

# CURRENT CONCEPTS REVIEW

## DIAGNOSIS OF PERIPROSTHETIC INFECTION

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- Periprosthetic infections are rare, but there is evidence to suggest that their frequency may be underestimated.
- No single laboratory test has perfect sensitivity and specificity for diagnosing infection. Most tests have better specificity when they are performed for patients in whom infection is suspected clinically rather than when they are used as screening tests.
- Screening test results that may suggest the possibility of infection include elevation of the erythrocyte sedimentation rate and/or serum C-reactive protein level more than three months after an arthroplasty. Most serologic tests are difficult to interpret when the patient has an underlying inflammatory arthropathy.
- Cultures of aspirated joint fluid can be especially helpful for patients who have symptoms suggestive of infection, but their results are best interpreted two weeks after administration of antibiotics has been discontinued. Joint fluid cell counts may also be helpful, but Gram stains of joint fluid have poor sensitivity and specificity.
- Criteria for diagnosing infection on the basis of frozen sections of implant membranes have not yet been standardized, but in many laboratories more than five neutrophils per high-power field in five or more fields (excluding surface fibrin) has been found to be suggestive of infection.
- Most polymerase chain reactions that detect the universal 16S rRNA bacterial gene have problems with false-positive results, but combining a universal polymerase chain reaction with subsequent bacterial sequencing can help improve specificity. Polymerase chain reactions can detect necrotic bacteria, so the clinical importance of positive results of this analysis in the absence of other features of infection remains to be determined.

There have been important improvements in total joint arthroplasty in terms of implant design, fixation, and control of periprosthetic infection. The use of prophylactic antibiotics, body exhaust systems, laminar airflow, and other precautions has helped reduce the prevalence of clinically recognized periprosthetic infection from nearly 10% in the early years in which arthroplasty was performed<sup>1</sup> to <1% in some series<sup>2,3</sup>. Despite this decline, periprosthetic infection remains one of the most challenging complications of joint arthroplasty and is associated with immense physiological, psychological, and financial costs. Furthermore, several recent observations have suggested, but have not proven, that some arthroplasty failures that were interpreted as being due to aseptic loosening might in fact have represented the consequence of inflammatory reactions to bacteria or bacterial products. These observations include (1) the finding that antibiotic-containing bone cement protects against so-called aseptic loosening<sup>4,5</sup>, (2) evidence of bacteria on a surprisingly high proportion of implants that had

been revised because of aseptic loosening<sup>6,7</sup>, (3) occasional cases in which implant membranes showed acute inflammation but intraoperative cultures were negative<sup>8,9</sup>, and (4) emerging data suggesting that bacterial endotoxin and related molecules may have a role in particle-induced bone resorption<sup>10-11</sup>.

The purpose of this article is to review our current understanding of periprosthetic infection with particular focus on the efficacy of various tests to help make the diagnosis.

### Definition of Infection

A fundamental issue in determining the prevalence of a disease is defining the criteria with which the disease can be diagnosed with certainty. Currently, periprosthetic infection is most frequently diagnosed by isolation of one or more organisms from the periprosthetic tissue or fluid with use of conventional microbiologic culture techniques, and the results of microbiologic culture are usually considered the standard with which other diagnostic tests are compared. However, or-

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TABLE I Intraoperative Cultures as a "Tarnished Gold Standard" for Diagnosing Infection

Reference	Discrepancies
Fehring and McAlister <sup>65</sup>	4 to 6 of 86 patients with negative cultures were thought to have an infection
Lonner et al. <sup>66</sup>	7 of 19 positive cultures were thought to have been due to contaminants
Athanasou et al. <sup>67</sup>	2 or 3 of 84 patients with negative cultures were thought to have an infection
Pandey et al. <sup>68</sup>	10 of 521 patients with negative cultures were thought to have an infection
Feldman et al. <sup>67</sup>	1 of 24 patients with negative cultures was thought to have an infection
Abdul-Karim et al. <sup>69</sup>	8 of 16 positive cultures were thought to have been due to contaminants
Padgett et al. <sup>15</sup>	30% of 142 hips treated with revision arthroplasty had at least 1 positive intraoperative culture, but a clinically important infection developed in only 1 hip
Barrack and Harris <sup>62</sup>	54 of 60 positive cultures in a consecutive series of 260 hip arthroplasties were thought to have been due to contaminants
Lachiewicz et al. <sup>50</sup>	2 of 21 positive cultures from sites of 142 total hip arthroplasties complicated by pain were thought to have been due to contaminants
Duff et al. <sup>50</sup>	1 of 19 positive cultures from sites of 64 total knee arthroplasties complicated by pain was thought to have been due to contaminant
Tunney et al. <sup>7</sup>	Conventional intraoperative cultures from sites of 5 of 120 total hip arthroplasties were positive, but cultures of material from the retrieved implants obtained with sonication were positive in 26 cases
Mirra et al. <sup>64</sup>	5 of 27 positive intraoperative cultures were thought to have been due to contaminants or so-called low-virulence organisms. One culture-negative joint was thought to have an infection

organisms are not always isolated from areas that ultimately prove to be infected, and sometimes positive cultures of specimens of periprosthetic tissue may not represent clinically important infections<sup>15</sup> (Table I) because specimens can become contaminated when the tissue is being harvested, being transported, or in the laboratory. In addition to microbiologic culture of tissue or fluid, other tests are used to help diagnose periprosthetic infection. However, all diagnostic tests have limitations, and the sensitivity, specificity, and predictive value of positive and negative test results are usually calculated with respect to an existing reference standard (the "gold standard") (Fig. 1)<sup>16-18</sup>. Because of the aforementioned

limitations of diagnostic tests, clinicians often utilize a combination of tests to confirm or exclude the diagnosis of periprosthetic infection. Developing a definition of infection that is robust enough to serve as a gold standard is an ongoing challenge that influences our perception of the value of any diagnostic test that is compared with that gold standard. The prevalence of infection in a cohort of patients also influences the predictive value of positive and negative test results. Recognizing the limitations of using a reference standard for comparison, many investigators have attempted to evaluate the efficacy of various tests for diagnosing periprosthetic infection, as discussed below.

Sensitivity = true positives / (true positives + false negatives)
Specificity = true negatives / (true negatives + false positives)
Accuracy = (true positives + true negatives) / (false negatives + false positives)
Predictive Value of a Positive Test = true positives / (true positives + false positives)
Predictive Value of a Negative Test = true negatives / (true negatives + false negatives)
Bayesian Positive Predictive Value = (Sensitivity X Prevalence) / ((Sensitivity X Prevalence) + ((1-Specificity) X (1-Prevalence)))
Bayesian Negative Predictive Value = ((Specificity X (1-Prevalence)) / ((1-Sensitivity) X Prevalence) + ((Specificity X (1-Prevalence)))

Fig. 1

Calculations commonly used to describe test efficacy. The equations for predictive value listed here are those most commonly used in the laboratory medicine, pathology, and orthopaedic literature. The predictive value of a test is strongly influenced by the prevalence of the disorder in the cohort of patients under investigation. Bayesian equations for predictive value include variables for estimated prevalence and are more commonly used in the epidemiology literature. More information about predictive value calculations is available in the Appendix.

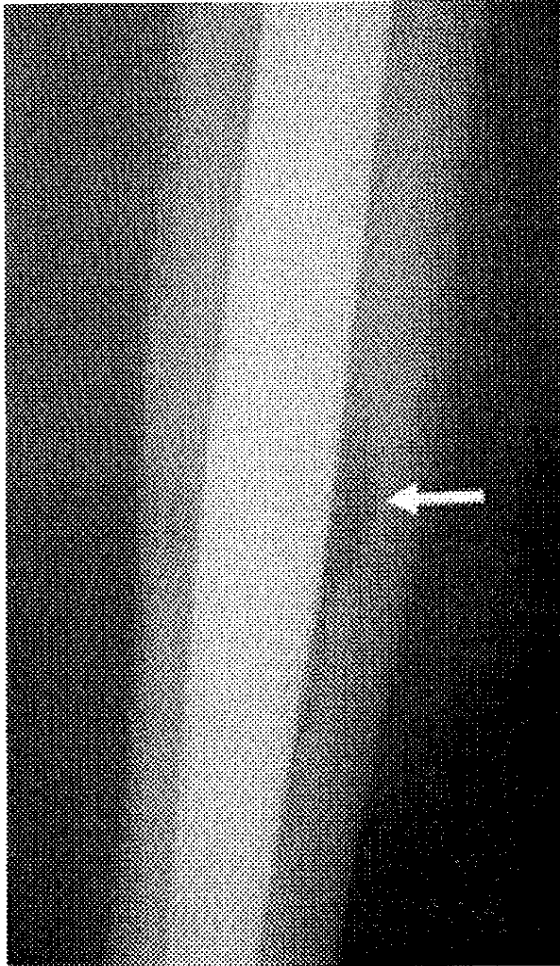


Fig. 2-A  
Plain radiograph showing an area of focal osteolysis (arrow) around the distal part of a well-fixed uncemented stem. This appearance is suggestive of periprosthetic infection, but it could also be related to particle-induced osteolysis.

### Classification of Periprosthetic Infection

Infections at the sites of total joint arthroplasties are sometimes categorized on the basis of the presumed mechanism and timing of the infection. So-called acute postoperative infections are thought to result from organisms that gained access to the joint during the operation, or soon after it, from the overlying skin or a draining wound. Infections of this type generally become symptomatic within a few days or weeks after the arthroplasty. So-called late chronic infections may result from organisms introduced during the operation, either from the air, from surgical instruments, or from the implant itself. The lag period is the time needed for the organisms to proliferate and induce symptoms that prompt recognition of the infection. Hematogenous infections are the result of the seeding of an arthroplasty site by organisms carried by the bloodstream from a different site (e.g., a urinary tract infection or a cutaneous or mucosal ulcer). The

distinction between these types of infection may be difficult and is somewhat arbitrary. While early reviews suggested that the majority of arthroplasty-related infections were the consequence of wound contamination<sup>19</sup>, more recent studies have suggested that late infections are much more common. For example, in a retrospective review of more than 6000 total knee replacements, Peersman et al.<sup>3</sup> reported an overall deep infection rate of 0.39% following primary arthroplasties and 0.97% following revision operations. One-third of the deep infections occurred within the first three months after the operation, and the remaining cases were considered late infections. In a study of more than 3000 total hip arthroplasties performed over a sixteen-year period, Schmalzried et al.<sup>20</sup> noted that the incidence of hematogenous arthroplasty-related infection increased during the time that the cohort was followed. This change from acute to chronic infections presumably reflects changes in surgical practice during recent decades, including the use of prophylactic antibiotics, the use of antibiotic-impregnated cement, and alterations in the operating room environment<sup>2,21-23</sup>.

### Tests for Diagnosing Arthroplasty-Related Infection

#### Clinical Factors

A detailed clinical history and physical examination constitute the most important ways to recognize a potential periprosthetic infection. The type and duration of symptoms, details of the postoperative course, the presence of comorbidities, and the types of treatments rendered should be discussed in detail. Periprosthetic infection may be diagnosed with reasonable certainty on the basis of the history and clinical presentation when there are classic signs of infection such as severe joint pain, fever, chills, or a draining periarticular sinus. In such cases, laboratory tests are used simply to confirm the diagnosis of the periprosthetic infection. However, periprosthetic infection has



Fig. 2-B  
Increased uptake in the corresponding area of focal osteolysis was noted on the technetium-99m bone scan.

an innocuous presentation in most patients and may be difficult to diagnose on the basis of the history and physical findings alone. Many of the symptoms and signs of infection overlap with those of other clinical conditions such as intra-articular hematoma, instability, and aseptic loosening. It is under these circumstances that additional diagnostic modalities play a critical role in the confirmation or exclusion of the diagnosis of periprosthetic infection.

#### *Radiographic Studies*

After a physical examination, evaluation of a patient with a loose or painful prosthetic joint commences with radiographic studies. There are a few nonspecific changes suggestive of infection that may be apparent on plain radiographs. These include periosteal reaction, scattered foci of osteolysis, or generalized bone resorption in the absence of implant wear (Fig. 2-A). In general, however, the majority of patients with periprosthetic infection, especially those with an acute presentation, do not have obvious radiographic findings suggestive of infection or may show features indistinguishable from those seen in association with aseptic loosening<sup>21</sup>. The main role of conventional radiographic evaluation of these patients is to rule out other conditions such as wear and osteolysis or fractures.

#### *Radionuclide Imaging*

Radionuclide studies currently have a role in the evaluation of many patients who have pain at the site of an arthroplasty (Fig. 2-B). In a study of seventy-two total joint replacements, Levitsky et al. reported that bone scintigraphy had a sensitivity of 33%, a specificity of 86%, a positive predictive value of 30%, and a negative predictive value of 88%<sup>25</sup>. Although false-positive results lead to low sensitivity, the relatively high predictive value of a negative result makes conventional bone scintigraphy useful as an initial screening test<sup>26</sup>. Combining technetium-99m bone scans with a review of conventional radiographs may slightly increase the sensitivity compared with that of a review of radiographs alone to diagnose infection or loosening<sup>27</sup>. Radioisotopes intended to target the white blood cells that are invariably present during infection can be helpful in some cases<sup>28</sup>. A scan employing indium-111, an isotope that labels leukocytes or immunoglobulin, is more sensitive than a routine technetium-99m scan<sup>29</sup>. Although one report suggested that indium-111 scanning has higher specificity than does 18F-FDG (fluorodeoxyglucose) imaging<sup>30</sup>, other studies have shown indium-111 scans to have relatively low sensitivity and specificity for diagnosing infections at the sites of arthroplasties<sup>31,32</sup>. For example, Scher et al. reported that indium-111 leukocyte scans had only 77% sensitivity, 86% specificity, 54% positive predictive value, and 95% negative predictive value when they were used to diagnose 143 patients with an infection rate of 17% who underwent an operation because of a painful joint implant<sup>31</sup>. Combining technetium-99m sulfur colloid marrow imaging with an indium-111-labeled leukocyte scan may improve specificity compared with that of either test alone<sup>33</sup>. The technetium scan is performed first to show all areas of high metabolic activity. The indium-111, as it

targets leukocytes, will accumulate in regions of inflammation. Combining the results of these two scans helps to distinguish true infection from uninflamed areas of high metabolic activity such as fracture or remodeling.

Gallium-67 is bound in serum to iron-transporting molecules such as transferrin. It is transported to tissues on the basis of vascularity, inflammation, and other factors. Gallium-67 scans alone have a low sensitivity for diagnosing infection<sup>27,31</sup>. The demonstration of congruent patterns by gallium-67 and technetium-99 scans often reflects aseptic changes around implants, but a lack of congruence (i.e., positive scans with different spatial distributions) can be seen when there is an infection<sup>35</sup>.

Technetium-99m-polyclonal IgG (immunoglobulin G) scintigraphy has been reported to have a high sensitivity for recognizing infections around hip and knee prostheses, but like many types of scans it has a low specificity<sup>36</sup>. The role of fluorodeoxyglucose-positron emission tomography (FDG-PET) scans in the diagnosis of infections at the sites of arthroplasties has been evaluated at some centers. Inflammatory cells metabolize predominantly glucose, and the uptake of glucose is enhanced when such cells are stimulated. Activated macrophages and neutrophils express high concentrations of glucose transporters, which facilitate the movement of FDG (as well as glucose) through the cell membrane. Deoxyglucose is phosphorylated to deoxyglucose-6-phosphate, which is not a substrate for glucose-6-phosphate dehydrogenase so it becomes trapped in tissue long enough to allow PET imaging. Thus, FDG reflects glucose utilization and can indicate areas of inflammation. Studies have shown combined FDG-PET imaging to have variable sensitivity and specificity for diagnosing periprosthetic infection<sup>30,37</sup>. One study, for example, demonstrated approximately 91% sensitivity and 72% specificity for diagnosing infections around knee prostheses and 90% sensitivity and 89% specificity for diagnosing infections around hip prostheses<sup>37</sup>. Although FDG-PET scans may have greater specificity than leukocyte-labeling bone scans, false-positive results may occur as a result of uptake of FDG in particle-induced inflammation around implants with aseptic loosening<sup>38</sup>.

#### *Serologic Tests*

Measurements of the Westergren erythrocyte sedimentation rate, the rate at which red blood cells sediment from whole blood, and of the level of C-reactive protein, a protein produced in the liver, are serologic tests that may be an important part of a diagnostic workup of patients with suspected periprosthetic infection. The erythrocyte sedimentation rate and the C-reactive protein level normally rise rapidly after joint arthroplasty, reaching peak levels several days after the operation, with the C-reactive protein level peaking slightly earlier than the erythrocyte sedimentation rate<sup>7,39-41</sup>. In the absence of an inflammatory arthropathy or infection, the serum level of C-reactive protein usually returns to normal by about three weeks after the arthroplasty<sup>40</sup>, although values may take longer to normalize after knee arthroplasty than after hip arthroplasty<sup>39</sup>. The erythrocyte sedimentation rate decreases more slowly than does the C-reactive protein level, may show some diurnal variation, and may re-

main slightly elevated for six weeks after the arthroplasty<sup>40</sup>. Elevations in the erythrocyte sedimentation rate and especially in the C-reactive protein level after three months suggest the possibility of infection<sup>42-46</sup>, but these levels need to be interpreted along with other findings. For example, both are elevated in patients who have an inflammatory condition without joint infection, and the tests can be used to monitor a variety of conditions such as inflammatory arthropathies<sup>47</sup>. C-reactive protein levels and erythrocyte sedimentation rates may be slightly elevated in patients in whom heterotopic ossification has developed<sup>48</sup>, are less predictive of infections in patients with underlying inflammatory arthropathies, may be elevated in patients with other postoperative complications such as bronchopneumonia<sup>49</sup>, and sometimes may not be elevated in the presence of periprosthetic infection. Measurements of the erythrocyte sedimentation rate in particular may have a high frequency of false-positive results<sup>50</sup>. In one of the relatively few studies that have provided enough information to calculate sensitivity and specificity, Spanghehl et al.<sup>15</sup> prospectively evaluated several different diagnostic tests that had been performed in a series of 202 revision hip arthroplasties. If inflammatory arthropathies were excluded, the erythrocyte sedimentation rate was found to have a sensitivity of 82% and a specificity of 85%. The predictive value of a negative test was only 58%, while the predictive value of a positive result was 95%. The C-reactive protein level was found to be a better indicator of infection than the erythrocyte sedimentation rate, with the C-reactive protein level having a sensitivity of 86%, a specificity of 92%, and predictive values for negative and positive tests of 74% and 99%, respectively. While neither the erythrocyte sedimentation rate nor the C-reactive protein level is diagnostic of infection, values that increase (or fail to decrease) three months after an arthroplasty should raise the suspicion of infection and prompt additional diagnostic studies.

Another serologic test that has shown promise for diagnosing infection is measurement of the serum level of interleukin-6 (IL-6), a factor produced by monocytes and macrophages. In a recent study, the serum level of IL-6 was found to be consistently elevated ( $>10$  pg/mL [ $>10$  ng/L]) in patients with periprosthetic infection, and it had a higher predictive value than most other serologic markers<sup>51</sup>. A potential advantage of measuring the IL-6 level is that the level returns to normal soon (within forty-eight hours) after the operation and is not likely to be elevated in patients with aseptic loosening. However, it may be elevated in patients with an underlying inflammatory arthropathy.

#### *Culture of Aspirated Joint Fluid*

One of the most important tests in the evaluation for potential periprosthetic infection is culture of the fluid aspirated from the joint. Our perception of the predictive value of this test, like that of most laboratory tests, is influenced by, among other things, the prevalence of infection in the cohort of patients under evaluation. This is illustrated by two studies by Barrack et al.<sup>52,53</sup>. In 1993, Barrack and Harris reported on a series of 270 consecutive patients who had undergone aspiration and culture shortly before revision total hip arthroplasty, even

when the clinical features did not necessarily suggest infection<sup>52</sup>. The results of 291 successful aspirations in 260 patients were evaluated. Six hips (2%) were eventually found to be infected. The cultures of the aspirates had six true-positive results, four false-negative results, and thirty-three false-positive results. The high frequency of false-positive results yielded a sensitivity of only 60% and a positive predictive value of only 15%, giving the impression that culture of aspirated fluid is a relatively poor test, at least when performed in a consecutive series of patients who had not been screened for features suggestive of infection. In a later study, however, Barrack et al. performed cultures of aspirated fluid obtained from sixty-nine patients with a symptomatic total knee replacement<sup>53</sup>. Twenty of the knees were ultimately diagnosed as being infected, whereas forty-nine were considered to be not infected. Some patients underwent multiple aspirations, but the initial series of cultures yielded eleven true-positive results, forty-seven true-negative results, two false-positive results, and nine false-negative results, with sensitivity and specificity values of 55% and 96%, respectively. The predictive value of a positive result in this series of knee arthroplasties was 85%, which was considerably better than the 15% predictive value of a positive result in the 1993 study of hip arthroplasties.

There are several possible reasons for the difference in the predictive values between the above studies<sup>52,53</sup>. One possible reason is that one study dealt with hips and the other, with knees. False-positive test results may be more common in fluids aspirated from hips than in those aspirated from knees. On the other hand, the prevalence of infection in the second study (29%) was much higher than that in the first (2%), presumably because the test was applied to all patients undergoing revision arthroplasty in the first study but was limited to patients with "symptomatic" knee replacements in the second. The important effect of prevalence on calculations of predictive values is illustrated by using the Bayesian equation to calculate the positive predictive value<sup>54</sup> (see Appendix). Including prevalence in the calculation yields a positive predictive value of only 15% in the 1993 study of hip fluid aspirations but a value of 72% in the 1997 study of knee aspirations. These calculations illustrate that the predictive value of a positive result of a culture of joint fluid is higher if the study is not used as a screening test for infection but is used instead as a confirmatory test for patients in whom clinical findings (or prior laboratory test results) have already raised the suspicion of infection.

Very similar findings were described by Spanghehl et al.<sup>15</sup>, who also recommended culture of aspirated fluid when a prior screening test, such as measurement of the erythrocyte sedimentation rate or C-reactive protein level, is positive. The sensitivity of cultures of aspirated fluid is increased by repeating the test for patients who had a negative result on prior culture of aspirated fluid but for whom there is a strong clinical suspicion of periprosthetic infection<sup>53</sup>. The sensitivity is greatly reduced when the test is performed for patients receiving antibiotic treatment<sup>53</sup>. To minimize the influence of antibiotics, joint aspiration is best performed at least two weeks after the last dose of antibiotics has been given. Although aspi-

TABLE II Leukocyte Counts in Aspirated Joint Fluid

Reference	Approximate Cutoff to Diagnose Infection	
	In Units Used in Publication*	Converted to Cells/ $\mu$ L
Spanghel et al. <sup>45</sup>	$50 \times 10^9$ /L	50,000
Kersey et al. <sup>59</sup>	2000/mL	2
Mason et al. <sup>60</sup>	2500/mL	2.5
Trampuz et al. <sup>61</sup>	$1.7 \times 10^3$ $\mu$ L	1700

\*The units used in the publication are not necessarily recommended by the authors of this Current Concepts Review.

ration of the knee can be performed without the use of fluoroscopy, the hip joint cannot be aspirated accurately unless fluoroscopy is utilized. Radiographic confirmation of appropriate needle placement is essential for joint aspiration of the hip and sometimes for aspiration of the knee.

#### Gram Stains of Aspirated Joint Fluid

Although Gram staining may be performed on joint fluid aspirated preoperatively or intraoperatively, this test in general has a relatively poor sensitivity and specificity<sup>45,55-57</sup>.

#### Joint Fluid Leukocyte Counts

In the absence of a joint implant, measurements of the concentration of leukocytes and the proportion of those leukocytes that are neutrophils in synovial fluid are important tests to help distinguish among osteoarthritis, infection, and noninfectious inflammatory arthropathies<sup>58</sup>. Several studies have indicated that cell counts of fluid aspirated from around total joint prostheses can also provide useful information, although the literature is somewhat difficult to interpret, in part because authors have used different units of volume to express values (Table II). For example, in a prospective study, Spanghel et al. included cell counts among other tests to diagnose infections at the sites of total hip arthroplasties<sup>45</sup>. Use of  $50 \times 10^9$  cells/L (50,000 cells/ $\mu$ L) as a cutoff point for the diagnosis of infection yielded a sensitivity of only 36%, reportedly because of frequent false-negative results, and use of 80% neutrophils as a cutoff resulted in a positive predictive value of only 52% because of a high frequency of false-positive findings<sup>45</sup>. Kersey et al. prospectively analyzed the white blood-cell count and differential of fluid from seventy-nine knees (seventy-four patients) prior to revision arthroplasties performed because of aseptic failure<sup>59</sup>. Patients who were thought to have an infection were excluded. The mean white blood-cell count in the joint fluid was 782/mL ( $<1/\mu$ L), with a mean differential of 13% neutrophils, but eight uninfected knees had a leukocyte count of  $>2000$ /mL (2/ $\mu$ L). Four of those knees were affected by rheumatoid arthritis, and three of the knees with rheumatoid arthritis had  $>50\%$  neutrophils. The authors concluded that synovial white blood-cell counts and differential counts from uninfected sites of total knee replacements are similar to the counts in fluid from knees without an im-

plant, and they suggested that  $<2000$  white blood cells/mL and  $<50\%$  neutrophils suggests the absence of infection<sup>59</sup>. It should be noted, however, that Kersey et al. did not include patients with infection in their series, and it is recognized that other conditions, such as crystalline arthropathies, can be associated with a high concentration of neutrophils in the joint fluid.

In 2003, Mason et al. retrospectively reviewed data on 440 revision total knee arthroplasties and identified eighty-six patients who had presented with clinical features suspicious for infection and had therefore undergone joint fluid aspirations<sup>60</sup>. The mean white blood-cell count for the fifty knees that were found to be uninfected was  $645 \pm 878$ /mL (about 6/ $\mu$ L), whereas the mean count for the thirty-six infected knees was 25,951/mL (260/ $\mu$ L). There was a mean of  $72.8\% \pm 28.6\%$  neutrophils in the infected knees and  $27\% \pm 24\%$  in the uninfected ones. The authors suggested that the optimum criteria for diagnosing infection included a white blood-cell count of  $>2500$ /mL and  $>60\%$  neutrophils<sup>60</sup>. Trampuz et al.<sup>61</sup> prospectively evaluated synovial fluid specimens from ninety-nine patients with aseptic failure of a total knee prosthesis and from thirty-four patients with an infection at the site of a total knee arthroplasty. Using receiver operator characteristic curves, the authors estimated that a synovial fluid leukocyte count of  $1.7 \times 10^3/\mu$ L or a differential count of  $>65\%$  neutrophils was the optimum cutoff for a diagnosis of infection<sup>61</sup>. As seen in Table II, the disparity in reported cell concentrations suggests that some authors may not have reported the correct units of volume. Setting aside the inconsistencies in units, there are still discrepancies with regard to the level at which the cell count in fluid from the site of a prosthetic joint may be considered abnormal. From a practical standpoint, we consider a white blood-cell count of  $>500/\mu$ L as suggestive of periprosthetic infection.

#### Efficacy of Analysis of Frozen Sections for Diagnosis

There are occasions when periprosthetic infection is suspected

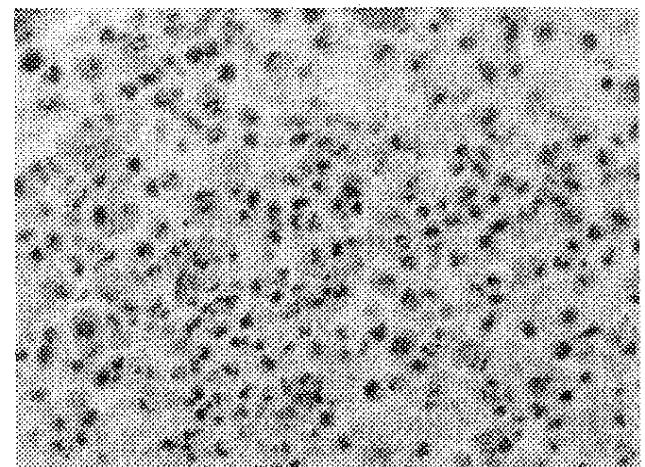


Fig. 3  
Photomicrograph of a peri-implant membrane, showing a very high concentration of neutrophils, which is essentially diagnostic of ongoing infection.

TABLE III Histologic Criteria for Interpretation of Frozen Sections as Diagnostic of Infection

Reference	Criteria
Mirra et al. <sup>65</sup>	>5 neutrophils in $\geq 5$ separate high-power fields*, excluding surface fibrin and inflammatory exudates
Abdul-Karim et al. <sup>69</sup>	>5 neutrophils in $\geq 5$ separate high-power fields, excluding surface fibrin and inflammatory exudates
Feldman et al. <sup>57</sup>	>5 polymorphonuclear leukocytes per high-power field in $\geq 5$ high-power fields
Fehring and McAlister <sup>66</sup>	Evidence of acute inflammation (no quantification). Excluded 3 cases with "moderate chronic inflammation"
Charosky et al. <sup>62</sup>	Acute or marked chronic inflammation
Lonner et al. <sup>66</sup>	>10 polymorphonuclear leukocytes per high-power field in $\geq 5$ high-power fields†
Athanasou et al. <sup>67</sup>	>5 polymorphonuclear leukocytes, lymphocytes, or plasma cells per high-power field in $\geq 10$ high-power fields
Pandey et al. <sup>68</sup>	One "inflammatory cell" per high-power field in $\geq 10$ high-power fields
Spanghel et al. <sup>45</sup>	$\geq 5$ stromal neutrophils in any single high-power field
Banit et al. <sup>70</sup>	>10 polymorphonuclear leukocytes per high-power field in $\geq 5$ high-power fields

\*The high-power field defined in this study was 500 $\times$ . The high-power field in all other studies either was 400 $\times$  or was not specified. †The authors also calculated results in terms of five polymorphonuclear leukocytes per high-power field but chose ten cells as optimum for diagnosis.

but cannot be confirmed by joint aspiration or the organism cannot be isolated. It would be valuable for surgeons to have access to tests that could be performed during revision surgery. The most frequently used intraoperative test for infection is the interpretation of frozen sections of tissue obtained from the joint capsule or periprosthetic membrane. Sometimes these specimens show marked acute inflammation and are essentially diagnostic of ongoing infection (Fig. 3). Other times, there is essentially no inflammation, an observation that suggests the absence of infection. However, implant membranes sometimes have a low concentration of neutrophils (Figs. 4-A and 4-B) or contain lymphocytes and plasma cells without neu-

trophils. The importance of this borderline inflammation is not obvious, and many investigators have attempted to establish histologic criteria that are diagnostic of infection (Table III). As will be described below, these authors have used different criteria for the histologic diagnosis of infection, have employed different reference standards with which to compare the histologic results, and have arrived at different conclusions, especially with respect to the importance of lymphocytes and plasma cells. Some authors have prospectively tested consecutive patients (thereby using frozen sections as a screening test), whereas others have evaluated frozen sections only when there was a suspicion of infection at the time of the opera-

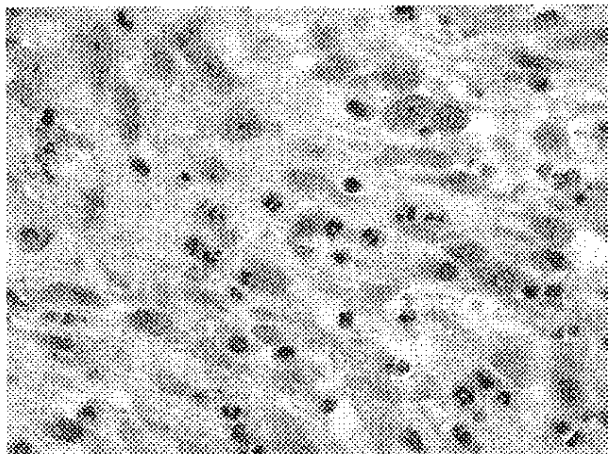


Fig. 4-A

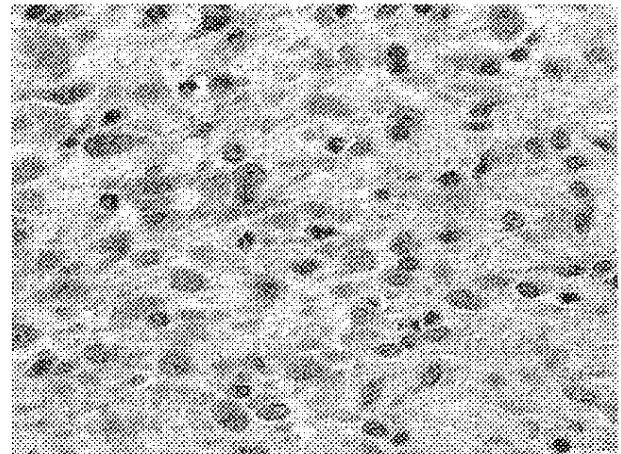


Fig. 4-B

**Figs. 4-A and 4-B** Low concentrations of neutrophils are best interpreted in conjunction with other clinical factors and laboratory tests. **Fig. 4-A** This photomicrograph shows more than fifteen neutrophils and, in the absence of an underlying inflammatory arthropathy, would strongly support the diagnosis of infection in most laboratories. **Fig. 4-B** This photomicrograph shows approximately six neutrophils, and at our laboratory, in the appropriate clinical setting, would be interpreted as being suggestive of ongoing infection. This amount of inflammation is below the threshold for a diagnosis of infection described in some other reports (Table II).



tion (thereby using frozen sections as a confirmatory test). As was true of the cultures of aspirated fluid described above, analyzing frozen sections from all patients undergoing revision arthroplasty is likely to reduce the specificity and predictive value of positive results compared with the values derived when frozen sections are analyzed only when there is clinical suspicion of infection at the time of surgery.

Perhaps the first study of the use of frozen sections to diagnose an infection at the site of an arthroplasty was reported by Charosky et al. in 1973<sup>62</sup>. Those authors described the results of analysis of frozen sections of implant membranes obtained from twenty patients, ten of whom had intraoperative cultures that were positive for organisms and ten of whom had negative cultures. Of the ten with positive cultures, five had acute inflammation that was "2+ or greater" (not otherwise defined) and the other five had chronic inflammation that was "2+ or greater." The authors concluded that acute inflammatory changes or "severe chronic inflammation" were presumptive evidence of infection.

Another early study, and probably the most frequently quoted (and misquoted), on this topic was performed by Mirra et al. and published in slightly different forms in 1976<sup>63</sup> and 1982<sup>64</sup>. In the first publication<sup>63</sup>, the authors noted that, of more than 550 total joint arthroplasties performed between 1970 and 1974 at a single center, an unspecified number were revision arthroplasties. The authors retrospectively reviewed the histologic findings in membranes around twenty-four failed hip prostheses and ten failed knee prostheses and attempted to correlate those findings with the presumed mechanism of failure. There was no single gold standard for diagnosing infection; instead, the diagnoses of septic and aseptic loosening appear to have been based on a combination of radiographic features and culture results. The authors did not describe the criteria that they used to select the thirty-four cases for review. The extent of inflammation was quantified as the average number of cells in five different microscopic fields obtained from areas of maximal inflammation. Interestingly, the high-power microscopic field used in the study was a net magnification of 500×. Although 60× lenses are also available, the majority of microscopes in use today have a 40× objective lens and a 10× ocular lens, yielding a final magnification of 400×—i.e., 20% lower than the magnification used in the study by Mirra et al. In the original publication by Mirra et al.<sup>63</sup>, acute inflammation was graded as absent, 1+ (one to five cells per high-power field), 2+ (six to forty-nine cells per high-power field), or 3+ (fifty or more cells per high-power field). Lymphocytes and plasma cells were quantified similarly. All fifteen patients with positive cultures had 2+ or 3+ acute inflammation, although one of them did not have clinical evidence of deep infection. Neutrophils were not present (at least not at the 2+ level) in patients for whom the cultures were negative. The authors noted that patients with rheumatoid arthritis can have up to ten neutrophils per high-power field, but apparently two infections in patients with coexisting rheumatoid arthritis still could be diagnosed on the basis of frozen sections.

In 1982, Mirra et al.<sup>64</sup> expanded their original series to in-

clude the results of biopsies from 1970 to 1978, including those done during fifty-four revision hip operations, thirty-nine revision knee operations, and one revision of a silicone toe implant. Ninety-four cases were studied, including the thirty-four that had been previously described<sup>63</sup>. Of those ninety-four biopsies, twenty-two demonstrated areas of acute inflammation with more than five neutrophils per high-power field in five fields. Twenty-one of the joints with a positive biopsy result had a positive culture and one had a negative culture but was thought to be infected on the basis of clinical findings. Five joints had positive intraoperative cultures (with growth of *Corynebacterium* in four and *Micrococcus* in one) but no substantial acute inflammation, and the organisms were thought to have been either contaminants or as causing a "low-virulence" infection. The two publications by Mirra et al. are the origin of the commonly quoted criterion of five neutrophils per high-power field. It should be noted that the original articles describe five neutrophils in each of five microscopic fields from the area of highest cellularity, excluding superficial fibrin, in a patient who does not have rheumatoid arthritis. To our knowledge, the influence of the variability in magnification (with 500× used by Mirra et al. compared with the more commonly used 400×) has not been previously noted.

Other authors have attempted to validate histologic criteria for the diagnosis of infection. For example, Fehring and McAlister<sup>65</sup> performed a study of 107 consecutive total joint revisions in which all patients had analysis of frozen sections of tissue obtained from multiple surgical sites. Intraoperative cultures were performed for all patients, and at least two tissue blocks representing four sites were evaluated in each case. Unfortunately, the results of the frozen-section analysis were somewhat compromised by the authors' exclusion of ten patients, in part because their cases were difficult to classify on the basis of the extent of inflammation. The authors did not try to determine the concentration of inflammatory cells that was predictive of infection. Instead, cases were interpreted as positive if there was "evidence of acute inflammation characterized by the presence of polymorphonuclear leukocytes." The authors emphasized the importance of an overall histologic interpretation, rather than relying solely on a count of neutrophil concentration. Using the results of intraoperative cultures as the reference standard, Fehring and McAlister calculated the sensitivity and specificity of the frozen-section interpretation as well as of an overall histologic diagnosis based on analysis of frozen and permanent sections. Of ninety-seven cases that were retained in the study, eleven were found to be infected and eighty-six were not infected. There were nine false-positive and nine false-negative frozen sections, yielding a specificity of 89.5% and a sensitivity of only 18.2%. On the basis of the complete histologic analysis, there were twelve false-positive and two false-negative results, yielding a sensitivity of 82% and a specificity of 86%. Interestingly, there was ultimately a high clinical suspicion of infection in six patients with negative intraoperative cultures: two had draining sinuses, one had a positive culture of fluid obtained with joint aspiration, and three had had prior resection arthroplasties



because of infection. Thus, this study could be interpreted as showing that frozen-section analysis has relatively poor sensitivity, especially if one considers the ten cases that were excluded. On the other hand, it also illustrates the problem of using intraoperative cultures as the reference standard instead of the final clinical diagnosis based on a combination of tests.

Lonner et al.<sup>66</sup> performed a prospective study similar to the one reported by Fehring and McAlister<sup>65</sup>. Frozen sections were obtained from at least two areas in each of 175 consecutive patients undergoing revision arthroplasty. The five most cellular fields were evaluated, and an infection was considered to be present if there was an average of five or more polymorphonuclear leukocytes in at least five high-power fields. The authors also recorded the cases with ten or more polymorphonuclear leukocytes per high-power field. An average of four or fewer polymorphonuclear leukocytes per high-power field was interpreted as indicating the absence of infection. Nineteen patients had positive intraoperative cultures. With the culture results used as the reference standard, there were three false-negative and seven false-positive histologic interpretations (a sensitivity of 84% and a specificity of 96%). Of the seven patients with a false-positive result, five had five to nine polymorphonuclear leukocytes per high-power field. If the authors had used ten cells per high-power field as the cutoff, there would have been only two false-positive histologic interpretations (specificity, 98%). Of note, seven of the positive intraoperative cultures were considered by the treating physicians to be probably due to contaminants. All of the patients with those cultures had negative histologic findings, and all were treated as if they did not have an infection. No signs of infection had developed in these seven patients after an average duration of twenty months of follow-up, a finding that illustrates the problem of using intraoperative culture results as the reference standard.

In 1995, Athanasou et al.<sup>67</sup> reported on a prospective study in which frozen sections from several different sites were obtained during each of 106 hip and knee revision arthroplasties performed between 1991 and 1993, and the results were compared with those of intraoperative cultures. In an evaluation of ten high-power fields with maximal inflammation, the authors quantified inflammatory cells into four tiers (absent, one, one to five, and more than five cells per field). Of note, lymphocytes and plasma cells were included along with neutrophils, but neutrophils entrapped in fibrin adherent to the surface of the membrane were excluded. Intraoperative cultures were considered positive if organisms grew on direct plating or if a similar strain grew on enrichment in more than one culture; single isolates from only one culture were considered to be negative findings. On the basis of the culture results, twenty-four arthroplasty sites were determined to be infected and eighty-four were considered to be not infected. Compared with these culture results, the frozen-section analysis yielded two false-negative and three false-positive results—a sensitivity of 90%, a specificity of 96%, and positive and negative predictive values of 88% and 98%. The authors noted that there were occasional lymphocytes in the thirty-six uninfected cases.

These cells were often perivascular and were not regarded as suspicious for infection. In addition, three patients with underlying rheumatoid arthritis had numerous lymphocytes and plasma cells, and five patients with aseptic loosening and abundant metal particles also had moderate numbers of lymphocytes. While these patients were recognized as probably not having an infection, the authors noted that: "in the absence of rheumatoid disease, plasma cells were a good marker of infection, being noted in eight of the infected cases." Of the two patients who were considered to have a "false-positive" frozen section on the basis of a negative intraoperative culture, one had loosening eighteen months later and was found to have an infection at the repeat revision arthroplasty. The second patient also had a clinical course suggestive of infection, which again emphasizes the limitation of using intraoperative culture results as a reference standard.

In 2000, Pandey et al.<sup>68</sup> reported a study that appears to have overlapped, in part, with the study by Athanasou et al.<sup>67</sup>. Pandey et al. retrospectively reviewed the results of histologic tissue analysis and intraoperative cultures of specimens from 617 revision arthroplasties performed between 1992 and 1996 at several hospitals affiliated with the Oxford Skeletal Infection Research and Intervention Service. Although there was overlap among the authors of the two studies<sup>67,68</sup>, different criteria were used for the histologic diagnosis of infection. At least ten high-power fields were evaluated, and an average score for the various inflammatory cells was calculated<sup>68</sup>. One inflammatory cell per high-power field in at least ten fields was considered to be consistent with infection. For the intraoperative cultures, isolation of the same organism from three or more culture specimens was considered diagnostic of infection. Organisms were considered contaminants if different strains grew in different broths and there was no growth on direct plating. A single isolate was considered to be unimportant. Of the 617 revision arthroplasty sites, 526 were clinically suspected to be aseptic and ninety-one were suspected to be infected. Eighty-one were proven to be infected according to the microbiologic criteria noted above. Five hundred and twenty-one cases had no growth on culture and had negative histologic findings as only scattered lymphocytes were present (true-negative histologic findings). Both the cultures and the histologic analysis showed features of infection in seventy-nine cases (true-positive histologic findings). Two cases had "significant growth of organisms" on culture but negative histologic findings (false-negative histologic findings), and ten cases had negative cultures but acute inflammation in the peri-implant membrane. Seven of the ten patients had received preoperative antibiotics, and all ten were treated clinically as if they had an infection. Finally, five cases showed inflammation in the tissue but negative cultures. Two of these patients had rheumatoid arthritis and loosening developed within two years.

As described above and in additional studies summarized in Table III<sup>57,69,70</sup>, criteria for interpreting microscope slides of frozen sections are not yet uniform. Considering a low number of neutrophils (for example, one cell per high-power field<sup>68</sup>) or even lymphocytes or plasma cells<sup>67</sup> to be diag-

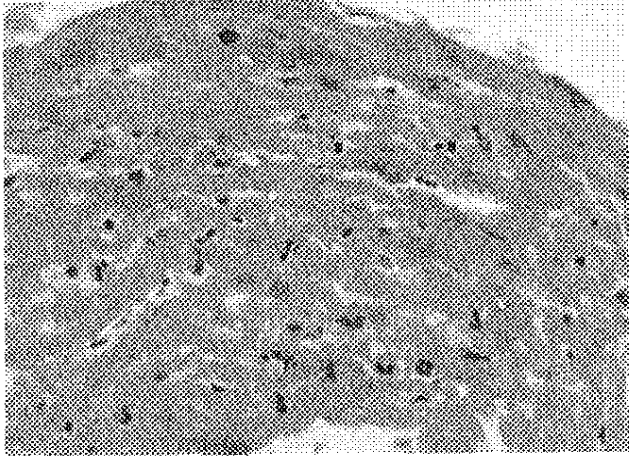


Fig. 5

Neutrophils entrapped in fibrin that is adherent to the surface of a periprosthetic membrane. Experience has shown that neutrophils in this location are not predictive of infection.

nostic of infection will provide maximum sensitivity but will be associated with false-positive diagnoses and hence decreased specificity. Use of more stringent criteria (for example, ten polymorphonuclear leukocytes per high-power field in at least ten high-power fields<sup>66</sup>) will improve specificity at the expense of sensitivity (Table III). Numeric criteria are complicated even more by differences in the visual field size of different microscopes. While most authors have used 10× ocular and 40× objective lenses (yielding a nominal net magnification of 400×), other differences in microscope and camera configurations can vary the visual field by as much as twofold. Therefore, the number of inflammatory cells per high-power field should be recognized as only an approximation.

Partly on the basis of the studies described above, we currently interpret a frozen section as being suggestive of infection if it contains at least five neutrophils in each of three 400× high-power microscopic fields located beneath the surface of the membrane (Figs. 2-A through 4-B). In the appropriate clinical setting, even fewer neutrophils should raise the suspicion of infection. Neutrophils entrapped in superficial fibrin (Fig. 5) or adherent to endothelial cells (marginating) are not thought to be diagnostic of infection, but neutrophils in fibrous tissue between the capillaries that compose granulation tissue may be predictive of infection. Frozen sections of tissue from a patient with an underlying inflammatory arthropathy such as rheumatoid arthritis are especially difficult to interpret because, in these patients, acute inflammation involves peri-implant membranes even in the absence of infection. Lymphocytes and plasma cells have been seen in biopsy specimens from patients who have been treated with antibiotics for infection, but these cells are currently thought to be nonspecific and in general not predictive of active infection. Inflammation is not uniformly distributed around the prosthesis, so frozen-section analysis of biopsy specimens taken from several different sites increases the sensitivity compared

with that of an analysis of a single biopsy specimen. It is also important for the tissue submitted for frozen-section analysis to adequately represent the fibrous membrane and not contain only superficial fibrin. Although we continue to use the same histologic criteria for diagnosing active infection at the second stage of a two-stage revision arthroplasty done because of infection, the predictive value of these observations in this clinical context (after the use of local and systemic antibiotics) requires further study (as described below). Communication and feedback between the surgeon and pathologist are key to help both physicians to determine the clinical importance of inflammation in any given case.

#### *Microbiologic Cultures of Tissue*

As noted above, the results of culture of tissue and/or fluid obtained during revision arthroplasty are usually considered the gold standard for determining the presence or absence of periprosthetic infection. While the clinical utility of intraoperative culture is clear, when viewed in the context of extended follow-up, the test still can yield false-negative and false-positive results (Table I). For example, in one study, 30% of 142 hips treated with revision arthroplasty had at least one positive intraoperative culture, but a clinically important infection later developed in only one case, suggesting a high frequency of false-positive cultures probably caused by contamination of the tissue samples<sup>15</sup>. Other authors have described cases in which, despite the presence of acute inflammation in the periprosthetic membrane and a clinical postoperative course consistent with infection, the intraoperative cultures remained negative (Table I). Some of the patients with negative cultures may have taken perioperative antibiotics. In a prospective study involving revision arthroplasty in 297 patients with a total of forty-one infections, Atkins et al. noted that only 65% of all samples obtained from the infected joints were culture-positive<sup>55</sup>. They recommended obtaining five or six culture specimens from each patient and suggested that the cutoff for a definite diagnosis of infection be growth of the identical organism on culture of three or more specimens. In general, it is recommended that surgeons take special precautions to minimize tissue contamination, such as obtaining multiple samples from deep tissues, using clean instruments for tissue retrieval, transferring tissue to the culture bottle without allowing contact with the operative field or gloves, and transferring of the culture samples to the laboratory for processing as quickly as possible. Levine and Evans recommended injecting fluid directly into blood culture vials instead of using swab samples to improve culture yield<sup>71</sup>. False-negative cultures are likely when the patient received preoperative or intraoperative antibiotics, when the offending organism cannot be isolated by the routine laboratory protocols, or when the submitted tissue samples were extensively cauterized. To minimize the incidence of false-negative cultures, representative samples should be obtained with sharp dissection, administration of antibiotics should be discontinued at least two weeks prior to the surgery, and intraoperative antibiotics should be withheld until the tissue samples are retrieved. Communica-

tion between the microbiologist and the orthopaedic surgeon is critical for isolation of rare and difficult-to-isolate organisms. The use of sonication may help to identify organisms that are adherent to implants or are contained within biofilm<sup>67,72-74</sup>.

#### *Diagnosing Infection at the Time of Reimplantation*

As described above, our understanding of the sensitivity and specificity of various observations and laboratory tests for the diagnosis of periprosthetic infection has been based mostly on the evaluation of patients who have undergone primary hip or knee arthroplasty. Criteria for diagnosing persistent infection at the time of reimplantation in a two-stage revision arthroplasty are even more ill-defined<sup>75</sup>. The inflammatory changes associated with resection arthroplasty reduce the specificity of radiographic studies, including indium-111 leukocyte scans<sup>71</sup>. In a review of the results of cultures of aspirated fluid obtained during thirty-four knee arthroplasties performed at the sites of previous infection, Lonner et al. found a high rate of false-negative findings<sup>76</sup>. The authors emphasized the importance of delaying aspiration until at least two weeks after antibiotic therapy has been terminated. Mont et al. found that the rate of persistent infection was lower when the timing of reimplantation was influenced by the results of cultures of fluid aspirated four weeks after completion of a six-week course of antibiotics than it was when patients underwent reimplantation without aspiration and culture<sup>77</sup>. To our knowledge, the use of frozen sections for diagnosing persistent infection at the time of reimplantation has been evaluated in only a single study<sup>78</sup>. Using intraoperative cultures as the gold standard and the morphologic criterion of ten neutrophils or more in each of five high-powered fields, Della Valle et al. recognized only one of four persistent infections in a series of sixty-four cases (sensitivity, 25%)<sup>78</sup>. While specificity was 95%, the sensitivity of frozen-section interpretation in this clinical setting seems to be lower than that in the setting of primary arthroplasty. Reducing the number of inflammatory cells needed to diagnose infection would be expected to increase sensitivity but might reduce specificity. Additional studies are needed to help clarify the most effective tests for diagnosing infection in this setting.

#### *Endotoxin*

Lipopolysaccharide is a component of the cell wall of gram-negative bacteria. It can be released during episodes of infection; it is pyrogenic; and, when present in high enough concentrations, it can induce the release of interleukins, tumor-necrosis factor, and other cytokines from monocytes and macrophages. Although "endotoxin" strictly refers to lipopolysaccharide from gram-negative organisms, similar molecules may also be associated with gram-positive organisms<sup>79</sup>. Although endotoxin is usually neutralized before causing systemic symptoms, there is increasing evidence that it may adhere to orthopaedic biomaterials, including particles of wear debris, and may enhance the inflammatory reaction to particles that is usually associated with aseptic loosening<sup>10-14</sup>. Therefore, contamination of implants or instruments with

bacterial endotoxin might yield an inflammatory reaction similar to that seen around infected implants. The potential clinical importance of endotoxin in periprosthetic infection and in cases of "aseptic" loosening requires further study.

#### *Molecular Techniques*

With the advances in molecular biology, several sophisticated techniques are being developed for the diagnosis of periprosthetic infection. One such technique is the use of the polymerase chain reaction for detecting evidence of organisms<sup>72,80-83</sup>. The technique relies on the use of forward and reverse primers designed to match specific sequences of target DNA. The most common target gene for bacterial identification is the 16S rRNA gene that is conserved in nearly all species of bacteria. For example, Tunney et al.<sup>72</sup> used polymerase chain reactions to test for evidence of bacteria in fluids obtained by sonication of 120 hip implants retrieved at revision arthroplasty. The implants were first placed in a water bath and then exposed to ultrasound to disrupt any biofilm and dislodge organisms. With use of primers for the 16S rRNA gene, 72% of their cases were interpreted as positive. The main problem with this technique is related to the apparently high prevalence of false-positive results, which have several possible sources<sup>84-86</sup>. First, polymerase chain reactions detect bacterial DNA from both viable and necrotic organisms, so traces of only a few necrotic bacteria dislodged by sonication from an implant surface may yield a positive test result. Second, one of the reagents employed in polymerase chain reactions (Taq polymerase) is derived from recombinant technology involving use of *Escherichia coli* organisms. Trace levels of DNA from the *Escherichia coli* contaminating the Taq polymerase reagent can also yield false-positive results of the polymerase chain reaction. Finally, the broad sensitivity of polymerase chain reactions directed against the 16S rRNA detects even trace contamination by clinically irrelevant organisms that occurs after specimen acquisition. One way to improve the specificity of polymerase chain reactions is to use primers and probes directed against a specific organism, or group of organisms, most likely to be involved in clinically important orthopaedic infections. For example, Sakai et al.<sup>87</sup> developed a polymerase chain reaction assay for staphylococci, in which post-amplification melting curve analysis allows distinction between *Staphylococcus aureus* and coagulase-negative staphylococci. Kobayashi et al.<sup>88</sup> used a combination of a modified universal polymerase chain reaction and sequencing technology to identify bacteria on the basis of DNA sequences that determine gram-positive versus gram-negative staining. Thus, combinations of specific polymerase chain reaction assays may ultimately prove to be more useful than broad-spectrum, so-called "universal" bacterial assays.

Other new techniques that may have a role in diagnosing infection include the use of microarray<sup>89</sup> and proteomics technologies. A microarray allows isolation and evaluation of numerous mRNA genes with a single test. Proteomics allows simultaneous isolation and evaluation of numerous proteins. The premise of these techniques is to identify organism-specific


genes or proteins. The challenge for all of the new molecular tests will be to distinguish clinically important infections from trace levels of necrotic bacteria or contaminants and to provide that information quickly enough to be of practical help in guiding patient care.

### Overview

The diagnosis of periprosthetic infection remains a challenging problem, as there is no single diagnostic modality with absolute sensitivity and specificity. Accurate diagnosis often requires the use of combinations of tests and a strong clinical suspicion. Serologic tests (measurements of white blood-cell count, erythrocyte sedimentation rate, and C-reactive protein level) represent the first-line investigation and generally have good sensitivity but lower specificity. Imaging, such as with a labeled white-blood-cell scan, may be used to further support a diagnosis of an infection when serologic findings are abnormal or in equivocal cases. Aspiration of the joint has high specificity and is especially valuable for diagnosing suspected infections of the knee. Intraoperative cultures should be performed for all patients suspected of having a periprosthetic infection. Extreme care should be exercised to prevent contamination of these samples. Analyses of intraoperative frozen sections have limitations, mostly related to the experience of the pathologist who interprets the sections and the sampling methods of the surgeon. In institutions with adequate pathology resources, interpretation of frozen sections can be very helpful at revision arthroplasty as well as at the time of reimplantation in a two-stage revision of an arthroplasty complicated by infection. Close communication between the surgeon and pathologist, with follow-up of borderline cases, helps the team of physicians to establish their own decision thresholds. Intraoperative cultures, although considered the gold standard, may be negative for some patients with clinically proven periprosthetic infection, and clin-

ical acumen should be employed to override the negative or equivocal findings of diagnostic modalities in some cases. New molecular diagnostic methods will help to diagnose infections in the future.

### Appendix

 A "predictive value calculator" is available on our web site at [jbjs.org](http://jbjs.org) (go to the article citation and click on "Supplementary Material").

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### References

- Charnley J, Eftekhari N. Postoperative infection in total prosthetic replacement arthroplasty of the hip joint. With special reference to the bacterial content of the air of the operating room. *Br J Surg*. 1969;56:641-9.
- Fitzgerald RH Jr. Total hip arthroplasty sepsis. Prevention and diagnosis. *Orthop Clin North Am*. 1992;23:259-64.
- Peersman G, Laskin R, Davis J, Peterson M. Infection in total knee replacement. A retrospective review of 6489 total knee replacements. *Clin Orthop Relat Res*. 2001;392:15-23.
- Espehaug B, Engesaeter LB, Vollset SE, Havelin LI, Langeland N. Antibiotic prophylactics in total hip arthroplasty. Review of 10,905 primary cemented total hip replacements reported to the Norwegian arthroplasty register, 1987 to 1995. *J Bone Joint Surg Br*. 1997;79:590-5.
- Havelin LI, Espehaug B, Vollset SE, Engesaeter LB. The effect of the type of cement on early revision of Charnley total hip prostheses. A review of eight thousand five hundred and seventy-nine primary arthroplasties from the Norwegian Arthroplasty Register. *J Bone Joint Surg Am*. 1995;77:1543-50.
- Tunney MM, Patrick S, Curran MD, Ramage G, Anderson N, Davis RI, Gorman SP, Nixon JR. Detection of prosthetic joint biofilm infection using immunological and molecular techniques. *Methods Enzymol*. 1999;310:566-76.
- Tunney MM, Patrick S, Gorman SP, Nixon JR, Anderson N, Davis RI, Hanna D, Ramage G. Improved detection of infection in hip replacements. A currently underestimated problem. *J Bone Joint Surg Br*. 1998;80:568-72.
- Chimento GF, Finger S, Barrack RL. Gram stain detection of infection during revision arthroplasty. *J Bone Joint Surg Br*. 1996;78:838-9.
- Fehring TK, Cohen B. Aspiration as a guide to sepsis in revision total hip arthroplasty. *J Arthroplasty*. 1996;11:543-7.
- Akitsu T, Bauer T, Farver CF, Mochida Y. The effect of particle wear debris on NF-kappaB activation and pro-inflammatory cytokine release in differentiated THP-1 cells. *J Biomed Mater Res*. 2002;59:507-15.
- Bi Y, Collier T, Goldberg V, Anderson J, Greenfield E. Adherent endotoxin mediates biological responses of titanium particles without stimulating their phagocytosis. *J Orthop Res*. 2002;20:696-703.
- Bi Y, Seabold JM, Kaar SG, Ragab AA, Goldberg VM, Anderson JM, Greenfield EM. Adherent endotoxin on orthopedic wear particles stimulates cytokine production and osteoclast differentiation. *J Bone Miner Res*. 2001;16:2082-91.
- Greenfield EM, Bi Y, Ragab AA, Goldberg VM, Nalepka JL, Seabold JM. Does endotoxin contribute to aseptic loosening of orthopedic implants? *J Biomed Mater Res B Appl Biomater*. 2005;72:179-85.
- Ragab AA, Van De Motter R, Lavish SA, Goldberg VM, Ninomiya JT, Carlin CR, Greenfield EM. Measurement and removal of adherent endotoxin from titanium particles and implant surfaces. *J Orthop Res*. 1999;17:803-9.
- Padgett DE, Silverman A, Sachjowicz F, Simpson RB, Rosenberg AG, Galante JO. Efficacy of intraoperative cultures obtained during revision total hip arthroplasty. *J Arthroplasty*. 1995;10:420-6.
- Bhandari M, Montori VM, Swiontkowski MF, Guyatt GH. User's guide to the surgical literature: how to use an article about a diagnostic test. *J Bone Joint Surg Am*. 2003;85:1133-40.

17. Galen RS. Predictive value and efficiency of laboratory testing. *Pediatr Clin North Am.* 1980;27:861-9.
18. Galen RS, Gambino SR. Beyond normality: the predictive value and efficiency of medical diagnoses. New York: Wiley; 1975.
19. Nelson JP. Deep infection following total hip arthroplasty. *J Bone Joint Surg Am.* 1977;59:1042-4.
20. Schmalzried TP, Amstutz HC, Au MK, Dorey FJ. Etiology of deep sepsis in total hip arthroplasty. The significance of hematogenous and recurrent infections. *Clin Orthop Relat Res.* 1992;280:200-7.
21. Hanssen AD, Osmon DR. The use of prophylactic antimicrobial agents during and after hip arthroplasty. *Clin Orthop Relat Res.* 1999;369:124-38.
22. Hanssen AD, Rand JA. Evaluation and treatment of infection at the site of a total hip or knee arthroplasty. *J Bone Joint Surg Am.* 1998;80:910-22.
23. Hanssen AD, Osmon DR, Nelson CL. Prevention of deep periprosthetic joint infection. *J Bone Joint Surg Am.* 1996;78:458-71.
24. Tigges S, Stiles RG, Roberson JR. Appearance of septic hip prostheses on plain radiographs. *AJR Am J Roentgenol.* 1994;163:377-80.
25. Levitsky KA, Hozack WJ, Balderston RA, Rothman RH, Gluckman SJ, Maslack MM, Booth RE Jr. Evaluation of the painful prosthetic joint. Relative value of bone scan, sedimentation rate, and joint aspiration. *J Arthroplasty.* 1991;6:237-44.
26. Stumpe KD, Notzli HP, Zanetti M, Kamel EM, Hany TF, Gorres GW, von Schulthess GK, Hodler J. PDG PET for differentiation of infection and aseptic loosening in total hip replacements: comparison with conventional radiography and three-phase bone scintigraphy. *Radiology.* 2004;231:333-41.
27. Alibadi P, Tumeq SS, Weissman BN, McNeil BJ. Cemented total hip prosthesis: radiographic and scintigraphic evaluation. *Radiology.* 1989;173:203-6.
28. Magnuson JE, Brown ML, Hauser MF, Berquist TH, Fitzgerald RH Jr, Klee GG. In-111-labeled leukocyte scintigraphy in suspected orthopedic prosthesis infection: comparison with other imaging modalities. *Radiology.* 1988;168:235-9.
29. Nijhof MW, Fleer A, Hardus K, Vogely HC, Schouls LM, Verbout AJ, Dhert WJ. Tobramycin-containing bone cement and systemic cefazolin in a one-stage revision. Treatment of infection in a rabbit model. *J Biomed Mater Res.* 2001;58:747-53.
30. Love C, Marwin SE, Tomas MB, Krauss ES, Tronco GG, Bhargava KK, Nichols KJ, Palestro CJ. Diagnosing infection in the failed joint replacement: a comparison of coincidence detection of 18F-FDG and 111In-labeled leukocyte/99mTc-sulfur colloid marrow imaging. *J Nucl Med.* 2004;45:1864-71.
31. Scher DM, Pak K, Lonner JH, Fenkel JE, Zuckerman JD, Di Cesare PE. The predictive value of indium-111 leukocyte scans in the diagnosis of infected total hip, knee, or resection arthroplasties. *J Arthroplasty.* 2000;15:295-300.
32. Teller RE, Christie MJ, Martin W, Nance EP, Haas DW. Sequential indium-labeled leukocyte and bone scans to diagnose prosthetic joint infection. *Clin Orthop Relat Res.* 2000;373:241-7.
33. Palestro CJ, Swyer AJ, Kim CK, Goldsmith SJ. Infected knee prosthesis: diagnosis with In-111 leukocyte, Tc-99m sulfur colloid, and Tc-99m MDP imaging. *Radiology.* 1991;179:645-8.
34. Kraemer WJ, Saplys R, Waddell JP, Morton J. Bone scan, gallium scan, and hip aspiration in the diagnosis of infected total hip arthroplasty. *J Arthroplasty.* 1993;8:611-6.
35. Rosenthal L, Lisbona R, Hernandez M, Hadjiplavou A. 99mTc-PP and 67Ga imaging following insertion of orthopedic devices. *Radiology.* 1979;133:717-21.
36. Demirkol MC, Adalet I, Unal SN, Tozun R, Cantez S. 99Tc(m)-polyclonal IgG scintigraphy in the detection of infected hip and knee prostheses. *Nucl Med Commun.* 1997;18:543-8.
37. Zhuang H, Duarte PS, Pourdehnad M, Maes A, Van Acker F, Shnier D, Garino JP, Fitzgerald RH, Alavi A. The promising role of 18F-FDG PET in detecting infected lower limb prosthesis implants. *J Nucl Med.* 2001;42:44-8.
38. Reinartz P, Mumme T, Hermanns B, Cremerius U, Wirtz DC, Schaefer WM, Neithard FU, Buell U. Radionuclide imaging of the painful hip arthroplasty: positron-emission tomography versus triple-phase bone scanning. *J Bone Joint Surg Br.* 2005;87:465-70.
39. Bilgen O, Atici T, Durak K, Karaeminogullari O, Bilgen MS. C-reactive protein values and erythrocyte sedimentation rates after total hip and total knee arthroplasty. *J Int Med Res.* 2001;29:7-12.
40. Larsson S, Thelander U, Friberg S. C-reactive protein (CRP) levels after elective orthopedic surgery. *Clin Orthop Relat Res.* 1992;275:237-42.
41. White J, Kelly M, Dunsmauir R. C-reactive protein level after total hip and total knee replacement. *J Bone Joint Surg Br.* 1998;80:909-11.
42. Carlsson AS. Erythrocyte sedimentation rate in infected and non-infected total-hip arthroplasties. *Acta Orthop Scand.* 1978;49:287-90.
43. Sanzen L, Carlsson AS. The diagnostic value of C-reactive protein in infected total hip arthroplasties. *J Bone Joint Surg Br.* 1989;71:638-41.
44. Sanzen L, Sundberg M. Periprosthetic low-grade hip infections. Erythrocyte sedimentation rate and C-reactive protein in 23 cases. *Acta Orthop Scand.* 1997;68:461-5.
45. Spangehl MJ, Masri BA, O'Connell JX, Duncan CP. Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. *J Bone Joint Surg Am.* 1999;81:672-83.
46. Virolainen P, Lahteenmaki H, Hiltunen A, Sipola E, Meurman O, Nelimarkka O. The reliability of diagnosis of infection during revision arthroplasties. *Scand J Surg.* 2002;91:178-81.
47. Maenpaa H, Laiho K, Kauppi M, Kaarela K, Kautiainen H, Lehto MU, Belt EA. A comparison of postoperative C-reactive protein changes in primary and revision hip arthroplasty in patients with rheumatoid arthritis. *J Arthroplasty.* 2002;17:108-10.
48. Sell S, Schleh T. C-reactive protein as an early indicator of the formation of heterotopic ossifications after total hip replacement. *Arch Orthop Trauma Surg.* 1999;119:205-7.
49. Ellitsgaard N, Andersson AP, Jensen KV, Jorgensen M. Changes in C-reactive protein and erythrocyte sedimentation rate after hip fractures. *Int Orthop.* 1991;15:311-4.
50. Lachlewicz PF, Rogers GD, Thomason HC. Aspiration of the hip joint before revision total hip arthroplasty. Clinical and laboratory factors influencing attainment of a positive culture. *J Bone Joint Surg Am.* 1996;78:749-54.
51. Di Cesare PE, Chang E, Preston CF, Liu CJ. Serum interleukin-6 as a marker of periprosthetic infection following total hip and knee arthroplasty. *J Bone Joint Surg Am.* 2005;87:1921-7.
52. Barrack RL, Harris WH. The value of aspiration of the hip joint before revision total hip arthroplasty. *J Bone Joint Surg Am.* 1993;75:66-76.
53. Barrack RL, Jennings RW, Wolfe MW, Bertot AJ. The value of preoperative aspiration before total knee revision. *Clin Orthop Relat Res.* 1997;345:8-16.
54. Streiner DL, Norman GR. PDQ epidemiology. 2nd ed. London: B.C. Decker; 1996.
55. Atkins BL, Athanasou N, Deeks JJ, Crook DW, Simpson H, Peto TE, McLardy-Smith P, Berend AR. Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. The OSIRIS Collaborative Study Group. *J Clin Microbiol.* 1998;36:2932-9.
56. Della Valle CJ, Scher DM, Kim YH, Oxley CM, Desai P, Zuckerman JD, Di Cesare PE. The role of intraoperative Gram stain in revision total joint arthroplasty. *J Arthroplasty.* 1999;14:500-4.
57. Feldman DS, Lonner JH, Desai P, Zuckerman JD. The role of intraoperative frozen sections in revision total joint arthroplasty. *J Bone Joint Surg Am.* 1995;77:1807-13.
58. Schumacher HR Jr, Klippel JH, Koopman WJ. Primer on the rheumatic diseases. 10th ed. Atlanta: The Arthritis Foundation; 1993.
59. Kersey R, Benjamin J, Marson B. White blood cell counts and differential in synovial fluid of aseptically failed total knee arthroplasty. *J Arthroplasty.* 2000;15:301-4.
60. Mason JB, Fehring TK, Odum SM, Griffin WL, Nussman DS. The value of white blood cell counts before revision total knee arthroplasty. *J Arthroplasty.* 2003;18:1038-43.
61. Trampuz A, Hanssen AD, Osmon DR, Mandrekar J, Steckelberg JM, Patel R. Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. *Am J Med.* 2004;117:556-62.
62. Charosky CB, Bullough PG, Wilson PD Jr. Total hip replacement failures. A histological evaluation. *J Bone Joint Surg Am.* 1973;55:49-58.
63. Mirra JM, Amstutz HC, Matos M, Gold R. The pathology of the joint tissues and its clinical relevance in prosthesis failure. *Clin Orthop Relat Res.* 1976;117:221-40.
64. Mirra JM, Marder RA, Amstutz HC. The pathology of failed total joint arthroplasty. *Clin Orthop Relat Res.* 1982;170:175-83.
65. Fehring TK, McAlister JA Jr. Frozen histologic section as a guide to sepsis in revision joint arthroplasty. *Clin Orthop Relat Res.* 1994;304:229-37.
66. Lonner JH, Desai P, DiCesare PE, Steiner G, Zuckerman JD. The reliability of analysis of intraoperative frozen sections for identifying active infection during revision hip or knee arthroplasty. *J Bone Joint Surg Am.* 1996;78:1553-8.

67. Athanasou NA, Pandey R, de Steiger R, Crook D, McLardy Smith PM. Diagnosis of infection by frozen section during revision arthroplasty. *J Bone Joint Surg Br.* 1995;77:28-33.
68. Pandey R, Berendt AR, Athanasou NA. Histological and microbiological findings in non-infected and infected revision arthroplasty tissues. The OSIRIS Collaborative Study Group. Oxford Skeletal Infection Research and Intervention Service. *Arch Orthop Trauma Surg.* 2000;120:570-4.
69. Abdul-Karim FW, McGinnis MG, Kraay M, Emancipator SN, Goldberg V. Frozen section biopsy assessment for the presence of polymorphonuclear leukocytes in patients undergoing revision of arthroplasties. *Mod Pathol.* 1998;11:427-31.
70. Banit DM, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res.* 2002;401:230-8.
71. Levine BR, Evans BG. Use of blood culture vial specimens in intraoperative detection of infection. *Clin Orthop Relat Res.* 2001;382:222-31.
72. Tunney MM, Patrick S, Curran MD, Ramage G, Hanna D, Nixon JR, Gorman SP, Davis RI, Anderson N. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. *J Clin Microbiol.* 1999;37:3281-90.
73. Dobbins JJ, Seligson D, Raff MJ. Bacterial colonization of orthopedic fixation devices in the absence of clinical infection. *J Infect Dis.* 1988;158:203-5.
74. Neut D, van Horn JR, van Kooten TG, van der Mei HC, Busscher HJ. Detection of biomaterial-associated infections in orthopaedic joint implants. *Clin Orthop Relat Res.* 2003;413:261-8.
75. Lonner JH. Identifying ongoing infection after resection arthroplasty and before second-stage reimplantation. *Am J Knee Surg.* 2001;14:68-71.
76. Lonner JH, Siliski JM, Della Valle C, DiCesare P, Lotke PA. Role of knee aspiration after resection of the infected total knee arthroplasty. *Am J Orthop.* 2001;30:305-9.
77. Mont MA, Waldman BJ, Hungerford DS. Evaluation of preoperative cultures before second-stage reimplantation of a total knee prosthesis complicated by infection. A comparison-group study. *J Bone Joint Surg Am.* 2000;82:1552-7.
78. Della Valle CJ, Bogner E, Desai P, Lonner JH, Adler E, Zuckerman JD, DiCesare PE. Analysis of frozen sections of intraoperative specimens obtained at the time of reoperation after hip or knee resection arthroplasty for the treatment of infection. *J Bone Joint Surg Am.* 1999;81:684-9.
79. Wang ZM, Liu C, Dziarski R. Chemokines are the main proinflammatory mediators in human monocytes activated by *Staphylococcus aureus*, peptidoglycan, and endotoxin. *J Biol Chem.* 2000;275:20260-7.
80. Mariani BD, Martin DS, Levine MJ, Booth RE Jr, Tuan RS. Polymerase chain reaction detection of bacterial infection in total knee arthroplasty. *Clin Orthop Relat Res.* 1996;331:11-22.
81. Yang S, Rothman RE. PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. *Lancet Infect Dis.* 2004;4:337-48.
82. Ince A, Rupp J, Frommelt L, Katzer A, Gille J, Lohr JF. Is "aseptic" loosening of the prosthetic cup after total hip replacement due to nonculturable bacterial pathogens in patients with low-grade infection? *Clin Infect Dis.* 2004;39:1599-603.
83. Trampuz A, Osmon DR, Hanssen AD, Steckelberg JM, Patel R. Molecular and antibiofilm approaches to prosthetic joint infection. *Clin Orthop Relat Res.* 2003;414:69-88.
84. Corless CE, Guiver M, Borrow R, Edwards-Jones V, Kaczmarek EB, Fox AJ. Contamination and sensitivity issues with a real-time universal 16S rRNA PCR. *J Clin Microbiol.* 2000;38:1747-52.
85. Meier A, Persing DH, Finken M, Bottger EC. Elimination of contaminating DNA within polymerase chain reaction reagents: implication for a general approach to detection of uncultured pathogens. *J Clin Microbiol.* 1993;31:646-52.
86. Newsome T, Li BJ, Zou N, Lo SC. Presence of bacterial phage-like DNA sequences in commercial Taq DNA polymerase reagents. *J Clin Microbiol.* 2004;42:2264-7.
87. Sakai H, Procop GW, Kobayashi N, Togawa D, Wilson DA, Borden L, Krebs V, Bauer TW. Simultaneous detection of *Staphylococcus aureus* and coagulase-negative staphylococci in positive blood cultures by real-time PCR with two fluorescence resonance energy transfer probe sets. *J Clin Microbiol.* 2004;42:5739-44.
88. Kobayashi N, Bauer TW, Togawa D, Lieberman IH, Sakai H, Fujishiro T, Tuohy MJ, Procop GW. A molecular gram stain using broad range PCR and pyrosequencing technology: a potentially useful tool for diagnosing orthopaedic infections. *Diagn Mol Pathol.* 2005;14:83-9.
89. Deirmengian C, Lonner J, Booth RJ. White blood cell gene expression: a new approach toward the study and diagnosis of infection. *Clin Orthop Relat Res.* 2005;440:38-44.
90. Duff GP, Lachiewicz PF, Kelley SS. Aspiration of the knee joint before revision arthroplasty. *Clin Orthop Relat Res.* 1996;331:132-9.