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CLINICAL RESEARCH

Blood Culture Flasks for Culturing Synovial Fluid in Prosthetic Joint Infections

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Abstract

Background Identifying the etiologic microorganism is essential to guide antimicrobial therapy in prosthetic joint infection.

Questions/purpose We (1) compared the frequency of positive cultures with synovial fluid inoculated in blood culture flasks (SF) with those of periprosthetic tissues or swabs in traditional cultures from patients with acute and chronic prosthetic joint infections (PJI) and (2) determined the sensitivity, specificity, and predictive values of the three methods.

Patients and Methods We retrospectively reviewed 87 patients with PJIs (54 knees, 33 hips) and 63 patients with aseptic loosening (34 knees, 29 hips). Two SF, periprosthetic tissue, and swab samples were taken for culture in all 150 patients except for 14 in whom only one SF fluid sample was obtained. Synovial fluid was inoculated in blood culture flasks and periprosthetic tissue and swab samples in standard media. Positive cultures were identified with standard biochemical procedures.

Results SF samples were positive in 78 of 87 infected cases (90%), periprosthetic tissue samples were positive in 71 (82%), and swab samples were positive in 59 (68%). SF, periprosthetic tissue, and swab samples were positive more frequently in acute than in chronic infections (96% versus 82% for SF, 87% versus 74% for periprosthetic tissue, and 87% versus 44% for swabs). The sensitivity, specificity, and positive and negative predictive values of SF were 91, 100, 100, and 93 for acute infections and 79, 100, 100, and 88 for chronic infections, respectively.

Conclusions SF samples cultured in flasks had higher sensitivity, specificity, and positive and negative predictive values for diagnosis of PJI when compared with standard tissue and swab samples. The usefulness of all samples was less in chronic than in acute infections.

Level of Evidence Level II, diagnostic study. See Guidelines for Authors for a complete description of levels of evidence.

Introduction

The bacteriologic diagnosis is essential for treatment of PJI. The low sensitivity of periprosthetic tissue samples in PJI is partly attributable to the fact that the majority of the microorganisms in these infections grow in biofilms attached to the implant surface (sessile bacteria) and only a low percentage are free-floating bacteria (planktonic) in the surrounding tissue dislodged from the sessile population [4].

To improve detection of microorganisms in chronic PJI, Trampuz et al. [15] compared cultures of samples obtained by sonication of explanted prostheses with conventional cultures of periprosthetic tissues among patients

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Each author certifies that his or her institution approved the human protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.

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undergoing revision or resection arthroplasty. The sensitivity of sonicate-fluid cultures was greater than the sensitivity of periprosthetic tissue cultures (78% vs 61%). However, this procedure is complex and time-consuming for microbiology laboratories; therefore, it is important to analyze the accuracy of different methods for culturing periprosthetic samples.

The superiority of blood culture bottles for detecting microorganisms in synovial fluid compared with conventional agar and broth methods for patients suspected of having septic arthritis was described by Hughes et al. [8]. They identified more pathogens (62 vs 51) and fewer contaminants (1 vs 11) using blood culture flasks than culturing on agar plates [8]. One previous study comparing SF grown in blood culture vials with tissue and swabs in patients with PJI concluded SF grown in vials had greater sensitivity, specificity, and accuracy than the other two methods [10]. That study, however, included a low number of patients (24, with nine being uninfected) and the authors did not differentiate between acute and chronic PJI. This information is important because in acute infections synovial fluid is abundant and the number of planktonic bacteria, responsible for typical signs of infection (redness, inflammation, fever) is high, whereas in chronic infections the volume of synovial fluid is low and the majority of bacteria are attached to the implant surface, which explains the scarcity of inflammatory signs. These data are supported by the low rate of negative culture results (6.25%) for patients with acute infections treated with open débridement [11] compared with the high rate (40%) seen for patients with chronic infections who underwent revision surgery [15].

To confirm and extend the previous literature we: (1) compared the frequency of positive cultures of SF with the results of periprosthetic tissue and swab samples cultured in standard media, (2) evaluated the three different samples according to the type of infection, acute or chronic, and the type of implant, knee or hip, and (3) determined the sensitivity, specificity, and predictive values of each sample in patients with a final diagnosis of aseptic loosening who underwent revision surgery and in whom samples were obtained for microbiologic analysis.

Patients and Methods

We retrospectively reviewed all 87 patients with acute or chronic knee (n = 54) and hip (n = 33) PJIs from February 2005 to November 2007. There were 48 acute and 39 chronic infections (Table 1). All 63 patients who underwent one-stage revision for aseptic loosening from January 2006 to November 2007 were studied as noninfected; 34 underwent knee revision and 29 had hip revision. Some patients required multiple operations; in these cases, only data from the first surgery were analyzed. The protocol for sampling was established by the orthopaedic department before February 2005 as described subsequently. The Institutional Review Board approved the study.

Acute PJI was suspected when local inflammatory signs, purulent drainage through the wound, and elevated C-reactive protein were present during the first 6 weeks after joint arthroplasty. All these patients underwent open débridement and the infection was confirmed if pus or devitalized tissue was found surrounding the prosthesis. Chronic PJI was suspected when the patient had pain, radiographic signs of loosening, elevated inflammatory markers (C-reactive protein of 10 mg/L or greater or erythrocyte sedimentation rate of 30 mm/hour or greater) [2], and (1) a sinus tract communicating with the prosthesis; (2) abnormal spots on leukocytes labeled with technetium-99 m scintigraphy; or (3) a positive culture of synovial fluid obtained using a percutaneous sterile technique. Plain radiographic findings of chronic PJI include aggressive nonfocal osteolysis, periosteal bone formation, and/or lysis of bone adjacent to the prosthesis [14]. Although rapid and progressive loosening of implants in the absence of any mechanical cause raises the possibility of infection, these findings are nonspecific for infection, because aseptic loosening secondary to wear debris produces similar radiographic signs [5]. Patients with chronic PJI underwent two-stage revision arthroplasty and the diagnosis was confirmed by the presence of five or more polymorphonuclear cells in at least five fields of a high-power field in periprosthetic tissue, or pus surrounding the prosthesis.

Aseptic failure was defined when the patient had pain and radiographic signs of loosening but inflammatory markers (C-reactive protein or erythrocyte sedimentation rate), leukocytes labeled with technetium-99 m scintigraphy were normal, and culture of synovial fluid obtained using a percutaneous sterile technique was negative. These patients underwent one-stage exchange and the diagnosis was confirmed by the presence of less than five polymorphonuclear cells in at least five fields of a high-power field in periprosthetic tissue [3, 6].

All operations were performed in a standard, nonlaminar airflow operating room. Following the protocol of our hospital, the antibiotic treatment always was delayed until deep samples for culture were obtained. The protocol for sampling during surgery (open débridement or revision surgery) consisted of obtaining samples just after arthrotomy as follows: (1) synovial fluid was aspirated and immediately inoculated half into aerobic and half into anaerobic blood culture flasks (BACTEC 9240 system; BD Diagnostic Systems, BD Corporation, NJ, USA). The volume inoculated in each flask was approximately 1 to 3 mL.

Solid samples from periprosthetic tissue with visual signs of inflammation, granulation, necrosis, or purulence were obtained and placed in sterile containers without medium or saline. Finally, swab cultures were obtained by passing a sterile swab over the intracapsular area, bone, or fluid and immediately placed in transport medium (AMIES transport medium). Two samples of tissue and swabs were obtained in all patients. In the case of synovial fluid, two samples were obtained in 136 patients and one sample in 14. Samples were processed and analyzed in the microbiology laboratory. Blood culture flasks containing the aspirated synovial fluid were incubated in the BACTEC 9240 system up to 5 days [8]. Positive cultures were Gram-stained and the microorganisms were identified by conventional microbiologic methods. The homogenized periprosthetic tissue and swabs were cultured in thioglycolate broth, blood agar in aerobic conditions, and Schaedler agar (in anaerobic conditions). All samples were incubated up to 5 days. Positive cultures were regrown in appropriate media. All isolated microorganisms were identified with standard biochemical procedures. A total of 933 samples were processed in the microbiology laboratory and were grouped according to the patients (infected or noninfected), the type of prosthesis (hip or knee), and the type of infection (acute or chronic) (Table 2).

Differences in the proportion of positive cultures between acute and chronic, and hip and knee PJIs were determined using a chi square test or Fisher's exact test when necessary. The results of cultures of each type of sample for patients with infections and patients with aseptic loosening were recorded and used to calculate the sensitivity, specificity, and positive and negative predictive values for each sample. The gold standard for defining infection was the clinical, radiographic, and histologic parameters previously described. In addition, these parameters were calculated and compared using the chi square test or Fisher's exact test according to the type of infection and type of prosthesis.

Table 1. Demographic characteristics of patients based on type of loosening

Parameter	Septic (87)		Aseptic (63)
	Acute (48)	Chronic (39)	
Males/females	20/28	21/18	19/44
Mean age (years)	69	72	71
Mean ASA scale	2.35	2.46	2.2
ID-DM	3 (6%)	3 (8%)	0
NID-DM	4 (8%)	7 (18%)	9 (14%)
Oral corticotherapy	6 (12%)	0	1 (1.5%)
Rheumatoid arthritis	2 (4%)	0	0
Chronic renal failure	0	5 (13%)	2 (3%)

ASA = American Society of Anesthesiologists; ID-DM = insulin dependent diabetes mellitus; NID-DM = non-insulin dependent diabetes mellitus.

Results

Synovial fluid samples were positive in 78 of 87 infected cases (90%), periprosthetic tissue samples were positive in 71 (82%), and swab samples were positive in 59 (68%) (Table 3). Synovial fluid samples were positive more frequently ($p = 0.001$) than swab samples. Synovial fluid samples were more frequently positive ($p = 0.03$) in acute (96%) than in chronic infections (82%), and swab samples were more frequently positive ($p < 0.0001$) in acute (87%) than in chronic infections (44%). There were no differences between hip and knee infections regarding synovial fluid and periprosthetic tissue specimens; however, swabs were positive less frequently ($p = 0.001$) in acute hip infections (54%) than in acute knee infections (97%). Three positive samples of the three materials (synovial fluid, periprosthetic tissue, and swab) were found more often ($p = 0.0001$) in acute than in chronic infections (79% versus 38%, respectively) (Table 3). In six cases (two patients with acute and four with chronic infections), the etiologic microorganism was identified only in other samples different from synovial fluid. The frequency of infected cases with all cultures negative (three) was greater ($p = 0.08$) in patients with chronic (8%) than with acute infections (0%). All these patients had positive intraoperative histologic samples that confirmed the diagnosis of infection. Among patients with aseptic loosening ($n = 63$), synovial fluid cultures were always negative, whereas 24 periprosthetic tissue cultures (21 patients) and one swab culture (one patient) were positive (Table 4). The microorganisms isolated in these cases were coagulase-negative staphylococci in 18 cases, Propionibacterium acnes in four, Clostridium spp in one, Micrococcus luteus in one, and Corynebacterium spp in one.

Taking into account infected and noninfected patients, synovial fluid had a sensitivity, specificity, and positive and negative predictive values of 86%, 100%, 100%, and 83%, respectively. The results for periprosthetic tissue were 69%, 81%, 85%, and 63%, respectively, and 61%, 99%, 99%, and 67% for swabs, respectively. The accuracy of each sample was greater for acute than chronic PJIs (Table 4). There were no differences in sensitivity, specificity, and predictive values between samples obtained from hip and knee prostheses except for swab samples. These samples had a lower sensitivity in hip infections (33%) than in knee infections (78%).

The most frequent isolated microorganism in chronic and acute PJIs was coagulase-negative staphylococci

Table 2. Number of samples from each group of patients according to the type of prosthesis (hip or knee) and type of infection (acute or chronic)

Group	Number of patients	Number of samples		
		SF (acute/chronic)	PT (acute/chronic)	S (acute/chronic)
Hip PJI	33	69 (24/45)	74 (26/48)	67 (20/47)
Hip, control subjects	29	55	60	68
Knee PJI	54	100 (69/31)	126 (88/38)	112 (73/39)
Knee, control subjects	34	62	66	74
Total	150	286	326	321

PJI = prosthetic joint infection; SF = synovial fluid; PT = periprosthetic tissue; S = swab.

Table 3. Profile of positive or negative samples in prosthetic joint infections and in aseptic loosening

Profile of sample			Number of acute infections (%)			Number of chronic infections (%)			Total (%)
SF	PT	S	Hip	Knee	Total	Hip	Knee	Total	
P	P	P	6 (54.4)	32 (86.5)	38 (79.2)	5 (22.7)	10 (58.8)	15 (38.5)	53 (60.9)
P	P	N	2 (18.2)	1 (2.7)	3 (6.2)	9 (41)	1(5.9)	10 (25.6)	13 (14.9)
P	N	N	3 (27.3)	0	3 (6.2)	3 (13.6)	2 (11.8)	5 (12.8)	8 (9.2)
N	N	N	0	0	0	2 (9.1)	1 (5.9)	3 (7.7)	3 (3.4)
N	N	P	0	1 (2.7)	1 (2.1)	0	0	0	1 (1.1)
N	P	P	0	1 (2.7)	1 (2.1)	0	0	0	1 (1.1)
N	P	N	0	0	0	2 (9.1)	2 (11.8)	4 (10.2)	4 (4.6)
P	N	P	0	2 (5.4)	2 (4.2)	1 (4.5)	1 (5.9)	2 (5.1)	4 (4.6)
Total			11	37	48	22	17	39	87

Profile of sample			Number of aseptic loosenings (%)		
SF	PT	S	Hip	Knee	Total
P	P	P	0	0	0
P	P	N	0	0	0
P	N	N	0	0	0
N	N	N	22 (75.86)	19 (55.88)	41 (65.07)
N	N	P	1 (3.44)	0	1 (1.5)
N	P	P	0	0	0
N	P	N	6 (20.68)	15 (44.11)	21 (33.33)
P	N	P	0	0	0
Total			29	34	63

SF = synovial fluid; PT = periprosthetic tissue; S = swab; P = culture-positive; N = culture-negative; HPJI = hip prosthetic joint infection; KPJI = knee prosthetic joint infection.

(Table 5). There were 19 (22%) polymicrobial infections, five (15%) in the group with hip infections and 14 (26%) in the group with knee PJIs. The majority of polymicrobial cases (15 of 19 [79%]) were acute infections. Three chronic PJIs (3%, one hip and two knees) had one positive culture. These cases were considered infections because histologic and clinical findings supported this diagnosis. Microorganisms isolated in these cases were coagulase-negative staphylococci in two and Propionibacterium acnes in one.

Discussion

Treatment of PJIs often is prolonged and reliable information regarding the etiologic microorganism is essential to select antimicrobial therapy. The protocols reported in one study for obtaining samples for microbiologic analysis have resulted in samples showing low sensitivity, most especially for chronic infections [15]. Previous data for septic arthritis suggested that inoculating synovial fluid in

Table 4. Sensitivity, specificity, PPV and NPV of each sample according to the type of infection (acute or chronic)

Type of infection	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy
Acute					
Synovial fluid	91.39	100	100	93.6	96.19
Periprosthetic tissue	78.94	80.95	78.95	80.95	80
Swab	80.65	99.3	98.68	88.68	91.91
Chronic					
Synovial fluid	78.94	100	100	87.96	91.7
Periprosthetic tissue	56.98	80.95	67.12	73.38	71.23
Swab	39.53	99.29	97.14	73.06	76.75

Table 5. Microorganisms isolated according to the type of infection and prosthesis

Microorganisms	Acute infections		Chronic infections		Total number‡
	Hip	Knee	Hip	Knee	
Gram-positive cocci					
Coagulase-negative staphylococci (methicillin-resistant)	11 (5)	17 (12)	8 (5)	12 (10)	48 (32)
Staphylococcus aureus (methicillin-resistant)	2 (1)	14 (2)	2 (1)	0	18 (4)
Streptococcus viridans	0	3	2	1	6
Enterococcus faecalis	1	3	2	1	7
Other*	0	3	0	2	5
Gram-negative bacilli					
Escherichia coli	0	5	1	1	7
Pseudomonas aeruginosa	1	3	0	0	4
Proteus spp	1	2	1	0	4
Enterobacter cloacae	1	1	0	1	3
Other†	0	3	1	0	4
Anaerobes					
Propionibacterium acnes	0	0	2	1	3
Peptostreptococcus spp	0	0	3	0	3
Yeast					
Candida albicans	1	0	0	0	1

* Staphylococcus saprophyticus, Streptococcus agalactiae, S. equisemilis, S. mitis, and Bacillus spp; †Klebsiella pneumoniae, Serratia marcescens, Stenotrophomonas maltophilia, and Campylobacter spp; ‡the total number of microorganisms was greater than the number of patients because of polymicrobial infections (see Results).

blood culture flasks increases the sensitivity of cultures. The aims of our study were (1) to compare the frequency of positive cultures of synovial fluid samples inoculated in blood culture flasks (SF) with the results of periprosthetic tissue samples and swab samples cultured in standard media, (2) to evaluate the results of the three different samples according to the type of infection, acute or chronic and the type of implant, knee or hip, and (3) to determine the sensitivity, specificity, and predictive values of each sample using a cohort of 63 patients with a final diagnosis of aseptic loosening who underwent revision surgery and in whom samples were obtained for microbiologic analysis.

Our study had some limitations. First, the selection of noninfected patients was based on presurgical clinical, serologic, and radiographic findings and intraoperative histology results, following recommended criteria [2, 6, 15], however, it is difficult to be sure all patients considered aseptic were really aseptic. Second, the incubation time for samples was 5 days. Some authors have reported a higher detection rate using longer incubation periods [13], although longer periods also can be associated with a greater risk of contamination.

Levine and Evans [10] studied 34 patients, 25 with infected PJIs and nine without infection. Synovial fluid in blood culture flasks was positive in 92% of infections, tissue biopsies in 77%, and swabs in 76%, with a sensitivity of 92%, 46%, and 64% respectively. The specificity of the three samples was 100%. However, they had a small

noninfected group (nine patients) and they did not evaluate the influence of the type of infection (acute or chronic) or the location of the implant (hip or knee). Our data confirmed that synovial fluid inoculated in blood culture flasks more frequently identified the etiologic microorganism than solid samples (periprosthetic tissue) and swabs. Results for all samples were better for acute than for chronic infections, probably as a result of the high density of planktonic bacteria in acute infections.

The contamination of samples obtained during surgery is frequent [7, 9, 12], and some authors have proposed a protocol based on multiple samples to rule out contaminants [1]. No synovial fluid culture and only one swab culture was positive in our patients who fulfilled the criteria of aseptic loosening. In contrast, 24 of 126 (19%) tissue samples were positive in our patients. These findings suggest the frequency of contamination of synovial fluid and swabs is less than for tissue cultures. As a result, the specificity for synovial fluid, swab cultures, and tissue cultures was 100%, 99%, and 81%, respectively. Despite previous recommendations of taking six different samples, based on sensitivity and specificity of periprosthetic tissue samples [13], it seems reasonable based on our results to recommend obtaining two synovial fluid samples (in blood culture flasks), one swab and one tissue sample in acute infections, and two or three SF (depending on the quantity of synovial fluid available) and three periprosthetic tissue samples in chronic PJIs. In chronic infections, swab could be avoided because in no cases the microorganism was isolated only in these samples. Based on our data with this protocol of sampling the microorganism would be identified in 100% of acute PJIs and 93% of chronic PJIs. The use of blood culture flasks means an additional cost; however, in our hospital, the cost of one blood culture flask is 2.50 Euros, which is reasonable.

Using conventional cultures, including SF in a blood culture flask, periprosthetic tissue, and swabs, the etiology of the infection was identified in 96.5% of cases; however, in three chronic PJIs, all cultures were negative. SF inoculated in blood culture flasks had greater sensitivity, specificity, and positive and negative predictive values for diagnosis of a PJI when compared with standard tissue and swab samples. The usefulness of all samples was less for chronic than for acute infections.

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