The value of synovial biopsy, joint aspiration and C-reactive protein in the diagnosis of late peri-prosthetic infection of total knee replacements

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We analysed the serum C-reactive protein level, synovial fluid obtained by joint aspiration and five synovial biopsies from 145 knee replacements prior to revision to assess the value of these parameters in diagnosing late peri-prosthetic infection. Five further synovial biopsies were used for histological analysis. Samples were also obtained during the revision and incubated and analysed in an identical manner for 14 days.

A total of 40 total knee replacements were found to be infected (prevalence 27.6%). The aspiration technique had a sensitivity of 72.5% (95% confidence interval (CI) 58.7 to 86.3), a specificity of 95.2% (95% CI 91.2 to 99.2), a positive predictive value of 85.3% (95% CI 73.4 to 100), a negative predictive value of 90.1% (95% CI 84.5 to 95.7) and an accuracy of 89%. The biopsy technique had a sensitivity of 100%, a specificity of 98.1% (95% CI 95.5 to 100), a positive predictive value of 95.2% (95% CI 88.8 to 100), a negative predictive value of 100% and an accuracy of 98.6%. C-reactive protein with a cut-off-point of 13.5 mg/l had a sensitivity of 72.5% (95% CI 58.7 to 86.3), a specificity of 80.9% (95% CI 73.4 to 88.4), a positive predictive value of 59.2% (95% CI 45.4 to 73.0), a negative predictive value of 88.5% (95% CI 81.0 to 96.0 CI) and an accuracy of 78.1%.

We found that biopsy was superior to joint aspiration and C-reactive protein in the diagnosis of late peri-prosthetic infection of total knee replacements.

The incidence of peri-prosthetic infection following total knee replacement (TKR) ranges from 1.1% to 12.4%, and in some reports it is reported to be the most frequent cause of failure during the first five years after implantation.1-4 Therefore, diagnostic accuracy of post-operative infection is paramount in patients with a painful and loose prosthesis.5,6

Whereas early infections (four weeks after implantation) usually cause a local and systemic inflammatory response, this is often missing in cases of late infection making the diagnosis difficult. Moreover, the classic clinical signs, laboratory tests, radiographs and bone scans are associated with a high level of false positives and negatives.7 Precise identification of bacteria and their antibiotic resistance patterns is helpful in planning treatment prior to surgery, and also provides the surgeon with an opportunity to add bacteria-specific antibiotics to the bone cement.8 Therefore, aspiration of the joint for bacteriological analysis of the synovial fluid is carried out routinely. However, the value of this investigation remains controversial. Sensitivity is reported to vary from 12% to 100% and specificity from 81% to 100% (Table I).9-13 This poor accuracy has been attributed to contamination of the aspirated fluid, bacteria that are difficult to grow in culture, such as facultative anaerobes and Gram-negative organisms, or to non-withdrawal of antibiotic therapy prior to aspiration.9,10,20,21

In an earlier study of 86 revision TKRs, we showed that pre-operative synovial biopsy was superior to joint aspiration for the diagnosis of peri-prosthetic infection.12 Aspiration had a sensitivity of 69%, a specificity of 97%, a positive predictive value of 85% and a negative predictive value of 92%. Synovial biopsy, on the other hand, had a sensitivity of 100%, a specificity of 94.7%, a positive predictive value of 87.4% and a negative predictive value of 100%. However, this particular study had a methodological flaw in that both the diagnostic methods were performed in only 15 TKRs.12

The objectives of the present study, therefore, were to carry out a new prospective examination of both the diagnostic procedures using a larger patient population, and to investigate the hypothesis that synovial biopsy was superior to joint aspiration and C-reactive protein for the diagnosis of late peri-prosthetic infection after TKR.
Patients and Methods

Between July 2004 and September 2007, 144 patients (145 knees) who needed a revision TKR for component loosening were prospectively included into the study following appropriate consent. The study was approved by the Local Ethics Committee prior to commencement. Prosthetic loosening was defined as proposed by Math et al.22 All the patients underwent diagnostic aspiration and synovial biopsy of the knee prior to revision. Of the 144 patients only eight showed local signs of infection with an accompanying fistula. The mean age of the patients was 68.4 years (30 to 87) and 81 were female. The primary diagnosis was osteoarthritis in 129 cases (89%) and rheumatoid arthritis in 16 (11%). The aspiration and biopsy were carried out a mean of 38.2 months (2 months to 21 years) after the primary implantation.

Values of C-reactive protein (CRP) were also determined before the operation. None of the patients were on any antibiotics for four weeks prior to aspiration and biopsy, as recommended by Burnett et al.24 Mont, Waldman and Hungerford,25 Lonner et al.26 and Gollwitzer et al.,27 to minimise the risk of antibiotic-induced false-negative results. In the eight patients with fistulae, antibiotic treatment was withdrawn and the patients were examined at weekly intervals up to the date of the procedure.

Aspiration and biopsy were carried out under aseptic conditions in the operating theatre under general anaesthesia without a tourniquet. The synovial fluid was collected in vials containing BD BACTEC-PEDS-PLUS/F Medium (Becton Dickinson, Heidelberg, Germany). This medium was originally designed to optimise blood cultures from children, and had proved superior to standard blood culture media in both recovery and time to detection of clinically significant microorganisms, even in low-volume samples.28

The biopsies were obtained using arthroscopic biopsy forceps introduced via an anterolateral approach. The samples were obtained from five different areas of the knee joint, close to the components. The biopsies for bacteriological analysis were obtained blind, without filling the joint with fluid, to avoid any dilution of infecting organisms. The knee was subsequently assessed arthroscopically for any bleeding and damage to the components caused by the biopsy forceps and, at the same time, five non-blind tissue samples were obtained for histological examination. A single dose of 2 g cefazoline (i.v) was given as prophylaxis once all samples had been obtained.

The biopsy samples were placed in sterile tubes and transferred together with the aspirated fluid to the microbiology laboratory within an hour of sampling. Specimens were processed immediately upon arrival. Synovial fluid culture vials were treated with fastidious organism supplement (Becton Dickinson), a growth enhancer which improves cultivation of fastidious organisms from normally sterile body fluids other than blood, according to the manufacturer’s instructions, and incubated using the BD BACTEC 9050 automatic blood culture system (Becton Dickinson). Cultures were discontinued and declared negative if there was no growth after 14 days.18,27,29,30 Cultivation of tissue samples was carried out as described in previous studies.7,18,27,29,31,32

The results of each series of tests (aspiration, bacteriology and histology, and serum CRP level) were evaluated individually. A joint was regarded as bacteriologically positive if the same bacterium was identified in at least two samples.33 A joint was considered to be histologically positive if at least five neutrophilic polymorph leucocytes per high-power field (×400) were identified in at least one of ten such fields.32,34,37 A CRP value > 13.5 mg/l was regarded as positive.38

### Table I. Value of joint aspiration in the diagnosis of the infected total knee replacement

<table>
<thead>
<tr>
<th>Authors</th>
<th>Number</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barrack et al10</td>
<td>53†</td>
<td>75</td>
<td>96</td>
<td>75</td>
<td>96</td>
<td>93</td>
</tr>
<tr>
<td>Duff et al11</td>
<td>39</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fuerst et al12</td>
<td>75</td>
<td>69</td>
<td>97</td>
<td>85</td>
<td>92</td>
<td>91</td>
</tr>
<tr>
<td>Glithero et al13</td>
<td>54†</td>
<td>89</td>
<td>97</td>
<td>94</td>
<td>95</td>
<td>94</td>
</tr>
<tr>
<td>Kordelle et al14</td>
<td>39</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>67</td>
</tr>
<tr>
<td>Johnson et al15</td>
<td>28†</td>
<td>12</td>
<td>81</td>
<td>25</td>
<td>65</td>
<td>58</td>
</tr>
<tr>
<td>Levitsky et al9</td>
<td>72†</td>
<td>67</td>
<td>96</td>
<td>75</td>
<td>94</td>
<td>91</td>
</tr>
<tr>
<td>Morrey et al16</td>
<td>73</td>
<td>45</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>Panousis et al17</td>
<td>92†</td>
<td>70</td>
<td>95</td>
<td>78</td>
<td>92</td>
<td>90</td>
</tr>
<tr>
<td>Steinbrink and Frommet18</td>
<td>2188†</td>
<td>82</td>
<td>96</td>
<td>87</td>
<td>94</td>
<td>92</td>
</tr>
<tr>
<td>Teller et al19</td>
<td>166†</td>
<td>28</td>
<td>99</td>
<td>83</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Virolainen et al7</td>
<td>69†</td>
<td>75</td>
<td>100</td>
<td>-</td>
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</tr>
</tbody>
</table>

* without previous antibiotic therapy
† knee and hip total replacement
‡ all infected
§ only hip total replacement
Based on the combined histology and microbiology results, a joint was considered to be infected if at least one of the following conditions was fulfilled: \(^7,31,32\) demonstration of the same micro-organism in at least two of the cultures; or demonstration of a micro-organism in at least one sample and at least five neutrophilic polymorph leucocytes per high-power field (×400) in the associated histological preparation. The growth of a microorganism in only one culture medium without any histological signs of infection was regarded as a contamination. \(^7\) No antibiotics were given in the time period between the biopsy and the first revision.

During revision, samples were obtained again from five different areas close to the components and were incubated for 14 days, as above. In addition, the synovium and peri-prosthetic connective tissue were obtained for histological assessment. Peri-operative antibiotics were administered only after all the samples had been obtained. At least ten high-power fields (×400) per synovium and peri-prosthetic tissue biopsy were analysed for neutrophilic polymorph leucocytes. The same criteria were used for the diagnosis of infection.

The diagnosis obtained from the revision surgery samples was regarded as the definitive result with respect to peri-prosthetic infection, and was used to evaluate the diagnostic methods (joint aspiration, biopsy and CRP).

The sensitivity, specificity and the positive and negative predictive values of each diagnostic method (aspiration, microbiological examination, histological examination, CRP and microbiological and histological examination in combination for the biopsy method) were determined. Bayes’ equation was used to calculate these values. \(^39\) The accuracy of the techniques was calculated from the sum of the true positives and the true negatives divided by the number of tests carried out. All calculations were carried out using SPSS for Windows, version 10.0 (SPSS Inc., Chicago, Illinois). A p-value < 0.05 was considered to be statistically significant.

**Results**

Of the 145 revision TKRs 40 were classified as infected, giving a prevalence of 27.6%. This is relatively high, but as our hospital is the reference centre for prosthetic joint infection, it attracts higher numbers of such cases. The microorganisms identified are shown in Table II.

Patients with a peri-prosthetic infection had significantly higher values of CRP, with a mean of 50.63 mg/l (SD 10.23) than those who did not have an infection, with a mean of

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**Table II.** Micro-organisms identified in the 40 cases of peri-prosthetic infection and their frequency of detection (one case with identification of *Staphylococcus aureus* and *Peptostreptococcus* species)

<table>
<thead>
<tr>
<th>Micro-organism identified</th>
<th>Number of infected joints</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>17</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Staph. hominis</em></td>
<td>4</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>4</td>
</tr>
<tr>
<td><em>Streptococcus dysgal ssp. equisimilis</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Enterobacter amnigenus</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Propionibacterium acnes</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Staph. capitis</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Staph. simulans</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Staph. warneri</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Peptostreptococcus species</em></td>
<td>1</td>
</tr>
</tbody>
</table>

**Table III.** Numbers of true positive, true negative, false positive and false negative examinations for the different diagnostic methods, as well as sensitivity, specificity, positive predictive value, negative predictive value and accuracy of these diagnostic tools

<table>
<thead>
<tr>
<th></th>
<th>CRP(^7)</th>
<th>Aspiration</th>
<th>Bacteriological examination</th>
<th>Histological examination</th>
<th>Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive</td>
<td>29</td>
<td>29</td>
<td>31</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>True negative</td>
<td>85</td>
<td>100</td>
<td>103</td>
<td>100</td>
<td>103</td>
</tr>
<tr>
<td>False positive</td>
<td>20</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>False negative</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>72.5</td>
<td>72.5</td>
<td>77.5 (64.6 to 90.4)</td>
<td>90.0 (80.7 to 99.3)</td>
<td>100</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>80.9</td>
<td>82.5</td>
<td>95.2 (91.2 to 99.2)</td>
<td>96.1 (95.5 to 100)</td>
<td>95.2</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>59.2</td>
<td>65.3</td>
<td>93.9 (82.7 to 100)</td>
<td>67.2 (77.8 to 97.8)</td>
<td>95.2</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>88.5</td>
<td>90.1</td>
<td>92.0 (87.0 to 98.0)</td>
<td>96.1 (92.4 to 99.8)</td>
<td>100</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>78.1</td>
<td>89</td>
<td>92.4</td>
<td>93.8</td>
<td>96.6</td>
</tr>
</tbody>
</table>

\* CRP, C-reactive protein  
† 95% CI, 95% confidence interval
A total of 29 patients from the infected group had a CRP ≥ 13.5 mg/l. However, 20 patients from the non-infected group had a CRP ≥ 13.5 mg/l, and 85 had a CRP < 13.5 mg/l. The diagnostic value of the CRP and the sensitivity, specificity, predictive values and accuracy of the various investigations are shown in Table III.

At a prevalence of 27.6% for infected TKRs in this study, the probability that a biopsy with a positive result really is infected amounts to 95.2% (95% confidence intervals (CI) 88.8 to 100, positive predictive value). The probability that a biopsy with a negative test result is actually not infected is 100% (negative predictive value). The accuracy is 98.6%. Therefore, biopsy as a combination of the bacteriological and histological examination showed the highest diagnostic value for identification of late peri-prosthetic infection.

During the arthroscopic examination of the knee that followed the biopsy there was no observable damage, such as scratches, to the components, nor any bleeding that required haemostasis. There were no instances where an additional anteromedial portal was required. No other complications occurred during this study; in particular, there were no infections or wound healing problems associated with the anterolateral incision.

The 40 infected TKRs subsequently underwent revision surgery whereby bacterium-specific antibiotics were added to the bone cement and bacterium-specific systemic antibiotic therapy was initiated. During the subsequent follow-up period of 23.4 months (9 to 40), only one patient had a recurrent infection.

Discussion
A pre-operative bacteriological examination should be performed prior to revision of a loose or painful TKR. Several methods of diagnosing infection have been described. The serum level of CRP is regarded as an important diagnostic parameter and has a sensitivity of 91%, a specificity of 86%, a positive predictive value of 74%, a negative predictive value of 95% and an accuracy of 88%.38 However, although we found significantly higher levels of CRP in the group with peri-prosthetic infections, the predictive power of the CRP in our study was not as good as that reported by others.38,40 A possible explanation may be that 16 patients with rheumatoid arthritis were included in our study and therefore had increased baseline CRP levels because of the nature of their disease. Only one patient with rheumatoid arthritis had an infected TKR. Moreover, the cut-off point for the value of CRP associated with an infection is probably influenced by the distribution of the individual values in any one cohort, so that different patient cohorts will exhibit different cut-off points. This might explain the different levels of significance attached to CRP as a diagnostic tool in the literature.2,27 A further disadvantage of CRP is that it does not identify the micro-organism in question and therefore does not provide the information necessary to develop a specific therapeutic antibiotic regimen.8,18,30 On the other hand, aspiration of the joint offers this advantage. Therefore, many authors recommend routine aspiration before a revision, even when there is no indication of an infection, because of its high diagnostic value.21,41-43

Our results support the hypothesis that synovial biopsy is superior to aspiration in the diagnosis of late peri-prosthetic infection. In particular, the sensitivity and the negative predictive value of biopsy were 100% in the present and our pilot study,12 and infection can be reliably ruled out with this method. We had no infections or other complications, although individual cases have been previously described.44

The length of the incubation period is important for identifying the micro-organism in aspirated fluid or biopsies because bacteria that lead to a peri-prosthetic infection exist in low numbers in the biofilm and are often in a sessile form which grows slowly.24,45,46 We chose to incubate our specimens for 14 days, based on previous studies.18,27,29,30 An inappropriate incubation period may be the reason for the poor sensitivity of tests as reported in previous studies.14,16 Moreover, patients should not be treated with antibiotics prior to the test. If this is not the case, then at least 14 days, and if possible four weeks, should elapse before any sampling occurs.9,11,24,25 However, if the patient develops symptoms of systemic infection during the antibiotic-free period, the TKR should be revised without delay. In these cases, an antibiotic-loaded spacer should be used in addition to broad-spectrum antibiotic therapy.24

One potential weakness of our study was that the biopsy was carried out in a blinded fashion with biopsy forceps, without filling the knee joint with fluid. The potential for damage to the surface of the components is a disadvantage of this technique. However, our study only addressed the patients with loose components, where revision surgery was indicated, and therefore any iatrogenic damage to the prostheses during the biopsy was of lesser importance. We do not recommend this technique for the diagnosis of peri-prosthetic infection in a well-fixed TKR. In addition, future studies should focus on examining data obtained via a blind biopsy compared with those obtained via a biopsy carried out by arthroscopic visualisation.

Tissue samples were also sent for histological examination. We found a sensitivity of 90% and a specificity of 95.2% with histology in our study, although some authors have reported these values to be approaching 100%.11,14,31

Our study shows that biopsy is superior to aspiration of the joint as a pre-operative diagnostic method in its ability to confirm or rule out the presence of late peri-prosthetic infection. It also offers the advantage of combining both bacteriological and histological examinations.

It is a minor procedure without major complications, with a high accuracy, and we use it routinely as a diagnostic procedure for peri-prosthetic infection in patients with TKRs. However, in patients with stable implants, where peri-prosthetic infection has to be ruled out, we recommend aspiration of the joint. Only in patients with a negative
aspiration but an increased CRP or clinical signs of infection, would we recommend a biopsy. Whether a blind technique gives better results than the arthroscopically-assisted method remains to be seen.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References