Research article/Raziskovalni prispevek

COMPARISON OF CULTURE OF SYNOVIAL FLUID, PERIPROSTHETIC TISSUE AND PROSTHESIS SONICATE FOR THE DIAGNOSIS OF KNEE PROSTHESIS INFECTION

PRIMERJAVA KULTURE SINOVIALNE TEKOČINE, OBPROTEZNEGA TKIVA IN SONIKATA PROTEZE V DIAGNOSTIKI OKUŽB KOLENSKIH PROTEZ

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Abstract – Background. *Synovial fluid and periprosthetic tissue specimens are the standard specimens cultured for the diagnosis of prosthetic joint infection (PJI). We hypothesize that ultrasonication of the explanted prosthesis may improve diagnosis of PJI by dislodging biofilm bacteria from the prosthesis surface and improve the sensitivity and specificity of diagnosis of PJI.*

Methods. *Included were patients undergoing knee prosthesis exchange for septic or biomechanical failure and have not received antimicrobial therapy in the last 2 weeks prior specimen collection. Cultures of synovial fluid and periprosthetic tissue specimens were performed per the usual clinical practice. Additionally, explanted joint components were sonicated for 5 minutes at frequency 40 kHz in sterile Ringer's solution; aliquots of 0.5 ml sonicate were plated onto five aerobic and five anaerobic blood agar plates, and incubated at 37 °C and examined for the next seven days. The number and identity of each colony morphology was recorded.*

Results. *35 patients undergoing knee replacement have been studied (24 for aseptic biomechanical failure and 11 for suspected PJI). In patients with PJI, coagulase-negative staphylococci (7 cases), Corynebacterium spp. (2 cases), Staphylococcus aureus (1 case), and viridans group streptococcus (1 case) were recovered. Culture sensitivity and specificity were for synovial fluid 88% and 100%, for periprosthetic tissue 83% and 81%, and for explant sonicate 91% and 100%, respectively. In sonicate cultures higher numbers of microorganisms than in periprosthetic tissue cultures were consistently detected.*

Ključne besede: *kolenska proteza; okužba; mikrobiološka diagnostika; sonikacija proteze*

Izvleček – Izhodišča. *Uvedba ortopedskih protez je revolucionalizirala medicino in izdatno izboljšala kakovost življenja mnogim osebam z boleznimi kosti in sklepov. Manj kot 10% bolnikov z vstavljeno protezo razvije po artroplastiki zaplete, ki zahtevajo ponovno operacijo. Aseptična biomehanska disfunkcija je najpogostejši razlog zapletov, sledijo jim okužbe sklepnih protez (OSP). Kljub temu da OSP predstavljajo relativno redek zaplet, sta ekonomska posledica za družbo in neprijetnost za bolnika ogromni. Diagnostika OSP je zapletena in zdravljenje dolgotrajno. Najpomembnejši razlog za težavno diagnostiko in pogosto neuspešno zdravljenje je dejstvo, da mnogi mikroorganizmi na umetnem materialu tvorijo biofilm, s katerim se zaščitijo pred zunanjimi vplivi, kot so na primer antibiotiki ali imunski odziv. Zato so različni avtorji poskušali izboljšati diagnostiko in zdravljenje OSP s postopki, ki delujejo proti razvoju biofilma ali ga razgradijo. Sinovialna tekočina in obprotezno tkivo predstavljata običajni vrsti vzorca za mikrobiološko preiskavo v diagnostiki OSP. Obe vrsti vzorca nista optimalni za ugotavljanje bakterij v obliki biofilma. Zato smo z raziskavo želeli preveriti hipotezo, ali je z ultrasonikacijo ortopedske proteze mogoče odstraniti na površini pritrjene bakterije v obliki biofilma in s tem izboljšati občutljivost in specifičnost diagnostike OSP.*

Metode. *Vključeni so bili hospitalizirani bolniki na kliniki Mayo (Rochester, Minnesota, USA), pri katerih je bila zaradi septičnega ali biomehaničnega vzroka med julijem 2001 in julijem 2002 zamenjana kolenska proteza. Dali so pristanek za sodelovanje v raziskavi ter v zadnjih dveh tednih pred odvzemom mikrobioloških vzorcev niso prejemali protimikrobnih zdravil. OSP smo definirali z najdbo makroskopsko vid-*

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Conclusions. *Using synovial fluid, periprosthetic tissue, and explant sonicate cultures, 12%, 17% and 9% of PJI were missed, respectively. Explant sonicate cultures were the most sensitive with respect to the diagnosis of PJI, indicating that explant ultrasonication may improve bacterial recovery. In sonicate cultures, infecting organisms were detected in high numbers, typically concentrated on only one of the prosthetic components. These findings indicate that explant ultrasonication may improve bacterial recovery and support the importance of biofilms in, and the focal nature of, PJI.*

nega gnoja ob odstranitvi proteze in/ali akutnega vnetja ob histološkem pregledu vzorcev obproteznega tkiva. Sinovialno tekočino, dobljeno med predoperativno aspiracijo sklepa, in vzorce obproteznega tkiva, odvzete med odstranitvijo proteze, smo zasadili na mikrobiološka gojišča po ustaljenem postopku. V raziskovalne namene smo dodatno odstranjeno protezo aseptično položili v sterilno vrečko in jo v posodi z anaerobnimi pogoji prenesli v mikrobiološki laboratorij. Tam smo protezo v 100 ml sterilne Ringerjeve raztopine soli sonificirali v vodni kopeli 5 minut pri frekvenci 40 kHz. Alikvote po 0,5 ml tako dobljenega sonikata smo zasadili na pet aerobnih in pet anaerobnih gojišč krvnega agarja ter gojišča inkubirali pri 37 °C. Gojišča smo dnevno pregledovali naslednjih sedem dni in zabeležili število kolonij ter mikrobiološko klasificirali vsako morfološko različno kolonijo.

Rezultati. *V raziskavo smo vključili 35 bolnikov z odstranjeno kolensko protezo (24 bolnikov z aseptičnim biomehanskim omajanjem in 11 bolnikov s sumom na OSP). Z izjemo prisotne fistule se klinični znaki niso razlikovali pri bolnikih z aseptičnim razlogom za menjavo proteze od tistih z OSP. Pri bolnikih z OSP smo osamili koagulaza-negativne stafilokoke (7 primerov), Corynebacterium spp. (2 primera), Staphylococcus aureus (1 primer) in streptokok iz skupine viridans (1 primer). Občutljivost in specifičnost preiskave s kulturo sta znašali za sinovialno tekočino 88% in 100%, za obprotezno tkivo 83% in 81% ter za sonikat proteze 91% in 100%. V kulturah sonikata proteze je dosledno porastlo večje število mikroorganizmov kot v kulturah obproteznega tkiva.*

Zaključki. *Kultura sonikata proteze se je ujemala z diagnozo OSP v 91%, kultura sinovialne tekočine se je ujemala v 88% in kultura obproteznega tkiva v 83%. Dejstvo, da se je kultura sonikata proteze najbolj ujemala z diagnozo OSP, podpira hipotezo, da je z ultrasonikacijo odstranjene proteze mogoče odstraniti pritrjene bakterije na površini umetnega materiala v biofilmu in na ta način izboljšati občutljivost preiskave za diagnozo OSP. V kulturah sonikata smo zaznali večje število bakterij kot v kulturah sinovialne tekočine in obproteznega tkiva. Patogene bakterije so bile najpogosteje v večjem številu lokalizirane le na eni od obeh proteznih komponent (tibialnem ali femoralnem delu), kar govori v prid fokalnosti okužbe in potrjuje pomen biofilma v patogenezi OSP.*

Introduction

Prosthetic joint implantation (arthroplasty) has greatly improved the quality of life for many individuals (1). Enthusiasm about the results of arthroplasty in elderly patients has led to gradual expansion of indications for arthroplasty, such that many relatively young, active patients now receive total joint replacements. Total joint replacement is one of the most successful operations currently available; less than 10% of patients develop complications during their lifetime that require revision surgery (2). Aseptic biomechanical loosening, often attributed to suboptimal patient selection, implant design and/or surgical technique, is the most common cause of prosthetic joint failure, followed by prosthetic joint infection (PJI) (3). Although PJI compromises the end result in only a small number of implant recipients, the economic impact of PJI is immense and devastating to both the patient and society. The diagnosis as well as the antimicrobial and surgical management of PJI is complex, and the recovery is arduous and prolonged (4).

Mechanisms of implant failure. It is well recognized that synthetic materials used to construct implants will not last forever. The functional implant life may be influenced by its material and design (e. g., ceramic, stainless steel, titanium, titanium alloy), technical aspects of device implantation (e. g., cementing, creation of a porous implant surface or hydroxyapatite cover for stimulating bone apposition), anti-adhesive implant coating (e. g., antiseptic or bioactive surface properties), quality of host bone, and extent of patient activity (5). The long-term implant stability depends mainly on good initial fixation (osseointegration) and factors maintaining sufficient local bone density around the prosthesis which is the net result of bone resorption (osteolysis) and new bone formation. Aseptic biomechanical failure is often difficult to distinguish from occult chronic PJI. Osteolysis induced by particulate wear debris from implant materials is recognized as a major cause of long-term failure (6). Other aseptic mechanisms of implant failure include inappropriate mechanical load, fatigue failure at bone-implant interfaces, implant micromotions, and perhaps synovial fluid hydrodynamic pressure (7).

Infections are responsible for approximately 1 to 5% of cases of primary arthroplasty failure (8–10). The incidence of PJI is higher after a revision arthroplasty, possibly due to the long operating time, scar formation, or unrecognized infection

present at the initial surgery. Other risk factors for PJI include rheumatoid arthritis, diabetes mellitus, malignancy, and surgical site infection not involving the prosthesis itself (11, 12). Microorganisms can colonize the prosthesis at the time of implantation (either through direct inoculation or as a result of airborne contamination of the wound) or may reach the implant postoperatively through hematogenous seeding or spread from a contiguous infectious focus. Once microorganisms attach to and grow on the surface of foreign bodies protected in a biofilm they are extremely difficult to eradicate.

Importance of biofilm formation in prosthetic joint infection. In natural ecosystems, the majority (> 99.9%) of bacteria grow in matrix-enclosed biofilms adherent to surfaces, in which they aggregate into durable communities rather than roaming as individuals (13). Formation of biofilms represents a basic survival strategy of bacteria, within which they have survived for millions of years in nature, protected from environmental influences. The widespread use of artificial implants and devices in health care settings render environmental and commensal organisms of relatively low virulence increasingly important in humans (14). Prosthetic joint infection is a typical low organism burden infection, the pathogenesis of which is related to bacteria growing in biofilms. Body fluids coat the prosthesis surfaces with a layer of host material, composed primarily of serum proteins and platelets (15). Bacteria can attach to the surface of foreign bodies and produce a highly-hydrated matrix of polysaccharide and protein. This extracellular polymeric substance together with embedded bacteria is collectively known as a biofilm (16). As bacterial colonies mature, surface-associated sessile bacteria on the periphery detach and disperse as suspended planktonic bacteria (17). Bacteria in the biofilm environment are associated with high levels of antimicrobial resistance. Table 1 shows three hypothetic resistance mechanisms of bacteria in biofilms (18). Biofilm-associated bacteria exhibit dramatically increased antimicrobial resistance with minimal inhibitory concentration values hundreds or even thousands of times higher that those of bacteria measured in a suspension culture (19, 20). Therefore, antimicrobial concentrations which are sufficient to kill free-floating planktonic organisms (e. g., in synovial fluid), may be inadequate to kill sessile bacterial cells embedded in an established biofilm which may act as nidus for recurrence of infection when antimicrobial therapy is discontinued. This can explain why, where antimicrobial therapy fails, removal of the infected implant is generally needed. The presence of organisms in biofilms also explains the poor sensitivity of Gram stain and culture of synovial fluid and periprosthetic tissue.

Diagnosis of prosthetic joint infection. The clinical presentation of PJI is highly variable. There are numerous preoperative and intraoperative tests currently in use in the routine diagnosis of PJI (Table 2); unfortunately none of these tests is ideal for the diagnosis of PJI and no standardized criteria for PJI exist. Although the presence of a sinus tract, or frank purulence in the joint aspirate or in tissue obtained at the time of surgery is specific for defining PJI, these signs are not sensitive (21). Because the management of PJI differs from that of aseptic failure, it is important to accurately differentiate these two entities. The consequences of misdiagnosis may be substantial. In particular, reimplantation of the prosthesis into an infected surgical site, without appropriate debridement and antimicrobial treatment, is likely to result in persistent infection, and ultimately in implant failure. Furthermore, a delay in the diagnosis and early treatment of PJI can permit the infection to become established at the bone-implant interface, thereby eliminating the possibility of using a debridement procedure with antimicrobial treatment and prosthesis retention (22–24).

Table 1. *Three hypothesis for mechanisms of antimicrobial resistance in biofilms (18).*

Razpr. 1. *Tri hipoteze o nastanku protimikrobne odpornosti v biofilmu (18).*

Mechanism hypothesis Hipoteza nastanka	Explanation Razlaga
Slow or incomplete penetration	Antimicrobial agents are adsorbed or inactivated in the biofilm.
Počasno ali nepopolno prodiranje	Protimikrobna zdravila se adsorbirajo ali inaktivirajo v biofilmu.
Altered chemical microenvironment	Nutrient depletion or waste product accumulation within the biofilm causes some bacteria to enter a non-growing (stationary) state, in which they are less susceptible to antimicrobial drugs.
Spremenjeno kemično mikrookolje	Pomanjkanje hranil ali kopičenje odpadnih proizvodov v biofilmu vpliva na nekatere bakterije, da se spremenijo v mirujoče (stacionarno) stanje, v katerem so manj občutljive na protimikrobna zdravila.
Phenotypic resistant state	A subpopulation of bacteria differentiate into a dormant, »spore-like« state.
Fenotipsko odporno stanje	Subpopulacija bakterij se spremeni v mirujočo, »spori-podobno« stanje.

Table 2. *Conventional preoperative and intraoperative tests for diagnosis of prosthetic joint infection.*

Razpr. 2. *Običajne predoperativne in intraoperativne preiskave za diagnostiko okužbe sklepne proteze.*

Test Preiskava	Description of the diagnostic tests Opis diagnostične preiskave
Preoperative tests Predoperativne preiskave	
Clinical history and examination	Persistent joint pain; fever, chills or rigors without known etiology; erythema, warmth or effusion of the joint; sinus tract.
Anamneza in klinični pregled	Stalna bolečina v sklepu; vročina ali mrzlica brez drugega vzroka; eritem, toplina ali izliv v sklepu; fistula.
Hematological tests	Leukocyte count and differential; erythrocyte sedimentation rate; C-reactive protein level (CRP).
Preiskave krvi	Število levkocitov in diferencialna krvna slika, hitrost sedimentacije eritrocitov, reaktivna beljakovina C (CRP).
Synovial fluid aspiration	Leukocyte count and differential; Gram stain and culture.
Aspiracija sinovialne tekočine	Število levkocitov in diferencialna krvna slika; barvanje po Gramu in kultura.
Radiographic imaging	Conventional radiography; computed tomography (CT) scan.
Radiološki slikovni prikaz	Konvencionalna radiografija; slikanje z računalniško tomografijo (CT).
Radionuclide bone scanning	Scintigraphy by a technetium ($Tc^{99}m$) scan, gallium citrate (Ga^{67}) scan, or indium (In^{111})-labeled leukocyte or immunoglobulin scan.
Radionuklidno slikanje kosti	Scintigrafija s tehnecijem ($Tc^{99}m$), galijevim citratom (Ga^{67}) ali z indijem (In^{111}) označenimi levkociti ali imunoglobulini.
Positron emission tomography	Fluorine-18 fluorodeoxyglucose (F-18 FDG) positron emission tomography.
Pozitronska emisijska tomografija	Fluor-18 fluorodeoksiglukoza (F-18 FDG) pozitronska emisijska tomografija.
Intraoperative tests Intraoperativne preiskave	
Periprosthetic tissue	Histopathology; Gram stain and culture.
Obprotezno tkivo	Histološka preiskava; barvanje po Gramu in kultura.
Explanted prosthesis	Culture
Odstranjena proteza	Kultura

The **preoperative diagnosis of PJI** is ideal to direct appropriate surgical management, and the initial choice of antimicrobial agents following removal of the infected prosthesis

as well as the choice of antimicrobial agent in bone cement beads or spacers. Clinical findings supportive of infection include fever and/or chills without known etiology, erythema, warmth or effusion of the joint. Routine hematological screening tests have not been particularly helpful in diagnosing PJI. Increased neutrophils and band forms, an elevated C-reactive protein level and erythrocyte sedimentation rate have also been associated with the presence of PJI, but are neither sufficiently sensitive nor specific. Arthrocentesis to obtain synovial fluid for leukocyte count and differential as well as for Gram staining and culture is frequently used in evaluating joint disorders (25). Table 3 shows the interpretation of synovial fluid cell count in patients **without** orthopedic implants set by the American College of Rheumatology (26). There are no such interpretive criteria for PJI. The sensitivity of synovial fluid aspirate culture has ranged from 33%–95% (27, 28). Cultures may be negative because of prior antimicrobial exposure, low number of organisms (because of adherence to the prosthesis surface), inappropriate culture media (e. g., anaerobes), or fastidious or atypical organisms (e. g., mycobacteria). In addition, synovial aspiration itself is associated with a small risk of introduction of infection, is not always possible (e. g., when no effusion is present), may be difficult (e. g., when a small effusion is present), or may be inconvenient (e. g., when a hip or sacroiliac joint is involved). Cultures of superficial sinus tract may be misleading as they may represent colonization with commensal organisms from the surrounding skin (29).

Table 3. *Synovial fluid cell count interpretation in patients* **without** *orthopedic implants (American College of Rheumatology) (26).*

Razpr. 3. *Interpretacija števila celic v sinovialni tekočini pri bolnikih* **brez** *ortopedskih vsadkov (American College of Rheumatology) (26).*

Characteristic Značilnost	Normal Normalno	Non-inflammatory Nevnetno	In-inflammatory Vnetno	Purulent Gnojno
Leukocytes (cells/mL) Levkociti (celic/ml)	< 150	< 3,000	3,000–50,000	> 50,000
Neutrophils (%) Nevtrofilci (%)	< 25	< 25	> 70	> 90

The **intraoperative diagnosis of PJI** can be made by histopathology or microbiology examination of the periprosthetic tissue. The presence of acute inflammation in periprosthetic soft-tissue specimens (e. g., joint capsule, arthroplasty membrane) at the time of surgery is indicative for infection. The degree of infiltration with inflammatory cells may vary considerably between specimens from the same patient, even within individual tissue sections. Therefore, areas with the most florid inflammatory changes should be assessed and at least ten high-power fields should be examined to obtain an average count (30). Sampling errors can lead to false-negative results, especially given the focal nature of PJI. Ideally therefore, multiple samples from the areas most suspicious for infection should be obtained. A major limitation of histopathology studies is that they do not identify the infecting organism, an essential element in selection of appropriate antimicrobial therapy. The sensitivity of Gram stain of periprosthetic tissue showed an extremely low sensitivity (0–23%) (31). Intraoperative tissue cultures provide the most accurate specimens for microbiological culture and are frequently used as the reference standard for diagnosing PJI. Prior or concurrent antimicrobial treatment may cause false-negative culture results in more than 50% of cases, emphasizing the importance of discontinuation of any antimicrobial therapy prior tissue sampling. Perioperative prophylaxis at revision surgery should not be started until after tissue specimens have been collected for culture. Multiple intraoperative tissue specimens should be sent to the microbiology laboratory for culture. If the implant is removed, the entire prosthesis can be cultured in enrichment broth media. The advantage of this approach is that the site of infection is sampled directly. However, care is needed interpretation of such culture results, because of the risk of contamination during processing. As discussed below, mild ultrasonication to dislodge bacteria present within adherent biofilms on the surface of the removed prosthesis may increase the sensitivity of the culture technique, and may be combined with molecular techniques (32).

Microbiology of prosthetic joint infection. In large series, staphylococci are the most important microorganisms involved in PJI, accounting for about 50% of infections overall, followed by streptococci, enterococci, *Enterobacteriaceae*, *Pseudomonas aeruginosa,* and anaerobes (2, 10, 33, 34). Early postoperative and hematogenous PJI is typically caused by relatively virulent organisms, such as *Staphylococcus aureus* and is often characterized by acute onset of symptoms and signs of infection. Late postoperative PJI is generally chronic (low-grade) in nature and is more commonly associated with relatively less-virulent organisms, such as coagulase negative staphylococci. Late postoperative PJI is generally characterized by more subtle signs of inflammation, chronic persistent postoperative pain, and/or early loosening of the implant (24).

Aim of the study. PJI are typically low organism burden and focal infections. We hypothesize that ultrasonication of the explanted prosthesis and use of sonicate culture instead of culture of synovial fluid or periprosthetic tissue may improve diagnosis of PJI by dislodging biofilm-associated bacteria from the prosthesis surface. To test this hypothesis, culture sensitivity and specificity of synovial fluid, periprosthetic tissue, and explant sonicate were compared in patients undergoing total knee replacement for an aseptic biomechanical failure (prosthesis revision) and for a suspected PJI (prosthesis resection).

Patients and methods

Inclusion and exclusion criteria. The study population consisted of hospitalized patients at the Mayo Clinic Rochester (Minnesota, USA) who underwent a total knee **revision** for aseptic biomechanical loosening *(one-stage exchange)* or **resection** for presumed infection *(two-stage exchange)* between July 2001 and July 2002 and gave prior informed consent. The study protocol was approved by the Institutional Review Board. Included were only patient who had not received any antimicrobial therapy 2 weeks prior to surgery. Patients undergoing revision arthroplasty for dislocation, bone or prosthetic component fracture, or technical issues were excluded.

Definition of prosthetic joint infection. For the purpose of this study, PJI was defined by culture-independent criteria: macroscopic visible purulence surrounding the prosthesis at the time of surgery and/or acute inflammation in periprosthetic tissue on histopathology examination.

Specimen collection and transport. Synovial fluid was aspirated preoperatively. Arthrocentesis was performed to obtain two ml of synovial fluid with a syringe and placed in a sterile container both of which have been irradiated from the side at a distance of 1 cm with long-wave (366 nm) UV at room temperature. Tissue in contact with the implant was collected and submitted to the microbiology laboratory for conventional culturing (1–6 specimens) and to the histopathology laboratory for histopathologic evaluation (1 specimen). For the pur-

pose of the study, the surgeon placed aseptically all of the explanted arthroplasty components in sterile bags. The tibial component and the spacer were placed in one bag, and the femoral component and the patellar component in another bag. The bags containing the components and the container containing the tissue were placed in an anaerobic jar for transport to the microbiology laboratory (Figure 1).

Figure 1. *Anaerobic jar for the transport of the prosthesis and periprosthetic tissue to the microbiology laboratory.*

Sl. 1. *Anaerobna posoda za transport proteze in obprotezne-ga tkiva do mikrobiološkega laboratorija.*

Specimen processing. Synovial fluid and periprosthetic tissue were cultured using standard techniques. Explanted arthroplasty components were double bagged. Then 100 ml Ringer's salt solution (25% [vol/vol]) containing cysteine (0.05% [vol/vol]) as a reducing agent was added and the air inside the bags was removed. The prosthetic components were sonicated for 5 minutes at 40 kHz in an ultrasonic bath (VWR brand, Aquasonic, VWR, USA). Aliquots (0.5 ml) of sonicate were plated onto each of five sheep blood agar (SBA) and five anaerobic sheep blood agar (ASBA) plates. One ml of the remaining volume of homogenized tissue was added to each 5 ml trypticase soy broth (TSB) and 10 ml thioglycollate broth (TGB) enriched with 1 ml deactivated rabbit serum. The SBA plates and TSB were incubated at 37°C aerobically and the ASBA plates and TGB at 37 °C anaerobically. The plates were examined for 7 days, the number of colonies counted and normalized for tissue weight. The TSB and TGB were subcultured on days 7 and 14 onto SBA (for aerobic incubation) and ASBA (for anaerobic incubation), respectively. Broth subcultures were incubated at 37°C for 48 hours. A positive explant sonicate culture was defined as the presence of the same organism on at least of 4 of 5 plates.

Statistical analysis. To evaluate differences between groups, the Mantel-Haenszel chi-square test was used (statistical package SPSS 10.0 for Windows, SPSS, USA). A P-value < 0.05 was considered significant.

Results

Table 4 summarizes clinical, radiological, laboratory and microbiological characteristics of 35 patients undergoing total knee replacement (24 for aseptic biomechanical failure and 11 for suspected PJI). With exception of sinus tract clinical signs were not helpful in differentiating patients with aseptic failure from those with PJI. In synovial fluid, total nucleated cells ($P < 0.001$) and neutrophils ($P < 0.001$) were significantly higher in patients with PJI.

Table 4. *Characteristics of 35 patients undergoing total knee replacement for aseptic failure (24 cases) and prosthetic joint infection (11 cases).*

Razpr. 4. *Značilnosti 35 bolnikov z zamenjano totalno kolensko protezo zaradi aseptičnega vzroka (24 primerov) in okužbe proteznega sklepa (11 primerov).*

	Characteristic Značilnost	Pat. with aseptic implant failure Bolniki z zamenjano protezo zaradi aseptičnega vzroka	Patients with prosthetic joint infection Bolniki z okužbo sklepne proteze	P[1]
	No. of patients (male/female) Štev. bolnikov (moški/ženske)	24 (16/8)	11 (7/4)	NS[2] NZ[3]
	Age (years), mean (range) Starost (leta), povprečje (razpon)	72 (46–88)	73 (46–80)	NS NZ
Preoperative Predoperativno	Local signs (swelling, redness) Lokalni znaki (oteklina, rdečina)	7/24	7/11	NS NZ
	Systemic signs (fever, chills) Sistemski znaki (vročina, mrzlica)	0/24	0/11	NS NZ
	Presence of sinus tract Prisotnost fistule	0/24	3/11	0.02 0,02
	Radiologic signs of loosening Radiološki znaki omajanja	14/24	7/11	NS NZ
Synovial fluid Sinovialna tekočina	Purulence of joint aspirate Gnojen aspirat sklepa	0/24	4/8	NS NZ
	Nucleated cells (/μL), mean (range); no. of patients Jedrne celice (/μl), povprečje (razpon); štev. bolnikov	1,016 (52–5,408); n = 16	47,387 (13,832–95,250); n = 6	< 0.001 < 0,001
	Neutrophils (%), mean (range); No. of patients Nevtrofilci (%), povprečje (razpon); štev. bolnikov	11 (1–42); n = 20	92 (84–100); n = 6	< 0.001 < 0,001
	Synovial fluid culture Kultura sinovialne tekočine	0/22	7/8	< 0.001 < 0,001
Intraoperative Intraoperativno	Periprosthetic purulence Gnoj ob protezi	0/24	9/11	< 0.001 < 0,001
	Acute inflammation in tissue Akutno vnetje v tkivu	0/22	7/11	< 0.001 < 0,001
	Periprosthetic tissue culture Obprotezna kultura tkiva	4/23	8/11	0.002 0,002
	Explant sonicate culture Kultura sonikata proteze	0/24	10/11	< 0.001 < 0,001

[1] P-value, Mantel-Haenszel chi-squares
[2] NS, not significant
[1] P-vrednost, Mantel-Haenszel hi-kvadrat
[3] NZ, ni značilno

Table 5 shows the culture results of synovial fluid, periprosthetic tissue, and prosthesis sonicate in 11 patients with PJI, indicating the number of colony-forming units and identification microorganism. In sonicate cultures higher numbers of microorganisms than in tissue cultures were consistently detected (Figure 2). In sonicate cultures, > 100 colonies were detected on the majority of blood agar plates in 7 of 11 patients with PJI (2 from femoral and 5 from tibial components). Thus, infecting organisms were typically concentrated on only one of the prosthetic components.

In patients with PJI, coagulase-negative staphylococci (7 cases), *Corynebacterium* spp. (2 cases), *Staphylococcus aureus* (1 case), and viridans group streptococcus (1 case) were recovered. The calculated culture sensitivity and specificity

Table 5. *Culture results of synovial fluid, periprosthetic tissue, and prosthesis sonicate in eleven patients with prosthetic joint infection.*

Razpr. 5. *Izvidi kulture sinovialne tekočine, obproteznega tkiva in sonikata proteze pri enajstih bolnikih z okužbo sklepne proteze.*

No. / Št.	Synovial fluid / Sinovialna tekočina	Periprosthetic tissue[1] / Obprotezno tkivo[1]	Prosthesis Sonicate[2] / Sonikat proteze[2] — Femur / Femur: Aerobic culture / Aerobne kulture	Femur / Femur: Anaerobic culture / Anaerobne kulture	Tibia / Tibija: Aerobic culture / Aerobne kulture	Tibia / Tibija: Anaerobic culture / Anaerobne kulture
1	ND[3]	2/4 (*Corynebacterium*[4])	0->100 (*Corynebacterium*)	0–7 (*Corynebacterium*)	0–1 (*Corynebacterium*)	0–1 (*Corynebacterium*)
2	SCN[4]	3/3 (SCN)	53–90 (SCN)	63–89 (SCN)	> 100 (SCN)	> 100 (SCN)
3	NG[5]	1/3 (SCN)	> 100 (SCN)	> 100 (SCN)	0–100 (SCN)	2–8 (SCN)
4	SVG[6]	0/3, NG	5–20 (SVG)	0–3 (SVG)	50–100 (SVG)	> 100 (SVG)
5	SCN	3/4 (SCN)	50–100 (SCN)	50–100 (SCN)	50–100 (SCN)	50 (SCN)
6	SCN	6/6 (SCN)	0–2 (SCN)	0–4 (SCN)	> 100 (SCN)	> 100 (SCN)
7	NG	0/2, NG	21–33 (SCN)	21–25 (SCN)	> 100 (SCN)	> 100 (SCN)
8	ND	3/3 (SCN)	0–2 (SCN)	0 NG	> 100 (SCN)	50->100 (SCN)
9	*S. aureus*	5/5 (*S. aureus*)	> 100 (*S. aureus*)	> 100 (*S. aureus*)	> 100 (*S. aureus*)	50->100 (*S. aureus*)
10	ND	1/3 (*Corynebacterium*)	0 NG	0 NG	0 NG	0–1 SCN
11	SCN	3/3 (SCN)	> 100 (SCN)	> 100 (SCN)	25–50 (SCN)	25–50 (SCN)

[1] Number of tissue specimens with growth/number of total number of tissue specimens collected (microorganism identification).
[2] Number of colony-forming units on blood agar plates (microorganism identification).
[3] ND = not done.
[4] SCN = *Staphylococcus,* coagulase-negative.
[5] NG = now growth.
[6] SVG = *Streptococcus,* viridans group.

[1] Število tkivnih vzorcev z rastjo/število vseh odvzetih tkivnih vzorcev (identifikacija mikroorganizma).
[2] Število enot, ki tvorijo kolonije na krvnih agarskih gojiščih (identifikacija mikroorganizma).
[3] ND = ni narejeno.
[4] SCN = *Staphylococcus*, koagulaza negativni.
[5] NG = ni rasti.
[6] SVG = *Streptococcus* iz skupine viridans.

from these data are summarized in Table 6. Culture sensitivity and specificity were for synovial fluid 88% and 100%, for periprosthetic tissue 83% and 81%, and for explant sonicate 91% and 100%, respectively. Explant sonicate cultures were more sensitive with respect to the diagnosis of PJI compared with synovial fluid or periprosthetic tissue cultures.

Discussion

Biofilm properties depend on attachment and aggregation of bacterial cells into microcolonies. Anti-biofilm diagnostic strategies are directed at disruption of adherent bacteria and their multicellular structure. By dispersing adherent bacteria from the surface the recovery efficiency and therefore the sensitivity of diagnostic assays may be increased. Free-floating planktonic organisms as opposed to their sessile surface-associated counterparts are likely preferentially cultured using conventional methods (e. g., synovial fluid and periprosthetic tissue culture). A variety of potential strategies to dislodge sessile bacteria in biofilms from foreign body surfaces, which may be of diagnostic relevance, have been tested including mechanical (e. g., ultrasonication, vortexing, shock wave), biochemical (e. g., enzymes), and electrical approaches (17). To date, only ultrasonication has been studied for improvement of recovery of adherent bacteria from explanted orthopedic devices, (35–37) and from vascular prosthetic grafts, ureteric stents or vascular and peritoneal catheters (38–41).

Ultrasound, transmitted at frequencies generally beyond the range of human hearing (> 20 kHz), has been used in many applications in medicine and research. Whereas in diagnostic and therapeutic applications high frequencies (MHz-range) are used, low frequencies (kHz-range) are employed for bacterial detachment (e. g., cleaning of medi-

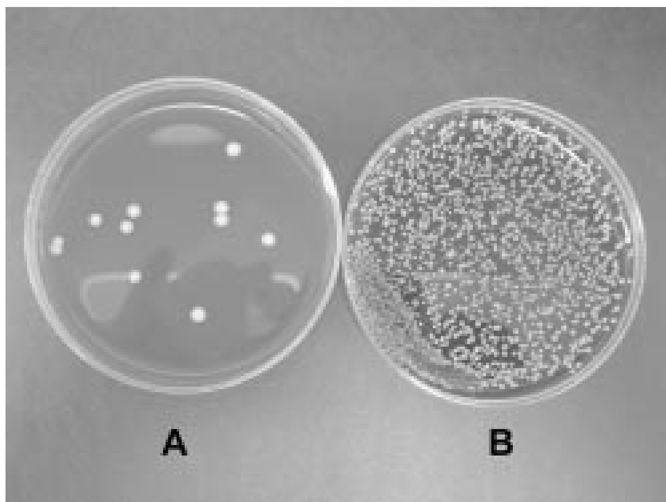

Figure 2. *In all sonicate cultures (B) higher numbers of microorganisms than in periprosthetic tissue cultures (A) were detected.*

Sl. 2. *V vseh kulturaah sonikata (B) smo zaznali večje število mikroorganizmov kot v kulturah obproteznega tkiva (A).*

Table 6. *Culture sensitivity and specificity (95% confidence interval) for synovial fluid, periprosthetic tissue, and prosthesis sonicate. Data demonstrate highest sensitivity and specificity of explant sonicate culture. Differences are not statistically significant.*

Razpr. 6. *Občutljivost in specifičnost kulture sinovialne tekočine, obproteznega tkiva in sonikata proteze. Rezultati kažejo največjo občutljivost in specifičnost za kulturo sonikata proteze. Razlike niso statistično značilne.*

Culture / Kultura	Sensitivity (95% CI) / Občutljivost (95% IZ[2])	Specificity (95% CI[1]) / Specifičnost (95% IZ)
Synovial fluid / Sinovialna tekočina	7/8 = 88% (49–93%)	22/22 = 100% (87–100%)
Periprosthetic tissue / Obprotezno tkivo	8/11 = 83% (35–95%)	11/23 = 81% (57–96%)
Prosthesis sonicate / Sonikat proteze	10/11 = 91% (63–94%)	24/24 = 100% (89–100%)

[1] CI = confidence interval
[2] IZ = interval zaupanja

cal instruments) (42). The ultrasound waves radiate through a solution and produce high and low pressure areas. During the low pressure stage, millions of microscopic vapor bubbles are formed (a process referred to as cavitation), which collapse during the high pressure stage releasing an enormous amount of energy on the surface of the objects (a process referred to as implosion). This agitation causes a vacuum-scrubbing action by releasing acoustic energy at the surface of objects.

Our preliminary study results support the hypothesis ultrasonication of the explanted prosthesis may improve diagnosis of PJI by dislodging biofilm-associated bacteria from the prosthesis surface. Using synovial fluid culture, 12% were missed, and using periprosthetic tissue cultures, 17% of PJI were missed. In this small sample size study, sonicate cultures agreed best with the diagnosis of PJI (91%). In sonicate cultures, > 100 colonies were detected on the majority of blood agar plates in 7 of 11 patients with PJI (2 from femoral and 5 from tibial components). The fact that infecting organisms were detected in high numbers typically concentrated on only one of the prosthetic components support the importance of biofilms in, and the focal nature of, PJI.

Mild ultrasonication has already been employed for dislodgement of adherent bacteria from surfaces of orthopedic devices for diagnostic purposes. Dobbins et al. performed ultrasonication for 10 minutes (and vortexing for 30 seconds) on internal fixation devices removed for reasons other than infection (35). 20 of 26 (77%) sonicate cultures were positive; in most cases (15 sonicates), coagulase-negative staphylococci were cultured. This was in contrast to swabs of adjacent tissues, where coagulase-negative staphylococci were isolated in only 3 of 26 (12%) cultures. Unfortunately, no negative controls were examined and the positive cultures could represent contamination.

Tunney et al. performed mild ultrasonication of explanted hip prostheses followed by sonicate culture (36), as well as by immunofluorescence microscopy and broad-range PCR amplification of the explant sonicate (32). In the first study (36), mild ultrasonication was performed on 120 removed hip prostheses. Sonicates were plated onto five aerobic and five strict anaerobic blood agar plates. Bacteria were cultured from 26 (22%) of 120 sonicate cultures, although the criteria used for definition of a positive culture result were not clear and vary between different publications by the same investigators describing the same patients (32, 36). *Propionibacterium acnes* was isolated either alone or in association with *Staphylococcus* spp. from 16 (62%) of 26 patients with positive sonicate cultures. Review of the records for 18 of the 26 patients with culture-positive implants revealed that infection was suspected as the cause of loosening in only six (33%) of 18 patients and, in all cases, histopathologic examination of the periprosthetic tissue showed inflammatory cells. Standard cultures of preoperatively aspirated synovial fluid or intraoperatively sampled periprosthetic tissue were positive in only two patients. The limitations of this study include the failure to document the diagnosis of PJI using well-formulated gold standard criteria and the incomplete clinical and histopathologic data.

Nguyen et al. used ultrasonication on 21 femoral components of hip and knee prostheses removed from patients without clinical evidence of infection (37). Swab cultures of the prostheses grew no organisms. The explant sonicate was passed through a 0.45-μm pore filter, and the filter residue was aerobically cultured. In one of 21 (5%) explant sonicates a coagulase-negative *Staphylococcus* sp. was cultured but the same organism was also found in one of 21 sonicate cultures from sterilized prostheses used in this study as a negative controls. Thus, sonicate cultures from aseptically failed prostheses did not yield more organisms than those from negative controls.

Other investigators have demonstrated that low-frequency continuous and pulsed ultrasound (28 to 67 kHz) for 6 to 24 hours enhances killing by antimicrobial agents of biofilm microorganisms *(P. aeruginosa* and *E. coli)* in vitro and in animal models (43–47). The mechanism by which ultrasound enhances antimicrobial action (the bioacoustic effect) is unclear as ultrasound with a power density of 100 to 300 W/cm^2 used in these studies has no inhibitory or bactericidal activity against bacteria when delivered alone. Many hypotheses exist to explain the mechanism of the bioacoustic effect, including increased transport through the membrane, a mechanical effect on the biofilm-associated bacteria, or promotion of specific gene expression (48). Whether or not the bioacoustic effect could be applied to the therapy of PJI remains to be determined.

Other potential anti-biofilm approaches. Similar to ultrasound, an acoustical **shock wave** is a transient pressure disturbance that propagates rapidly in three-dimensional space. In contrast to ultrasound, a shock wave is a sonic pulse with a high peak pressure (50–100 MPa) and a broad frequency spectrum (16 Hz to 20 MHz). Shock waves produce high stresses that act on boundary interfaces between two media (e. g., tissue and prosthesis) generating cavitation bubbles. During collapse of the bubbles a high amount of acoustic energy is released in the form of water jets and high temperature (49). Originally applied clinically as lithotripsy to pulverize calcific deposits within the body (e. g., renal stones), shock waves have gained increasing usage in musculoskeletal disorders (orthotripsy), especially in treating enthesiopathies and delayed fracture healing (50–52). The primary advantage of shock wave therapy is its noninvasive nature. Mechanical bacterial removal with **vortexing** has been used in the diagnosis of infections of vascular catheters and vascular grafts, mostly as an adjunct to ultrasonication (38, 39, 53). Vortex agitation with glass beads is an efficient method to mechanically disrupt and remove biofilm-associated bacteria (54). However, in diagnosis of PJI, vortex agitation with beads may represent a risk for prosthesis contamination. Low **electric current** combined with an antimicrobial agent has been shown to enhance the killing of biofilm-associated bacteria as compared to the antimicrobial agent alone (the bioelectric effect) (55–58). A mixture of **enzymes** has been shown to be bactericidal and effective in eradicating biofilms of several different organisms by dissolving the matrix polymers of the biofilm. Other **chemical substances** showed blockage of biofilm matrix synthesis. For example, Yasuda et al. demonstrated that clarithromycin enhanced the therapeutic efficacies of other antimicrobial agents against infections caused by clarithromycin-resistant strains of *P. aeruginosa* and *S. epidermidis* (59, 60). Lactoferrin, a ubiquitous constituent of human secretions, was shown to block biofilm development by *P. aeruginosa*. By chelating iron, lactoferrin stimulates twitching, a specialized form of surface motility, causing the bacteria to wander across the surface instead of forming cell clusters and biofilms (61). As both the formation and detachment of biofilms has been found to be controlled by **chemical signals** (diffusible homoserine lactones), a specific anti-biofilm defense mechanism might act on disruption of quorum-sensing signaling molecules, involved in biofilm architecture (16, 62). At sufficient population density, these signals reach concentrations required for activation of genes involved in biofilm differentiation. During biofilm formation different **genes** may be activated or repressed. Up- and down-regulation of genes in bacteria provides an additional possible antibiofilm strategy through inhibition of gene transcription (63–66). These studies reveal potential anti-biofilm defense mechanisms acting at a critical juncture in biofilm development but to date, none of these methods have been used in the setting of PJI.

Conclusions

Our preliminary study supports the hypothesis that ultrasonication of the explanted prosthesis may improve diagnosis of PJI by dislodging biofilm-associated bacteria from the prosthesis surface. Using joint aspirate, periprosthetic tissue, and explant sonicate cultures, 12%, 17% and 9% of PJI were missed, respectively. Explant sonicate cultures were the most sensitive with respect to the diagnosis of PJI, indicating that explant ultrasonication may improve bacterial recovery. In contrast, clinical signs (with exception of the presence of sinus tract) were not helpful in differentiating patients with aseptic failure from those with PJI. In synovial fluid, total nucleated cells ($p < 0.001$) and neutrophils ($p < 0.001$) were significantly higher in patients with PJI. In sonicate cultures, infecting organisms were detected in high numbers, typically concentrated on only one of the prosthetic components. These findings support the importance of biofilms in, and the focal nature of, PJI. Further studies are needed to determine the optimal specimen processing and interpretation of results. In addition, other anti-biofilm approaches may be useful in the future, including shock-waves, vortex agitation, electric current, gene therapy, enzymes and other chemical substances.

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