MUSCULOSKELETAL SURGERY (2021) 105:39–42
https://doi.org/10.1007/s12306-019-00626-x

ORIGINAL ARTICLE

Unyvero ITI® system for the clinical resolution of discrepancies in periprosthetic joint infection diagnosis

Silvia Zannoli[1] · Andrea Sambri[2,4] · Manuela Morotti[1] · Martina Tassinari[1] · Arianna Torri[1] · Giuseppe Bianchi[2] · Massimiliano De Paolis[2] · Davide Maria Donati[2,4] · Vittorio Sambri[1,3]

Received: 11 September 2019 / Accepted: 1 October 2019 / Published online: 8 October 2019

Abstract

Objectives The Unyvero molecular assay was tested for the clinical resolution of discordant results, evaluating its role in prosthetic joint infection diagnosis.

Methods Multiplex PCR was performed on 45 samples from prosthesis treatment (either sonication or dithiothreitol). Analytical performance was compared to that of biofilm culture using Musculoskeletal Infection Society criteria as gold standard.

Results Unyvero and biofilm culture showed similar agreement rates compared to the gold standard (34/43 and 32/43, respectively). Both methods showed six additional identifications compatible with true infection; five positive results from biofilm culture were deemed contaminations.

Conclusions The Unyvero system showed good performances and a significantly shorter turnaround time compared to cultural methods, presenting an added value to PJI diagnosis even when performed following a composite approach.

Keywords Prosthetic joint infection · Diagnosis · PCR

Introduction

Periprosthetic joint infection (PJI) is one of the most frequent complications of arthroplasty, associated with an increased risk of long treatments, additional surgery and protracted hospitalization [1].

The current best evidence suggests the use of several diagnostic criteria to improve early diagnosis and treatment, although only microbiological identification provides the highest level of certainty [2]. However, cultures from PJI patients may be negative, e.g., due to ongoing antibiotic treatment [3]. Additionally, periprosthetic culture takes several days and is challenging for biofilm-embedded bacteria [4].

✉ Andrea Sambri
andrea_sambri@libero.it

1 Unit of Microbiology, The Great Romagna Area Hub Laboratory, Pievesestina, Cesena, Italy

2 Department of Orthopaedic Oncology, Istituto Ortopedico Rizzoli, Via Pupilli 1, 40136 Bologna, Italy

3 Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy

4 Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, Bologna, Italy

Alternative analytical techniques are being explored, including molecular methods. Multiplex PCR (mPCR) assays have been developed to rapidly and simultaneously identify multiple pathogens as well as their resistance genes [5]. Availability of reliable molecular diagnostic tools that can provide pathogen detection within hours might help prevent inappropriate treatment.

Here, we focus on the in-depth analysis of a selected pool of samples obtained within a previous larger study [6]. The aim is the evaluation of the clinical significance of discrepant results between pre-operative assessment and final diagnosis of PJI and the potential role of an automated multiplex PCR commercial assay within an algorithm targeted at improving the timing and accuracy of such diagnosis.

Methods

Study design

This study was performed as a retrospective single-center study, focused on the clinical resolution of selected, discrepant results obtained within a previous study, compatibly with the availability of leftover samples [6].

Patients were selected if pre-operative assessment (suspected septic or aseptic prosthesis failure, based on clinical presentation and laboratory tests) was unmatching with post-operative diagnosis of PJI according to the Musculoskeletal Infection Society (MSIS) guidelines (Group A, $n=19$). A pool of patients with matching results (Group B, $n=26$) was randomly selected as a control group [2].

Samples were tested retrospectively with a commercial mPCR assay (Unyvero ITI® cartridge system, Curetis®). Analytical performance was evaluated using MSIS guidelines as gold standard and compared to biofilm culture results, obtained previously [6]. Broad-range PCR targeted to the 16S rRNA gene and, where available, culture on joint aspirate were used as confirmation tests.

Microbiological methods

For each patient, five specimens of periprosthetic tissue were cultured for up to 14 days, following a protocol previously described [6]. As per MSIS guidelines, culture results were analyzed in combination with pre-operative assessment to reach a diagnosis of PJI.

Additionally, biofilm culture (either from sonication or dithiothreitol fluid) for each patient was performed for up to 14 days on fluid obtained by prosthesis treatment with either sonication ($n=23$) or dithiothreitol (DTT, $n=22$) as previously described [6].

Unyvero assay (mPCR)

For each patient, Curetis® analysis was performed on 180 µl of fluid obtained by prosthesis treatment over a three-month period (May–July 2017). Each specimen was transferred into a sample tube, which was closed with a sample tube cap containing proteinase K and an internal control gene as the quality control. Sample lysis was performed on a lysator module, including mechanical, thermal, chemical, and enzymatic treatment. The pre-treated samples were then transferred, together with a mastermix tube, into cartridges containing reagents for DNA purification, PCR primers, and probes and inserted into the analyzer module. For each sample, the system performed eight multiplex nucleic acid amplifications by end-point PCR, associated with qualitative amplicon detection by array hybridization. Time to result was approximately 5 h [7–9].

16S rRNA gene PCR

16S rRNA gene PCR assays were performed in parallel with mPCR from the same aliquot of sonicated/DTT-treated sample.

DNA automated extraction (Maxwell system, Promega) was followed by end-point PCR to target the 16S rRNA gene (forward primer 27F, 5′-AGA GTT TGA TCM TGG CTC AG-3′; reverse primer 1492R, 5′-GGT TAC CTT GTT ACG ACT T-3′; 1465-bp amplification product; GenBank accession number J01859.1) as previously described [10]. The corresponding amplicons were sequenced in both strands and assembled, and the consensus sequences were inserted in the BLAST database. The rates of concordance between 16S rRNA gene PCR and bacteriological results were based on results at the genus (≥96% similarity) and species (≥98% similarity) levels. A negative control and a positive control with *E. coli* DNA were assayed in parallel with the samples.

Results

Of 43 valid results, 29 were in agreement between mPCR, biofilm culture and the gold standard (20 positive and 9 negative). Of those, 10 concordant results were found in Group A (3 positive and 7 negative) and 19 in Group B (17 positive and 2 negative).

Table 1 illustrates unmatching results. No significant difference in the number of samples treated with DTT ($n=6$) or sonication ($n=8$) was found. Three specimens were negative with the mPCR but positive in culture both on biofilm and periprosthetic samples (Table 1, cases 1–3). Six samples showed detections with both mPCR and biofilm culture, but no growth in culture on biopsy: in five instances pathogen identification matched (cases 4–9).

In five cases, biofilm culture was positive but mPCR and culture on biopsy was negative (cases 10–14); in one instance, the microorganism detected was not included in the Unyvero panel. In the remaining, the culture showed growth of coagulase-negative staphylococci (CoNS), which no other method confirmed.

Discussion

In this study, we evaluated the potential added value of the Curetis® test within an algorithm targeted at improving the timing and accuracy of PJI diagnosis.

Unyvero and biofilm culture showed a similar agreement rate compared to the gold standard (respectively, 34 and 32 concordant results over 43). mPCR was negative in three cases, where MSIS criteria were suggestive of PJI, two of which sustained by pathogens within the Unyvero panel; in one case, culture was positive only after enrichment: this might suggest a low bacterial load in the sample which, coupled with the small volume required for mPCR, put the number of target sequences under the limit of detection. The remaining sample showed a double positive result for *S. hominis* in conventional culture, sufficient for diagnosis of PJI; however, the microorganism

Table 1 Overview of unmatching results

Case no	Group	Pre-operative indication of infection	Periprosthetic culture	Diagnosis of PJI*	Unyvero result	Biofilm culture	16S rRNA PCR
1	B	Yes	*Staphylococcus aureus* (2/5)	Yes	Neg	*S. aureus* (after enrichment)	Neg
2	A	No	*Serratia marcescens* (5/5)	Yes	Neg (out of panel)	*S. marcescens*	Neg
3	A	No	*Staphylococcus hominis* (2/5) *Staphylococcus epidermidis* (1/5)	Yes	Neg	*S. aureus*	Neg
4	B	No	Neg	No	*Enterococcus faecalis*	*E. faecalis*	*E. faecalis*
5	B	No	*S. epidermidis* (1/5)	No	CoNS	*S. epidermidis*	*S. epidermidis*
6	A	Yes	Neg	No	CoNS	*Staphylococcus haemolyticus*	*S. haemolyticus*
7	A	Yes	Neg	No	CoNS	*Staphylococcus lugdunensis*	Neg
8	A	Yes	Neg	No	CoNS	*S. epidermidis*	*S. epidermidis*
9	A	Yes	Neg	No	*Streptococcus agalactiae*	*S. haemolyticus*, *Corynebacterium* spp.	*S. agalactiae*
10	B	No	Neg	No	Neg (out of panel)	*Pseudomonas oryzihabitans*	*P. oryzihabitans*
11	B	No	Neg	No	Neg	*Staphylococcus capitis*	Neg
12	B	No	Neg	No	Neg	*S. hominis*	Neg
13	B	No	Neg	No	Neg	*S. epidermidis*	Neg
14	A	Yes	Neg	No	Neg	*S. capitis*	Neg

*Prosthetic joint infection

identified is a common contaminant, and biofilm culture only identified a low number of *S. aureus* colonies, while pre-operative assessment not suggestive of infection: in this case, the data are conflicting and not sufficient to reach a conclusion either way.

In five cases, mPCR and biofilm culture were concordant in the identification of a pathogen not detected by conventional culture; in four of those, identification was further supported by 16S rRNA gene sequencing. Furthermore, *S. agalactiae* and *P. oryzihabitans* identifications in two additional samples, respectively, through mPCR and culture, were confirmed by 16S PCR, culture on aspirate and pre-operative criteria.

However, five additional detections over 11 found by culture-based methods were likely contaminations, while all six from mPCR were confirmed through alternative methods and highly suggestive of true PJI.

Even considering the inherent limitation within this study, i.e., the sample pool size, these data suggest a higher sensitivity of both mPCR and biofilm culture compared with conventional culture, but a higher specificity of Unyvero with respect to culture-based methods. This is in accord with the previous literature [7, 8].

Additionally, while the presence of a pool of samples with unmatching pre-operative and final assessment was to be expected, a higher number of additional detections compatible with true PJI were found in Group A (4/17) with respect to Group B (2/26), despite its smaller size.

While combining clinical and laboratory data proves necessary in the diagnosis of PJI, choosing the appropriate technique for microbiological identification is paramount. The mPCR assay tested here proved very promising under this aspect, providing an added value to PJI diagnosis even when performed following the recommended composite approach. Although unable to identify some of the less common pathogens, the test is able to reduce the turnaround time from potentially weeks to hours and presents a fairly wide panel of resistance genes, allowing for a prompt treatment of the patient with targeted antibiotics. An added value is the ability to identify multiple pathogens simultaneously, as opposed to 16S PCR, and to analyze pre-treated as well as primary samples.

Acknowledgements The authors wish to thank Curetis® for providing free of charge the devices and consumables necessary for the study as a trial run. The company played no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Funding statement This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest All authors declare no conflicts of interest and no competing financial interests.

Ethical approval Ethical approval or informed consent was not required, since the study has been performed using exclusively anonymized leftover samples derived from a previous study. The anonymization was achieved by using the current procedure (AVR-PPC P09, rev.2) checked by the local Ethical Board.

References

1. Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J (2012) Economic burden of periprosthetic joint infection in the United States. J Arthroplast 27:61.e1–65.e1
2. Parvizi J, Gehrke T, International Consensus Group on Periprosthetic Joint Infection (2014) Definition of periprosthetic joint infection. J Arthroplast 29:1331
3. Parvizi J, Erkocak OF, Della Valle CJ (2014) Culture-negative periprosthetic joint infection. J Bone Joint Surg Amer 96:430–436
4. Berbari EF, Marculescu C, Sia I, Lahr BD, Hanssen AD, Steckelberg JM et al (2007) Culture-negative prosthetic joint infection. Clin Infect Dis 45:1113–1119
5. Malandain D, Bémer P, Leroy A, Léger J, Plouzeau C, Valentin AS et al (2018) Assessment of the automated multiplex-PCR Unyvero i60 ITI® cartridge system to diagnose prosthetic joint infection: a multicenter study. Clin Microbiol Infect 24:83.e1–83.e6
6. Sambri A, Cadossi M, Giannini S, Pignatti G, Marcacci M, Neri MP et al (2017) Is treatment with dithiothreitol more effective than sonication for the diagnosis of prosthetic joint infection? Clin Orthop Relat Res 00:1–9
7. Prieto-Borja L, Rodriguez-Sevilla G, Auñon A, Pérez-Jorge C, Sandoval E, Garcia-Cañete J et al (2017) Evaluation of a commercial multiplex PCR (Unyvero i60®) designed for the diagnosis of bone and joint infections using prosthetic-joint sonication. Enferm Infecc Microbiol Clin 35:236–242
8. Hischebeth GT, Randau TM, Buhr JK, Wimmer MD, Hoerauf A, Molitor E et al (2016) Unyvero i60 implant and tissue infection (ITI) multiplex PCR system in diagnosing periprosthetic joint infection. J Microbiol Methods 121:27–32
9. Borde JP, Häcker GA, Guschl S, Serr A, Danner T, Hübner J et al (2015) Diagnosis of prosthetic joint infections using UMD-Universal Kit and the automated multiplex-PCR Unyvero i60 ITI(®) cartridge system: a pilot study. Infection 43:551–560
10. Lane DJ (1991) 16S/23S rRNA Sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematic. Wiley, New York, pp 115–175