Original Research Article

HIP International

HIP International
2020, Vol. 30(1S) 72–77

DOI: 10.1177/1120700020938576
journals.sagepub.com/home/hpi
SAGE

How reliable is the next generation of multiplex-PCR for diagnosing prosthetic joint infection compared to the MSIS criteria? Still missing the ideal test

Christian Lausmann[1], Kim Niklas Kolle[1], Mustafa Citak[1], Hussein Abdelaziz[1], Juan Schulmeyer[1,5], Gio D Delgado[1,2], Thorsten Gehrke[1], Matthias Gebauer[3] and Akos Zahar[4]

Abstract

Introduction: Identification of the pathogen in case of a periprosthetic joint infection (PJI) remains 1 of the greatest challenges in septic surgery. Rapid germ identification enables timely, specific, antimicrobial therapy. The first multiplex PCR (polymerase chain reaction) generation (Unyvero-i60) enables germ detection within 5 hours with a sensitivity of 78.8% and a specificity of 100%. The aim of this study is to investigate the performance of the new generation of cartridges (Unyvero-ITI) of multiplex PCR in the case of a PJI.

Methods: In a prospective study, intraoperatively aspirated synovial fluid from 97 patients with aseptic or septic hip or knee revision surgery (49 aseptic, 48 septic) was examined with the multiplex PCR system (Unyvero-ITI) and the results were compared with the MSIS criteria. In addition, the time until the microbiological result was obtained in the event of a germ detection was documented.

Results: The multiplex PCR showed a germ detection with a sensitivity of 85.1% and a specificity of 98.0%. In 7 cases a false negative result was found and in one patient a false positive result was found. The general accuracy of this test procedure was 91.8%. The detection of germs was carried out within 5 hours with the multiplex PCR compared to 4.9 days in conventional microbiological diagnostics.

Conclusions: The new generation of multiplex-PCR was able to improve germ detection. The possibility of prompt detection of germs offers the option of faster, targeted antimicrobial therapy. This diagnostic tool offers significant advantages, particularly in the context of an acute periprosthetic infection.

Keywords

Diagnosing PJI, multiplex-PCR, periprosthetic joint infection, revision arthroplasty

Date received: 22 January 2020; accepted: 4 March 2020

Introduction

Diagnosing periprosthetic joint infection (PJI) remains challenging. The infection rates after total joint arthroplasty (TJA) are reported to be up to 3.0% and are likely to increase within the next decades.[1–3] Conventional diagnostic methods for PJI show a broad range of accuracy but proof of germ is not possible with every method. Recently, novel methods have been established to improve the diagnostic of PJI. Alpha-defensin, performed as ELISA, or lateral flow-test, can detect PJI from the synovial fluid. The ELISA test provides a sensitivity and specificity over 95%.[4–8] The lateral flow test showed lower performance

[1]Department of Orthopaedic Surgery, Helios ENDO-Klinik Hamburg, Hamburg, Germany
[2]Department of Orthopaedics, Philippine General Hospital, University of the Philippines Manila, Manila, Philippines
[3]Department of Orthopaedic Surgery, Roland Klinik, Bremen, Germany
[4]Department of Orthopaedic Surgery and Traumatology, Helios Klinikum Emil von Behring, Berlin, Germany
[5]Department of Orthopaedic Surgery, Hospital Barros Luco Trudeau, Santiago, Chile

Corresponding author:
Christian Lausmann, Department of Orthopaedic Surgery, Helios ENDO-Klinik Hamburg, Holstenstraße 2, Hamburg, 22767, Germany.
Email: christian.lausmann@helios-gesundheit.de

with sensitivity and specificity of 84–97% and 96–100% respectively.[9–11] Furthermore, Serum D-Dimer showed promising results as a possible marker for PJI with a sensitivity of 89% and specificity of 93%, respectively.[12] These techniques can detect PJI within short time but are not able to provide the causative germ. To date, the detection of pathogens can only be achieved by culturing and recently by multiplex polymerase chain reaction (mPCR). Most of the diagnostic methods are time consuming but the fast diagnosis of PJI including the proof of germ is one of the key issues of the PJI's management. The mPCR is a rapid molecular diagnostic tool and is able to detect pathogens and their antibiotic susceptibility within several hours. The polymerase chain reaction (PCR) is able to amplify DNA-fragments by using synthetic primers complementary to the target DNA. After several amplification cycles the DNA sequences are duplicated. Afterwards the specific DNA is isolated by electrophoresis or hybridization. For the Multiplex-PCR these reactions can be performed simultaneously in one cartridge to isolate the DNA of the causative germ.

The first generation of mPCR (Unyvero i60) showed encouraging results with a broad range of accuracy but the ideal diagnostic test is still missing.[13–15] The aim of the current study was to investigate if the next generation of mPCR is able to improve the performance in diagnosing PJI and to compare the results with the MSIS criteria including Alpha-Defensin.[16,17]

Patients and methods

The current study is a prospective single-centre study which was approved by the local ethical committee and written informed consent was given by all patients before inclusion. We included patients 18 years old or older with a painful hip or knee arthroplasty who had to undergo a revision surgery. In the outpatient clinic every patient underwent the standard ENDO-Klinik protocol routinely in order to select the procedure for septic or aseptic revision.[18] In case of an indication for a revision surgery blood test for C-reactive protein (CRP) as well as a joint aspiration with bacterial culture, detection of cell count and granulocyte percentage, leukocyte esterase test and Alpha-defensin ELISA testing were preoperatively performed for every patient. Prior to aspiration patients were not allowed to take antibiotics for at least 2 weeks and all aspirated synovial fluids were cultured for 14 days in the Microbiology Laboratory of University Hospital Schleswig-Holstein, Kiel, Germany. Depending on these results all patients were discussed in the institutional interdisciplinary team meeting and were selected for aseptic or septic revision according to the Musculoskeletal Infection Society (MSIS) criteria.[16,17] No patient with indication for acute revision $<$90 days after primary arthroplasty was included in this study.

At surgery (septic or aseptic revision) we performed an intraoperative aspiration of the affected joint directly after skin incision and subcutaneous preparation prior to capsulotomy. The surgeon used an 18-gauge needle and a 20-mL syringe for aspiration and avoided an admixture of blood. If not enough amount ($<$2.5 mL) of synovial fluid could be obtained the patient was excluded from this study.

Intraoperatively taken tissue samples as well as a part of the synovial fluid were sent to the Microbiology Laboratory for standard microbiology cultures with culturing time of 14 days, and for cell count and granulocyte percentage. 1–2 ml of the intraoperatively obtained synovial fluid was sent to an independent laboratory (Labor Dr. Fenner und Kollegen, Hamburg, Germany) to run the ELISA Alpha-defensin test within the next 24 hours. We used 1 drop of the synovial fluid to perform the leukocyte esterase test intraoperatively and from the remaining aspirate 180 µl were used for the mPCR. After surgery and intraoperative aspiration, 97 patients (46 females and 51 males) could be included. With the synovial fluid the mPCR as well as all other diagnostic tests were performed a second time and the results of the mPCR and the ELISA Alpha-defensin levels were compared to the culture results of the intraoperatively taken tissue samples as well as the other MSIS criteria. In case of germ detection by the mPCR the time interval between the mPCR result and the microbial result was documented. 50 patients had an aseptic revision, 47 patients had a septic revision due to PJI. In case of the septic revisions, 46 patients underwent a 1-stage septic exchange, in 1 case a 2-stage septic procedure was necessary due to a massive infection which could not be treated by a one-stage procedure.

In order to analyse the synovial samples the Unyvero Implant and Tissue Infection cartridge application (ITI) (Curetis N.V., Holzgerlingen, Germany) was used. This provides a semi-quantitative DNA test performing several different mPCR reactions parallely to detect pathogen-associated nucleic acids and resistance markers in solid, fluid and highly viscous samples.[15]

The new generation of Unyvero ITI Cartridge has an extended panel and increased sensitivity. It has added a universal bacterial primer, several *Candida* species, various types of *Streptococci, Klebsiella variicola*, and other new diagnostic targets. In addition, clinical sensitivity has been significantly improved for many key pathogens. In case of PJI with coagulase-negative *Staphylocci* the system makes no detailed distinction.

Statistical analysis

The distributions of the continuous variables are presented by count (n), mean and extrema (min, max). The distribution of categorical data is described by absolute and relative frequencies. For the quantification of the diagnostic power the following measures and the related 95% confidence intervals (95% CI) were calculated: sensitivity,

specificity, negative and positive predictive value, as well as overall accuracy. For the statistical analysis SAS 9.3 for Windows was used.

Results

We could enroll 126 patients. In two cases, surgery was cancelled because of medical complications and 27 patients were excluded due to insufficient volume of aspirated synovial fluid. A total of 97 synovial fluids of 51 hip joint, 45 knee joints and 1 total femur replacement could be included for final investigation of this study. 50 patients were considered as aseptic and 47 patients as infected. The mean age was 69.3 ± 10.0 years (range 43–87 years). From 97 aspiration fluids, the mPCR could detected germs in 41 cases and in 56 cases no germ could be found. After matching these results with the cultures of intraoperatively taken samples as a gold standard 7 cases were false negative and 1 case was false positive. After statistical analyses the mPCR showed a sensitivity of 85.1% (95% CI, 71.7–93.8%), a specificity of 98% (95% CI, 89.4–99.9%), a positive predictive value (PPV) of 97.6% (95% CI, 87.1–99.9%) and a negative predictive value (NPV) of 87.5% (95% CI, 75.9–94.8%). The overall accuracy of the mPCR was 91.8% (95% CI, 84.4–96.4%). The flowchart of the included patients is shown in Figure 1.

Aseptic group

In the group of aseptic patients, the mPCR showed no proof of germ in 49 cases, in only 1 case the mPCR detected a coagulase negative *Staphylococcus*, which was not confirmed by any other diagnostic method. It was considered as contamination. The LE-test could be performed in 49 cases and showed negative results, only in 1 case this test was haemorrhagic. Cell count analysis was possible in 35 cases and in only 2 cases the cell count was over 3000/µl. 1 of those cases showed a massive metallosis. Differential was possible in 35 patients and showed in every case a PMN% <80%. CRP levels could be determined in 49 out of 50 cases, in 1 case CRP determination was not possible due to technical problems. Only 1 aseptic patient showed a low rise of 15 mg/l. The Alpha-defensin level was slightly elevated only in 1 patient with a fracture of the femoral stem (Alpha-defensin level 1.2, cut off >0.99). All other aseptic patients showed normal Alpha-defensin levels. Culture of synovial fluid was possible in every aseptic case and showed no growth of germ after 14 days.

PJI group. In the group of septic patients, the mPCR could be performed in every 47 cases. In 1 case (2.1%) the mPCR showed an invalid result and therefore a germ could not be detected. Matching the other mPCR results with the microbial investigation in 40 out of 47 septic patients at least one germ could be detected (85.1%). Only in 1 of those cases the mPCR detected coagulase-negative *Staphylococci* and the microbial result showed *Streptococcus mitis*. 5 polymicrobial infections detected by the mPCR could be confirmed only in 2 cases. In 6 cases the mPCR was able to achieve a proof of germ whereas the detection of the synovial fluid failed. The time to gain the mPCR result was about 5 hours whereas the mean investigation time for the microbial results was 4.9 days. The distribution of all germs detected by mPCR is shown in Table 1.

The LE strip test was ++ or +++ in 43 cases (91.5%), in 4 cases (8.5 %) the LE test was haemorrhagic and was not applicable. Due to lack of volume or clotted synovial fluid cell count analysis and PMN% was only possible in 33 (70.2%) out of 47 septic cases. With the MSIS criteria limit of 3000 leukocytes/uL and PMN% >80%, 30 cases of the septic patients (63.8%) had an elevated cell count and 32 cases (68.1%) an elevated PMN%. The CRP level was elevated (>10 mg/L) in 36 cases out of 47. The microbial investigation of the intraoperatively taken synovial fluid could detect a germ in 37 cases, only in 2 patients the aspiration was culture positive whereas the mPCR lacked.

The Alpha-defensin levels were elevated in every septic patient and showed no false positive or false negative results. The statistical results of all diagnostic criteria are shown in Table 2.

Discussion

When the diagnosis of a periprosthetic joint infection (PJI) is determined, proper surgical intervention and antibiotic therapy is initiated. However, despite significant improvements in diagnostic modalities, the diagnosis of PJI remains difficult. 1 of the mainstays for PJI pathogen identification is a traditional microbial culture, but there are a few important disadvantages to consider. First, the long incubation period may take as long as 5–14 days in the clinical setting. This long waiting period is not ideal especially for acute PJIs wherein a prompt intervention is critical in order to avoid further local spread and systemic compromise.[19] In contrast, the mPCR test is a simple and automated method analysing the specimen directly and does not require an incubation period. Based on this study, the mPCR test results were available within 5 hours as compared to a mean time of 4.9 days for positive microbial cultures. Thus, the mPCR test affords a significantly shorter time frame to obtain results and consequently prompts an immediate intervention or a faster treatment adjustment.[13–15]

The occurence of culture-negative PJI, reported to be as high as 22%, is another phenomenon which highlights the weakness of traditional microbial cultures.[20] This usually happens in cases where patients with suspected PJI are inappropriately treated with antibiotics resulting in false-negative cultures. Another explanation is that some bacteria are more difficult to culture than others.[21] While the sensitivity of traditional microbial cultures for PJI is low, varying from 39% to 70%, the mPCR test is able to identify

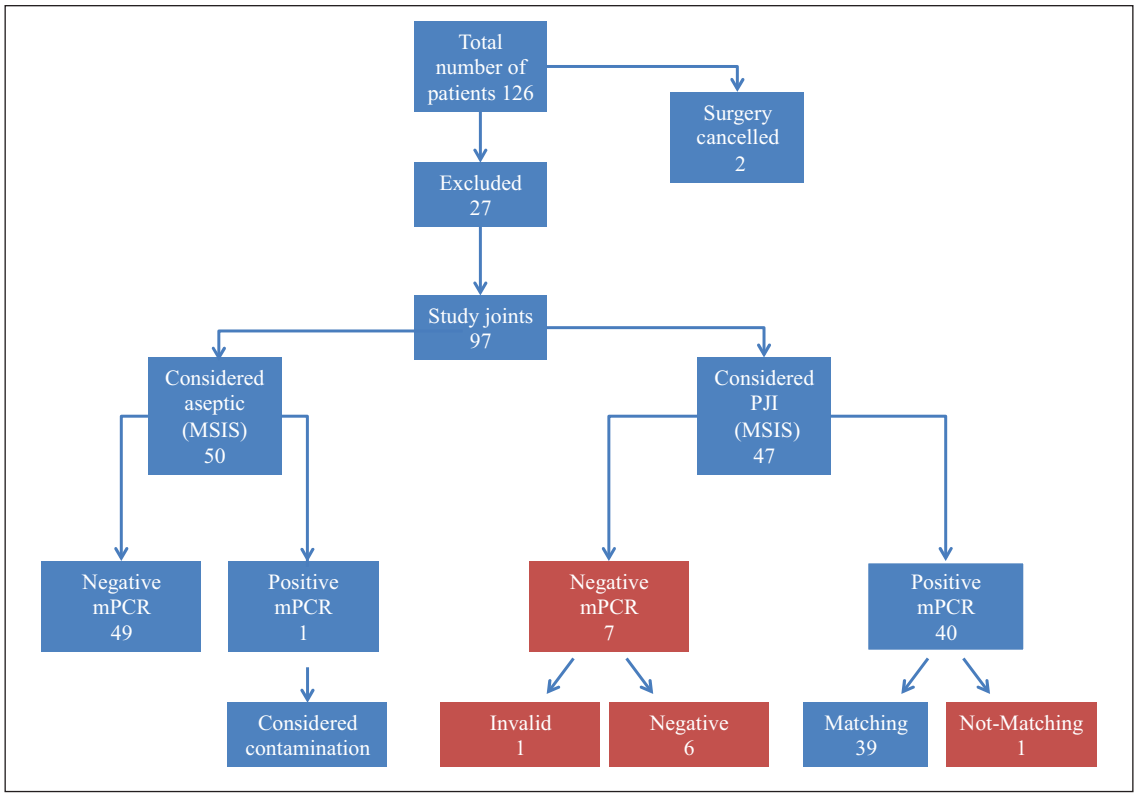

Figure 1. Flowchart of the patients included in this study.
MSIS, Musculoskeletal Infection Society; PJI, periprosthetic joint infection; mPCR, multiplex polymerase chain reaction.

Table 1. Number and distribution of pathogens detected by the mPCR in septic patients including polymicrobial PJIs.

Pathogen	Number	Distribution %
Coagulase-negative *staphylococci*	31	65.9
Cutibacterium acnes	3	6.4
Finegoldia magna	2	4.3
Streptococci	4	8.5
Abiotrophia detectiva	2	4.3
Enterococci	1	2.1
Proteus species	1	2.1

pathogens even during culture-negative PJI.[13] Also in this study in 6 cases the mPCR detected a pathogen considered as PJI by the MSIS criteria whereas the synovial fluid investigation showed a culture-negative result. In 1 of these patients a preoperative antibiotic therapy was given. Similar results can be found in recent literature.[22]

The main purpose of this study was to show the reliability of the new generation of mPCR compared to the previous one. The first generation of mPCR showed sensitivity rates lower than 80%.[13,14] In this study an increased performance of the next generation of mPCR in analyzing the synovial fluid with a sensitivity of 85.1% and a specificity of 98% could be proofed. In the literature variable results are described. For example, the work group of Morgenstern et al.[22] showed a lower performance of the mPCR with a sensitivity of 60%, but the mPCR was superior to culture for detection of low-virulent pathogens.

Renz et al.[23] combined the mPCR with the sonication of synovial fluid and found a sensitivity of 51% and a specificity of 94% respectively. Similar results were found by Hischebeth et al.[24] presenting a sensitivity of 66.7% and a specificity of 100%. In their study mPCR and fluid sonication were also investigated together.

The statistical results of the other diagnostic tests used in this study show a higher overall accuracy for Alpha-defensin (99%), LE-Test (98.9%), WBC (92.6%) and PMN (98.5%) than for mPCR (91.8%). These tests may be better in indicating the presence of an infection but do not have the capability to identify the specific pathogens, unlike the mPCR. Furthermore, CC and PMN are dependent of a suitable volume of synovial fluid which is not always easy to aspirate whereas for the mPCR a sample of 180µl is sufficient. Therefore, these diagnostic methods were only available in 68 (70.1%) cases in this study. The low availability of synovial fluid is a common problem in the diagnostic algorithm of PJI.[25] Nevertheless, the high accuracy of the diagnostic test methods may be affected by the fact that all aspirations were safely performed intraoperatively. Another limitation of the mPCR is that in case of missing the proper primer for the causative germ the investigation will show a negative result and so diagnosis of PJI is not possible. Furthermore this investigation technique is more

Table 2. Statistical results of all diagnostic criteria investigated in this study.

	mPCR	Alpha- defensin	LE	CC	PMN %	CRP	Aspiration
Availability	97/97	97/97	92/97	68/97	68/97	96/97	97/97
Sensitivity	85.1%	100%	100%	90.9%	97%	76.6%	78.7%
Specificity	98%	98%	98%	94.3%	100%	98%	100%
NPV	87.5%	100%	100%	91.7%	97.2%	81.4%	83.3%
PPV	97.6%	97.9%	97.7%	93.8%	100%	97.3%	100%
OA	**91.8%**	**99%**	**98.9%**	**92.6%**	**98.5%**	**87.5%**	**89.7%**

mPCR, multiplex polymerase chain reaction; LE, leukocyte esterase test; CC, cell count; PMN%, polymorphonuclear neutrophil percentage; CRP, C-reactive protein; OA, overall accuracy.

expensive than most of the other diagnostic test and thus it might be only affordable for specialised centres.

Finally the new generation of mPCR can even identify multiple pathogens from a single specimen and likewise detect antibiotic resistance markers, which was not the purpose of this study.[26,27] It is therefore appropriate to use a combined scoring system to determine PJI, as described by the MSIS Criteria.[16,17]

Conclusion

The new generation of multiplex PCR has improved the detection of bacteria. The option of timely detection of germs offers the option of time saving, targeted antimicrobial therapy. Especially in the context of an acute periprosthetic infection or culture-negative cases this diagnostic tool offers significant advantages. In our institution the mPCR is used routinely in cases of a suspected PJI with a previous culture-negative aspiration or even in patients with an acute periprosthetic infection under antibiotic therapy. But the performance of mPCR is lower than other diagnostic methods for PJI so the ideal test is still missing.

The development of the system as well as the infection diagnostics have to be further improved. The mPCR is a useful additional tool for diagnosing PJI in special cases.

Authors' note

Gio D Delgado is also affiliated with Department of Orthopaedics, Philippine General Hospital, University of the Philippines Manila, Manila, Philippines. Juan Schulmeyer is also affiliated with Department of Orthopaedic Surgery, Hospital Barros Luco Trudeau, Santiago, Chile.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: TG and AZ received personal fees from the company Zimmer Biomet for consulting work.
CL: received personal fees from Curetis as an invited speaker.
All other authors declare that there is no conflict of interest.

Funding

The author(s) received no financial support for the research, authorship and/or publication of this article.

References

1. Hanssen AD, Osmon DR and Nelson CL. Prevention of deep periprosthetic joint infection. *Instr Course Lect* 1997; 46: 555–567.
2. Kurtz SM, Ong KL, Schmier J, et al. Future clinical and economic impact of revision total hip and knee arthroplasty. *J Bone Joint Surg Am* 2007; 89(Suppl. 3): 144–151.
3. Salvati EA, Gonzalez Della Valle A, Masri BA, et al. The infected total hip arthroplasty. *Instr Course Lect* 2003; 52: 223–245.
4. Deirmengian C, Kardos K, Kilmartin P, et al. Combined measurement of synovial fluid alpha-Defensin and C-reactive protein levels: highly accurate for diagnosing periprosthetic joint infection. *J Bone Joint Surg Am* 2014; 96: 1439–1445.
5. Bingham J, Clarke H, Spangehl M, et al. The alpha defensin-1 biomarker assay can be used to evaluate the potentially infected total joint arthroplasty. *Clin Orthop Relat Res* 2014; 472: 4006–4009.
6. Frangiamore SJ, Gajewski ND, Saleh A, et al. α-defensin accuracy to diagnose periprosthetic joint infection-best available test? *J Arthroplasty* 2016; 31: 456–460.
7. Kanwar S, Al-Mansoori AA, Chand MR, et al. What is the optimal criteria to use for detecting periprosthetic joint infections before total joint arthroplasty? *J Arthroplasty* 2018; 33: S201–S204.
8. Bonanzinga T, Zahar A, Dutsch M, et al. How reliable is the Alpha-defensin immunoassay test for diagnosing periprosthetic joint infection? A prospective study. *Clin Orthop Relat Res* 2017; 475: 408–415.
9. Gehrke T, Lausmann C, Citak M, et al. The accuracy of the Alpha defensin lateral flow device for diagnosis of periprosthetic joint infection: comparison with a gold standard. *J Bone Joint Surg Am* 2018; 100: 42–48.
10. Renz N, Yermak K, Perka C, et al. Alpha defensin lateral flow test for diagnosis of periprosthetic joint infection: not a screening but a confirmatory test. *J Bone Joint Surg Am* 2018; 100: 742–750.
11. Berger P, Van Cauter M, Driesen R, et al. Diagnosis of prosthetic joint infection with Alpha-defensin using a lateral flow device: a multicentre study. *Bone Joint J* 2017; 99–B: 1176–1182.
12. Shahi A, Kheir MM, Tarabichi M, et al. Serum D-dimer test is promising for the diagnosis of periprosthetic joint infection and timing of reimplantation. *J Bone Joint Surg Am* 2017; 99: 1419–1427.
13. Lausmann C, Zahar A, Citak M, et al. Are there benefits in early diagnosis of prosthetic joint infection with multiplex

polymerase chain reaction? *J Bone Jt Infect* 2017; 2: 175–183.
14. Hischebeth GT, Randau TM, Buhr JK, et al. Unyvero i60 implant and tissue infection (ITI) multiplex PCR system in diagnosing periprosthetic joint infection. *J Microbiol Methods* 2016; 121: 27–32.
15. Borde JP, Häcker GA, Guschl S, et al. Diagnosis of prosthetic joint infections using UMD-Universal Kit and the automated multiplex-PCR Unyvero i60 ITI® cartridge system: a pilot study. *Infection* 2015; 43: 551–560.
16. Cats-Baril W, Gehrke T, Huff K, et al. International consensus on periprosthetic joint infection: description of the consensus process. *Clin Orthop Relat Res* 2013; 471: 4065–4075.
17. Parvizi J, Gehrke T and Chen AF. Proceedings of the international consensus on periprosthetic joint infection. *Bone Joint J* 2013; 95–B: 1450–1452.
18. Gehrke T, Zahar A and Kendoff D. One-stage exchange: it all began here. *Bone Joint J* 2013; 95–B(Suppl. A): 77–83.
19. Springer BD. The diagnosis of periprosthetic joint infection. *J Arthroplasty* 2015; 30: 908–911.
20. Tan TL, Kheir MM, Shohat N, et al. Culture-negative periprosthetic joint infection: an update on what to expect. *JB JS Open Access* 2018; 3: e0060.
21. Parikh MS and Antony S. A comprehensive review of the diagnosis and management of prosthetic joint infections in the absence of positive cultures. *J Infect Public Health* 2016; 9: 545–556.
22. Morgenstern C, Cabric S, Perka C, et al. Synovial fluid multiplex PCR is superior to culture for detection of low-virulent pathogens causing periprosthetic joint infection. *Diagn Microbiol Infect Dis* 2018; 90: 115–119.
23. Renz N, Feihl S, Cabric S, et al. Performance of automated multiplex PCR using sonication fluid for diagnosis of periprosthetic joint infection: a prospective cohort. *Infection* 2017; 45: 877–884.
24. Hischebeth GTR, Gravius S, Buhr JK, et al. Novel diagnostics in revision arthroplasty: implant sonication and multiplex polymerase chain reaction. *J Vis Exp* 2017; 130: 55147.
25. Zahar A, Lausmann C, Cavalheiro C, et al. How reliable is the cell count analysis in the diagnosis of prosthetic joint infection? *J Arthroplasty* 2018; 33: 3257–3262.
26. Burrack-Lange SC, Personne Y, Huber M, et al. Multicenter assessment of the rapid Unyvero Blood Culture molecular assay. *J Med Microbiol* 2018; 67: 1294–1301.
27. Personne Y, Ozongwu C, Platt G, et al. 'Sample-in, answer-out'? Evaluation and comprehensive analysis of the Unyvero P50 pneumonia assay. *Diagn Microbiol Infect Dis* 2016; 86: 5–10.