Prolonged Bacterial Culture to Identify Late Periprosthetic Joint Infection: A Promising Strategy

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Background. The value of microbiological culture to diagnose late periprosthetic infection is limited, especially because standard methods may fail to detect biofilm-forming sessile or other fastidious bacteria. There is no agreement on the appropriate cultivation period, although this period is a crucial factor. This study was designed to assess the duration of culture that is necessary for reliable detection.

Patients and methods. Ten periprosthetic tissue specimens each were obtained during revision from 284 patients with suspected late hip or knee arthroplasty infection. Five samples were examined by microbiological culture over a 14-day period, and 5 were subjected to histologic analysis. To define infection, a pre-established algorithm was used; this included detection of indistinguishable organisms in $\geqslant$2 tissue samples or growth in 1 tissue sample and a positive result of histologic analysis (>5 neutrophils in at least 10 high-power fields). The time to detection of organisms was monitored.

Results. Infection was diagnosed in 110 patients. After 7 days (the longest incubation period most frequently reported), the detection rate via culture was merely 73.6%. Organisms indicating infection were found for up to 13 days. "Early"-detected species (mostly staphylococci) emerged predominantly during the first week, whereas "late"-detected agents (mostly *Propionibacterium* species) were detected mainly during the second week. In both populations, an unequivocal correlation between the number of culture-positive tissue samples and positive results of histologic analysis was noted, which corroborated the evidence that true infections were detected over the entire cultivation period.

Conclusions. Prolonged microbiological culture for 2 weeks is promising because it yields signs of periprosthetic infection in a significant proportion of patients that would otherwise remain unidentified.

Periprosthetic infection is probably the most feared complication of joint arthroplasty. For therapeutic reasons, it is important to distinguish between early and late infections. According to 1 widely accepted classification [1, 2], early infections occur within the first 4 weeks after primary implantation and are most often caused by highly virulent organisms (e.g., *Staphylococcus aureus* or Enterobacteriaceae) acquired during or shortly after implantation. In contrast, late manifestations comprise low-grade infections that are also predominantly attained during implantation but that are caused by less-virulent agents that belong to the normal skin flora (e.g., coagulase-negative staphylococci, *Propionibacterium* species, and coryneform bacteria) or infections that result from hematogenous spreading from remote sites [1–3]. Although in cases of early infection, the implant can remain with a promising prospect of success, late infections require replacement of the prosthesis [2, 4]. Thus, the unequivocal diagnosis of infection is crucial for ensuring adequate treatment of affected patients.

The classification of periprosthetic infections is sometimes confusing, because there is a different nomenclature that subdivides the late infections into delayed or low-grade and late manifestations (depending on whether they occur 3 months–2 years or >2 years after primary implantation, respectively) [23]. However, this distinction does not have direct therapeutic consequences, because both kinds of infection require replacement arthroplasty. Therefore, it is clinically fea-

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sible to consider all infections that emerge >4 weeks after implantation to be late infections.

Early infections usually cause local and systemic inflammatory reactions; however, these reactions are often missing in cases of late infection, which makes the diagnosis of late infections much more difficult. Classic clinical diagnosis, laboratory tests, and imaging techniques, such as radiography and scintigraphy, are hampered by high rates of false-positive and false-negative results [1]. Therefore, invasive methods that comprise bacterial culture of joint aspirate or periprosthetic tissue samples and histologic analysis play a pivotal role in the recognition of late arthroplasty infections. Reliable cultivation of infectious organisms is especially important, because local (via supplementation of bone cement with antibiotics) and systemic postoperative antibiotic therapy can be designed individually for each patient, depending on the cultured species and its antimicrobial susceptibility profile.

Because the causative agents predominantly belong to the normal skin flora, the criteria used to define late periprosthetic infection are crucial. The algorithm that we favor in our hospital is based on bacterial culture and histologic analysis [5] and has proven to be feasible in various other clinical settings [1, 6–9]. However, no binding consensus criteria for diagnosis exist to date. The definitions of infection, the number of tissue samples obtained for diagnostic purposes, and the technical performance and interpretation of culture and histologic analysis vary considerably among studies. As a result, reported ranges of sensitivity and specificity of microbiological tissue culture are 65%–94% and 81%–100%, respectively [1, 7, 9–21]. Except for in the case of classic infection caused by a planctonic agent, it is well established that biofilm formation on the implant by sessile bacteria is a major pathogenetic factor, especially in low-grade arthroplasty infection [3, 22, 23]. However, standard culture techniques do not meet the requirements for reliable identification of such slowly growing organisms. In particular, it seems that the need for an appropriate period of incubation has been underestimated. Previous studies were conducted with culture durations of 4–7 days [1, 7, 8, 17, 21], and most conveyed no information with regard to the incubation periods [11, 14–16, 18, 20]. A few authors have recommended incubation for 14 days to improve sensitivity [24–26], but the culture duration that is actually necessary for reliable diagnosis was never assessed systematically.

The present study fills the gap of missing studies. It is postulated that, for diagnosis of late periprosthetic infection, a 2-week duration of culture is superior to cultivation for a shorter period. To validate this hypothesis, the amount of time necessary for infectious organisms to become detectable was monitored in samples from patients who met the classification criteria for having infection according to a pre-established definition. The findings strongly suggest that this approach can markedly improve the identification of late arthroplasty infections.

PATIENTS AND METHODS

Study design. Infections were assessed in all patients who underwent revision of loose hip and knee arthroplasty from August 2004 through July 2007 in a prospective study. All patients gave informed consent to participate, and the protocol was approved by the local research ethics board. To minimize the risk of false-negative culture results, no patient was treated with antibiotics within 4 weeks before the revision operation, according to previous recommendations [27–29]. During intervention, samples of the periprosthetic soft tissue and the loosening membrane were obtained for microbiological culture and histologic analysis from 5 different sites each and were transferred to sterile transport vials. Perioperative antibiotic prophylaxis or therapy was not administered until samples had been obtained. Sufficient tissue material was available for 284 patients (who underwent 145 hip and 139 knee interventions). The mean age of the patients was 69 years; 153 of the patients were male, and 131 were female. Revision arthroplasty was performed a mean of 38 months after primary implantation (range, 2–252 months). In cases of bacterial growth in patient samples, the time to detection of organisms was analyzed.

Definition of infection. In accordance with a previously established algorithm [1, 7, 8], infection was predefined as (1) isolation of indistinguishable organisms (according to the biochemical profile and the antimicrobial susceptibility pattern) from at least 2 tissue samples or (2) detection of bacterial growth in 1 tissue sample and positive results of histologic analysis. Tissue samples were considered to be positive by histologic analysis if a 3+ neutrophil polymorph infiltrate (>5 cells in at least 10 high-power fields; original magnification, $\times$400) was present [6, 8, 9]. Analogously, organisms isolated from only 1 tissue sample without histologic signs of infection were interpreted as contaminants [1, 7, 8].

Culture procedures. Patient specimens were processed immediately after their arrival at the laboratory. Cultivation of tissue samples was performed essentially as described elsewhere [26], under the sterile conditions of a class 2 laminar flow workbench. In brief, tissue specimens were thoroughly minced. Aerobic and anaerobic culture and Gram stain were performed with all tissue lysates. For aerobic culture, sample aliquots were applied to trypticase-soy agar containing 5% sheep blood (Oxoid), chocolate agar (Becton Dickinson), MacConkey II agar (Becton Dickinson), and brain-heart infusion broth (Oxoid) and were incubated at 36 $\pm$ 1°C under standard conditions. For anaerobic culture, Schaedler agar containing vitamin K1 and 5% sheep blood (Becton Dickinson) and Schaedler broth (Oxoid) were inoculated and cultured at 36 $\pm$ 1°C in an anaerobic atmosphere.

Table 1. Spectrum and relative frequencies of isolated species.

Organism	No. (%) of samples	
	Infection (n = 110)	No infection (n = 47)
Staphylococcus aureus	12 (10.9)	2 (4.3)
Coagulase-negative staphylococci	60 (54.5)	27 (57.4)
Enterococcus species	6 (5.5)	…
Streptococcus species	5 (4.5)	1 (2.1)
Enterobacteriaceae	2 (1.8)	1 (2.1)
Coryneform bacteria[a]	6 (5.5)	6 (12.8)
Propionibacterium species	15 (13.6)	6 (12.8)
Peptostreptococcus species	4 (3.6)	1 (2.1)
Other[b]	…	3 (6.4)

[a] *Corynebacterium* species, *Rothia* species, *Actinomyces* species, and *Bacillus* species.
[b] *Micrococcus* species and *Aspergillus* species. There were no statistically significant differences between infected and uninfected samples.

Media were checked daily for bacterial growth. Broth cultures were discontinued and declared negative if no growth was visible at 14 days. Clouded broths were Gram stained and subcultured onto appropriate agar plates. Grown organisms were identified by standard microbiological procedures, including biochemical characterization with the API system (BioMérieux). Antibiotic susceptibility testing was performed by disk diffusion or dilution methods according to the recommendations of the Clinical and Laboratory Standards Institute.

Statistical analysis. All analyses were performed with the BIAS for Windows software package, version 8.4.1 (Epsilon-Verlag). Comparison of relative frequencies of bacterial species was performed with the Mantel-Haenszel test. The association of the number of culture-positive tissues with histologic findings was compared by the χ^2 test. For overall time to detection of infectious organisms, 95% Hall-Wellner CIs were calculated. Differences in the detection rates between groups were analyzed by the χ^2 test. Time to detection of different species was compared by the Kruskal-Wallis test and Dunn's test. In all procedures, a P value <.05 was rated as statistically significant.

RESULTS

Infections and isolated species. Because no statistically significant differences were found between hip and knee interventions with regard to the following data, all samples were treated as belonging to the same collective. In accordance with the aforementioned algorithm, 110 patient specimens (38.7%) were classified as being infected. In 47 cases, cultured organisms were rated as contaminants. The range and relative frequencies of isolated agents were virtually the same among infected and uninfected specimens (table 1). Although some species were isolated solely from infected samples (enterococci) or as contaminants (*Micrococcus* species and *Aspergillus* species), the differences in frequencies did not reach statistical significance.

Table 2 shows in which measure results of culture and histologic analysis contributed to the diagnosis of infection. There was an unequivocal association between the number of culture-positive tissue samples and histologic signs of infection. The rates of positive results of histologic analysis among samples did not differ significantly among the species of isolated organisms (data not shown).

Time to diagnosis of infection. The number of days until bacterial growth became detectable in infected samples was monitored (figure 1). Although the median time to diagnosis was only 4 days, organisms that enforced the rating of infection according to the diagnostic algorithm were found up to 13 days. Of note, the detection rate after 7 days of culture, which is the longest incubation period reported in most previous investigations, was merely 73.6%. The deficit in culture positivity was statistically significant for up to 6 days of cultivation.

Contaminants were detected significantly later than were infecting organisms (P = .015). However, the median time to detection was only 7 days. Thus, as many as 52% of the contaminating strains emerged during the first week of culture (data not shown).

Early-detected versus late-detected organisms. The time to detection of organisms was further interpreted with respect to the different species (figure 2). The isolated agents were separated into 2 major categories: an "early" population (e.g., staphylococci, enterococci, streptococci, and Enterobacteriaceae), found predominantly within the first week of culture (n = 116), and a "late" population (e.g., *Propionibacterium* species, aerobic gram-positive bacilli, and *Peptostreptococcus* species), detected predominantly during the second week (n = 41).

The clear-cut correlation between the number of culture-positive tissue samples and histologic evidence of infection was reflected both in the early-detected and in the late-detected populations of agents (table 3). Although the relative frequency

Table 2. Overall association of culture with histologic findings.

Result of histologic analysis	Total no. of samples	No. of culture-positive tissue samples,[a] no. of samples		
		⩾2[b]	1	0
Positive	104	80	18	6
Negative	180	12	47[c]	121
All	284	92	65	127

[a] For ⩾2 positive tissue samples versus 1 positive tissue sample, P< .001. For 1 positive tissue sample versus no positive tissue sample, P< .001.
[b] Growth of indistinguishable organisms.
[c] Contaminating strains.

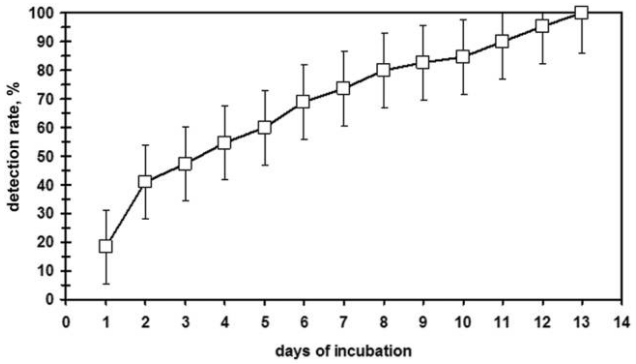

Figure 1. Time to diagnosis of infection by culture. Whisker lines span the 95% Hall-Wellner CI.

of infection was higher in the early-infection population (85 [73.3%] of 116 cases) than in the late-infection population (25 [61.0%] of 41 cases), the difference was not statistically significant.

DISCUSSION

To our knowledge, this is the first study to provide clear evidence (1) that there is a significant risk that periprosthetic infections will not be detected if a prolonged (14-day) microbiological culture period—as recommended elsewhere [24–26]—is not applied and (2) that organisms isolated >1 week after the start of culture do not overproportionally reflect contaminating strains. As expected for cases of late periprosthetic infection, the spectrum of the isolated species was very similar in infected and contaminated tissue samples (according to the definition) (table 1). Therefore, it was essential to ensure that the diagnostic strategy allowed discrimination between infectious agents and contaminants as accurately and efficiently as possible, so that it was applicable to the routine clinical setting. The prolonged culture period that we used was potentially cumbersome, because the number of detected contaminating strains also increases with increasing culture duration. In the studies from which we adopted the definition criteria for infection, however, culture incubation was not performed for >7 days. Therefore, because of the longer incubation period in our study, repetitive isolates detected >7 days after the start of culture could have been contaminants that were misinterpreted as infectious organisms. Indeed, the overall infection rate (38.7%) was comparatively high. Although this is not surprising in general because our hospital is a reference center for periprosthetic infections, this question had to be addressed. Our data suggest that our diagnostic approach was adequate to detect true infections for 3 reasons.

Firstly, repetitive isolation of phenotypically identical clones was more frequently associated with positive results of histologic analysis than was growth in 1 tissue sample (table 2) and, therefore, could be interpreted as highly indicative of infection. The clear-cut association of ⩾2 culture-positive tissue samples with positive results of histologic analysis is remarkable, because the adopted threshold of 3+ neutrophil infiltrate, as established in the algorithm to diagnose infection on the basis of histologic findings, is comparatively high. However, this threshold is advantageous because it rules out a number of cases of inflammation of noninfectious origin [6, 8, 9, 31]. Indeed, in our setting, positive results of histologic analysis of sterile tissue samples was uncommon (table 2) and was mostly observed for patients who underwent knee arthroplasty and had rheumatoid arthritis (data not shown). Theoretically, with this threshold, the number of infections may have been underestimated, because infected samples may have been mischaracterized as contaminated samples (growth in only 1 tissue sample). However, this also is unlikely because histologic signs of infection were much more frequent in tissue samples with bacterial growth than in sterile samples (table 2).

Secondly, the correlation of repetitively isolated indistinguishable organisms with positive results of histologic analysis

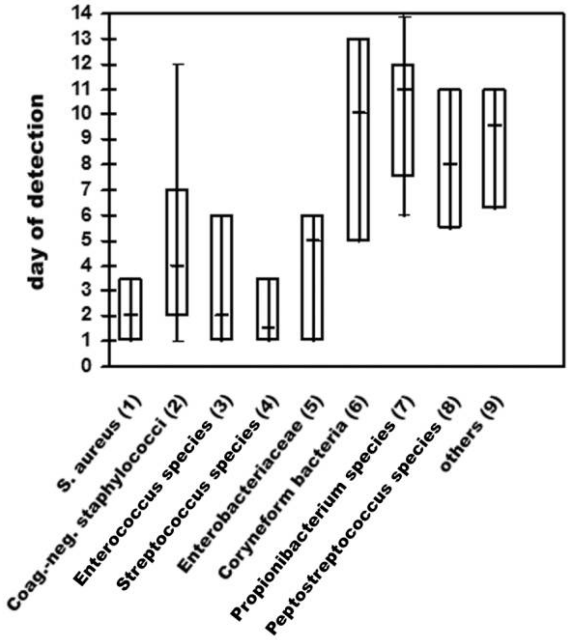

Figure 2. Time to detection of the different species in all patient samples ($n = 284$). Horizontal bars, boxes, and whisker lines indicate median values, 25%–75% percentiles, and 5%–95% percentiles, respectively. Early-detected species included *Staphylococcus aureus,* coagulase-negative (Coag.-neg.) staphylococci, *Enterococcus* species, *Streptococcus* species, and Enterobacteriaceae. Late-detected species included coryneform bacteria, *Propionibacterium* species, *Peptostreptococcus* species, and other species. There were no statistically significant differences among the late-detected species with regard to time to detection.

Table 3. Association of early- and late-detected organisms with histologic findings.

	Early-detected organisms			Late-detected organisms		
		No. of culture-positive tissue samples,[a] no. of samples			No. of culture-positive tissue samples,[b] no. of samples	
Result of histologic analysis	Total no. of samples	⩾2[c]	1	Total no. of samples	⩾2[c]	1
Positive	76	65	11	21	14	7
Negative	40	9	31[d]	20	4	16[d]
All	116	74	42	41	18	23

NOTE. Early-detected species included *Staphylococcus aureus,* coagulase-negative staphylococci, *Enterococcus* species, *Streptococcus* species, and Enterobacteriaceae. Late-detected species included coryneform bacteria, *Propionibacterium* species, *Peptostreptococcus* species, and other species.

[a] For early-detected organisms grown in ⩾2 tissue samples versus 1 tissue sample, $P < .001$.
[b] For late-detected organisms grown in ⩾2 tissue samples versus 1 tissue sample, $P = .007$.
[c] Growth of indistinguishable organisms.
[d] Contaminating strains.

was almost as strong for the late-detected organisms as for the early-detected organisms (table 3). This implies that a significant amount of agents that were detected >7 days after the start of culture reflected true infection.

Thirdly, more than one-half of the contaminating species emerged within the first week of culture. This contradicts the apprehension that the contamination problem may have increased dramatically with prolonged cultivation.

Taken together, the data suggest that the criteria to define infection were also applicable in the context of a 2-week cultivation period. Thus, it is reasonable to conclude that the definition of contaminants (bacterial growth in only 1 tissue sample and negative results of histologic analysis) was realistic as well. Therefore, whether contaminating strains were detected after 1 day or after 2 weeks was not significant.

A possible disadvantage of the definition criteria that we used is that phenotypic characterization of organisms (with use of biochemical and antibiotic-resistance profiles) to identify repetitive isolates is assuredly inaccurate and, therefore, inferior to molecular typing methods, especially for coagulase-negative staphylococci [30]. However, the aim of the present study was not to evaluate definition criteria for periprosthetic infection but was solely to determine the influence of culture duration on the diagnostic results based on pre-established criteria. The algorithm adopted at our clinic has already shown broad clinical feasibility [1, 5–9] and is based on phenotypic characterization, most likely because routine molecular typing is not practicable in the clinical setting. Moreover, in spite of the shortcomings of phenotypic differentiation of isolates, our data strongly suggest that, from the clinical standpoint, true infections can be identified with adequate accuracy by using our approach.

A substantial proportion of patients (26.4%) were classified as being infected when the period of culture was 2 weeks but would not have been rated as such if the culture duration had been only 1 week (figure 1). One week, however, is the longest cultivation period reported by the vast majority of investigations [1, 7, 8, 17, 21]. Often, the culture period is not even mentioned [11, 14–16, 18, 20]; thus, presumably, standard conditions (incubation for up to 5 days) were applied in these studies. It is quite likely that the necessity for adequate culture conditions has been underestimated in the past and that, thus, insufficient durations of culture, at least in part, account for the varying significance of culture results.

With regard to the time to growth of early and late species (figure 2), patients harboring fastidious organisms would not be classified as being infected if the incubation period is inadequately short. This has grave clinical consequences, because the treatment of patients with "aseptic" implant failure is completely different. *Propionibacterium* species were isolated with high frequency in our setting, compared with other investigations [7, 17, 21, 32–35]. The 2-week incubation period implemented in our study provides a reasonable explanation for this finding, because propionibacteria were detected almost exclusively during the second week of culture. On the other hand, even with regard to the early organisms (e.g., staphylococci, enterococci, streptococci, and Enterobacteriaceae), incubation for 7 days would not have been sufficient to rule out periprosthetic infection. For example, nearly one-fourth of the coagulase-negative staphylococci isolated from infected samples were not detected until the second week (data not shown). This delayed growth may have reflected low concentrations of sessile bacteria, which are often present in biofilms [23].

Sensitive cultivation of an organism enables subsequent antimicrobial-susceptibility testing, which is essential for the choice of an adequate antibiotic regimen. Moreover, this enables the surgeon to add specific antibiotics to the bone cement during 1-stage or 2-stage revision operations [36]. To make use of these advantages, many authors have practiced preoperative

aspiration of the joint and bacteriological analysis of the harvested fluid specimens. However, a study of the relevant literature reveals a large variance in the value of this method [1, 11, 14, 17, 18, 20, 37, 38]. Again, an insufficient culture duration is a likely cause of these discrepancies. Because the data presented in this study were collected from specimens that were obtained during prosthetic revision surgery, it is reasonable to conclude that incubation for 2 weeks should also be advantageous to predict infection in preoperative biopsy or joint aspirate samples obtained for culture. Indeed, in accordance with the same algorithm used to define infection as the one used in the present study, a cultivation period of 2 weeks for preoperative culture of synovial biopsy specimens from patients who underwent knee arthroplasty and were treated in our hospital provided results that are highly concordant (100% sensitivity and 98.1% specificity) with the definitive findings from tissue samples obtained during revision surgery [5]. Similarly, phenotypically indistinguishable organisms have been detected in preoperative hip joint aspirate specimens with 73.5% sensitivity and 94.3% specificity by culture after replacement surgery (unpublished data). This high repeatability of preoperative and intraoperative culture results further corroborates our interpretation that contaminating strains are not a major problem with regard to accurate prediction of infection.

To overcome the drawbacks of microbiological culture, PCR-based methods to detect and discriminate bacteria by 16S rRNA gene analysis have been used. However, these techniques are cost-intensive and methodically cumbersome, and for various reasons, no noteworthy advantages over culture have been demonstrated to date [25, 39, 40]. Furthermore, the significance of genotypic characterization is severely restricted with regard to the evaluation of antibiotic susceptibility. Thus, at present, there is no reasonable alternative to classical microbiological culture. As shown in the present study, the incubation period of 2 weeks is a promising approach toward optimization of periprosthetic infection diagnostics.

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