14% most frequent ≤ 36m
  - *Streptococcus pyogenes* 10%
  - *Streptococcus pneumoniae* 10%
- Increasing number of reports of *K. kingae* OAI in young children, as result of improved isolation techniques: liquid media increases rate of positive cultures compared to standard media (P=0.0001)

Moumille K et al Acta Pediatr 2005; 94: 419-20
Bocchini CE et al Pediatrics 2006;117:433-40
Kingella kingae

- Small gram-negative nonencapsulated coccobacillus in pairs or short chains
- Fastidious aerobes
- Colonies on agar may be small, smooth and translucent or appear as larger colonies which look pitted
- Only species to produce beta–hemolysis but not all strains are beta-hemolytic
Pathogens Recovered from 100 Synovial Fluid Specimen in which Both Conventional (CC) and BACTEC (BC) Cultures were Performed

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC+ BC+</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>7</td>
</tr>
<tr>
<td>Kingella kingae</td>
<td>1</td>
</tr>
<tr>
<td>Brucella melitensis</td>
<td>1</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>2</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus group C</td>
<td>1</td>
</tr>
<tr>
<td>Haemophilus influenzae type b</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>15</strong></td>
</tr>
</tbody>
</table>

Increased Recovery of *Kingella kingae* in Joint Fluid from Children with Septic Arthritis

- 10/11 *Kingella kingae* isolates recovered from BACTEC cultures only; for other pathogens recovery by BACTEC and conventional method comparable
  

- *K. kingae* recovered from 2 cases of septic arthritis by using BacT/Alert, not from conventional cultures
  
Age Distribution of 85 Patients with Invasive K. kingae Infections Diagnosed in Southern Israel in the 15-year Period 1988-2002

- *K. kingae* causesd 19/40 (48%) episodes of bacteriologically proven joint infections among children younger than 2 in southern Israel.

- *K. kingae* was the most common bacterial isolate in children younger than 3 in Atlanta after the conugate *H.influenzae* vaccine.

Increased Recovery of Organisms Causing Septic Arthritis Using Blood Culture Systems

- Prospective study on 137 adult and pediatric patients with clinical arthritis
- Inoculation of synovial fluid into an Isolator 1.5 microbial tube improves the recovery rate: total 21.5% positives:
  - 100% in Isolator
  - 78.9% in conventional cultures ($P<0.02$)
- Time to detection: similar 93.5% Isolator and 83.3% conventional cultures positive by the second day ($P>0.05$)
- Gram stain revealed causative organism in 56.0% culture

Contribution of Broad Range PCR to Diagnosis of Osteoarticular Infections

• Patients
  - Prospective study:
    171 children with osteoarticular infections between 01/2001 – 02/2004

• Methods: osteoarticular fluids of biopsy samples:
  - Cultures on BA, chocolate agar
  - BACT ALERT 3D blood culture bottles
  - 16S rDNA amplification culture neg samples

**Distribution of Bacterial Species Detected by Culture or PCR in Children with Primary OAI**

<table>
<thead>
<tr>
<th>Species</th>
<th>Culture</th>
<th>% of positive OAI (n=64)</th>
<th>Culture and PCR</th>
<th>% of Positive OAI (n=79)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td></td>
<td>Number</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>30</td>
<td>47</td>
<td>30</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>16</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>ß-Hemolytic streptococci, group A (n=7), group B (n=2), group C (n=1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Kingella kingae</em></td>
<td>9</td>
<td>14</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>7</td>
<td>11</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td><em>Propionibacterium acnes</em></td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><em>Moraxella</em> spp.</td>
<td>1</td>
<td>1.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Gemella morbillorum</em> + <em>Streptococcus gordonii</em></td>
<td>1</td>
<td>1.5</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Contribution of Broad Range PCR to Diagnosis of Osteoarticular Infections: CONCLUSIONS

• Only 79/171 (46%) of cases of OAI were bacteriologically documented by culture on PCR.
• The use of blood culture bottles strongly increases the diagnostic yield.
• *Kingella kingaeae* accounted for 14% of culture-positive cases.
• Molecular methods increased the identification of *Kingella kingaeae* in OAI up to 30%.

Do we need PCR for the Diagnosis of Osteoarticular Infections?
Usefulness of PCR for the Diagnosis of Bone and Joint Infections?

- 525 bone and joint samples collected by needle aspiration or by surgical biopsy
- Conventional culture and 16S rRNA PCR followed by sequencing
- Interpretation of results:
  - Isolation of the organism at least twice
  - If negative culture: true PCR positive if same microorganism at least twice with different PCR’s

⇒ Very strict criteria

Schematic Strategy for the Reconciliation of Conventional Culture and PCR Assays

Comparison of the Data Obtained for the Analysis of 525 Bone or Joint Samples Using Conventional Culture and 16S rRNA gene PCR followed by sequencing

Usefulness of PCR for the Diagnosis of Bone and Joint Infections | Results

- 89 concordant results:
  - 40% S. aureus
  - 20% CNS
  - 12% Streptococci
  - 12% Enterobacteriaceae
  - 6% Ps. aeruginosa
  - 7% anaerobes, HACEK, M. tuberculosis
- Culture false negative (n=16): 44% due to antibiotic treatment:
  - 25% S. aureus
  - 50% Streptococci
  - 25% Gram negatives and fastidious organisms
- PCR false negative (n=9): most S. aureus and CNS: inhibitors
- Polymicrobial (n=7): rarely or previously unreported: most anaerobes
Comparison of Sensitivity, Specificity, Positive Predictive Value, and Negative Value between Culture and 16S rRNA gene PCR Followed by Sequencing

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>No. of samples with definite conclusion</th>
<th>% sensitivity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% specificity&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Predictive value</th>
<th>Predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
<td>% Pos.</td>
</tr>
<tr>
<td>Culture</td>
<td>Positive</td>
<td>105</td>
<td>13</td>
<td>86.7</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>16</td>
<td>391</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR</td>
<td>Positive</td>
<td>112</td>
<td>5</td>
<td>92.5</td>
<td>95.7</td>
<td>95.7</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>9</td>
<td>389</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>121</td>
<td>424</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> P = 0.13 (chi-square test).

<sup>b</sup> P = 0.06 (chi-square test).

Strategy for the Use of 16S rRNA gene PCR for the Diagnosis of Bone or Joint Infections

PCR is complimentary to culture and should be used in patients highly suspected for whom culture was negative or in cases of suspected polymicrobial infections.

Osteomyelitis in Diabetic Foot Infections

Difficult and controversial

- Lack of consensus on the diagnosis of foot osteomyelitis
- Many available diagnostic tests, but they often yield equivocal results
- Osteomyelitis ↑ the likelihood of surgical intervention, including amputation
- Osteomyelitis impairs healing of the overlying wound and acts as a focus for recurrent infections

⇒ Diagnosis is important

# Percutaneous Bone Biopsy versus Swab Cultures for Diagnosis of Diabetic Foot Osteomyelitis

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Total</th>
<th>From bone biopsy sample only</th>
<th>From swab sample only</th>
<th>From both bone biopsy and swab samples</th>
<th>Concordance %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>49</td>
<td>13</td>
<td>15</td>
<td>21</td>
<td>42.8</td>
</tr>
<tr>
<td>CNS</td>
<td>35</td>
<td>30</td>
<td>4</td>
<td>1</td>
<td>2.8</td>
</tr>
<tr>
<td>Streptococci</td>
<td>31</td>
<td>11</td>
<td>12</td>
<td>8</td>
<td>25.8</td>
</tr>
<tr>
<td>Enterococci</td>
<td>15</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>6.67</td>
</tr>
<tr>
<td>Corynebacteria</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gram-negative bacilli</td>
<td>42</td>
<td>12</td>
<td>18</td>
<td>12</td>
<td>28.5</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>191</td>
<td>79</td>
<td>65</td>
<td>43</td>
<td><strong>22.5</strong></td>
</tr>
</tbody>
</table>

Percutaneous Bone Biopsy versus Swab Cultures for Diagnosis of Diabetic Foot Osteomyelitis

• Largest population of consecutive patients with diabetic foot osteomyelitis studied with surgical percutaneous bone biopsy

• Overall concordance for all isolates was 22.5%: 42.8% for *Staphylococcus aureus*.

• Distribution in bone and swab cultures were similar, except for CNS which were more prevalent in bone samples (*P*<0.001)

⇒ Superficial swab cultures do not reliably identify bone bacteria

Microbiology versus MRI and Labelled Leucocyte Scanning in the Diagnosis of Osteomyelitis of the Diabetic Foot

- 31 patients with foot lesions ≥ grade 3 Wagner classification
- Histopathological examination as gold standard
- *Pseudomonas aeruginosa* (33%) most common, MRSA (24%), *Acinetobacter* spp (12%); anaerobic cultures only *Peptostreptococcus* spp (3%)

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiology</td>
<td>92%</td>
<td>60%</td>
<td>92%</td>
<td>60%</td>
</tr>
<tr>
<td>Leucocyte scanning</td>
<td>91%</td>
<td>67%</td>
<td>95%</td>
<td>50%</td>
</tr>
<tr>
<td>MRI</td>
<td>78%</td>
<td>60%</td>
<td>90%</td>
<td>37.5%</td>
</tr>
</tbody>
</table>

⇒ Microbiology is effective and less costly

Needle Puncture vs Superficial Swab in Infected Diabetic Foot with Osteomyelitis

- Prospective study in 21 diabetic patients
- Mean number of microorganisms isolated by needle puncture compared to superficial swabbing: 1.09 vs 2.04 ($P<0.02$)
- *S. aureus* : 70% of cases
- In 76% of patients, microbiological confirmation with needle aspiration
- Needle puncture
  = more specific
  = safe, minimally invasive

**IDSA Grading System for ranking Recommendations in Clinical Guidelines**

<table>
<thead>
<tr>
<th>Strength of recommendation</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Good evidence to support a recommendation for use</td>
</tr>
<tr>
<td>B</td>
<td>Moderate evidence to support a recommendation for use</td>
</tr>
<tr>
<td>C</td>
<td>Poor evidence to support a recommendation; optional</td>
</tr>
<tr>
<td>D</td>
<td>Moderate evidence to support a recommendation against use</td>
</tr>
<tr>
<td>E</td>
<td>Good evidence to support a recommendation against use</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Quality of evidence</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Evidence from at least one properly randomized, controlled trial</td>
</tr>
<tr>
<td>II</td>
<td>Evidence from at least 1 well-designed clinical trial without randomization, from cohort or case-controlled analytic studies, from multiple time-series studies, or from dramatic results in uncontrolled experiments</td>
</tr>
<tr>
<td>III</td>
<td>Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.</td>
</tr>
</tbody>
</table>

Guidelines on Diagnosis of Osteomyelitis in Diabetic Foot Infections

• **Bone biopsy** is valuable for establishing the diagnosis of osteomyelitis: B II
  - for defining the pathogenic organism
  - for determining the antibiotic susceptibility

• **Further research is much needed**: adequately powered prospective studies should be undertaken to elucidate and validate: A III
  - systems for classifying infection
  - diagnosis osteomyelitis
  - defining optimal antibiotic regimes
  - clarifying the role of surgery in treating osteomyelitis

### Simple Puncture versus Notch Needle Biopsy

- Prospective study on 54 patients with deep infections of knees, ankles, elbows, shoulder
- Surgical prelevement was gold standard

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple puncture</td>
<td>31.25%</td>
<td>97%</td>
<td>83%</td>
</tr>
<tr>
<td>Notch needle biopsy</td>
<td>69%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

⇒ Notch needle biopsy is superior to simple puncture

Osteoarticular Tuberculosis

• Review of 26 patients over last 10 years
• Positive skintest in 15/18 patients: sensitivity 83.3%
• Positive, negative tuberculin test does not rule out skeletal tuberculosis
• Yield in different samples:
  - bone tissue: 86%
  - synovial tissue: 69%
  - abscess: 66%
  - synovial fluid: 66%
⇒ Tissue samples yield best results and should be available
⇒ Case reports of PCR proven MTb bone infections

Fungal Osteoarticular Infections

- Most studies consist of case reports or small case series
- Increasing with growing use of prosthetic joints and ↑ immunosuppressed patients
- Largest review → in cancer patients
- Moulds are most common pathogens: 24/28 (86%)
  - *Aspergillus* spp (n=10)
  - *Fusarium* spp. (n=6)
  - Zygomycetes (n=5)
  - *Scedosporium apiospermum* (n=2)

⇒ Diagnosis by culture of bone tissue

Osteoarticular Involvement in Brucellosis

- Documented extensively from Middle East and Spain, infrequently in this region
- In 42% of cases of acute brucellosis: osteoarticular involvement
- 95% of patients: positive serology with culture proven brucellosis
- Overall, 82% of bloodcultures and 100% of other body fluid cultures positive

Prosthetic Osteoarticular Infections

- 60% by direct contamination during operative procedure
- 50% of all isolates: *S. aureus* and *S. epidermidis*
- ESR and CRP: non specific
- Gram staining of synovial fluid and periprosthetic tissue: low sensitivity: 25-30%
- Culture from synovial fluid of periprosthetic tissue = GOLD STANDARD: sensitivity : 65-94%
- PCR for rRNA: experimental but ↑ sensitivity

Osteoarthritis and Anaerobes:

- *Peptostreptococcus magnus*
  - After orthopaedic prothesis or implantation of fixation devices
  

- *Pripionibacterium acnes*
  - Spondylodiscitis following lumbar punction
    
  
  - Spontaneous infection or secondary to implantation of foreign material
    

⇒ ANAEROBIC CULTURE SHOULD BE CONSIDERED
Which « procedures » for an optimal Microbiological Diagnosis of Osteoarticular Infections: CONCLUSIONS

- **ESR and CRP**: quite sensitive but low specificity

- **Correct sampling is crucial**: percutaneous or surgical biopsy material required in case of bone infections, synovial fluid for arthritis

- Inoculation of samples into **blood culture systems** increases significantly the diagnostic yield

- **PCR** may be considered in culture negative cases but so far not introduced into routine management