Diagnostics in prosthetic joint infections

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Prosthetic joint infection (PJI) poses a significant burden on patients, clinicians and the healthcare economy. Although various tests have been established for the diagnosis of PJI, the diagnosis remains challenging. In this review, established and potential future diagnostic tests are presented, some of which could provide stepping stones towards improved diagnosis, identification of aetiological agents and efficacious therapeutic options for the management of PJI.

Keywords: PJI, aseptic loosening, biomarkers, sonication

Introduction

The diagnosis of prosthetic joint infection (PJI) continues to be a major challenge in clinical practice. As therapeutic success rates for PJI can be improved tremendously if the condition is diagnosed early, diagnostic techniques have received increasing consideration from multidisciplinary teams involved in managing PJI. In general, tests can be grouped into pre-operative and intra-/post-operative diagnostics, some of which are excessively overused and others are underutilized for a number of reasons, including conflicting results in the literature, lack of availability and cost. The purpose of this article is to provide an overview of a range of diagnostic techniques that have been established in the diagnosis of PJI and some novel techniques that could become key diagnostics in the future (Figure 1).

Pre-operative diagnostics

Biochemical, haematological, serological and microbiology studies

White blood cell (WBC) counts, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels tend to be first-line screening tests for evaluating patients with suspected PJI, probably due to their relatively low cost and widespread availability. However, they are not essential when making a diagnosis of PJI, particularly in clinically apparent infections. Indeed, increased WBC, ESR and CRP levels are neither particularly sensitive nor specific for PJI.1–6 False-negative or low values could occur in the context of suppressive antimicrobial therapy, low-virulence pathogens, chronic infections and/or infections with a fistula, which are all common occurrences in PJI.7,8 Equally, they may be elevated due to concomitant inflammatory conditions or after primary uncomplicated arthroplasty.9 Furthermore, the diagnostic effectiveness of CRP and ESR can also be different according to the type of prosthesis or surgery. Piper et al.10 found CRP and ESR values were higher in knee arthroplasty and spine implant patients than in hip arthroplasty patients with infection and showed the lowest sensitivity for diagnosis of shoulder arthroplasty infection. Some advocate measuring baseline levels followed by serial measurements to assess trends, while others suggest that corroborating ESR and CRP values may provide the best positive (PPV) and negative predictive values (NPV) for the diagnosis of PJI.8,11–14 However, in a study of >3500 CRP measurements in >250 PJI patients, CRP values were neither sensitive nor specific as indicators for infection following second-stage revision surgery or for debridement, antibiotic and implant retention (DAIR) procedures. More importantly, the authors concluded that routine CRP monitoring should not be recommended in the prosthetic joint setting as the results can lead to inappropriate management decisions and increased cost.15

A number of other biomarkers have been evaluated or studied in the diagnosis of PJI (Table 1).16–21 Studies have shown that the serum concentration of interleukin 6 (IL-6) is significantly higher in patients with septic loosening compared with aseptic loosening: cut-off levels of 8 or 9 pg/mL provided sensitivities of 40% and 80% and specificities of 81% and 77%, respectively,16,17 with the latter cut-off providing a PPV of 65%, an NPV of 50% and 78% accuracy for the diagnosis of PJI.17 Some have suggested that elevated IL-6 levels >12 pg/mL combined with ‘high’ CRP levels provide a good screening test to identify patients with PJI.16,21 However, the normal range of serum IL-6 varies, which may reflect a considerable variation in cut-off ranges in different studies.16,17,20,22–24 Furthermore, like CRP, IL-6 is not a specific marker for bacterial infection and its concentration in the peripheral blood increases after trauma, chronic inflammatory conditions and arthroplasty.1,25–27

Since the early 1990s, there has been much interest in procalcitonin (PCT). Although studies have often given conflicting results regarding the superiority of PCT, it is largely believed that PCT is a more accurate indicator of bacterial infection than the biomarkers mentioned above.28–31 Unlike other surgical procedures, it appears that serum PCT levels are not significantly elevated.
Figure 1. Diagnostic algorithm for PJI, including some novel science, tests and technologies (shown in italics) that may not be available in many centres or require further investigations. PJI, prosthetic joint infection; WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL-6, IL-8, CRP, α2-macroglobulin and vascular endothelial growth factor.
following arthroplasty. In one study, serum PCT was evaluated within 10 days after orthopaedic surgery and was useful in differentiating infectious from non-infectious causes of fever. However, in other studies, despite striking specificity, the sensitivity of serum PCT has not been found to be of value in the diagnosis of PJI (Table 1) and hence, at present, serum PCT cannot be considered a superior marker to identify patients with PJI.

The association of tumour necrosis factor α (TNF-α) with other cytokines was previously reported in knee synovial tissue from patients with rheumatoid arthritis, however, there is insufficient information on the value of TNF-α as a diagnostic marker for infection or PJI.

Soluble intercellular adhesion molecule 1 (sICAM-1) is a member of the immunoglobulin superfamily. Expression of the gene encoding sICAM-1 can be induced by cytokines and/or bacteria. Worthington et al. found that median sICAM-1 concentrations in the serum of patients with septic loosening (330 ng/mL) were significantly higher than in those with aseptic loosening (180 ng/mL) (Table 1). Drago et al. considered sICAM-1 to be a good marker for distinguishing cases of PJI from comparison groups consisting of patients without infection or those with previous infections that had been cleared.

As the majority of cases of PJI are due to coagulate-negative staphylococci (CoNS), serum IgG to short-chain exocellular lipoteichoic acid (sce-LTA) (previously termed lipid S) produced by CoNS could represent a valuable diagnostic marker in patients with device-related infections including PJI due to CoNS.

A staphylococcal IgM ELISA has been adapted for the diagnosis of delayed PJI. The test detects serum IgM antibodies to staphylococcal biofilm polysaccharide antigens and researchers found a significant difference in levels between delayed PJI cases, non-infected implants and cases without prosthesis and infection. Using a cut-off value of 0.35 ELISA units, the test showed sensitivity of 90% and specificity of 95%. Other serological tests have also been adapted for use, with LumineX technology able to detect anti-Staphylococcus aureus and anti-Staphylococcus epidermidis IgG in 2 h.

Studies evaluating these biomarkers in PJI are limited. The majority are small studies with different designs and inclusion and exclusion criteria. They fail to provide information regarding assay reproducibility and other factors that can affect the kinetics of some these markers, e.g. presence of comorbidities and treatment with antibiotics, steroids and other immunomodulators at the time of testing. Hence, to a diagnostican, the role of these biomarkers in the diagnosis of PJI still remains to be fully defined. Undertaking tests with higher sensitivity followed by tests with higher specificity may prove to be of more value in assessing patients where there is a clinical suspicion of PJI.

**MRSA screening, blood cultures and swabs**

Methicillin-resistant S. aureus (MRSA) screening, especially prior to orthopaedic implantation, is normal practice in UK hospitals. Documented concomitant or past MRSA carriage acts as a surrogate marker for clinicians to treat empirically with an MRSA-active antibiotic. However, a number of reports suggest current or past colonization with MRSA does not necessitate empirical antibiotic coverage for MRSA in PJI.

Blood cultures should be performed to exclude concomitant bacteraemia in suspected cases of PJI or if the patient is febrile and/or if there are concerns of metastatic infection. However, they often remain negative due to prior empirical antimicrobial therapy. Ideally, two or more sets or repeat samples should be taken prior to commencing antibiotic therapy to ascertain significance, particularly in cases of positive cultures yielding skin flora. Superficial and sinus tract swabs are not helpful, as the organisms cultured do not predict those causing deep infection.

**Imaging studies**

Plain radiographs are widely used in the initial evaluation of painful arthroplasties, despite the lack of sensitivity and specificity. They are most helpful in the diagnosis of PJI when studied serially over time, after implantation, and may guide further diagnostics. Ultrasonography could also be helpful in detecting joint effusions and guide arthrocentesis.

Magnetic resonance imaging (MRI) and CT are not considered to be first-line imaging modalities for evaluating PJI. They can help detect sinus tracts, soft tissue abscesses, bone erosion and peri-prosthetic lucency. Detecting periostitis has 100% sensitivity but only 16% specificity for PJI. Associated joint distension and soft tissue fluid collections around arthroplasties increase this specificity to 87%. However, MRI should only be performed in patients with implants that are safe for this technique.

**Pre-operative arthrocentesis**

Pre-operative arthrocentesis is a valuable procedure for the investigation of PJI or failed arthroplasties. It must be performed aseptically and ideally in all patients with suspected PJI unless this is contraindicated (e.g. uncontrolled coagulopathy) or when the diagnosis of PJI is obvious prior to planned surgery.

**Possible future strategies**

**Advances in diagnostic laboratory techniques**

In the future, biomarkers may be able to provide additional information to support an initial clinical suspicion of PJI. Increased understanding of the mechanisms of PJI may offer the opportunity to develop new assays. For example, the development of assays that detect serum IgM antibodies to CoNS, and short-chain exocellular lipoteichoic acid (sce-LTA) is already established.

**The role of imaging studies**

The role of imaging studies should be reviewed in the context of advances in surgical techniques and technologies. Ultrasonography and CT are becoming increasingly important and are likely to play an increasing role in the diagnosis and management of PJI.

**Conclusion**

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 interleukin-6; PCT, procalcitonin; sICAM-1, soluble intercellular adhesion molecule 1; sce-LTA, short-chain exocellular lipoteichoic acid; MRI, magnetic resonance imaging; CT, computed tomography; FDG-PET, 18F-fluoro-2-deoxyglucose positron emission tomography; SPECT, single-photon emission computed tomography; PCR, polymerase chain reaction; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; FISH, fluorescence in situ hybridization; PNA, peptide nucleic acid probe; ETGA, enzymatic template generation and amplification.
In revision total knee arthroplasty (TKA), it was demonstrated that synovial fluid WBC counts of \( \geq 1100/\mu\text{L} \) containing \( \geq 64\% \) neutrophils resulted in 99.6% NPV for excluding PJI. In contrast, a synovial fluid WBC count of \( \geq 1700/\mu\text{L} \) containing \( \geq 65\% \) neutrophils had sensitivities for knee PJI of 94% and 97%, respectively, and specificities of 88% and 98%, respectively, in patients without underlying inflammatory joint diseases and who were \( > 6 \) months from TKA implantation.

Table 1. Biomarkers other than CRP, ESR and WBC that have been used or studied in the diagnosis of PJI

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Cut-off</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV</th>
<th>NPV</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (^{1,6})</td>
<td>( \geq 40 ) ng/mL</td>
<td>43</td>
<td>94</td>
<td>75</td>
<td>85</td>
<td>testing needs to be done within 1 h and is time-consuming routine test that can be performed on routine immunoassay platforms; however, it lacks sensitivity, particularly in localized infections routine test; however, its normal range varies in adults, which may reflect a considerable variation of cut-off ranges in different studies</td>
</tr>
<tr>
<td>Serum PCT (^{16,20})</td>
<td>( \geq 0.3 ) ng/mL</td>
<td>&lt;30 – 33</td>
<td>98 – 100</td>
<td>87</td>
<td>80</td>
<td>insensitive information regarding clinical utilization in routine diagnostic laboratories specific only for coagulase-negative staphylococci; insufficient information regarding clinical utilization in routine diagnostic laboratories specific only for coagulase-negative staphylococci</td>
</tr>
<tr>
<td>IL-6 (^{16,17,20})</td>
<td>8 – 12 pg/mL</td>
<td>40 – 95</td>
<td>80 – 87</td>
<td>65 – 74</td>
<td>50 – 98</td>
<td>insensitive information regarding clinical utilization in routine diagnostic laboratories specific only for coagulase-negative staphylococci</td>
</tr>
<tr>
<td>sICAM-1 (^{17})</td>
<td>250 ng/mL</td>
<td>94</td>
<td>74</td>
<td>65</td>
<td>65</td>
<td>insensitive information regarding clinical utilization in routine diagnostic laboratories specific only for coagulase-negative staphylococci</td>
</tr>
<tr>
<td>Serum IgG to sce-LTA (^{17})</td>
<td>3 out of 4 cases with CoNS PJI showed elevated levels</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-staphylococcal IgM (^{19})</td>
<td>( \geq 0.35 ) unit</td>
<td>90</td>
<td>95</td>
<td>90</td>
<td>95</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Diagnostic and clinical value of more advanced imaging techniques

<table>
<thead>
<tr>
<th>Imaging techniques</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radionuclide imaging and (^{18})F-fluoro-2-deoxyglucose positron emission tomography (^{51})</td>
<td>91 – 100</td>
<td>9 – 97</td>
<td>specificity varies depending on diagnostic criteria used</td>
</tr>
<tr>
<td>Combined (^{111})In-WBC and (^{99m})Tc-sulphur colloid single-photon emission computed tomography/CT (^{51})</td>
<td>96 – 100</td>
<td>91 – 97</td>
<td>reported diagnostic accuracy of 95%–97%; appear to be promising tools in diagnosis of PJI routine test; however, its normal range varies in adults, which may reflect a considerable variation of cut-off ranges in different studies</td>
</tr>
<tr>
<td>Antigranulocyte scintigraphy with monoclonal antibodies or antibody fragments (^{52})</td>
<td>83</td>
<td>80</td>
<td>routine test; however, its normal range varies in adults, which may reflect a considerable variation of cut-off ranges in different studies</td>
</tr>
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</table>

In revision total knee arthroplasty (TKA), it was demonstrated that synovial fluid WBC counts \(<1100/\mu\text{L}\) containing \(<64\%\) neutrophils resulted in 99.6% NPV for excluding PJI. In contrast, a synovial fluid WBC count of \(>1700/\mu\text{L}\) or \(>65\%\) neutrophils had sensitivities for knee PJI of 94% and 97%, respectively, and specificities of 88% and 98%, respectively, in patients without underlying inflammatory joint diseases and who were \(>6\) months from TKA implantation. WBC counts of \(>27800/\mu\text{L}\) and 89% neutrophils have also been predictive of early TKA infections.

With regard to hip arthroplasty, in a study of \(>200\) patients with painful hip arthroplasties, a synovial fluid WBC count \(>4200/\mu\text{L}\) was 84% sensitive and 93% specific, and 80% neutrophils was 84% sensitive and 82% specific to detect all those with hip PJI. In general, the leucocyte differential varies between septic and aseptic loosening of prosthetic joints, with a predominance of neutrophils in PJI compared with lymphocytosis in inflammatory conditions. However, the interpretation of synovial WBC is complex when there is bleeding into the joint or in concomitant inflammatory arthritis; like other investigations, the synovial WBC count and its ability to predict or confirm PJI must be interpreted in light of clinical findings, the aspirated joint, the type of prosthesis and the time from implantation.

In a novel application, synovial aspirates were tested for the presence of leucocyte esterase enzyme using a simple colorimetric strip test. In 30 cases of PJI of the knee, when the leucocyte esterase reading was considered positive by the investigators, the test was 80.6% sensitive and 41.7%–100% specific with a PPV of 41.7%–100% and an NPV of 92.3%. Although analysis of the colorimetric strip test is subjective, these results are encouraging, especially as a point-of-care test in the diagnosis of PJI. Furthermore, in our institution, we have studied synovial PCT levels in pre-defined cases of joint infections and control groups including cases of PJI and aseptic loosening. We observed higher synovial PCT levels in infection cases including PJI compared with control cases.
aseptic loosening and other inflammatory arthritids. However, larger prospective studies are needed to further validate these preliminary findings in both native and prosthetic joints before synovial PCT becomes part of any diagnostic pathway.

Intra-/post-operative diagnostics

Histopathological studies

Histopathological examination of intraoperative samples could provide additional information to gross surgical appearance and aid in the diagnosis of PJI and acute inflammation, although such examination is unlikely to identify causative organisms. A further consideration is that the presence of particular pathogens and previous antibiotic therapy could modify the nature of the inflammatory response and alter the results. A neutrophil count of 5–10 cells per high power field, at a magnification of ×400, has a sensitivity of 50%–93% and specificity of 77%–100% for predicting PJI and has been used to decide between the need for revision versus resection arthroplasty when other pre-operative evaluation has failed to confirm PJI. Withholding or stopping antimicrobial therapy (if possible for ≥2 weeks) prior to collecting the specimens increases the yield of recovered organisms.

The importance of at least five separate biopsy samples for bacterial culture, taken in proximity to hip prostheses for the optimal diagnosis of PJI, was first propounded in the 1980s and later confirmed by other investigators. Combination of synovial fluid and periprosthetic tissue may provide the best sensitivity, specificity and accuracy. Each specimen should be obtained with a separate set of sterile instruments and placed into a separate sterile container. At this stage, a frozen section, if available, may also be performed. Samples should be transferred as soon as possible to the laboratory for culture, but if delays are inevitable, they can be kept either at 4°C or at room temperature. Maintaining anaerobic conditions during transport and, if needed, using Amies transport medium may yield better viability.

Various preparatory methods have been applied in the processing of tissue samples prior to culture on assorted media, e.g. partitioning bigger samples into smaller pieces with surgical knives, homogenization using Ballotini beads or grinding with a mortar and pestle or a Seward stomacher. Gram staining of tissue samples can be performed but has low sensitivity. Inoculating synovial fluid into paediatric blood culture bottles (BCBs) was reported to detect more pathogens than direct culture methods. A prospective study evaluated four different culture media used in the diagnosis of PJI: BACTEC and CM. Equally, others demonstrated higher sensitivity and specificity for synovial fluid inoculation into aerobic and anaerobic BCBs. However, despite the above reports, this practice has not been featured in more recent guidelines.

Most studies recommend 5 days of incubation for aerobic cultures and 7 days for anaerobic cultures, but prolonged incubation for up to 13–14 days may help with pathogen isolation, particularly Propionibacterium spp. and small colony forms, e.g. in Escherichia coli. Special culture techniques for fungi and mycobacteria are necessary if clinically isolated. Isolation of identical organism(s) from two to three or more independent specimens is highly predictive of infection (sensitivity: 65%; specificity: 99.6%). The prevalence of small colony forms in PJI is unclear. These organisms may be present in areas near the prosthesis with low concentrations of antibiotic diffusing from the cement. Hence, the UK Standards for Microbiology Investigations recommends examination of culture plates with a plate microscope to detect small colony forms of staphylococci.

False-negative culture results in PJI may be due to sampling error, prior antibiotic therapy, low quantity of microorganisms, fastidious organisms, use of inappropriate culture media and delays in processing samples. Furthermore, organisms may be concentrated in the periprosthetic tissue and in biofilm-related implant infections and, for these reasons, conventional culture methods developed for planktonic bacteria are not reliable and have led to the erroneous diagnosis of ‘aseptic loosening or failure’ in what were genuine infections. Hence, obtaining samples from the prosthesis could improve the diagnosis of PJI. The explanted prosthesis, joint components, bones, pins and screws can be placed in a sterile, airtight container and covered with Ringer’s solution or saline prior to submission to the laboratory for sonication and subsequent centrifugation and subculture of sonication fluid. Studies have demonstrated that the diagnostic sensitivity and specificity of Gram staining of sonication fluid are ~45% and ~100%, respectively. Subculture of sonication fluid has shown a sensitivity of 79% compared with 60% for conventional cultures. Additionally, the sensitivities of periprosthetic tissue and sonication fluid culture in patients receiving antimicrobial therapy within 14 days before surgery also differed significantly (45% and 75%, respectively).

The specificities of sonication fluid culture, tissue culture and synovial fluid culture are ~99%, ~99% and ~98%, respectively. Optimizing sonication parameters such as duration, temperature and centrifugations are critical for better microbial yield. Vortexing samples for 30–60 s before and after 5 min of low-frequency sonication also leads to better pathogen recovery. However, sonication is not widely available in routine diagnostic centres; moreover, it may damage bacteria, especially Gram negatives and anaerobes, and there is a risk of contamination during the procedure.

A recent prospective study compared the efficacy of vortexing alone for 1 min versus vortexing plus sonication for biofilm disruption in the diagnosis of PJI. Among 135 removed prostheses, 35 were diagnosed with infection and 100 with aseptic failure. Using a cut-off of ≥50 cfu/mL, vortexing plus sonication showed higher sensitivity than vortexing alone (60% versus 40%) while the specificity was 99% for both methods. At this cut-off, the sensitivities of sonication and vortexing fluid culture were reduced to 39% and 30%, respectively, in patients who previously received antibiotics. However, at a lower cut-off of ≥1 cfu/mL, vortexing
alone and the combined method were nearly identical in both sensitivity (69% versus 71%) and specificity (92% versus 93%). Therefore, the authors advocated using the lower cut-off. Vortexing is not technically challenging, does not appear to be harmful to bacteria and, for laboratories that cannot perform sonication, vortexing a resected device without sonication is probably a reasonable alternative.

**Novel technologies, tests and potential future diagnostics**

Various molecular tools have been used in the diagnosis of PJI, including PCR amplification and sequencing analysis of 16S rRNA, specific PCRs and reverse transcription PCR. These are attractive tools especially in culture-negative infections or in the presence of fastidious microorganisms. These techniques will be discussed in more detail elsewhere in this Supplement. Other novel technologies are listed and discussed in Figure 2. Synovial fluid or intraoperative tissue samples after sonication or bead mill homogenization could potentially be investigated by these techniques. Other potentially useful technologies under development include microarray, phage-induced impedance fluctuation analysis, nanomedicine and metabolomics. These may potentially be applied in the routine management of PJI in the future, not only to identify pathogens, virulence factors and antimicrobial susceptibilities, but also to provide information on disease process and progression as well as response to therapy.

**Conclusion**

A prompt diagnosis and recognition of the aetiological agent are crucial for the effective management of PJI. The goal of optimally replacing culture methods with more rapid and informative methods for diagnosing aetiological agents in PJI has advanced dramatically over the past 10–20 years. Koch’s culture methods are still widely used for microbiological investigation of PJI, even in many modern diagnostic microbiology laboratories. However, it is increasingly apparent that traditional methods are not fit for purpose, especially where biofilm infections are concerned, highlighting the urgent need for a change in the way we investigate and diagnose these types of infections. It is perhaps time to have a more detailed assessment of new technologies and to standardize technology performance. Until such time, rapid surgical exploration and microbiological sampling remains the recommended ‘gold standard’ diagnostic technique to achieve the optimal outcome.

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