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KNEE ARTHROPLASTY

Preoperative PCR analysis of synovial fluid has limited value for the diagnosis of periprosthetic joint infections of total knee arthroplasties

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Abstract
Preoperative diagnosis of periprosthetic joint infection (PJI) is important because of the therapeutic consequences. This prospective study was designed to answer the question, if preoperative PCR analysis of the synovial fluid in addition to the culture and the CRP analysis of the blood are helpful for the diagnosis of PJI in knee arthroplasties. Before revision CRP analysis of the blood, cultivation and PCR analysis of synovial fluid of 116 knee endoprostheses were performed. During revision surgery, five tissue samples of the periprosthetic tissue were cultured and five further samples subjected to histological analysis. These analyses of the periprosthetic tissue were used to verify the results of the preoperative diagnostic methods. Twenty-seven prostheses were identified as infected (prevalence 23.3%). The combined analyses of the joint fluid cultivation and the CRP blood level resulted in a sensitivity of 77.8%, a specificity of 95.5%, a positive-predictive value of 84.0%, a negative-predictive value of 93.4% and an accuracy of 91.4%. The PCR analysis of the synovial fluid resulted in a sensitivity of 55.6%, a specificity of 82.0%, a positive-predictive value of 48.4%, a negative-predictive value of 85.9% and an accuracy of 75.9%. The sensitivity for culture of the aspirate and PCR analysis in combination with an elevated CRP level was 85.2%, the specificity 82.0%, the positive-predictive value 58.9%, the negative-predictive value 94.8% and the accuracy 82.7%. The preoperative PCR analysis of synovial fluid has only limited value in addition to the standard culture analysis.

Keywords Periprosthetic joint infection · Knee arthroplasty · Diagnosis · PCR · Culture

Introduction

Periprosthetic joint infection (PJI) is a severe complication of primary knee replacement surgery and has many consequences. The level of incidence is around 2% [1] and in some reports this type of infection is claimed to be the most frequent cause of implant failure during the first 5 years following surgery [2–4]. Thus, the accuracy of the preoperative diagnosis of possible periprosthetic joint infection becomes especially important in cases of loosened or painful knee endoprostheses [5, 6].

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Whereas early infections, i.e., those occurring within the first 4 weeks of implantation, usually cause local and systemic inflammatory reactions, these are often missing in cases of late periprosthetic joint infection with low-grade symptoms, occurring later than 4 weeks after implantation [7]. This makes the diagnosis of late periprosthetic infections very much more difficult. The classical clinical signs, laboratory tests and imaging techniques such as X-ray and scintigraphy are associated with a high level of false positives and false negatives [8, 9].

A preoperative diagnostic workup before revision surgery is helpful because the therapeutic strategy differs between septic revisions and aseptic revision. Local and systemic antibiotic therapy can be planned specifically before surgery takes place and can be started at a time before new biofilm formation on a new prosthesis has taken place [10–12].

For this reason, many authors have carried out preoperative aspiration besides CRP analysis of the blood [9–11]. The synovial fluid can be used for microbiological cultivation and measurement of CRP, leukocyte count, alpha-defensin and leukocyte esterase. Because no diagnostic method has an accuracy of 100%, a combination of different methodologies has an advantage, whereby cultivation is necessary because of the important possibility of identifying the microorganism and its susceptibility against antibiotics [12].

PCR is another method for identifying microorganisms and has the advantage that the results are available after a relatively short time period and that uncultured microorganisms can be detected [13, 14]. The value of the PCR analyses differs very much between the published studies. A review of the English literature was performed using the keywords "PCR" and "diagnostic" and "periprosthetic joint infection" showing sensitivities between 36 and 100% for synovial fluid and between 20 and 100% for tissue analyses, specificities between 49 and 100% for synovial fluid and between 45 and 100% for tissue analyses, positive-predictive values between 34 and 100% for synovial fluid and between 19 and 100% for tissue analyses and negative predictive values between 59 and 100% for synovial fluid and between 35 and 97% for tissue analyses (Table 1). Hereby, the PCR analyses were done mostly using intraoperatively taken periprosthetic tissue, synovial fluid or postoperatively performed fluid by sonication. PCR analyses of preoperatively taken synovial fluid via aspiration are very rare and the results of these view studies are inhomogeneous (Table 1). Therefore the value of an additional PCR analysis of the synovial fluid preoperatively in combination with the gold standard of culture technique of the synovial fluid and CRP analysis of the blood for diagnosing late periprosthetic knee infections before the revision surgery is not clear.

Table 1 Significance of PCR for the diagnosis of total knee arthroplasty infections

References	*N*	Technique	Time	Material	Sensitivity (%)	Specifity (%)	PPV (%)	NPV (%)	Accuracy (%)
Mariani et al. [38]	50 K	16S-rRNA	Preop	Aspirate	100	49	41	100	62
Bergin et al. [39]	64 K	rRNA RT-qPCR	Preop	Aspirate	71	100	100	93	94
Kordelle et al. [40]	50H + K	16S-rRNA	Intraop	Aspirate + tissue	36	100	100	61	68
Kordelle et al. [41]	22 K	16S-rRNA	Intraop	Tissue	20	100	100	60	62
Panousis et al. [42]	92 H + K	16S-rRNA	Intraop	Aspirate	92	74	34	98	76
Rak et al. [43]	67 H + K	BR 16S-rRNA	Intraop	Tissue	75	94.1	80	92.3	
Rak et al. [44]	100 H + K	BR-PCR	Intraop	Tissue	83.3	100			
Rak et al. [45]	87 H + K	16S-rRNA	Intraop	Tissue Sonication	76 93	93 93	85 87	89 96	
Cazanave et al. [13]	434 H + K	10-assay real-time PCR Sonication	Intraop	Sonication	77.1	97.9	94.9	89.6	
Miyamae et al. [36]	86 H + K	16S-rRNA real-time PCR	Intraop	Tissue	90	45	19	97	
Ryu et al. [14]	95 K	8 assay real-time PCR	Intraop	Tissue	15.6	96.8	90.9	35.7	
Suda et al. [46]	30 H + K	Multiplex-PCR	Intraop	Tissue	30.8	100	87	65	73
Marin et al. [47]	122 dif	16S-rRNA	Intraop	Tissue	67.1	97.8	94.3	84.8	87.1
Hischebeth et al. [35]	31 dif	Multiplex-PCR	Preop	Aspirate	55.6	100	100	61.9	
			Intraop	Sonciation	50	100	100	59.1	

H hip, *K* knee, *dif.* different joints, *BR-PCR* broad-range PCR

Therefore, the objectives of the present study were to carry out a prospective examination of the analytical procedure of preoperative aspiration for bacteriological and PCR analysis using a larger patient collective and to answer the following questions:

What is the value of PCR alone for the preoperative diagnosis of late periprosthetic infections of knee prostheses?

Does preoperative PCR bring additional information to the laboratory test of blood C-reactive protein and the gold standard culture technique of the aspirate?

Materials and methods

This prospective study included all 116 patients (61 women, 55 men) who required knee prosthesis revision surgery between January 2016 and June 2017. They all underwent a prior aspiration of the knee. The mean age of the patients was 67.6 ± 9.3 years (41–91 years). The primary diagnosis in 112 cases was osteoarthritis of the knee and in 4 cases rheumatoid arthritis. The revision operation was carried out 38.6 ± 38.1 months (2–230 months) after the primary implantation.

None of the patients took any antibiotics in the 4 weeks preceding the aspiration. The joint aspiration techniques were carried out under sterile conditions. The harvested fluid was immediately aspirated into pediatric blood culture bottles containing BD BACTEC-PEDS-PLUS/F-Medium (Becton Dickinson, Heidelberg, Germany) and was incubated for 14 days [15].

Molecular pathogen detection was performed from joint aspirate fluids by the UMD-Universal test kit (Molzym, Bremen, Germany) according to the manufacturer's instructions. Briefly, human cells present in patient samples were digested using a chaotropic buffer, followed by incubation with DNase to remove host cell DNA. Enhanced bacterial and fungal pathogens were enriched and then treated with an additional lysis reagent to break down cell walls. Pathogen DNA was extracted and purified for PCR analysis. Two separate PCR assays were performed with each sample using primers targeting conserved gene regions of bacterial 16S rRNA and fungal and eukaryote 18S rRNA, respectively. Adequate positive and negative controls were included in each assay according to the manufacturer's instructions. PCR products were analyzed by agarose gel electrophoresis and ethidium bromide staining, and positive reactions were documented under ultraviolet light examination. Amplification products were purified with the *QIAquick* PCR Purification Kit (Qiagen, Hilden, Germany) and sequencing was performed by GATC (Biotech, Konstanz, Germany) using the same primers as those provided in the UMD-Universal test kit. For identification of pathogens, sequence data were analyzed using the online BLAST search tool [16]. The turnaround time of tests results ranged from 48 to 72 h, whereby the PCR itself took 2.5 h. Compared to reference strains included in the database, sequence identities of ≥ 97 and ≥ 99% from the study samples were defined as pathogen identification to the genus and the species levels, respectively. Samples with positive PCR reactions but no sequencing result were classified as not evaluable.

During the revision surgery itself, samples were taken from five different areas close to the prosthesis (synovium and bone). In addition, five samples from the synovium and the periprosthetic connective tissue membrane associated with the loosened prosthesis were obtained for histological assessment. Perioperative antibiotics were only administered once all the samples had been taken. The biopsy samples were each placed in sterile tubes and transferred together with the aspirated fluid to the microbiological laboratory within an hour of sampling. The samples were streaked onto blood agar and inoculated into special nutrient broth for anaerobic organisms. All the samples were incubated for 14 days [15, 17, 18]. The results were analyzed according to Atkins et al. [19], Virolainen et al. [8], Pandey et al. [20] and the criteria of the Musculoskeletal Infection Society (MSIS) [21], whereby a synovial membrane sample was regarded as positive when at least one of the following conditions had been fulfilled:

1. Demonstration of the same pathogen in at least two of the samples.
2. Demonstration of a pathogen in at least one sample and demonstration of at least five neutrophilic polymorph leukocytes per high power field (400×) in the associated histological preparation as described by Mirra et al. [22, 23], Feldman et al. [24], Lonner et al. [25] and Pandey et al. [20].

The presence of bacteria in only one sample without any histological confirmation was regarded as a result of contamination during the sampling procedure or during the incubation period, in accordance with Virolainen et al. [8].

The diagnosis obtained from the revision surgery samples was regarded as the definitive result with respect to periprosthetic infection and was then used to evaluate the diagnostic methods (CRP, culture and PCR analysis of the joint aspiration).

The conditional probabilities "sensitivity" and "specificity" were determined as characteristic parameters of the diagnostic methods. In the case of sensitivity, this represents the proportion of infections that the test detects as infected (the positive test results) and, in the case of specificity, the proportion of tests that have negative results. The probability that in cases of positive or negative test results sepsis does or does not exist is represented by the positive and negative prediction percentages. They are dependent on the

prevalence, i.e., the proportion of infected prostheses in the whole collective or, in other words, the pre-test probability of infection. The Bayes' equation was used to calculate these statistics [26]. The accuracy of the techniques was calculated from the sum of the true positives and the true negatives divided by the number of tests carried out. All subjects gave informed consent to participate in the study and the protocol was approved by the research ethics boards of the respective institution.

Results

Of the 116 revision operations carried out, 27 were classified during surgery as infected according to the criteria described in "Materials and methods" (prevalence 23.3%). The bacteria that were identified are listed in Table 2, whereby it should be noted that in one case two microorganisms were identified.

Of the cultivations of the synovial fluid, 86 were true negatives, 20 true positives, 3 false positives and 7 false negatives. Transformation of these data results in a sensitivity of 74.0% and a specificity of 96.6% (Table 3). Considering the 23.3% prevalence of infected knee endoprostheses, the probability that a patient with a positive test result from the aspirated fluid really does have an infected knee prosthesis is 86.9% (positive-predictive value). The probability that a loosened knee prosthesis with a negative test result is actually not infected amounts to 92.5.0% (negative predictive value). The proportion of correct test results is 91.4% (accuracy) (Table 3).

Seventy-three patients without infections had a CRP level under 10 mg/l (true negative) and 16 with PJI over 10 mg/l (true positive). Eleven patients showed false negative and 16 false positive results. These lead to a sensitivity for the CRP level over 10 mg/l as a sign of PJI of 59.3%, a specificity of 82.0%, a positive-predictive value of 50.0%, a negative-predictive value of 86.9% and an accuracy of 76.7% (Table 3).

Combining the parameters of culture of the synovial fluid and CRP level in the blood showed 21 true-positive and 85 true-negative results. False positive results using both parameters in combination were four and false negative results five. Therefore, the sensitivity for culture of the aspirate in combination with an elevated CRP level was 77.8%, the specificity 95.5%, the positive-predictive value 84.0%, the negative-predictive value 93.4% and the accuracy 91.4% (Table 3).

Fifteen of the PCR analysis were true positives, 73 true negatives, 16 false positives and 12 false negatives (Tables 4, 5). The sensitivity was therefore 55.6% and the specificity 82.0% (Table 3). At a prevalence of 23.3% infected knee prostheses in this study, the probability that a biopsy with a positive test result really is infected amounts to 48.8% (positive-predictive value). The probability that a biopsy with a negative test result is actually not infected is 85.9% (negative predictive value). The proportion of correct test results is 75.9% (accuracy) (Table 3). Two periprosthetic joint infections that were rated as negative in the combined analysis of synovial fluid cultivation and CRP level could be detected with the PCR analyses.

The combination of PCR analysis with the cultivation and the CRP level showed 23 true positives, 73 true negatives, 16 false positives and 4 false negatives (Tables 4, 5). Therefore the sensitivity for culture of the aspirate and PCR analysis in combination with an elevated CRP level was 85.2%, the specificity 82.0%, the positive-predictive value 58.9%, the negative-predictive value 94.8% and the accuracy 82.7% (Table 3).

Table 2 Identified bacteria and their frequency of detection

Bacterium	*N*
Staph. epidermidis	4
MRSE	2
Staph. aureus	3
Staph. hominis	3
Staph. capitis	4
Staph. lugdonensis	2
Staph. warneri	1
Enterococcus faecalis	3
Cutibaterium acnes	3
Propionibacterium species	1
Klebsiella pneumoniae	1
Proteus mirabilis	1

Table 3 Results of CRP, culture of the synovial fluid and PCR analysis of the synovial fluid

N = 116	CRP > 10 mg/l (%)	Aspiration culture (%)	CRP + culture (%)	Aspiration PCR (%)	PCR + CRP + culture (%)
Sensitivity	59.3	74.0	77.8	55.6	85.2
Specificity	82.0	96.6	95.5	82.0	82.0
Positive-predictive value	50.0	86.9	84.0	48.4	58.9
Negative-predictive value	86.9	92.5	93.4	85.9	94.8
Accuracy	76.7	91.4	91.4	75.9	82.7

Table 4 Findings of the 27 cases with periprosthetic joint infection

Patient	CRP (mg/l)	Aspiration culture	Aspiration PCR	Microorganism	Intraop histology	Intraop culture
1	45.1	+	–	*Staph. epidermidis*	+	5 of 5
2	55.0	+	–	*Staph. capitits*	+	5 of 5
3	6.0	+	–	*Staph. captitis*	–	5 of 5
4	28.5	+	+	*Staph. aureus*	+	5 of 5
5	65,5	+	+	*Staph. aureus*	+	4 of 5
6	2.9	+	–	*Staph. capitis*	+	2 of 5
7	23.1	+	+	*Staph. epidermidis*	+	4 of 5
8	2.2	–	+	*Enterococcus faecalis*	+	5 of 5
9	61,8	+	+	*Proteus mirabilis*	+	4 of 5
10	68.6	+	+	*Streptooccus agalactiae*	+	5 of 5
11	87.7	+	+	*Staph. aureus*	+	5 of 5
12	46.4	+	+	*Staph. epidermidis*	+	5 of 5
13	89.1	+	+	*Gemella sanuinis*	+	5 of 5
14	8.0	–	–	*Staph. hominis*	–	4 of 5
15	53.4	+	+	*Staph. lugdunensis*	+	5 of 5
16	2.2	+	–	Cutibacterium agnes	+	3 of 5
17	11.7	+	–	*Staph. hominis*	+	4 of 5
18	6.5	+	+	Propionibacterium species + *Staph. warneri*	–	3 of 5
19	6.0	–	+	*Staph. epidermidis*	–	3 of 5
20	38.3	–	+	*Staph. epidermidis*	+	2 of 5
21	7.3	–	–	*Corynebacterium urealyticum*	+	2 of 5
22	4.0	+	–	Cutibacterium agnes	–	5 of 5
23	43.0	+	+	*Staph. epidermidis*	+	4 of 5
24	49.3	+	+	*Enterococcus faecalis*	+	5 of 5
25	4.0	–	–	Cutibacterium agnes	–	3 of 5
26	4.0	–	–	*Staph. capitis*	+	4 of 5
27	19.4	+	–	*Staph. hominis*	+	2 of 5

Table 5 Findings of the 16 cases with false positive PCR results

Patient	CRP (mg/l)	Aspiration culture	Aspiration PCR	Intraop histology	Intraop culture
1	14.1	–	*Citrobacter koseri*	–	–
2	13.0	–	Actinobacter species	–	–
3	2.7	–	*Anoxybacillus flavithermus*	–	–
4	16.8	–	*Staph. hominis*	–	–
5	1.9	–	*Staph. hominis*		
6	2.1	–	Actinobacter species	–	–
7	7.4	–	*Staph. epidermidis*	–	–
8	5.0	–	*Staph. epidermidis*	–	–
9	4.0	–	*Staph. epidermidis*	–	–
10	6.9	–	*Sphingomonas paucimobilis*	–	–
11	3.8	–	*Malassezia restricta*	–	–
12	7.8	–	*Malassezia restricta* + *Stegi-noporella truncata*	–	–
13	23.9	–	*Staph. epidermidis*	–	–
14	2.8	–	*Staph. hominis*	–	1 of 5
15	2.4	–	*Malassezia sympodialis*	–	–
16	13.0	–	Actinobacter species	–	–

Discussion

A preoperative bacteriological examination should be carried out before a loose or painful knee prosthesis is exchanged, because the presence of a periprosthetic joint infection would result in significant changes in the nature of the subsequent therapeutic procedures. Therefore, many authors recommend joint aspiration with culture analysis on a regular basis besides CRP analysis of the blood before a revision operation is carried out [26–30]. Because no single diagnostic analysis of the synovial fluid has an accuracy of 100%, a combination of different parameters is helpful [12]. The aim of the present study was to answer the question, if preoperative PCR analysis of the synovial fluid in addition to the culture of the synovial fluid and the CRP analysis of the blood is helpful for the preoperative diagnosis of PJI in knee arthroplasties.

The results of the present study of 116 knee prosthesis revision operations showed that the preoperative PCR analysis of synovial fluid has only limited value in addition to the standard culture analysis. The sensitivity of the PCR analysis was inferior to the combined examination of synovial culture and blood CRP and only two additional cases that were negative in the culture could be detected with the PCR analysis. Moreover the addition of PCR to the culture and CRP analysis did not improve the results; it worsened the specificity, the positive-predictive value and the accuracy, whereas the sensitivity and negative-predictive value were improved only slightly.

The culture is necessary, because it is the only method that allows the identification of the susceptibility of the microorganisms against antibiotics. This is in our opinion necessary to choose the specific local and systemic antibiotic therapy to the susceptibility of the microorganisms, because not all bacteria can be successfully treated with broad-spectrum systemic antibiotics and the same antibiotics are constantly used in the cement spacers (e.g., some Gram-negative organisms). So, this is an argument for investigating the antibiotic resistance pattern of the isolated bacteria and selecting a specific local and systemic antibiotic therapy before revision surgery take place.

The disadvantage of culture technique for the diagnosis is that patients should not be treated with antibiotics beforehand or the antitbiotic therapy should be stopped for at least 14 days, if possible 4 weeks, before any sampling occurs [31–34]. Despite this, the identification of bacteria was made more difficult in these cases by pre-treatment with antibiotics. This disadvantage can be addressed with the PCR analysis, because the PCR panel is less affected by 14 days prior antibiotics [13]. Another advantage of the PCR analysis is the fast result of the analysis within hours and not up to 14 days in the culture technique [13, 15].

The disadvantage of the PCR analysis is the high level of false-positive results, as we could also find in our study. The reason is that PCR does also detect killed and not only live organisms [13, 35]. Moreover, the differentiation between contaminant and causative microorganisms for the same PCR result is also difficult [13, 36, 37]. The high price is another disadvantage of the PCR technique [13]. Therefore, other analyses of the synovial fluid such as alpha-defensin and leukocyte count in addition to the cultivation seem to be more helpful in analyzing PJI.

Though the study had a relatively high number of patients, it still has some limitations. We only analyzed the synovial fluid with culture and PCR and not with leukocyte count and alpha-defensin. The focus of the study was to identify the value of PCR alone and in addition to the standard examinations of CRP in the blood and culture of the synovial fluid and not the value of other tests of the synovial fluid. However, in our opinion the cultivation of the aspirate is always necessary, because it is the only examination that allows the preoperative determination of the microorganism and its susceptibility. If the value of PCR is not significant, in addition to cultivation and CRP, it would not be better if we would have examined also the leukocyte count and/or alpha-defensin in the synovial fluid. However, another prospective study analyzing all possible diagnostic techniques of the synovial fluid may be helpful to identify the test combinations with the highest value for daily use. This study included only knee arthroplasties, because the aspiration always resulted in a sufficient amount to perform both the cultivation and PCR analysis, which may not be the case on a regular basis for hip arthroplasties. In theory, PCR results can be obtained within 24 h upon receipt of the sample if sequence analysis is available in the same laboratory. In our setting, the turnaround time of test results ranged from 48 to 72 h. However, this is no disadvantage in the authors' experience, since the vast majority of culture-negative infections, which are the very ones that would primarily call for PCR analysis, are low-grade infections that do not necessarily require immediate identification of the causative agents. Therefore, 48- to 72-h turnaround times of PCR results are completely acceptable from the clinical standpoint.

Summing up, because of the limited value of PCR analysis, it should not be used on a regular basis and may only be helpful in cases with antibiotic treatment, where this antibiotic treatment cannot be stopped and a rapid detection of the microorganism is necessary.

References

1. Koh CK, Zeng I, Ravi S, Zhu M, Vince KG, Young SW (2017) Periprosthetic joint infection is the main cause of failure for

modern knee arthroplasty: an analysis of 11,134 knees. Clin Orthop Relat Res 475:2194–2201
2. Fehring TK, Griffin WL (1998) Revision of failed cementless total knee implants with cement. Clin Orthop Relat Res 356:34–38
3. Saleh KJ, Rand JA, McQueen A (2003) Current status of revision total knee arthroplasty: how do we assess results? J Bone Jt Surg Am 85(Suppl 1):18–20
4. Scuderi GR, Insall JN, Windosr RE, Moran MC (1989) Survivorship of cemented knee replacements. J Bone Jt Surg Br 71:798–803
5. Hanssen AD (2002) Managing the infected knee: as good as it gets. J Arthroplast 17(4 Suppl 1):98–101
6. Della Valle CJ, Zuckermann JD, Di Cesare PE (2004) Periprosthetic sepsis. Clin Orthop Relat Res 420:26–31
7. Tsukayama DT, Estrada R, Gustilo RB (1996) Infection after total hip arthroplasty. A study of the treatment of one hundred and six infections. J Bone Jt Surg Am 78:512–523
8. Virolainen P, Lahteenmaki H, Hiltunen A, Sipola E, Meurman O, Nelimarkka O (2002) The reliability of diagnosis of infection during revision arthroplasties. Scan J Surg 91:178–181
9. Trampuz A, Zimmerli W (2005) Prosthetic joint infections: update in diagnosis and treatment. Swiss Med Wkly 135:243–251
10. Munjal S, Phillips MJ, Krackow KA (2001) Revision total knee arthroplasty: planning, controversies, and management-infection. Instr Course Lect 50:367–377
11. Fink B, Makowiak C, Fuerst M, Berger I, Schäfer P, Frommelt L (2008) The value of synovial biopsy, joint aspiration and C-reactive protein in the diagnosis of late peri-prosthetic infection of total knee replacements. J Bone Jt Surg Br 90:874–878
12. Fink B, Lass R (2014) A diagnostic algorithm for painful total knee replacement. Z Orthop Unfall 152:189–202
13. Cazanave C, Greenwood-Quaintance KE, Hanssen AE, Karau MJ, Schmidt SM, Gomez Urena EO, Mandrekar JN, Osmon DR, Lough LE, Pritt BS, Steckelberg JM, Patel R (2013) Rapid molecular microbiologic diagnosis of prosthetic joint infection. J Clin Micorobiol 51:2280–2287
14. Ryu SY, Greenwood-Quaintance KE, Hanssen AD, Mandrekar JN, Patel R (2014) Low sensivity of periprosthetic tissue PCR for prosthetic knee infection diagnosis. Microbiol Infect Dis 79:448–453
15. Schäfer P, Fink B, Sandow D, Margull A, Berger I, Frommelt L (2008) Prolonged bacterial culture to identify late periprosthetic joint infection: a promising strategy. Clin Infect Dis 47:1403–1409
16. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410
17. Steinbrink K, Frommelt L (1995) Treatment of periprosthetic infection of the hip using one-stage exchange surgery. Orthopäde 24:335–343
18. Ince A, Rupp J, Frommelt L, Katzer A, Gille J, Löhr JF (2004) Is "aseptic" loosening of the prosthetic cup after total hip replacement due to nonculturable bacterial pathogens in patients with low-grade infection? Clin Infect Dis 39:1599–1603
19. Atkins BL, Athanasou N, Deeks JJ, Crook DW, Simpson H, Peto TE, McLardy-Smith P, Berendt AR (1998) Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. J Clin Microbiol 36:2932–2939
20. Pandey R, Drakouilakis E, Athanasou NA (1999) An assessment of the histological criteria used to diagnose infection in hip revision arthroplasty tissues. J Clin Pathol 52:118–123
21. Parvizi J, Zmistowski B, Berbari EF, Bauer TW, Springer BD, Della Valle CJ, Garvin KL, Mont MA, Wongworawat MD, Zalavras CG (2011) New definition of periprosthetic joint infection: from the workgroup of the Musculoskeletal Infection Society. Clin Orthop Relat Res 469:2992–2994
22. Mirra JM, Amstutz HC, Matos M, Gold R (1976) The pathology of the joint tissues and its clinical relevance in prosthetic failure. Clin Orthop Relat Res 117:221–240
23. Mirra JM, Marder RA, Amstutz HC (1982) The pathology of failed total joint arthroplasty. Clin Orthop Relat Res 170:175–183
24. Feldman DS, Lonner JH, Desai P, Zuckerman JD (1995) The role of intraoperative frozen sections in revision total joint arthroplasty. J Bone Jt Surg Am 77:1807–1813
25. Lonner JH, Desai P, Dicesare PE, Steiner G, Zuckerman JD (1996) The reliability of analysis of intraoperative frozen sections for identifying active infection during revision hip or knee arthroplasty. J Bone Jt Surg Am 78:1553–1558
26. Heinecke A, Hultsch E, Repges R (eds) (1992) Medizinische Biometrie. Springer, Berlin
27. Saleh KJ, Clark CR, Rand JA, Brown GA (2003) Modes of failure and preoperative evaluation. J Bone Jt Surg Am 85(Suppl. 1):21–25
28. Pellegrini VD Jr (1997) Management of the patient with an infected knee arthroplasty. Instr Course Lect 46:215–219
29. Simmons TD, Stern SH (1996) Diagnosis and management of the infected total knee arthroplasty. Am J Knee Surg 9:99–106
30. Tsukayama DT, Goldberg VM, Kyle R (2003) Diagnosis and management of infection after total knee arthroplasty. J Bone Jt Surg Am 85(Suppl.1):75–80
31. Duff GP, Lachiewics PF, Kelly SS (1996) Aspiration of the knee joint before revision arthroplasty. Clin Orthop Relat Res 331:132–139
32. Levitsky KA, Hozack WJ, Balderston RA, Rothman RH, Gluckman SJ, Maslack MM, Booth RE Jr (1991) Evaluation of the painful prosthetic joint. Relative value of bone scan, sedimentation rate, and joint aspiration. J Arthroplast 6:237–244
33. Barrack RL (1997) The value of preoperative knee aspiration: don't ask, don't tell. Orthopedics 20:862–864
34. Mont MA, Waldman BJ, Hungerford DS (2000) Evaluation of preoperative cultures before second-stage reimplantation of a total knee prosthesis complicated by infection. A comparison-group study. J Bone Jt Surg Am 82:1552–1557
35. Hischebeth GT, Randau TM, Buhr JK, Wimmer MD, Hoerauf A, Molitor E, Bekeredjian-Ding I, Gravius S (2016) Unyvero i60 implant ant tissue infection (ITI) multiplex PCR system in diagnosing periprosthetic joint infection. J Microbiol Methods 121:27–32
36. Miyamae Y, Inaba Y, Kobayashi N, Choe H, Yukizawa Y, Ike H, Saito T (2013) Different diagnostic properties of C-reactive protein, real-time PCR, and histopathology of frozen and permanent sections in diagnosis of periprosthetic joint infection. Acta Orthop 84:524–529
37. Zegaer BH, Ionnidis A, Babis GC, Ionnidou V, Kossyvakis A, Bersimis S, Papaparskevas J, Petinaki E, Pliatsika P, Chatzipanagiotou S (2014) Detection of bacteria bearing resistant biofilm forms, by using the unsiversal and specific PCR is still unhelpful in the diagnosis of periprosthetic joint infections. Front Med (Lausanne) 1:30
38. Mariani BD, Martin DS, Levine MJ, Booth RE Jr, Tuan RS (1996) Polymerase chain rection detection of bacterial infection in total knee arthroplasty. Clin Orthop Relat Res 331:11–22
39. Bergin PF, Doppelt JD, Hamilton WG, Mirick GE, Jones AE, Sritulanondha S, Helm JM, Tuan RS (2010) Detection of periprosthetic infections with use of ribosomal RNA-based polymerase chain reaction. J Bone Jt Surg 92-A:654–663
40. Kordelle J, Klett R, Stahl U, Hossain H, Schleicher I, Haas H (2004) Diagnostic of infection after total knee replacement. Z Orthop 142:337–343
41. Kordelle J, Hossain H, Stahl U, Schleicher I, Haas H (2004) Usefulness of 16S rDNA-polymerase- chain-reaction (PCR) in the

intraoperative detection of infection in revison of failed arthroplasties. Z Orthop Ihre Grenzgeb 142:571–576
42. Panousis K, Grigoris P, Butchler I, Rana B, Reilly JH, Hamblen DL (2005) Poor predictive value of broad-rand PCR for the detection of arthroplasty infection in 92 cases. Acta Orthop 76:341–346
43. Rak M, Barlic-Maganja D, Kavcic M, Trebse R, Cör A (2014) Comparison of molecular and culture method in diagnosis of prosthetic joint infection. FEMS Microbiol Lett 343:42–48
44. Rak M, Barlic-Maganja D, Kavcic M, Trebse R, Cor A (2015) Identification of the same species in at least two intra-operative samples for prosthetic joint infection diagnsotics yields the best results with broad-range polymerase chain reaction. Int Orthop 39:975–979
45. Rak M, Kavcic M, Trebse R, Cör A (2016) Detection of bacteria with molecular methods in prosthetic joint infection: sonication fluid better than periprosthetic tissue. Acta Orthop 87:339–345
46. Suda A, Tinelli M, Beisemann ND, Weil Y, Khoury A, Bischel OE (2017) Diagnosis of periprosthetic joint infection using alpha-defensin test or multiplex-PCR: ideal diagnostic test still not found. Int Orthop 41:1307–1313
47. Marin M, Garcia-Lechuz JM, Alonso P, Villanueva M, Alcala L, Gimeno M, Cercenado E, Sanchez-Somolinos (2012) Role of universal 16S rRNA gene PCR and sequencing in diagnosis of prosthetic joint infection. J Clin Microbiol 50:583–589