Journal of Molecular Diagnostics, Vol. 10, No. 6, November 2008

DOI: 10.2353/jmoldx.2008.070137

Amplification-Based DNA Analysis in the Diagnosis of Prosthetic Joint Infection

Bernard Vandercam,* Sabine Jeumont,† Olivier Cornu,‡ Jean-Cyr Yombi,* Frédéric Lecouvet,§ Philippe Lefèvre,‖ Léonid M. Irenge,‖ and Jean-Luc Gala†¶

From the Division of Infectious Diseases, the Center for Applied Molecular Technologies,† Department of Orthopedic Surgery,‡ and the Department of Radiology,§ St-Luc's University Hospital, Université catholique de Louvain, Brussels; the Defence Laboratories Department,¶ Belgian Armed Forces, Brussels; and the Department of Mycobacterial Enzymology,‖ Institut Pasteur, Brussels, Belgium*

Microbiological cultures are moderately sensitive for diagnosing prosthetic joint infection (PJI). This study was conducted to determine whether amplification-based DNA methods applied on intraoperative samples could enhance PJI diagnosis compared with culture alone in routine surgical practice. Revision arthroplasty was performed for suspected PJI ($n = 41$) and osteoarthrosis control ($n = 28$) patients, and a diagnosis of PJI was confirmed in 34 patients. Amplification by polymerase chain reaction was performed on both 16S ribosomal DNA universal target genes and *femA Staphylococcus*-specific target genes. Species identification was achieved through amplicon sequencing. Amplification of the *femA* gene led to subsequent testing for methicillin resistance by amplification of the *mecA* gene. Microbiological and molecular assays identified a causative organism in 22 of 34 patients (64.7%) and in 31 of 34 patients (91.2%), respectively. In 18 of the 22 culture-positive patients, molecular and microbiological results were concordant for bacterial genus, species, and/or methicillin resistance. Bacterial agents were identified only by molecular methods in nine PJI patients, including seven who were receiving antibiotics at the time of surgery and one with recent but not concomitant antibiotherapy. DNA-based methods were found to effectively complement microbiological methods, without interfering with existing procedures for sample collection, for the identification of causative pathogens from intraoperative PJI samples, especially in patients with recent or concomitant antibiotherapy. ***(J Mol Diagn 2008, 10:537–543; DOI: 10.2353/jmoldx.2008.070137)***

Orthopedic implants have become an essential component of modern medicine. Since the number of trauma cases is steadily rising and the percentage of patients aged >65 years is on the increase in industrialized countries, the number of patients requiring implants will continue to grow, as will the risk for orthopedic device-related infections. The most common pathogens involved in prosthetic joint infections (PJIs) are coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus*. Other microorganisms, such as streptococci, Gram-negative bacilli, enterococci, and anaerobes, are less frequently isolated.[1,2]

Given the wide spectrum of potential pathogens, definitive identification of the microbial agent is mandatory to optimize the surgical strategy and initiate appropriate long-term antibiotherapy.[3,4] The gold standard for the diagnosis of infection is still culture and isolation of the causative pathogen. However, standard microbiological cultures are only moderately sensitive and specific for PJI diagnosis. The most frequently encountered limitations of conventional microbiological methods are previous antimicrobial treatment, prophylaxis before sampling, and methodology problems.[5]

Nonculture molecular approaches based on amplification and/or sequence analysis of conserved or species-specific genomic targets have shown high sensitivity in identifying non-growing or slow-growing bacterial agents responsible for bone or prosthetic infections.[6–9] However, the true impact of molecular methods on PJI diagnosis in routine clinical practice has yet to be confirmed. In a previous study, we addressed this issue by studying infectious discitis.[9] In the current study, we set out to assess the ability of these molecular methods to identify the causative pathogen in PJI compared to conventional microbiological testing, without interfering with existing procedures for sample collection.

Supported by the Department of Management of Scientific and Technological Research of Defence, Royal High Institute for Defence supporting research and development grants (MED-03 and MED-08) and by the Region Wallonne, Belgique (Nanotic/DEDICCAS 51/6250).

B.V. and S.J. contributed equally to this work.

Accepted for publication July 21, 2008.

Address reprint requests to Jean-Luc Gala, Center for Applied Molecular Technologies, 30.46, Clos Chapelle-aux-Champs, UCL/30.46, B-1200 Brussels, Belgium. E-mail: jean-luc.gala@uclouvain.be.

Table 1. Patients Presenting with a Presumed Diagnosis of PJI or Revision Arthroplasty for Osteoarthrosis: Clinical, Microbiological and Molecular Data

Patient	Age/sex	Prosthetic site	Prior joint infection	Culture results (methicillin resistance status)	*16S rDNA*, G+/G-	*femA*	*mecA*	DNA-based identification (methicillin resistance status)	Antibiotherapy before surgery (duration or time elapsed since discontinuation of antibiotics)	Comments
Patients matching a diagnosis of PJI										
Discordant positive results: positive culture and molecular results but discordant identification										
1	67/F	Knee	Yes	*S. faecalis* and *S. pyogenes*	G+	0	ND	*S. pyogenes*	No	Rare growing colonies; probable *S. faecalis* contamination during the subculture process; *S. pyogenes* subsequently identified by culture after molecular results were known
2	61/F	Hip	Yes	*Bacillus* sp	G+	1	0	*Staphylococcus* sp. (S)	Ongoing (4 W)	Samples for culture and DNA analysis not collected from the same sites
3	55/M	Hip	Yes	*P. aeruginosa*	G-	ND	ND	*Serratia* sp. and *P. aeruginosa*	Discontinued (48 W)	Past history of PJI due to *P. aeruginosa*
4	62/M	Hip	Yes	CoNS (S)	G-	ND	ND	*Clostridium* sp.	Ongoing (5 D)	Past history of PJI communicating within an endopelvic abscess infected by polymicrobial flora
Discordant results: negative culture results and positive molecular results										
5	41/F	Hip	No	NI	G+	1	0	*S. aureus* (S)	Ongoing (4 W)	Purulence at the time of surgery
6	50/M	Hip	Yes	NI	G+	1	0	*S. epidermidis* (S)	Ongoing (5 W)	CoNS cultured in preoperative joint aspirate
7	71/M	Hip	Yes	NI	G+	1	0	*S. epidermidis* (S)	Discontinued (10 D)	PJI with positive culture (CoNS) diagnosed 6 months before
8	62/F	Knee	No	NI	G+	0	ND	*S. anginosus*	Ongoing (3 W)	Preoperative joint aspirate positive for *S. anginosus*
9	60/F	Knee	No	NI	G+	1	0	*S. hominis* (S)	No	Included at third episode of aseptic loosening
10	53/M	Hip	Yes	NI	G+	1	1	*S. epidermidis* (R)	Ongoing (2 D)	Methicillin resistant CoNS cultured 3 and 6 months later
11	46/F	Hip	No	NI	G+	0	ND	*Serratia* sp	Ongoing (10 D)	Purulence at the time of debridement
12	77/F	Hip	No	NI	G+	1	0	*S. hominis* (S)	Ongoing (10 D)	Purulence at the time of surgery
13	62/M	Hip (right)	No	NI	G+	0	ND	*S. bovis*	Ongoing (20 D)	Aortic endocarditis caused by *S. bovis*, complicated by left and right PJI; preoperative joint aspirate positive for *S. bovis*
Concordant positive results										
14	56/F	Hip	No	*S. agalactiae*	G+	0	ND	*S. agalactiae*	Discontinued (15 D)	
15	79/F	Hip	Yes	*S. aureus* (R)	G+	1	1	*S. aureus* (R)	Discontinued (52 W)	
16	72/F	Hip	No	*S. aureus* (R)	G+	1	1	*S. aureus* (R)	No	
17	70/M	Hip	Yes	*S. aureus* (S)	G+	1	1	*S. aureus* (S)	Discontinued (7 D)	
18	72/M	Hip	No	CoNS (R)	G+	1	1	*S. epidermidis* (R)	No	
19	57/M	Hip	No	*S. aureus* (S)	G+	1	0	*S. aureus* (S)	No	
20	77/F	Hip	No	*S. aureus* (R)	G+	1	1	*S. aureus* (R)	Discontinued (7 W)	Previous antibiotherapy for soft tissue MRSA infection
21	80/M	Hip	No	*L. monocytogenes*	G+	0	0	*L. monocytogenes*	Ongoing (2 W)	Ongoing antibiotherapy justified by earlier positive blood cultures for *L. monocytogenes*
22	62/M	Hip (left)	No	*S. bovis*	G+	0	ND	*S. bovis*	No	Aortic endocarditis caused by *S. bovis* complicated by left and right PJI
23	58/F	Hip	No	*S. bovis*	G+	0	ND	*S. bovis*	Ongoing (7 D)	
24	42/F	Knee	Yes	*S. anginosus*	G+	0	ND	*S. anginosus*	Discontinued (3 W)	
25	66/M	Hip	No	*S. haemolyticus* group G	G+	0	ND	*S. dysgalactiae*	No	
26	48/M	Knee	Yes	*P. aeruginosa*	G-	ND	ND	*P. aeruginosa*	No	
27	72/M	Hip	Yes	CoNS (R)	G+	1	1	*S. epidermidis* (R)	No	
28	73/M	Hip	No	*S. sanguis*	G+	0	ND	*S. anginosus*	No	
29	59/F	Hip	Yes	*S. aureus* (S)	G+	1		*S. aureus*	Discontinued (10 D)	

(table continues)

Table 1. *Continued*

Patient	Age/sex	Prosthetic site	Prior joint infection	Culture results (methicillin resistance status)	*16S rDNA*, G+/G-	*femA*	*mecA*	DNA-based identification (methicillin resistance status)	Antibiotherapy before surgery (duration or time elapsed since discontinuation of antibiotics)	Comments
30	63/M	Hip (right)	No	*S. bovis*	G+	0	ND	*S. bovis*	No	Relapse after arthrotomy debridement
31	63/M	Hip (left)	No	*S. bovis*	G+	0	ND	*S. bovis*	No	Relapse after arthrotomy debridement
Concordant negative results										
32	53/F	Knee	Yes	NI	0	ND	ND	NI	NA	Culture positive for *S. agalactiae* 6 weeks later
33	43/M	Knee	No	NI	0	ND	ND	NI	Ongoing (10 W)	*S. faecalis* cultured 6 weeks after antibiotic discontinuation
34	82/M	Hip	No	NI	0	ND	ND	NI	Ongoing (10 W)	Aortic endocarditis caused by *S. bovis*
Patients with unconfirmed diagnosis of PJI										
1¢	60/F	Hip	No				ND		No	Follow-up: >2 years
2¢	68/F	Knee	No				ND		No	Follow-up: >2 years
3¢	67/F	Knee	No		G+	1	1	*S. hominis* (R)	No	Follow-up: >2 years; allergic reaction to wire
4¢	49/M	Knee	No				ND		No	Follow-up: >2 years
5¢	63/F	Knee	No				ND		No	Follow-up: 9 months
6¢	78/M	Hip	No				ND		No	Follow-up: >2 years
7¢	74/F	Knee	No				ND		No	Revision arthroplasty 15 months later; all cultures negative except one for *Corynebacterium* sp
Controls (revision arthroplasty for osteoarthrosis) $n = 28$										

Controls included patients who presented for hip ($n = 16$) or knee ($n = 12$) revision arthroplasty for osteoarthrosis. Sex ratio (M/F), 11/17; mean age, 63.5. NI, no identification of the causative bacteria; 0, no amplification of the target gene; 1, amplification of the target gene; (S), methicillin susceptible; (R), methicillin resistant; ND, not done; D, days; W, weeks.

Materials and Methods

Patient Characteristics

This prospective study included 41 patients (21 men and 20 women) with a median of age 63 years (range, 41–82 years), who underwent revision arthroplasty by the same surgeon for suspected PJI (hip, 29; knee, 12) (Table 1). It should be pointed out that one patient underwent surgery on both hips over a 1-month period and was readmitted 1 year later. For the purposes of the study, he was assessed as four distinct cases (patients 13, 22, 30, and 31). Patient enrollment was based on presumed PJI, including patients with or without previous infections, and with or without ongoing or recent antibiotherapy. All tissue samples were collected concurrently for molecular and microbiological assays. Inclusion criteria for presumed diagnosis of PJI were as follows: local or systemic clinical symptoms suggestive of PJI; local and/or referred persistent pain at the site of the device with or without implant loosening; and exclusion of any other unrelated confounding etiological factors.

PJI was diagnosed in the presence of one of the following conditions: 1) a sinus tract communicating with the prosthesis, confirmed either by arthrography or direct examination during surgery; 2) evidence of purulence surrounding the prosthesis at the time of debridement and subsequent identification of a microorganism after culture, although this criterion was not mandatory; 3) two or more positive cultures on intraoperative specimens with at least one of the following criteria: inflammation on histopathological examination of periprosthetic tissue, clinical and laboratory signs of infection, and radiological or nuclear signs of infection; 4) at least one of the following criteria: inflammation on histopathological examination of periprosthetic tissue, clinical and laboratory signs of infection, and radiological or nuclear signs of infection but negative culture. The latter was considered retrospectively as PJI on the basis of pre- and/or postoperative microbiological results. Regarding CoNS, three positive specimens were required to be considered as a positive result. All cases with insufficient clinical follow-up or biological data ($n = 5$) were excluded from this study.

Sample Collection

Routine intraoperative culture was systematically performed on biopsies, swabs, or aspirates. Samples were obtained from tissues in close proximity to the surface of the prosthesis, as well as tissues presenting with signs of inflammation. A swab of joint fluid was also taken on entering the capsule. Specimens ($n = 3–6$) were sampled from different sites and were not placed into specific anaerobic containers. To avoid interfering with routine surgical practice for sample collection, two aliquots were dispatched to the molecular laboratory for pathogen genotyping using a molecular-based method, while all other specimens were sent to the microbiology unit. The personnel of the two units were blinded to each other's results. A single investigator assessed all of the cases by recording demographic data, orthopedic case history and follow-up, preoperative joint aspiration results, type of infection, signs and symptoms, laboratory parameters

of inflammation (white blood cell count, neutrophil percentage, C-reactive protein), microbiology, histology, imaging procedures, intraoperative surgical findings, and antimicrobial therapy.

Blind Control Analysis

Patients ($n = 28$) undergoing primary hip or knee arthroplasty for osteoarthrosis in the absence of any laboratory, histopathological, microbiological, or intraoperative signs of infection were considered as negative controls.

Microbiological Analysis

Synovial fluid was streaked onto sheep blood agar plates (BD BBL Columbia agar with 5% sheep blood) and chocolate agar plates (BD BBL chocolate II agar). Agar plates were incubated in aerobic (5% to 7% CO_2) and anaerobic conditions at 35°C to 37°C. Residual liquid aspirates were inoculated into Bactec bottles (Bactec Plus aerobic/F medium and Bactec Plus anaerobic/F medium, Becton Dickinson, Franklin Lakes, NJ). They were incubated at 35°C and followed up for 1 week to detect bacterial activity by a rise in CO_2 levels using a Bactec 9240 machine (Becton Dickinson). Subcultures were performed from positive bottles on Columbia agar sheep blood (Becton Dickinson) and on thioglycolate medium broth (Difco, Detroit, MI) at 35°C for 2 days, with a first examination after 24 hours. Additional blind subcultures were performed after 7 days. Organisms and antibiotic sensitivities were identified by standard methods, as previously described.[10] The same procedure was performed for tissue specimens, except that they were first homogenized in 3 ml of BHI broth (BD BBL BHI broth) for 1 minute.

Molecular Identification of Bacteria

Preanalytical processing of biological samples, the DNA extraction procedure for clinical samples, duplex polymerase chain reaction (PCR), control of PCR contamination and inhibition, and molecular species identification have been extensively detailed elsewhere by our group.[9] Briefly, duplex-specific amplification of the 16S ribosomal DNA gene was performed to identify signals corresponding to the presence of Gram-positive, Gram-negative, or mixed associated Gram-positive and Gram-negative bacteria. It was based on the use of a consensus forward primer common to Gram-positive and Gram-negative bacteria and reverse primers specific for Gram-positive and Gram-negative bacteria (see Trampuz et al[14] for the description of primers and PCR conditions). Discrimination between Gram-positive bacteria and Gram-negative bacteria was performed by running PCR products on a 2% agarose gel stained with ethidium bromide. According to bacterial species, expected size of the amplicons was variable and ranged from 206 to 217 bp for Gram-positive bacteria, and from 405 to 412 bp for Gram-negative bacteria. The corresponding amplicons were sequenced in both directions on an automated ABI 377 A apparatus (Applied Biosystems, Nieuwekerk, The Netherlands), using the Taq Dye Deoxy Terminator cycle sequencing kit from the same manufacturer. Weak bands were sequenced after extraction from the agarose gel and cloning using the TOPO XL PCR cloning kit (Invitrogen, Carlsbad, CA), according to the manufacturer's protocol. Sequences were compared with public databases for similarity-based species identification using the BLAST program from the National Center for Biotechnology Information database, according to scores proposed by Bosshard et al.[11] A Gram-positive signal led to a *femA* PCR amplification for *Staphylococcus* species and methicillin resistance detection (*mecA*), as previously described.[9]

Special attention was paid to the quality control process. Each clinical specimen was processed concomitantly with an extraction control (DNA-free Tris-HCl, 10 mmol/L buffer, pH 8). To prevent contamination, PCR was performed under stringent conditions. All DNA manipulations pre- and post-PCR were performed in separate designated rooms with separate pipetting devices to avoid contamination of the samples with foreign DNA. Furthermore, all equipment used in the preamplification steps was DNA-free and further irradiated by ultraviolet light to avoid false-positive results from contaminating DNA. Master mixture water controls and DNA extraction controls were used for every batch of samples processed.

In each case without DNA amplification, PCR inhibition was investigated. Briefly, 10^4 copies of a TOPO-XL plasmid carrying the 16S ribosomal DNA of *S. aureus* was mixed with the sample and added to the PCR mixture; amplification was performed as described above. PCR inhibition was considered relevant when no amplification or markedly decreased amplification of 10^4 plasmid copies of *S. aureus* was observed in the mixture.

Results

Microbiological Findings

In the cohort of patients with a presumed diagnosis of PJI, seven patients who did not fulfill the PJI criteria were excluded. Altogether, there were 34 cases with confirmed PJI, among which 22 (64.7%) had a positive culture result (Table 1). No microorganisms were detected by culture in the 12 remaining cases (35.3%). Only single pathogens were isolated by culture: CoNS in three cases (identification at the species level is not routinely performed), *S. aureus* in six, *Bacillus* species in one, *Streptococcus agalactiae* in one, *S. bovis* in four, *S. anginosus* in one, *S. sanguis* in one, *S. haemolyticus* in one, *S. faecalis* in one, *Pseudomonas aeruginosa* in two, and *Listeria monocytogenes* in one). Methicillin resistance was detected in five staphylococci, among which three were *S. aureus* and two were CoNS. No bacterial growth was observed on samples from the 28 control patients.

Molecular Analysis

Altogether, the molecular markers allowed bacterial agent identification in 31 (91.2%) of the 34 cases with PJI

Table 2. Comparison between DNA-Based and Culture Results: Details on Concordant and Discordant Results

Culture and DNA-based methods: result comparison	Global rate (percentage)	Details			
		Microbiology	Molecular assay	Rate per category (percentage)	Patient number
Concordance	21/34 (61.8)				
		+	+	18/34 (52.9)	14–31
		−	−	3/34 (8.8)	32–34
Discrepancy	13/34 (38.2)				
		+	+	4/34 (11.8)	1–4
		−	+	9/34 (26.5)	5–13

Clinical, microbiological, and molecular results for each patient are detailed in Table 1.

(Table 1). No significant inhibition was observed. A single pathogen was identified at the species level in 30 cases, including 15 staphylococci (among which seven were *S. aureus*), 11 streptococci, one *Clostridium* species, one *L. monocytogenes*, one *P. aeruginosa* and one *Serratia* species. In patient 3, the original sequences were suggestive of a mixed population of bacteria. Accordingly, cloning was necessary to resolve mixed sequences (TOPO XL kit) (*Serratia* sp. and *P. aeruginosa*). The extraction controls remained negative in all of the samples from presumed PJI. None of the 28 control patient samples were PCR positive, but one of the samples from the seven patients with ruled out infection was positive by PCR for methicillin-resistant *S. hominis*.

Bacterial DNA was amplified using *16S rDNA*, *femA,* and *mecA* in 31, 15, and six of the 34 patient samples, respectively. Among the 31 *16S rDNA*-positive samples, 28 were Gram-positive/*16S rDNA* and three were Gram-negative/*16S rDNA*. Fifteen of the Gram-positive/*16S rDNA* amplicons matched staphylococcal sequences and all of the corresponding samples appeared to be *femA*-positive. Sequencing the 15 *16S rDNA* and *femA* amplicons allowed to identify 14 staphylococcal species, among which seven were *S. aureus,* five were *S. epidermidis*, and two were *S. hominis.* Sequence analysis of the 13 non-staphylococcal Gram-positive/*16S rDNA* identified *S. bovis* (five), *S. anginosus* (two), *S. agalactiae* (one), *S. haemolyticus* (one), *S. sanguis* (one), *S. pyogenes* (one), *Clostridium* sp. (one), and *L. monocytogenes* (one).

Comparison of Bacteriological and Molecular Results

In case of PJI, concordance was observed between bacteriological and molecular analyses in 18 patients with positive results and three patients with negative results (Table 2). Discrepant results between the two methods were obtained in 13 patients (patients 1 to 13). In four patients (patients 1 to 4), both analyses identified a pathogen, but the presumed causative organism was different. In patient 1, *S. faecalis* was identified from a blind subcultures of the Bactec bottle, while PCR was positive for *S. pyogenes* alone. On the basis of this result, new subcultures were performed on rare growing colonies and *S. pyogenes* was ultimately identified. Accordingly, this *S. faecalis* was felt to be a probable contaminant. In patient 2, *Bacillus* sp. was identified by culture, but molecular testing revealed *Staphylococcus*. Identification at the species level could not be completed due to the poor quality of the sequence. In patient 3, only *P. aeruginosa* was cultured, while DNA analysis identified *P. aeruginosa* and *Serratia* sp. Patient 4 presented with a PJI relapse 9 months after a polymicrobial (fecal flora) infection of the hip. At relapse, culture identified *S. epidermidis* in three samples from this patient, but *Clostridium* sp. was found by molecular testing in a further two samples.

Bacteria were identified by DNA analyses in nine samples (patients 5 to 13) in which conventional microbiological testing consistently produced negative results. In patients 5, 11, and 12, purulence was found at the time of surgery. In patients 6 and 8, the molecular diagnosis was confirmed by microbiological results on preoperative joint aspirate (CoNS in patient 6 and *S. anginosus* in patient 8). Patient 7, with genotypic identification of *S. epidermidis,* had already been treated 6 months earlier for microbiologically proven (CoNS) PJI. In patient 9, with genotypic identification of *S. hominis*, reimplantation was justified because of a third episode of hip arthroplasty loosening in the presence of increased leukocytosis and an elevated C-reactive protein level, consistent with septic loosening. In patient 10, with genotypic identification of *S. epidermidis*, follow-up data confirmed the molecular results, and this patient was readmitted 3 months later for microbiologically proven (CoNS) PJI. In patient 13, the positive PCR result was confirmed by prior concordant positive culture in joint aspirate obtained 20 days before initiating antibiotherapy (aortic endocarditis caused by *S. bovis*, complicated by left and right PJI). All but one patient had recently received or were still receiving antibiotics at the time of surgery (median duration, 3 weeks; range, 2 days to 5 weeks).

Concordant false-negative results were obtained in three patients. In patient 32, the diagnosis of PJI was supported by the presence of a fistula, an elevated C-reactive protein level, and a positive leukocyte scan. Six weeks after surgery, conventional cultures became positive for *S. agalactiae.* The other two patients (patients 33 and 34) were receiving ongoing antibiotherapy for more than 10 weeks at the time of surgery. In patient 33, the diagnosis of PJI at enrolment inclusion was borne out *a posteriori* by the growth of *S. faecalis* 6 weeks after antibiotic discontinuation. In patient 34, the diagnosis was

supported by the clinical occurrence of PJI during the treatment of *S. bovis* endocarditis.

Discussion

In PJI, appropriate antimicrobial therapy and an optimal surgical approach depend on identification of the specific etiological microorganism(s) involved and adequate antimicrobial susceptibility testing.[3–6] Culture of periprosthetic tissue provides the most reliable means of detecting the pathogen and is considered the technique of choice for diagnosing PJI. However, the sensitivity of culture ranges from 65% to 94%.[12–14] Numerous factors can lead to false-negative results: prior antimicrobial treatment, perioperative prophylaxis administered before tissue specimen collection, a small inoculum of bacteria in the stationary growth phase, the presence of fastidious organisms such as nutritionally variant streptococci or small-colony variants of staphylococci or *Escherichia coli*,[15,16] as well as technical problems like prolonged transport time to the microbiology laboratory, inappropriate culture medium or difficulty dislodging bacteria growing in biofilms on the prosthesis surface.

Nonculture molecular methods based on amplification and/or sequence analysis of conserved or species-specific genomic targets are expected to overcome some of the above-mentioned limitations. Several studies have addressed the usefulness of PCR in the diagnosis of PJI. Most were based on bacterial 16S rDNA as the genetic target for detection of medically significant bacteria in culture-negative or polymicrobial samples.[7,9,17,18] The major shortcomings of these studies were the paucity of clinical data and the lack of follow-up. Nonetheless, Fenollar et al recently showed that this strategy may detect previously unknown pathogens and potentially novel bacterial species.[18] However, the overriding concern is the risk of generating false-positive results when using broad-range PCR assays, as in the current study. Hoeffel et al[19] attempted to reduce the false-positive amplification of broad-range PCR by developing genus-specific PCR primers, which target a subgroup of Gram-positive cocci and exclude *E. coli*, a common contaminant of test reagents.[19] To address the issue of false-positive molecular results, special attention was paid to sample collection, processing, and elimination of exogenous bacterial DNA, which could be present in the polymerase, as previously reported.[9] It is worth noting that all extraction controls remained negative throughout the study, both in samples from presumed and ruled out PJI, as well as in negative control samples.

Full concordance between positive and negative molecular results and conventional microbiological findings was observed in 18 of 34 and three of 34 patients with PJI, respectively. Good concordance between DNA-based and culture-positive samples was noted either for genus and/or species identification or detection of methicillin resistance. While a high prevalence of staphylococci was confirmed in case of PJI, DNA assays allowed them to be identified at the species and methicillin resistance level, except in one sample. Whether or not CoNS molecular species identification is of clinical value remains an issue,[20] but mounting evidence suggests that some CoNS species, such as *S. lugdunensis*, may have virulence factors that closely resemble those of *S. aureus* and, consequently, could cause similarly aggressive and destructive infections.[21] Regarding positive but discrepant species identification by culture and DNA assay, the discrepant phenotype (*S. faecalis* and *S. pyogenes*) and genotype (*S. pyogenes*) found in patient 1 were clearly attributed to contamination by *S. faecalis*. In patient 2, DNA analysis failed to detect the *Bacillus* sp. on two consecutive samples, but contamination during either culture or molecular sample processing cannot be excluded. In patient 3, who suffered a microbiologically (*P. aeruginosa*) proven relapse, both *P. aeruginosa* and *Serratia* sp. were detected at the DNA level. Evidence of a double sequence led us to clone the PCR amplicons to separately analyze amplicons of distinct origin. In patient 4, *Clostridium* sp. was detected by DNA analysis, whereas *S. epidermidis* was cultured from three intraoperative specimens. This patient, who had not been receiving ciprofloxacin for 5 days at the time of surgery, had a past history of PJI communicating within an endopelvic abscess and superinfection by a polymicrobial flora (*Citrobacter freundii*, *Enterococcus faecium, Lactobacillus* sp., and *Candida glabrata*). While the DNA-based assay and cultures were clearly discordant, a *Clostridium*-negative culture at the time of surgery could have resulted from the lack of an anaerobic container for transport of the clinical samples to the laboratory. Ciprofloxacin, which exhibits some activity against *Clostridium* sp., could also have been a contributing factor. The reason why *S. epidermidis* was not genotypically identified remains unclear, but it could have been related to the sampling procedure.

The use of molecular diagnostics to complement culture methods increased the identification rate of pathogens from 22 of 34 (64.7%) to 31 of 34 (91.2%). It should be noted that seven of the nine culture-negative PCR-positive patients had been receiving antibiotics for a period of 2 days to 5 weeks at the time of surgery. In patient 3, antibiotics were discontinued only 10 days before surgery. These findings confirm previous data reporting the persistence of bacterial DNA in samples collected from septic arthritis up to 22 days after antibiotic initiation.[22] Using pathogen-specific primers, persistence of *S. aureus* DNA was also identified up to 10 weeks after initiation of therapy in a patient with knee PJI.[23] Likewise, specific nucleic acids were shown to persist in gonococcal arthritis a few days after the start of therapy.[24] These converging data corroborate current observations and highlight the interest of molecular testing in patients receiving antibiotherapy, in whom the rate of false-negative culture can reach 50%.[5]

The current protocol involved collecting and sending separate samples for molecular and bacteriological analyses. Our aim was to avoid interfering with the usual procedure followed in the operating theater, ie, undue sample manipulation by the surgical personnel charged with dispatching the samples to the laboratories. The objective was to assess the performance of molecular

assays in a real surgical setting without affecting the normal workload. While it could be argued that this experimental design does not compare results of different methods on identical samples, it does compare results from the same infected or presumed infected surgical sites. An alternative would have been to send the samples to a specially designated receiving area, and then assign them for respective molecular and microbiological analyses.

The cost of implementing amplification-based DNA analyses in a clinical setting was another major issue. Accordingly, and despite the preliminary and experimental design of the current study, we attempted to assess the financial constraints potentially related to these methods. Only the variable costs of processing a single specimen were considered, therefore excluding all expenses related to human resources, equipment, and infrastructure. The assessment simply took into account the DNA-based identification strategy applied in our study. The variable cost was $15.50 per sample extraction or PCR assay and $42.50 per amplicon sequence. Altogether, 145 PCRs were performed on samples collected from 60 patients (mean, 2.4 PCRs/sample), including 82 Gram-positive/Gram-negative *16rDNA*, 47 *femA* and 16 *mecA,* as well as 55 sequence analyses. The cost of the whole study was therefore $5590 (euro), giving a mean of $93 (euro)/sample, which should be compared with the cost of current microbiological identification methods. In the near future, the outlay could be substantially reduced with automation and processing of several samples concurrently.

In conclusion, current data reveal the high sensitivity and specificity of DNA-based methods and support their use as an adjunct to microbiological approaches for identification of causative agents in intraoperative PJI samples. Indeed, in the present study, the combined use of molecular and culture methods increased the bacterial detection rate from 73.5% to 91.2%, proving especially effective in case of non-growing pathogens due to recent or concomitant antibiotherapy.

Acknowledgments

We thank Prof. Delloye and our collaborators from the Orthopedic Surgery Department, Cliniques universitaires St Luc, for providing all of the intraoperative samples.

References

1. Trampuz A, Steckelberg JM, Osmon DR, Cockerill FR, Hanssen AD, Patel R: Advances in the laboratory diagnosis of prosthetic joint infection. Rev Medical Microbiol 2003, 14:1–14
2. Berbari EF, Hanssen AD, Duffy MC, Steckelberg JM, Ilstrup DM, Harmsen WS, Osmon DR: Risk factors for prosthetic joint infection: case-control study. Clin Infect Dis 1998, 27:1247–1254
3. Zimmerli W, Trampuz A, Ochsner PE: Prosthetic-joint infections. N Engl J Med 2004, 351:1645–1654
4. Zimmerli W, Ochsner PE: Management of infection associated with prosthetic joints. Infection 2003, 31:99–108
5. Spangehl MJ, Masri BA, O'Connell JX, Duncan CP: Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. J Bone Joint Surg Am 1999, 81:672–683
6. Widmer AF: New developments in diagnosis and treatment of infection in orthopedic implants. Clin Infect Dis 2001, 33 Suppl 2: S94–S106
7. Tunney MM, Patrick S, Curran MD, Ramage G, Hanna D, Nixon JR, Gorman SP, Davis RI, Anderson N: Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. J Clin Microbiol 1999, 37:3281–3290
8. Trampuz A, Osmon DR, Hanssen AD, Steckelberg JM, Patel R: Molecular and antibiofilm approaches to prosthetic joint infection. Clin Orthop Relat Res 2003, 414:69–88
9. Lecouvet F, Irenge L, Vandercam B, Nzeusseu A, Hamels S, Gala JL: The etiologic diagnosis of infectious discitis is improved by amplification-based DNA analysis. Arthritis Rheum 2004, 50:2985–2994
10. Stokes EJ, Ridgway GL, Wren MWD: Laboratory Control of Antimicrobial Chemotherapy. Edited by Microbiology C. London 1993, pp 239–250
11. Bosshard PP, Abels S, Zbinden R, Bottger EC, Altwegg M: Ribosomal DNA sequencing for identification of aerobic gram-positive rods in the clinical laboratory (an 18-month evaluation). J Clin Microbiol 2003, 41:4134–4140
12. Atkins BL, Athanasou N, Deeks JJ, Crook DW, Simpson H, Peto TE, McLardy-Smith P, Berendt AR: Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty: The OSIRIS Collaborative Study Group. J Clin Microbiol 1998, 36:2932–2939
13. Bernard L, Lubbeke A, Stern R, Bru JP, Feron JM, Peyramond D, Denormandie P, Arvieux C, Chirouze C, Perronne C, Hoffmeyer P: Value of preoperative investigations in diagnosing prosthetic joint infection: retrospective cohort study and literature review. Scand J Infect Dis 2004, 36:410–416
14. Trampuz A, Piper KE, Hanssen AD, Osmon DR, Cockerill FR, Steckelberg JM, Patel R: Sonication of explanted prosthetic components in bags for diagnosis of prosthetic joint infection is associated with risk of contamination. J Clin Microbiol 2006, 44:628–631
15. Proctor RA, Kahl B, von Eiff C, Vaudaux PE, Lew DP, Peters G: Staphylococcal small colony variants have novel mechanisms for antibiotic resistance. Clin Infect Dis 1998, 27(Suppl 1):S68–S74
16. Roggenkamp A, Sing A, Hornef M, Brunner U, Autenrieth IB, Heesemann J: Chronic prosthetic hip infection caused by a small-colony variant of Escherichia coli. J Clin Microbiol 1998, 36:2530–2534
17. Tunney MM, Patrick S, Gorman SP, Nixon JR, Anderson N, Davis RI, Hanna D, Ramage G: Improved detection of infection in hip replacements: a currently underestimated problem. J Bone Joint Surg Br 1998, 80:568–572
18. Fenollar F, Roux V, Stein A, Drancourt M, Raoult D: Analysis of 525 samples to determine the usefulness of PCR amplification and sequencing of the 16S rRNA gene for diagnosis of bone and joint infections. J Clin Microbiol 2006, 44:1018–1028
19. Hoeffel DP, Hinrichs SH, Garvin KL: Molecular diagnostics for the detection of musculoskeletal infection. Clin Orthop Relat Res 1999, 360:37–46
20. Seo SK, Venkataraman L, DeGirolami PC, Samore MH: Molecular typing of coagulase-negative staphylococci from blood cultures does not correlate with clinical criteria for true bacteremia. Am J Med 2000, 109:697–704
21. Seenivasan MH, Yu VL: Staphylococcus lugdunensis endocarditis: the hidden peril of coagulase-negative staphylococcus in blood cultures. Eur J Clin Microbiol Infect Dis 2003, 22:489–491
22. van der Heijden IM, Wilbrink B, Vije AE, Schouls LM, Breedveld FC, Tak PP: Detection of bacterial DNA in serial synovial samples obtained during antibiotic treatment from patients with septic arthritis. Arthritis Rheum 1999, 42:2198–2203
23. Canvin JM, Goutcher SC, Hagig M, Gemmell CG, Sturrock RD: Persistence of Staphylococcus aureus as detected by polymerase chain reaction in the synovial fluid of a patient with septic arthritis. Br J Rheumatol 1997, 36:203–206
24. Muralidhar B, Rumore PM, Steinman CR: Use of the polymerase chain reaction to study arthritis due to Neisseria gonorrhoeae. Arthritis Rheum 1994, 37:710–717