Detection of Prosthetic Joint Infection Using Synovial Fluid Polymerase Chain Reaction-Electrospray Ionization Mass Spectrometry

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Polymerase chain reaction coupled with electrospray ionization mass spectrometry applied to synovial fluid had an 81% sensitivity and a 95% specificity for the diagnosis of prosthetic joint infection.
The number of cases of prosthetic joint infection (PJI) is increasing. Microbiologic diagnosis of PJI has traditionally been made by culture of synovial fluid, periprosthetic tissue and/or the implant itself. However, cultures are not universally positive (1-6); this has spawned an interest in using molecular strategies to diagnose PJI (7-13). We have recently shown that a technique that couples PCR with electrospray ionization mass spectrometry (PCR-ESI/MS), used previously in a variety of settings (14-18), can be applied to materials dislodged from explanted orthopedic implants (sonicate fluid) to diagnose of PJI with increased sensitivity compared with culture (19). Jacovides et al. used an older version of this technology than we had used and detected organisms in synovial fluid in 88% of presumed noninfectious arthroplasty failures (20). Although we also found a lower specificity of PCR-ESI/MS (94%) than culture (99%) when applied to sonicate fluid (21), we did not find nearly the proportion of positive specimens in presumed noninfectious failure reported by Jacovides et al. (20). Herein, we evaluated synovial fluid specimens collected in containers treated to minimize background DNA using the same version of the PCR-ESI/MS assay we used to study sonicate fluid (21).

The PCR-ESI/MS BAC protocol (Ibis Biosciences, Carlsbad, CA) was used to test synovial fluid collected by sterile arthrocentesis from subjects with knee arthroplasty failure at Mayo Clinic, Rochester MN. The research application PCR-ESI/MS BAC assay studied detects more than 3,400 species of bacteria, 40 species of Candida and four antibiotic resistance markers, bla\text{KPC}, vanA, vanB and mecA. Syringes, vacutainer collection tubes, and freezer storage vials used for collection and storage of specimens were pretreated to minimize contaminating DNA by irradiation in a self-contained \textsuperscript{137}Cs gamma irradiator with a total dose of 1 Gy (22). Specimens were collected between 2001 and 2012 and stored at -70°C until PCR-ESI/MS testing in 2012. At the time of specimen collection, cultures had been performed using previously-described
methods (23). Subjects were classified as having PJI or aseptic failure (AF) using the 2011 Musculoskeletal Infection Society (MSIS) criteria, a combined clinical/laboratory classification system (24). Accordingly, a case was considered as having PJI if a sinus tract communicating with the prosthesis was found; or, the same organism was isolated from two non-synovial fluid samples (i.e., periprosthetic tissue, sonicate fluid) obtained from the affected joint at the time of revision surgery; or, if four of the following six minor criteria were present: Elevated erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP); elevated synovial fluid white blood cell (WBC) count; elevated synovial neutrophil percentage (%PMN); presence of purulence in the affected joint; isolation of a microorganism from a single periprosthetic tissue specimen or the implant itself; and acute inflammation on periprosthetic tissue histologic examination. Cases that did not meet the above criteria were considered to have AF.

DNA was extracted from 1 ml of synovial fluid using magnetic bead-beating with the KingFisher DNA Extraction instrument (Thermo Scientific, Waltham, MA), as previously described (17). Following PCR amplification, the molecular mass of the amplified DNA was determined by mass spectrometry. The organism detected (assessed based on the PCR assay that was positive and the base composition of the amplified product inferred from its molecular mass) was determined by the system’s software with two parameters additionally reported, Q-score and level of detection (25). The Q-score, a rating between 0 (low) and 1 (high), represents a relative measure of the strength of the data supporting identification. Per the system software, only organisms with Q-scores $\geq 0.90$ were reported. The level of detection is a semi-quantitative measure of the amount of amplified DNA calculated relative to an internal calibrant, and reported in genome equivalents (GE)/well. We performed an ROC curve analysis to determine the level of detection that would best discriminate PJI from AF. Sensitivity and specificity for
synovial fluid culture and PCR-ESI/MS were compared using McNemar’s test of paired proportions.

One hundred and three synovial fluids from 21 PJI and 82 AF cases were evaluated. No differences in age, gender, time from synovial fluid collection to revision surgery or specimen storage time were found between the groups (Table 1). A cutoff of \( \geq 24 \) GE/well was determined to be the ideal discriminator to separate infected and non-infected cases (AUC, 0.88).

In the PJI group, PCR-ESI/MS was positive in 17, and synovial fluid culture in 18 subjects (sensitivities 81 and 86%, respectively, \( p = 0.56 \)) and in the AF group, PCR/ESI-MS was negative in 78, while synovial fluid culture was negative in all 82 subjects (specificities 95% and 100%, respectively, \( p = 0.045 \)).

Four PJI cases had negative PCR-ESI/MS results, two of which had positive synovial fluid cultures (cases 180 and 181, Supplemental Table 1). The other two had negative synovial fluid PCR-ESI/MS and culture results; in one, ten months had elapsed between synovial fluid collection and surgery (i.e., periprosthetic tissue specimen collection), suggesting that this subject may not have been infected at the time of synovial fluid collection. In the fourth case, only one month had elapsed between synovial fluid collection and surgery, the patient had not previously received antibiotics, periprosthetic tissue and synovial fluid cultures were negative but prosthesis sonication fluid was positive for \( >100 \) cfu/mL \( P. \) acnes. During surgery, purulence was encountered, although pathologic review of the periprosthetic tissue specimen revealed synovial hyperplasia with chronic inflammation.

Supplemental Table 2 shows clinical follow up information on all subjects with aseptic failure who had microorganisms detected at any level by PCR-ESI/MS (n=10). Six (cases 221, 277,
285, 331, 332 and 350) had levels of detection below what was considered positive (<24 GE/well). The remaining four positive PCR-ESI/MS cases had levels of detection ranging from 24 GE/well (P. acnes and S. warneri) to 160 GE/well (mecA-positive S. epidermidis). No evidence of infection was found in any of these ten cases.

The most common organism detected by PCR-ESI/MS was Staphylococcus epidermidis (11 specimens, Table 2). In the PJI group there was microbiologic concordance between PCR-ESI/MS and synovial fluid culture in 15 cases; one discordant case grew oxacillin-resistant coagulase-negative Staphylococcus species (CoNS) from synovial fluid culture as well as from all three periprosthetic tissue specimens cultured, while Propionibacterium acnes and Candida tropicalis were detected by PCR-ESI/MS. Of the remaining three discordant cases, one was positive by PCR-ESI/MS and negative by synovial fluid culture, and two were negative by PCR-ESI/MS and positive by culture (Supplemental Table 1).

We also compared results of PCR-ESI/MS with results of cultures of non-synovial fluid specimens. Seven PJI subjects had undergone implant culture using vortexing-sonication (3, 4, 6), five of whom had concordant results with PCR-ESI/MS, one of whom had an additional organism (CoNS 51-100 cfu/10 ml) detected by culture, and one of whom was positive only by sonicate fluid culture (P. acnes >100 cfu/10 ml). Eighteen PJI subjects had had specimens submitted for periprosthetic tissue culture; complete microbiologic concordance was found in 12, PCR-ESI/MS was positive and culture negative in one, culture was positive and PCR-ESI/MS negative in two, tissue culture grew two additional organisms in one case (PCR-ESI/MS detected only S. epidermidis while culture was positive for CoNS, Lactobacillus species and Klebsiella species - all grew only from broth). One case grew CoNS in tissue culture while PCR-ESI/MS and synovial fluid culture were positive for Enterococcus faecalis, and in last case, mentioned
above, PCR-ESI/MS was positive for *P. acnes* and *C. tropicalis* whereas periprosthetic tissue and synovial fluid culture grew CoNS.

Twelve subjects (9 PJI and 3 AF) had received antibiotics within 30 days prior to synovial fluid aspiration. Of the nine PJI cases, PCR-ESI/MS was positive in eight, and synovial fluid culture was positive in nine; PCR-ESI/MS and culture were negative in all three AF cases.

There was high level of concordance between synovial fluid PCR-ESI/MS detection of antibiotic resistance markers *mecA* and *vanA/vanB* and traditional phenotypic antimicrobial susceptibility testing of cultured isolates. Out of the 11 staphylococci detected by PCR-ESI/MS, 10 had concordant results between detection of *mecA* by PCR-ESI/MS and resistance to oxacillin by phenotypic antimicrobial susceptibility testing of the isolated organism. The single discordant case was a CoNS isolated from synovial fluid culture; the isolate had an oxacillin minimum inhibitory concentration of 1 µg/ml (interpreted as resistant by current Clinical and Laboratory Standards Institute guidelines, (26) but the synovial fluid was *mecA*-negative but positive for *S. epidermidis/caprae* by PCR-ESI/MS. Oxacillin resistance in the isolate may have been mediated by a mechanism other than *mecA*, more than one CoNS strains may have been present in the specimen, or the oxacillin susceptibility breakpoint may not correlate with the presence of *mecA*.

Only one subject had *E. faecalis* detected in synovial fluid by culture; *vanA/B* testing was negative by PCR-ESI/MS and the isolate vancomycin-susceptible by phenotypic antimicrobial susceptibility testing.

Twenty-two subjects (5 PJI, 17 AF) had prosthesis sonicate fluid PCR-ESI/MS done as part of our prior study (21). The five PJI cases had concordant positive results by PCR-ESI/MS in both specimen types, and 15 of 17 AF cases were PCR-ESI/MS negative in both specimen types. One
case was PCR-ESI/MS positive for *Micrococcus lylae/luteus* (level, 15; Q-score, 0.90) in synovial fluid and negative in sonicate fluid, and the final case was PCR-ESI/MS positive for *Hyphomicrobium denitrificans* (level, 31; Q-score, 0.95) in sonicate fluid and negative in synovial fluid.

As mentioned above, review of the medical records of all presumed non-infected cases with a positive PCR-ESI/MS result with follow-up as long as nine years after synovial fluid collection (Supplementary Table 2) did not reveal any evidence of PJI. In the study by Jacovides et al. (20), a high rate of false positivity was reported, with 49 out of 57 AF subjects having a positive PCR-ESI/MS result. The use of different PCR-ESI/MS system criteria for considering results positive and/or collection of specimens in such a way as to eliminate contaminating DNA may account for the differences found.

A finding common to both our study and that of Jacovides et al. is that some of the AF subjects with a positive PCR-ESI/MS result had a history of prior infection. In our study, two such cases were found (cases 214 and 285 in Supplemental Table 2), one of which was a subject with a history of *S. aureus* PJI three years prior to synovial fluid collection who had a positive PCR-ESI/MS for *S. aureus* (level, 3; Q score, 0.9). This suggests that microbial DNA may persist in synovial fluid following successful treatment and therefore its presence may not always indicate persistent infection.

Limitations of our study include the relatively small sample size and prolonged storage of some of our specimens prior to PCR-ESI/MS testing. In addition, because of the retrospective nature of the medical record review, some data, such as antecedent use of antimicrobial agents, may be incomplete. PCR-ESI/MS and culture may complement one another in microorganism detection.
in PJI, as PCR-ESI/MS detected a microorganism in one culture-negative PJI case and synovial
fluid culture was positive in two PCR-ESI/MS-negative PJI cases. Failure of PCR-ESI/MS to
detect an organism in four PJI cases may be explained by the time elapsed between specimen
collection and the surgical procedure in one case, the low organism burden of chronic infection,
and/or prolonged storage of synovial fluid specimens in the other cases.

In summary, our study shows that PCR-ESI/MS of synovial fluid has a similar sensitivity to
synovial fluid culture albeit a lower specificity. PCR-ESI/MS can be performed in approximately
12-16 hours and provides not just microbial identification but also information on selected
antimicrobial resistance markers.
ACKNOWLEDGEMENTS

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REFERENCES


### TABLE 1: Demographic and Laboratory Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Aseptic Failure (n=82)</th>
<th>Prosthetic Joint Infection (n=21)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [Mean (SD)] years</td>
<td>68.6 (11.7)</td>
<td>67.76 (10.1)</td>
<td>0.62</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male %</td>
<td>57.2</td>
<td>76.2</td>
<td>0.11</td>
</tr>
<tr>
<td>Female %</td>
<td>42.9</td>
<td>53.8</td>
<td></td>
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<tr>
<td>Synovial cell count mean [cells/µl (SD)]</td>
<td>1498.1 (2579.3)</td>
<td>39263 (37574)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Neutrophil percentage mean [% (SD)]</td>
<td>18.4 (21.7)</td>
<td>88.9 (7.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sedimentation rate mean [mm/hr (SD)]</td>
<td>13.4 (14.7)</td>
<td>69.5 (34.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C-reactive protein mean [mg/dl (SD)]</td>
<td>0.79 (1.1)</td>
<td>8.26 (5.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time from synovial fluid collection to revision surgery mean [days (SD)]</td>
<td>45.8 (65.1)</td>
<td>45.1 (71.8)</td>
<td>0.96</td>
</tr>
<tr>
<td>Synovial fluid storage time mean (from collection to PCR-ESI/MS analysis) [months (SD)]</td>
<td>88.2 (30.3)</td>
<td>88.7 (39.6)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

SD = standard deviation
### TABLE 2: Microbiology of PCR-ESI/MS and Culture of Synovial Fluid

<table>
<thead>
<tr>
<th>Organism</th>
<th>PCR-ESI/MS (number of positive results)</th>
<th>Synovial Fluid Culture (number of positive results)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PJI</td>
<td>AF</td>
</tr>
<tr>
<td>Coagulase negative Staphylococcus sp.</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Viridans group Streptococcus sp.</td>
<td>1(^b)</td>
<td>0</td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Methylobacterium mesophilium</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Micrococcus lylae/luteus</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Capnocytophaga canimorsus</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Polymicrobial</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

- \(^a\) S. epidermidis (7), S. epidermidis/caprae (1), S. capitis (1), S. warneri (1), and S. haemolyticus (1). Six (all S. epidermidis) were mecA positive.
- \(^b\) Streptococcus salivarius/thermophilus.
- \(^c\) Polymicrobial detections by PCR-ESI/MS included P. acnes plus C. tropicalis (PJI subject), and P. acnes plus Streptococcus salivarius species group plus Streptococcus mitis species group (AF subject) and E. cloacae complex plus A. baumannii (AF subject).

PJI, prosthetic joint infection; AF, aseptic failure