Aus dem

CharitéCentrum für Orthopädie und Unfallchirurgie

Centrum für Muskuloskeletale Chirurgie

Ärztlicher Direktor: Univ.-Prof. Dr. med. Carsten Perka

Geschäftsführender Direktor: Univ.-Prof. Dr. med. Ulrich Stöckle

Habilitationsschrift

Novel Diagnostic Methods in Periprosthetic Joint Infections

zur Erlangung der Lehrbefähigung

für das Fach Experimentelle Orthopädie und Unfallchirurgie

vorgelegt dem Fakultätsrat der Medizinischen Fakultät

Charité - Universitätsmedizin Berlin

von Dr. med. Nora Renz

Eingereicht: August 2021

Dekan: Prof. Dr. med. Axel R. Pries

1. Gutachter: Prof. Dr. med. Dirk Jäger

2. Gutachter: Prof. Dr. med. Reinhard Hoffmann

Table of contents

Abbreviations

ADLF, alpha defensin lateral flow

AF, aseptic failure

AUC, area under the curve

CFU, colony-forming unit

CRP, C-reactive protein

EBJIS, European Bone and Joint Infection Society

ELISA, enzyme-linked immunosorbent assay

ESR, erythrocyte sedimentation rate

IDSA, Infectious Diseases Society of America

IL-6, interleukin 6

MSIS, Musculoskeletal Infection Society

LDH, lactate dehydrogenase

(m)PCR, (multiplex) polymerase chain reaction

PJI, periprosthetic joint infection

SLIM, synovia like interface membrane

spp., species

WBC, white blood cell

1. Introduction

1.1 Epidemiology of periprosthetic joint infection

Joint replacement is one of the most revolutionary procedures among all surgical interventions. Arthroplasty remains the only treatment for damaged articulations, which is able to completely relieve pain and restore function and thereby improve quality of life of millions of people worldwide. Nowadays already being a frequently performed procedure, the number of implanted prostheses is expected to continue to rise due to increasing life expectancy (1). Despite considerable efforts to prevent periprosthetic joint infections (PJI), the incidence of this severe complication was shown to rise in multiple national registries (2, 3). The absolute number of PJI cases will surely increase due to the growing number of primary implantations being performed and the cumulative time the implants remain in place and consecutively are at risk of infection. Due to heterogeneous definition criteria used, the reported incidence of PJI varies widely among institutions and publications and is probably largely underestimated (4). PJI incidence is reported to be 0.5-2% for primary hip and knee prostheses and slightly higher for shoulder and elbow prostheses (5, 6). The infection rate of revision prostheses is even higher. Since most joint replacements are performed to treat degenerative cartilage damage in patients with osteoarthritis, the affected population is of advanced age and generally holds multiple comorbidities.

1.2 Definition of periprosthetic joint infection

There is no unanimously accepted and validated definition to diagnose PJI in scientific and clinical practice. To date, no single test provides absolute accuracy to confirm PJI.

Classification systems including various diagnostic criteria such as clinical findings, systemic inflammatory markers, leukocyte count and biomarkers in synovial fluid, histopathology of periprosthetic tissue and conventional culture of synovial fluid, periprosthetic tissue and sonication have been proposed and used in different publications (6-12). The two most commonly used classification systems in the United States are the Infectious Disease Society of America (IDSA) - criteria (8), and the Musculoskeletal Infection Society (MSIS) - criteria (9), which have been modified repeatedly (10, 11, 13). Both definitions are not ideal for clinical use, as they are rather specific than sensitive, and a considerable number of low-grade infections is probably missed (10). In clinical practice, the sensitivity should be as high as possible, in order not to miss any PJI.

Criteria published by the Swiss Orthopaedics and Swiss Society of Infectious Diseases were increasingly used across Europe. In September 2017, they were proposed to the European Bone and Joint Infection Society (EBJIS) in a modified version as a working PJI definition for the society, and thereafter these definition criteria have been referred to as “proposed EBJIS criteria” in many studies. In contrast to the MSIS and IDSA criteria, the „proposed EBJIS criteria“ also consider sonication of the removed implant in the diagnosis and use lower cut-off values for synovial fluid leukocyte count, allowing for better detection of low-grade PJI (12). In 2019, the working draft was further modified and finally published in 2021 as “EBJIS criteria” (14). The “proposed EBJIS criteria” were used at the institution, where this work was conducted. Due to the time lag between conduction of the studies and the publication of the definitive EBJIS criteria, the forerunning version of definition criteria served as institutional criteria and were therefore used in all the projects.

Table 1. Different definitions of PJI (modified from (12))

MSIS † (11, 15) (≥1 of the 2 Major Criteria OR ≥3 of 5 Minor Criteria)	**IDSA‡ (8) (≥1 of the Following 4 Criteria)**	**Institutional criteria§ (16) (≥1 of the Following 4 Criteria)**
Major criteria:	Sinus tract communicating with the prosthesis	Purulence around the prosthesis or sinus tract
2 positive periprosthetic cultures	Purulence without other etiology surrounding the prosthesis	Increased synovial fluid leukocyte count
Sinus tract communicating with the prosthesis	Acute inflammation seen on histopathological examination of the periprosthetic tissue	Positive histopathology
Minor criteria:	≥2 intraoperative cultures or combination of preoperative aspiration and intraoperative cultures yielding an indistinguishable organism	Confirmatory microbial growth in synovial fluid, periprosthetic tissue, or sonication culture
Elevated CRP† and ESR (>30 mm/hr)		
Elevated synovial fluid leukocyte count or positive leukocyte esterase strip test (++ or +++)		
Elevated synovial fluid percentage of granulocytes		
A single positive culture		
Positive histological analysis of periprosthetic tissue		

†For the MSIS criteria, elevated CRP was indicated by >10 mg/L in chronic infections or >100 mg/L in acute infections; elevated synovial fluid leukocyte count, by >3,000 leukocytes/μL in chronic infections or >10,000 leukocytes/μL in acute infections; elevated synovial fluid percentage of granulocytes, by >80% in chronic infections or >90% in acute infections; and positive histological analysis of periprosthetic tissue was defined as >5 neutrophils per high-power field (HPF) in 5 HPFs observed on periprosthetic tissue at ×400 magnification.
‡For IDSA, growth of a virulent microorganism (e.g., *Staphylococcus aureus*) in a single specimen of a tissue biopsy or synovial fluid may also represent PJI.
§For the institutional criteria, increased synovial fluid leukocyte count was indicated by a leukocyte count of >2,000/μL or >70% granulocytes; not interpretable within 6 weeks of surgery, in rheumatic joint disease, or after periprosthetic fracture or dislocation. Positive histopathology was defined as a mean of >23 granulocytes per 10 HPF (type II or type III). Confirmatory microbial growth in periprosthetic tissue culture was considered positive if ≥1 specimen was positive in highly virulent organisms or ≥2 specimens showed microbial growth of a low-virulent pathogen, and sonication culture was considered positive if >50 colony-forming units/mL of sonication fluid grew.

1.3 Classification and pathogenesis of periprosthetic joint infection

Traditionally, PJIs are classified according to their temporal appearance as early (< 3 months after surgery), delayed (3–24 months after surgery), and late infections (> 2 years after surgery) (7, 8). Early and delayed infections are mainly exogenously acquired in the perioperative period, whereas most late PJIs are hematogenously acquired. Hematogenous PJI represent a distinct subgroup of PJI and develop by seeding of bacteria during bacteremia, mostly originating from another primary focus or (para-)medical interventions (17). They account for approximately 20-35% of all PJI and present predominantly as acute infections (7, 17, 18). Acute PJI are defined as infections occurring within 3 - 4 weeks after surgery (in case of perioperative infections) or with a symptom duration of less than 3 - 4 weeks in case of late hematogenous infections. PJI presenting after 4 weeks or with symptoms lasting longer than 4 weeks are considered chronic (7, 16). The acuity of infection is particularly relevant in implant-associated infections, as the biofilm age (“maturity”) guides the surgical management.

1.4 Microbiology of PJI

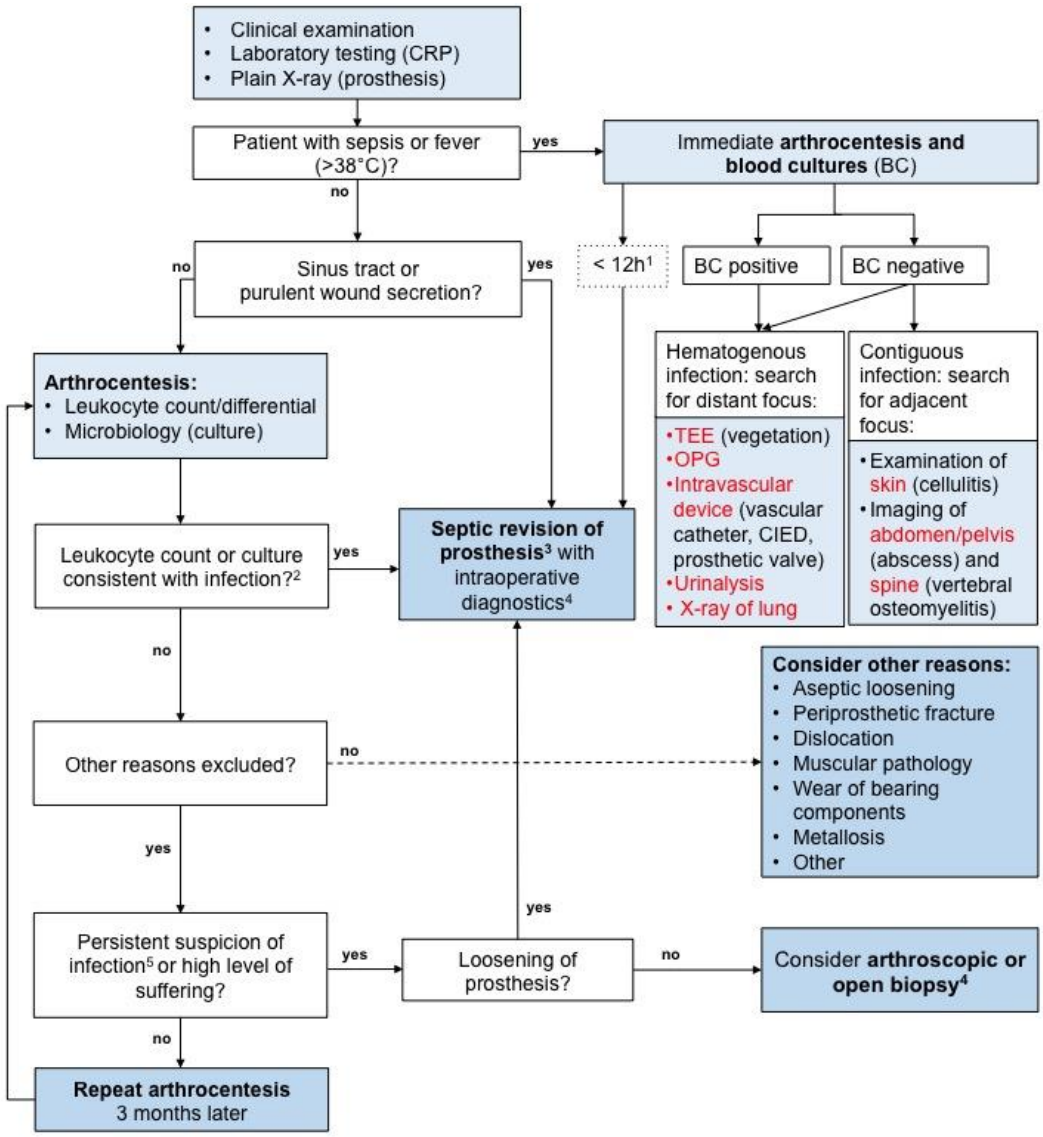
Most pathogens causing PJI originate either from the skin or mucosal surfaces of the oral cavity, urogenital and intestinal tract which represent bacteria of the patient’s microbiome. The majority of bone and joint infections are caused by gram-positive cocci, predominantly by *Staphylococcus* spp. accounting for 50-60% of all infections (6). The remaining percentages are compiled by *Streptococcus* spp, *Enterococcus* spp, gram-positive anaerobes (e.g., *Cutibacterium* spp.) and gram-negative anaerobes (e.g., *Bacteroides* spp.), and other rare pathogens such as fungi,

mycobacteria or obligatory intracellular bacteria (19). The frequency of individual pathogens depends on the pathogenesis of infection, the anatomic location and the time of occurrence in case of postoperative infections. While hematogenous and early postoperative PJI are mainly caused by highly virulent pathogens such as *Staphylococcus aureus*, streptococci and gram-negative rods, the causative agents of delayed perioperative infections inherit a low virulence and include coagulase-negative staphylococci or *Cutibacterium* spp.. They are known to cause low-grade infections which present with subtle clinical signs and symptoms of infection and therefore represent a considerable diagnostic challenge. They are frequently misdiagnosed as aseptic failure (AF).

1.5 Diagnosis of periprosthetic joint infection

In view of the clear clinical findings, the diagnosis of acute PJIs is straightforward with most diagnostic tests inheriting a high sensitivity. In contrast, chronic low-grade infections are difficult to differentiate from aseptic prosthetic failures. Therefore, a comprehensive algorithmic approach combining preoperative and intraoperative results is required to reliably confirm or exclude infection (Figure 1). The management of PJI including diagnostic work-up and treatment require the cooperation of an interdisciplinary team including orthopedic surgeons, infectious diseases specialists, microbiologists and plastic surgeons. Therewith, the treatment outcome can be significantly improved (20, 21). There are numerous errors regarding diagnostic measures, their indication and interpretation, the treating team should be aware of and do everything to avoid (22).

Figure 1. Diagnostic algorithm for periprosthetic joint infection (23)



[1] Surgery should be performed as soon as possible by an experienced orthopedic surgeon
[2] Leukocyte count: >2000/µl leukocytes or >70% granulocytes; microbiology: for highly virulent organisms (e.g. *Staphylococcus aureus, Escherichia coli*) already one positive sample confirms infection, for low-virulent organisms (e.g. *Staphylococcus epidermidis,Cutibacterium acnes*) ≥2 positive samples are required to confirm infection
[3] According to the treatment algorithm for PJI
[4] Leukocyte count/differential, histopathology, microbiology (+/- sonication)
[5] Increased CRP, risk history (prolonged secretion or revision surgery after primary implantation), early loosening of prosthesis
BC: blood cultures, TEE: transesophageal echocardiography, OPG: orthopantomogram, CIED: cardiac implantable electronic device

1.5.1 Preoperative diagnostic methods

Every painful prosthetic joint should be assessed for infection, preferably before revision surgery (16). Initial examinations include clinical examination of the patient,

determination of systemic inflammatory parameters and imaging. Systemic inflammatory parameters such as C-reactive protein (CRP), white blood cell (WBC) count, erythrocyte sedimentation rate (ESR) are neither sufficiently sensitive nor specific for the diagnosis of PJI (19, 24-27). Nevertheless, they represent a relevant puzzle stone in the diagnostic work-up.

In patients presenting with chronic symptoms suggestive for periprosthetic joint infection, the initial focus lies on the preoperative assessment, as ideally, the infection is diagnosed (or excluded) before revision. Knowledge of the cause of failure allows planning the most appropriate treatment strategy. In the preoperative setting, purulent wound secretion and/or sinus tract communicating with the prosthetic joint confirm PJI (8). In this case no further diagnostic measures are needed and revision surgery to complete the diagnostic work-up to identify the pathogen should be scheduled. In absence of confirmatory signs, the most important and sensitive diagnostic measure in the preoperative setting is joint aspiration with a comprehensive analysis of the harvested synovial fluid. It should be performed according to standard aseptic technique, preferably in the operating or intervention room.

Culture of synovial fluid has a relatively low sensitivity, as only planktonic bacteria are detected, and bacteria embedded in the biofilm on the implant surface remain unrecognized (28). The positivity rate of culture considerably depends on the underlying pathogen and its virulence, the acuity of the infection and the administration of antimicrobials before joint aspiration (28). In a recent meta-analysis including 34 studies using heterogeneous definition criteria for PJI, the pooled sensitivity of synovial fluid culture was 72% (29). In studies including more infections caused by low-virulent pathogens such as *Cutibacterium* spp. and coagulase-negative

staphylococci, the sensitivity is even lower (30, 31).

Synovial fluid leukocyte count analysis is more sensitive, as it reflects the host reaction against the microorganisms. The absolute leukocyte count and the differential with a special focus on the percentage of neutrophils are of interest. However, in situations associated with aseptic inflammatory changes of the joint (i.e., healing process in the first 4-6 weeks after surgery, inflammatory changes after trauma, recurrent dislocations and underlying inflammatory arthropathies) the specificity is compromised (12, 32). Furthermore, the optimal cut-off for the diagnosis of infection is subject to debate and numerous cut-offs ranging from 1.000 to 4.200/µl for absolute leukocyte count and 65-80% for neutrophils have been proposed (32-36). Generally, at this stage of the diagnostic algorithm, a test with a high sensitivity is preferred, therefore lower leukocyte count (e.g., 2.000/µl) is advocated, risking “overdiagnosing” rather than underdiagnosing infection. Still, if infection is not confirmed in intraoperative diagnostic tests, which are more accurate, the diagnosis and respective treatment should be revised.

1.5.2 Intraoperative diagnostic methods

Intraoperative diagnostics including periprosthetic tissue histopathology and microbiology as well as sonication fluid culture of the removed implants are more sensitive than preoperative arthrocentesis, as the interface between bone and prosthesis and the implant itself are accessible for diagnostic investigations.

Histopathology of periprosthetic tissue represents a standard procedure in the intraoperative diagnosis of PJI. Granulocytes are detected through immuno-histochemical techniques and validated using histopathological scores. A synovial-like

interface membrane (SLIM) classification for periprosthetic joint infection assessing the histopathological changes in periprosthetic membrane was introduced by Krenn and Morawietz (37). Recently, the CD15 focus score was developed and validated (38). The identification and quantification of neutrophil granulocytes by immunohistochemical detection of antigen CD15 is considered a high-quality method characterised by the ease of use to diagnose infection and subclassify infection into low- and high-grade infection. High diagnostic accuracy with a sensitivity of 91% and specificity of 92% was demonstrated when compared to microbiological findings (39). Analysis of periprosthetic tissue from representative areas (i.e., bone-prosthesis interface and joint pseudocapsule ("neosynovial membrane") is one of the most valuable components in the routine microbiological diagnosis of PJI. At least three to five specimens should be harvested to attain a high diagnostic yield and allow for discrimination of contaminants and real pathogens (8, 40). The sensitivity of intraoperative tissue culture ranges from 65% to 94% (40, 41).

Sonication is a method using low-frequency ultrasound waves to detach the biofilm and its microorganisms from the explanted prosthesis or its modular parts. The resulting sonication fluid can be plated onto solid media (aerobic and anaerobic plates) or inoculated into blood culture bottles (42-44). Sonication was shown to be a useful additional method further improving the pathogen detection rate of intraoperative diagnostics (40). The landmark study on sonication by Trampuz et al. showed a sensitivity of 79% and specificity of 99% when using a cut-off of 50 colony-forming units per millilitre (CFU/ml). It was significantly more sensitive in patients treated with antibiotics within 2 weeks before sampling (75% versus 45%, $p < 0.001$) (42). A recent analysis of the influence of the prosthesis biomaterial on microbial detection by

sonication showed larger bacterial counts after sonication of polyethylene liners than of metal alloys, suggesting intrinsic differences in the ability of microorganisms to form biofilms on various biomaterials (45).

The pathogen detection rate in intraoperatively collected specimens is significantly higher compared to synovial fluid culture in delayed and late infections, in mixed infections and in infections caused by low-virulent pathogens (28).

1.6 Novel diagnostic methods

Diagnostic approaches target either the causative pathogen (i.e., cultures of different specimens) or the inflammatory host response directed against the pathogen (i.e., systemic inflammatory serum markers, leukocyte count in synovial fluid or histopathology of periprosthetic tissue). Routinely used diagnostic tests lack sensitivity and specificity, having led to an enormous effort regarding exploration of novel diagnostic methods in the past decades. Numerous new biomarkers reflecting the host-response to infection were investigated in synovial fluid, among them most promising interleukin-6 (IL-6) (46, 47), calprotectin (48, 49), C-reactive protein (50, 51), alpha defensin (52-59) and leukocyte esterase (60-63) in synovial fluid. Regarding pathogen detection, the focus has been set on molecular diagnostic methods such as polymerase chain reactions detecting fragments of bacterial DNA and next generation sequencing (64-67). Commercially available or home designed multiplex PCR-kits were applied in synovial fluid, periprosthetic tissue and sonication fluid (59, 68-71).

1.6.1 Alpha defensin in synovial fluid

Alpha defensin is an anti-microbial peptide, which is released by neutrophils in response to the presence of bacterial pathogens. It induces depolarization of the microbial cell membrane and thereby has antimicrobial activity against various microorganisms (72). It has been proposed as novel diagnostic biomarker in synovial fluid for the diagnosis of PJI by several research groups (52, 54-56, 73, 74). The accuracy of two test methods using alpha defensin have been extensively investigated in the last decade. Quantitative determination of alpha defensin using an enzyme-linked immunosorbent assay (ELISA) showed high sensitivity (97%-100%) and specificity (95%-100%) (54, 56, 57, 73, 74). However, this test method requires a laboratory facility and has a long turnaround time. To overcome these drawbacks, a qualitative bedside immunoassay test producing results within 10 minutes was designed. With this advantage, this test method gained attention as a potential decision aid during surgery. However, the performance of the qualitative alpha defensin lateral flow (ADLF) test was shown to be poorer than the previously demonstrated observations for the ELISA test with a sensitivity of 67-77% and specificity of 82-94% (53, 59, 75). The accuracy of this novel biomarker was only evaluated against the diagnosis of PJI using MSIS criteria. Its capability of diagnosing low-grade infections has never been assessed.

1.6.2 D-lactate in synovial fluid

D-Lactate is a metabolite, almost exclusively produced by bacteria. Its L-rotatory and D-rotatory isomers are both metabolic products of organisms, however, mammalian cells can produce almost exclusively L-lactate, as they contain only the enzyme L-lactate dehydrogenase (LDH). Consequently, the serum concentration of D-lactate in

humans is barely present (nanomolar to micromolar range) and if present, it is mainly produced by intestinal microbiota (76). In contrast, bacterial species possess both D-LDH and L-LDH enzymes and, therefore, produce measurable amounts of D-lactate and L-lactate. Several studies were carried out to measure the D-lactate concentration in primary sterile body fluids such as cerebrospinal and synovial fluids in order to discriminate infection from aseptic inflammation (77-79). D-lactate was shown to be a promising marker for the diagnosis of bacterial meningitis and native joint arthritis (77, 80). Two methods for D-lactate measurements are available, namely spectrophotometry and chromatography. For the commercial D-lactate test kit, 50 µl of synovial fluid is required, the turnaround time is 30 - 45min. To date, its ability to discriminate septic from aseptic prosthetic failures has never been assessed.

1.6.3 Multiplex polymerase chain reaction (PCR)

Polymerase chain reaction is a non-culture-based method for detection of microbial deoxyribonucleic acid (DNA), which has been investigated extensively for the diagnosis of PJI in the past decade. Two recent meta-analyses reported an overall sensitivity of 69-81% and a specificity of 91-94% (81, 82). Due to a high sensitivity and short turnaround time, it has been considered a reliable and accurate method for detection of PJI (82, 83). In addition, it requires a small amount of biologic material and is not affected by antimicrobial pre-treatment since even non-viable microorganisms are detectable (83). Different PCR assays have been elaborated in recent years. Whereas specific PCR assays are only able to detect predefined microorganisms, broad-range 16S ribosomal DNA PCR is prone to a lower specificity due to DNA contamination and was shown to have a lower sensitivity (81).

Furthermore, it was shown to inherit difficulties detecting polymicrobial infections and requires subsequent sequencing (84-86). Multiplex PCR with a predefined primer set allows simultaneous detection of most common pathogens of a specific infection. Numerous commercial test kits have been developed for pathogen detection in infections of the central nervous system, respiratory tract, gastrointestinal tract or implant and tissue infections. Multiplex PCR assays were investigated in several different specimens for the diagnosis of PJI, showing sensitivities and specificities of 56% and 92%, respectively, for synovial fluid (68), 31% and 100%, respectively, for periprosthetic tissue (59), and 78-96% and 100%, respectively, for sonication fluid (69, 70). Based on previous results of clinical studies evaluating multiplex PCR kits, manufacturers further improved test kits by including additional primers, modifying existing primers and adjusting the detection threshold.

Another advantage of PCR method is the rapid detection of clinically relevant genotypic resistances of identified pathogens to allow for early targeted antimicrobial treatment. However, limited data exists on the performance of multiplex PCR of antimicrobial resistance markers and their correlation with conventional culture (87).

1.7. Aim of the work

Despite considerable efforts in research to improve the accuracy of diagnostic methods, it remains still a challenge to differentiate aseptic prosthetic failures due to inflammatory or mechanical conditions from periprosthetic joint infection. Causing only subtle clinical signs and symptoms, low-grade infections remain often unrecognized and are mistaken for aseptic failures, which entails huge consequences for the patient and the healthcare system. In addition, biofilm-associated infections are difficult to

diagnose, as traditional microbiological tests mainly detect planktonic (i.e., free-floating) and not sessile (i.e., adherent) bacteria in the biofilm. The aim of this work was to assess the diagnostic performance of the aforementioned novel diagnostic tests and estimate their value for the diagnosis of PJI.

2. Results

2.1 Alpha defensin

First, we scrutinized alpha defensin in synovial fluid, a host-specific marker, which was previously extensively tested in cohorts, where infection was defined based on definition criteria with insufficient sensitivity for low-grade infections.

2.1.1 Performance of alpha defensin lateral flow test for the diagnosis of PJI

By using more sensitive diagnostic criteria such as IDSA criteria and proposed EBJIS (i.e., institutional) criteria, we aimed at assessing its performance in general and with a special focus on low-grade infections. In addition, the accuracy of this novel test had never been compared to the leukocyte count, which was a further aim of the work.

The following text corresponds to the abstract from the publication:

Renz N, Yermak K, Perka C, Trampuz A

Alpha defensin lateral flow test for diagnosis of periprosthetic joint infection. Not a screening but a confirmatory test.

J Bone Joint Surg Am. 2018 May 2;100(9):742-5

https://doi.org/10.2106/JBJS.17.01005

"Background: Determination of alpha defensin in synovial fluid has shown promising results for diagnosing periprosthetic joint infection (PJI). The purposes of our study were to assess the performance of alpha defensin lateral flow (ADLF) test for the diagnosis of acute and chronic PJI using 3 classification systems and to compare its performance with the synovial fluid leukocyte count.

Methods: Patients in whom aspiration of a prosthetic hip or knee joint was performed before revision arthroplasty were prospectively included. In addition to standard diagnostic tests, the ADLF test was performed in synovial fluid. Patients were classified as having PJI or aseptic failure according to the definition criteria of the Musculoskeletal Infection Society (MSIS), the Infectious Diseases Society of America (IDSA), and the proposed criteria of the European Bone and Joint Infection Society (EBJIS). The performance of the ADLF test and the leukocyte count was compared using the McNemar chi-square test.

Results: Of 212 included patients, 151 (71%) had a knee prosthesis and 61 (29%) had a hip prosthesis. PJI was diagnosed in 45 patients (21%) using the MSIS criteria, in 55 patients (26%) using the IDSA criteria and in 79 patients (37%) using the proposed EBJIS criteria. The sensitivity of the ADLF test was 84% (95% confidence interval [CI], 71% to 94%) with the MSIS criteria, 67% (95% CI, 53% to 79%) with the IDSA criteria, and 54% (95% CI, 43% to 66%) with the proposed EBJIS criteria. The ADLF test showed high specificity using all classification criteria (96% to 99%) and represented the most specific preoperative test for PJI, especially in the early postoperative period (91%; 95% CI, 59% to 100%). Using the proposed EBJIS definition criteria, the sensitivity of the leukocyte count was significantly higher than that of the ADLF test (86% [95% CI, 76% to 93%] compared with 54% [95% CI, 43% to 66%]; $p < 0.001$), particularly in chronic PJI (81% compared with 44%, respectively; $p < 0.001$).

Conclusions: The ADLF test was rapid and highly specific for diagnosing PJI (>95%). However, its sensitivity was limited (54% to 84%) and it should therefore not be used for screening, but rather as a confirmatory test for PJI."

2.1.2 Comparison of qualitative and quantitative alpha defensin test

In the second article, we compared the performance of qualitative (ADLF) and quantitative alpha defensin (ELISA) tests.

The following text corresponds to the abstract from the publication:

Sigmund IK, Yermak K, Perka C, Trampuz A, Renz N

Is the enzyme-linked immunosorbent assay more accurate than the lateral flow alpha defensin test for diagnosing periprosthetic joint infection?

Clin Orthop Relat Res. 2018 Aug; 476(8):1645-1654

https://doi.org/10.1097/CORR.0000000000000336

“Background: Alpha defensin was proposed as a new biomarker in synovial fluid for the diagnostic workup of failed joint prostheses. To our knowledge, no comparative study of the performance of the quantitative enzyme-linked immunosorbent assay (ELISA) and qualitative lateral flow alpha defensin test has been reported.

Questions/purposes: (1) Using the proposed European Bone and Joint Infection Society (EBJIS) criteria for defining periprosthetic joint infection (PJI), is there a difference in the diagnostic accuracy of quantitative ELISA and qualitative lateral flow alpha defensin tests? (2) Is there a difference in the performance of the two alpha defensin tests when using three definition classification systems (Musculoskeletal Infection Society [MSIS], Infectious Diseases Society of America [IDSA], and proposed EBJIS)?

Methods: In this retrospective study of samples collected earlier as part of a related longitudinal study, we included patients in whom aspiration of the prosthetic hip or knee was performed as routine investigation before every revision arthroplasty. Between October 2016 and April 2017, a total of 73 patients were eligible for inclusion. As a result of an insufficient fluid volume for analysis (< 5 mL), two patients were excluded. Among the 71 patients in the final analysis, 54 had a knee and 17 a hip arthroplasty. Using the proposed EBJIS criteria, PJI was diagnosed in 22 patients (31%) and aseptic failure in 49 (69%). The alpha defensin ELISA and lateral flow tests were performed in synovial fluid. Patients were classified as having PJI or aseptic failure using the MSIS, the IDSA, and the proposed EBJIS criteria. Sensitivity and specificity of ELISA and the lateral flow alpha defensin test were calculated. Based on receiver operating characteristic analysis, area under the curve values were compared.

Results: When measured against the proposed EBJIS criteria, the sensitivity of alpha defensin ELISA and the lateral flow test was low and not different from one another with the numbers available at 50% (95% confidence interval [CI], 31%-69%) and 46% (95% CI, 27%-65%; p = 0.857), respectively, whereas both methods showed high specificity (98% [95% CI, 88%-100%]; p = 1.000). For sensitivity, the highest values were seen when compared against the MSIS criteria (ELISA: 85% [95% CI, 56%-97%], lateral flow: 77% [95% CI]; p = 0.871), intermediate with IDSA criteria (ELISA: 73% [95% CI, 48%-89%], lateral flow: 67% [95% CI]; p = 0.867), and lowest with proposed EBJIS criteria (ELISA: 50% [95% CI, 31%-69%], lateral flow: 46% [95% CI]; p = 0.763). Specificity, however, was high regardless of the criteria used, where ELISA and lateral flow produced results that were not different (MSIS: 98% [95% CI, 90%-100%], IDSA:

98% [95% CI, 90%-100%], EBJIS: 98% [95% CI, 88%-100%]; p = 1.000). The area under the curve of alpha defensin ELISA and the lateral flow test was similar, regardless of the definition criteria used (EBJIS: p = 0.566; IDSA: p = 0.425; MSIS: p = 0.339).

Conclusions: There is no difference between the quantitative and qualitative alpha defensin test for confirmation of PJI, irrespective of applied definition criteria. Having the advantage of providing results within 10 minutes without the need for a laboratory facility, the qualitative test may be of interest in the intraoperative setting, however, at a cost of higher test expense."

2.2 D-lactate

2.2.1 Performance of D-lactate in synovial fluid

Secondly, we evaluated the diagnostic performance of D-lactate - a pathogen-specific biomarker - in synovial fluid and aimed to discover potential factors influencing the accuracy of this test.

The following text corresponds to the abstract from the publication:

Yermak K, Karbysheva S, Perka C, Trampuz A, Renz N

Performance of synovial fluid D-lactate for the diagnosis of periprosthetic joint infection: A prospective observational study.

J Infect. 2019 Aug;79(2):123-129

https://doi.org/10.1016/j.jinf.2019.05.015

“Objectives: Synovial fluid leukocyte count is the current standard test for diagnosing periprosthetic joint infection (PJI). As D-lactate is almost exclusively produced by bacteria, it represents a useful biomarker for bacterial infection. We evaluated the performance of synovial fluid D-lactate for the diagnosis of PJI and compared it with the synovial fluid leukocyte count.

Methods: Consecutive patients with joint aspiration of a prosthetic hip, knee or shoulder joint were prospectively included. PJI was diagnosed according to the working criteria of the European Bone and Joint Infection Society (EBJIS). The synovial fluid D-lactate was determined spectrophotometrically at 570 nm, synovial fluid leukocytes were counted by flow cytometry. The receiver operating characteristic

(ROC) analysis was performed to assess the diagnostic performance of investigated parameters.

Results: Of 148 patients, 44 (30%) were diagnosed with PJI and 104 (70%) with aseptic failure. For diagnosis of PJI, the sensitivity of synovial fluid D-lactate (at cut-off 1.263 mmol/l) was 86.4% [95% CI, 75.0-95.5%] and the specificity was 80.8% [95% CI, 73.1-88.5%]. The AUCs of D-lactate concentration and leukocyte count were 90.3% [95% CI 85.7-95.0%] and 91.0% [95% CI 85.1-96.8%], respectively ($p = 0.8$). Virulence of the pathogen did not influence the D-lactate concentration ($p = 0.123$). The synovial fluid erythrocyte concentration correlated with D-lactate in patients with aseptic failure ($\rho = 0.339$, $p < 0.01$).

Conclusion: Synovial fluid D-lactate showed similar performance to the leukocyte count for diagnosis of PJI. Advantages of D-lactate test are requirement of low synovial fluid volume, short turnaround time and low cost.”

2.2.2 Performance of D-lactate in synovial fluid with different classifications

Furthermore, we aimed at defining the optimal cut-off of this metabolite using different definition criteria for the diagnosis of PJI and analysed the influence of the causing pathogen on the detected D-lactate level.

The following text corresponds to the abstract from the publication:

Karbysheva S, Yermak K, Grigoricheva L, Perka C, Renz N, Trampuz A

Synovial Fluid d-Lactate-A Novel Pathogen-Specific Biomarker for the Diagnosis of Periprosthetic Joint Infection.

J Arthroplasty. 2020 Aug;35(8):2223-2229.e2

https://doi.org/10.1016/j.arth.2020.03.016

“Background: Synovial fluid d-lactate may be useful for diagnosing periprosthetic joint infection (PJI) as this biomarker is exclusively produced by bacteria. We evaluated the performance of synovial fluid d-lactate using 2 definition criteria and determined its optimal cutoff value for diagnosing PJI.

Methods: Consecutive patients undergoing joint aspiration before prosthesis revision were prospectively included. Synovial fluid was collected for culture, leukocyte count, and d-lactate concentration (by spectrophotometry). Youden's J statistic was used for determining optimal d-lactate cutoff value on the receiver operating characteristic curve by maximizing sensitivity and specificity.

Results: A total of 224 patients were included. Using Musculoskeletal Infection Society criteria, 71 patients (32%) were diagnosed with PJI and 153 (68%) with aseptic failure (AF), whereas using institutional criteria, 92 patients (41%) were diagnosed with PJI and 132 (59%) with AF. The optimal cutoff of synovial fluid d-lactate to differentiate PJI from AF was 1.3 mmol/L, independent of the used definition criteria. Synovial fluid d-lactate had a sensitivity of 94.3% (95% confidence interval [95% CI], 86.2-98.4) and specificity of 78.4% (95% CI, 66.8-81.2) using Musculoskeletal Infection Society criteria, whereas its sensitivity was 92.4% (95% CI, 84.9-96.9) and specificity 88.6% (95% CI, 81.9-93.5) using institutional criteria. The concentration of d-lactate was higher in infections caused by Staphylococcus aureus ($P < .001$) and streptococci ($P = .016$) than by coagulase-negative staphylococci or in culture-negative PJI.

Conclusion: The synovial fluid d-lactate showed high sensitivity (>90%) for diagnosis of PJI using both definition criteria and correlated with the pathogen virulence. The high sensitivity makes this biomarker useful as a point-of-care screening test for PJI."

2.3 Multiplex Polymerase chain reaction (PCR)

Third, we were interested in the performance of a commercially available multiplex PCR test kit in different specimens. Previous generations of this fully automated test with a short turnaround time (compared to conventional culture) were analysed by other researchers and the test was further improved.

2.3.1 Multiplex PCR in synovial fluid

The purpose of the first study was to assess the ability of pathogen detection of multiplex PCR in the preoperative setting, i.e., in synovial fluid.

The following text corresponds to the abstract from the publication:

Morgenstern C, Cabric S, Perka C, Trampuz A, Renz N

Synovial fluid multiplex PCR is superior to culture for detection of low-virulent pathogens causing periprosthetic joint infection.

Diagn Microbiol Infect Dis. 2018 Feb;90(2):115-119.

https://doi.org/10.1016/j.diagmicrobio.2017.10.016

“Introduction: Analysis of joint aspirate is the standard preoperative investigation for diagnosis of periprosthetic joint infection (PJI). We compared the diagnostic performance of culture and multiplex polymerase chain reaction (PCR) of synovial fluid for diagnosis of PJI.

Patients and Methods: Patients in whom aspiration of the prosthetic hip or knee joint was performed before revision arthroplasty were prospectively included. The performance of synovial fluid culture and multiplex PCR was compared by McNemar's chi-squared test.

Results: A total of 142 patients were included, 82 with knee and 60 with hip prosthesis. PJI was diagnosed in 77 patients (54%) and aseptic failure in 65 patients (46%). The sensitivity of synovial fluid culture and PCR was 52% and 60%, respectively, showing concordant results in 116 patients (82%). In patients with PJI, PCR missed 6 highvirulent pathogens (*S. aureus*, streptococci, *E. faecalis, E. coli*)
which grew in synovial fluid culture, whereas synovial fluid culture missed 12 pathogens detected by multiplex PCR, predominantly low-virulent pathogens (*Cutibacterium acnes* and coagulase-negative staphylococci). In patients with aseptic failure, PCR detected 6 low-virulent organisms (predominantly *C. acnes*).

Conclusion: While the overall performance of synovial fluid PCR was comparable to culture, PCR was superior for detection of low-virulent bacteria such as *Cutibacterium* spp. and coagulase-negative staphylococci. In addition, synovial fluid culture required several days for growth, whereas multiplex PCR provided results within 5 hours in an automated manner.”

2.3.2 Multiplex PCR in sonication fluid

The second study investigated the accuracy of multiplex PCR in the intraoperative setting, i.e., in sonication fluid of retrieved prostheses.

The following text corresponds to the abstract from the publication:

Renz N, Feihl S, Cabric S, Trampuz A

Performance of automated multiplex PCR using sonication fluid for periprosthetic joint infection: a prospective cohort.

Infection. 2017 Dec; 45(6):877-884

https://doi.org/10.1007/s15010-017-1073-5

"Purpose: Sonication of explanted prostheses improved the microbiological diagnosis of periprosthetic joint infections (PJI). We evaluated the performance of automated multiplex polymerase chain reaction (PCR) using sonication fluid for the microbiological diagnosis of PJI.

Methods: In a prospective cohort using uniform definition criteria for PJI, explanted joint prostheses were investigated by sonication and the resulting sonication fluid was analyzed by culture and multiplex PCR. McNemar's Chi-squared test was used to compare the performance of diagnostic tests.

Results: Among 111 patients, PJI was diagnosed in 78 (70%) and aseptic failure in 33 (30%). For the diagnosis of PJI, the sensitivity and specificity of periprosthetic tissue culture was 51 and 100% of sonication fluid culture 58 and 100%, and of

sonication fluid PCR 51 and 94%, respectively. Among 70 microorganisms, periprosthetic tissue culture grew 52 (74%), sonication fluid culture grew 50 (71%) and sonication fluid PCR detected 37 pathogens (53%). If only organisms are considered, for which primers are included in the test panel, PCR detected 37 of 58 pathogens (64%). The sonication fluid PCR missed 19 pathogens (predominantly oral streptococci and anaerobes), whereas 7 additional microorganisms were detected only by PCR (including *Cutibacterium* spp. and coagulase-negative staphylococci).

Conclusions: The performance of multiplex PCR using sonication fluid is comparable to culture of periprosthetic tissue or sonication fluid. The advantages of PCR are short processing time (< 5 h) and fully automated procedure. However, culture technique is still needed due to the low sensitivity and the need of comprehensive susceptibility testing. Modification of primers or inclusion of additional ones may improve the performance of PCR, especially of low-virulent organisms."

2.3.3 Genotypic resistance testing with multiplex PCR

Furthermore, we were interested in the ability of detecting resistance genes by PCR, which may have an impact on the accuracy of early antimicrobial treatment. Therefore, we compared phenotypic and genotypic resistance testing of 3 prospective cohorts including osteosynthesis-associated infections, septic arthritis and PJI (31, 88-90).

The following text corresponds to the abstract from the publication:

Sigmund IK*, Renz N* (equal contribution), Feihl S, Morgenstern C, Cabric S, Trampuz A

Value of multiplex PCR for detection of antimicrobial resistance in samples retrieved from patients with orthopaedic infections.

BMC Microbiology. (2020) 20:88

https://doi.org/10.1186/s12866-020-01741-7

“Background: The performance of multiplex PCR (mPCR) for detection of antimicrobial resistance from clinical isolates is unknown. We assessed the ability of mPCR to analyse resistance genes directly from clinical samples. Patients with orthopedic infections were prospectively included. Phenotypical and genotypical resistance was evaluated in clinical samples (synovial and sonication fluid) where identical pathogens were identified by culture and mPCR.

Result: A total of 94 samples were analysed, including 60 sonication fluid and 34 synovial fluid samples. For coagulase-negative staphylococcus strains, mPCR detected resistance to oxacillin in 10 of 23 isolates (44%) and to rifampin in none of 6

isolates. For S. aureus isolates, detection rate of oxacillin and rifampin-resistance was 100% (2/2 and 1/1, respectively). Fluoroquinolone-resistance was confirmed by mPCR in all 3 isolates of Enterobacteriaceae, in enterococci resistance to aminoglycoside-high level was detected in 1 of 3 isolates (33%) and in streptococci resistance to macrolides/lincosamides in none of 2 isolates. The overall sensitivity for different pathogens and antimicrobials was 46% and specificity 95%, the median concordance was 80% (range, 57-100%). Full agreement was observed for oxacillin in *S. aureus*, vancomycin in enterococci, carbapenems/cephalosporins in Enterobacteriaceae and rifampin in *Cutibacterium* species.

Conclusion: The overall sensitivity for detection of antimicrobial resistance by mPCR directly from clinical samples was low. False-negative mPCR results occurred mainly in coagulase-negative staphylococci, especially for oxacillin and rifampin. However, the specificity of mPCR was high and a positive result reliably predicted antimicrobial resistance. Including universal primers in the PCR test assay may improve the detection rate but requires additional sequencing step."

3. Discussion

Knowledge of the underlying cause for prosthetic failure is of utmost importance and considerably influences the treatment and its outcome. Since medical and surgical treatment strategies of septic and aseptic failures vary widely, infection should be confirmed or excluded preferably prior to revision surgery. As to date no reliable diagnostic test exists to provide this crucial information, the scientific community made huge efforts to find a novel discriminative test. Conventional tests such as leukocyte count and culture of synovial fluid were shown to have limited accuracy in specific situations, such as low-grade infections, in case of early postoperative period, metallosis or in patients with underlying inflammatory conditions (e.g., rheumatic disease) (12, 28, 32). In the past decade, several new biomarkers have been investigated. However, none of them was exclusively assessed regarding its ability to detect low-grade and early postoperative infections, which belong to the most challenging entities regarding diagnosis.

A further drawback of current scientific data focusing on diagnostic tests is the lack of uniformly accepted definition criteria and the insensitive character of proposed definition criteria by different societies. While in clinical practice, the sensitivity should be as high as possible in order not to miss any PJI, formally, definition criteria should have a very high specificity for clinical studies to permit a reliable comparison of published results. However, by using highly specific definition criteria in scientific analyses of diagnostic tests, low-grade infections are neglected. This contrasts the need of a discriminative test particularly in this specific and difficult-to-identify subgroup of PJI.

Therefore, the aim of this work was to evaluate novel diagnostic approaches with a special focus on low-grade infections. This information is only gained by using more sensitive definition criteria such as the criteria used in our institution, where all the studies were carried out. These criteria served as a basis for the development of the EBJIS criteria, which were published 2021 after several modifications (14). For the interpretation of the results and the implementation of the new information into the clinical practice, one should consider, that septic failures are rather “overdiagnosed” than underdiagnosed, and test performance is rather underestimated than overestimated.

3.1 Alpha defensin in synovial fluid

In our prospective cohort study on prosthetic failures, we found a considerable difference of the alpha defensin test sensitivity depending on the applied definition criteria. The more sensitive the applied definition criteria were, the less sensitive was the ADLF test (sensitivity of 84% with MSIS vs. 54% with institutional criteria). This observation may be explained by the assumption, that institutional criteria to a larger extent include also chronic low-grade infection compared to MSIS criteria. This hypothesis was corroborated by the sub-analysis of the number of episodes reflecting different situations according to their acuity. Whereas MSIS criteria only confirmed 26 chronic PJI cases (i.e., symptom duration of more than 4 weeks), institutional criteria identified 59 chronic PJI. For acute infections (symptom duration of less than 4 weeks), there was no considerable difference (16 with MSIS and 17 with institutional criteria). The test sensitivity of ADLF was significantly lower in chronic infections that in acute PJI when applying institutional criteria (44% vs. 88%). This concludes that the test is

not useful to reliably detect or screen for chronic low-grade infections. Furthermore, this observation explains the difference regarding test sensitivity reported in previous studies ranging from 67-77% (53, 59, 75). However, due to the high specificity irrespective of the applied definition criteria (96-99%), it may be used as a rule in, i.e., confirmatory test. Whereas overall, the ADLF test had an inferior sensitivity compared to leukocyte count (86% vs. 54%), it was found to be more specific for infection, especially in the early postoperative setting. Infections within the first 6 weeks after surgery are another diagnostic challenge, as the healing process may mimic infection due to the physiologically high leukocyte count. In the subgroup analysis of this subgroup ADLF demonstrated a significantly higher specificity than synovial fluid leukocyte count. Consecutively, in this distinct setting of prolonged wound discharge, ADLF may be helpful to discriminate early infection from aseptic conditions. This is a relevant new finding, as to date, there is no accurate diagnostic test to confirm PJI at this early stage (19). There are reports of false-positive results in case of metallosis lowering specificity of ADLF in this specific setting (73, 91).

Advantages of ADLF test as a bedside test are the easy use without need of a laboratory facility, the rapid turnaround time (approximately 10 minutes) and the lack of interference with previous antimicrobial treatment or blood contamination (54, 55). The latter is an important advantage compared to the leukocyte esterase strip test. Leukocyte esterase strip test was proposed by several authors, however, its use is limited by the inconclusive reading of the test result in case of admixed blood in synovial fluid (60, 62, 63).

The ADLF test was developed based on the results of its forerunner, the laboratory based, quantitative alpha defensin ELISA test. Previous studies showed high accuracy

with 97-100% sensitivity and 95-100% specificity (52, 56, 57, 73), which is higher to the performances shown for the qualitative ADLF test. However, no comparative study of the two test modalities in the same patient population has been performed to date. In our analysis, there was no significant difference between qualitative and qualitative alpha defensin test for the diagnosis of PJI, irrespective of the used definition criteria. In situations with inconclusive preoperative diagnostic work-up, a reliable intraoperative test is needed. The ELISA test is not suitable due to the need of a laboratory infrastructure and long turnaround time. Therefore, the rapid ADLF test was developed to provide results within minutes. However, the costs are considerably (approximately sevenfold) higher and only positive results should guide further treatment strategies (based on the high specificity). The ELISA test may be of advantage as a cost-saving approach especially in the pre-operative setting, where the turnaround time is negligible. However, both tests cannot be used as a single test to exclude PJI and cannot replace leukocyte count in synovial fluid in clinical routine.

3.2 D-lactate

In our two prospective studies we report first observations of and experiences with D-Lactate as pathogen-specific diagnostic test for detection of PJI. Previous analysis investigated its value for discrimination of septic and aseptic conditions in native joints and reported a sensitivity of 86% and specificity of 96% (79). The performance in prosthetic joints, which are more commonly affected by low-grade infections caused by low-virulent pathogens than native joints, was unknown. The optimal cut-off for the diagnosis of PJI was 1.3mmol/l in both our cohorts, irrespective of the used definition criteria. Because D-lactate is determined quantitatively and is not a dichotomous

positive/negative test, the physician is allowed to decide whether to use it as sensitive (screening, “rule out”) or more specific (confirmatory, “rule in”) test by applying different cut-off values.

The elaborated sensitivity was 86-92% with a specificity of 82-89%. Compared to leukocyte count -the gold standard host-specific test in synovial fluid- D-lactate was numerically more sensitive (86% vs. 80%) but less specific (82% vs. 89%). This trend was observed predominantly in chronic infections. Both tests were more sensitive but less specific in early infections than in delayed and late infections.

The low specificity was unexpected, as the marker is almost exclusively a product of bacterial metabolism. Based on the positive correlation between erythrocytes and D-lactate in the aseptic group, false-positive D-lactate test due to the similar absorbance wave lengths of hemoglobin (i.e., 540nm for hemoglobin and 570nm for D-lactate) is hypothesized. The poorer specificity in the early postoperative period might corroborate this assumption, as in this setting residual blood in the operated joint is present. Pre-test centrifugation of the sample might improve the specificity, however, we did not elaborate this hypothesis in our study. Other explanations for false-positive D-lactate test may be short bowel syndrome and severe uncontrolled diabetes mellitus with insulin deficiency (92). Due to incomplete assessment of underlying comorbidities, we could not elaborate these potential confounding factors. Therefore, further studies exploring interfering conditions which explain the limited specificity are needed.

In previous reports, D-lactate production was described for several bacterial species including *Staphylococcus* spp., *Streptococcus* spp., Enterobacterales and *Bacteroides fragilis* as well as for Lactobacillales and gut microbiota (77, 93, 94). In PJI caused by high-virulent bacteria such as *S. aureus* and streptococci, D-lactate

concentration was higher as compared with PJI caused by low-virulent pathogens (i.e., coagulase-negative staphylococci), and no difference was observed comparing low-grade PJIs and culture-negative infections. The D-lactate concentration most likely mirrors the virulence of the pathogen and the microbial burden.

Of note, increased D-lactate concentration (2.7 mmol/L) was detected in one patient with PJI caused by *Candida parapsilosis*, although D-lactate is assumed to be specific for bacteria. It was previously hypothesized, that local oxygen limitation may lead to alcoholic fermentation in yeasts with resulting production of glycerol, pyruvate, and D-lactate (95). Due to the low number of fungal PJI included in our analyses, the value of D-lactate in this setting could not be explored. Further studies are needed.

3.3 Multiplex PCR

Knowledge of the causing pathogen is crucial not only for antimicrobial treatment but also to determinate the best available treatment strategy. Many experts proclaim the necessity of isolation of the microbial agent prior to revision surgery and suggest repeating arthrocentesis until the pathogen is identified. As culture of synovial fluid has only limited sensitivity (7), molecular diagnostics gained attention in recent years.

In our comparison of culture and PCR of synovial fluid harvested in preoperative arthrocentesis, we found a similar performance of both tests with a poor sensitivity (52-60%) but high specificity (89-98%) and a concordance of results in 82% (116 of 142 episodes). The short turnaround time of 5 hours represents a valuable advantage in this context. By showing improved detection of low-virulent pathogens in synovial fluid using multiplex PCR, we revealed another benefit since low-virulent pathogens such as *Cutibacterium* spp. or coagulase-negative staphylococci usually require

longer incubation periods due to their slow growth (30). In contrast, the missed high-virulent pathogens indeed represent a concern. Whereas the missed identification of *Streptococcus mitis/oralis* was expected due to the lack of a corresponding primer in the test kit, the failure to detect the other missed isolates (*S. aureus, E. coli, E. faecalis)* is not explained.

In addition, the poorer specificity, i.e., higher false-positive rate, when compared to the gold standard of culture remains unclear. The challenge of positive microbiological findings in presumed aseptic patients has been described in several previous studies involving different medical devices (42, 96, 97). Whether the positive result represents contamination in the laboratory or true “silent” colonization with development of clinical symptoms at a later stage is unclear.

By combining two novel methods for pathogen detection, i.e., dislodgement of the biofilm by sonication and consecutive analysis of the resulting fluid by multiplex PCR, we expected an improvement of the pathogen detection rate. However, the sensitivity of sonication fluid PCR was comparable to culture of sonication fluid (51% vs. 58%). These results are in line with a similar recent cohort study assessing the same multiplex PCR test kit (50%) (98). The limited sensitivity may be explained by the multiplex PCR design, which allows only detection of pathogens, for which a primer is included in the test kit. Therefore, by only considering organisms, for which primers are included in the test kit, we additionally calculated the analytical performance, which showed a numerically higher detection rate of 64% (as compared to 53%). To overcome this test-set-up specific drawback of the test, an added universal primer for broad-range 16S DNA could further increase the detection rate of the PCR kit. However, this method requires an additional step for pathogen identification of the

isolated pathogen (e.g., sequencing). Also detecting DNA from nonviable organisms, we expected the PCR method to show a higher sensitivity than in conventional cultures. One explanation for this could be the detection limit of the PCR method, estimated at ~ 10^4 DNA pathogen fragments/ml of specimen. In chronic, low-grade PJI with a low bacterial burden, this threshold value might not be reached. Therefore, we (2017, p.882) suggested, that "*additional bacterial DNA concentration methods in sonication fluid other than centrifugation (i.e., filtration of sonication fluid, DNA binding) may increase the sensitivity of PCR and modified preanalytical processing to concentrate DNA should be systematically tested in future studies*" (89).

From the clinical standpoint, early pathogen identification and knowledge of its susceptibility is of considerable relevance in order to optimize the antibiotic treatment and therewith avoid development of resistance. In our analysis including isolates of 4 cohorts, the overall sensitivity of genotypic resistance detection for all pathogens was 46% and its specificity 95% with a median concordance of 80%. Whereas the genotypic and phenotypic resistances for rifampin and oxacillin corresponded fully for *S. aureus* isolates, the sensitivity was poor in coagulase-negative staphylococci (0% and 44%). We (2020, p. 5) assume, that "*the limited detection of oxacillin resistance in coagulase-negative staphylococci by mPCR may be explained by the low microbial burden usually seen in low-grade infections caused by less virulent staphylococci, which probably does not reach the detection limit of the mPCR system of mecA and mecC, estimated at ~ 104 and ~ 106 DNA copies/ml, respectively. This hypothesis is corroborated by the fact, that the concordance in infections caused by S. aureus – usually acute infections - was considerably higher. Furthermore, a great diversity of the staphylococcal cassette chromosome mec in coagulase-negative staphylococci*

may contribute to a reduced detection rate of oxacillin resistance (99)" (100). In terms of rifampin resistance, which is clinically highly relevant due to its impact on the surgical strategy, the results were disappointing for coagulase-negative staphylococci. However, the test kit is not validated for rifampin susceptibility testing for this subgroup of staphylococci. When analysing only staphylococci, for which the system is authenticated (i.e., *S. aureus*), a high concordance (93%) with a negative predictive value of 100% was obtained. Analogously, fluoroquinolones are used to treat biofilms in implant-associated infections due to gram-negative bacteria (101). For this constellation (tested for *E. coli* in our analysis) the concordance was satisfying with an agreement of 89% with a negative predictive value of 100%.

The high specificity of genotypical resistance testing (95%) is of clinical relevance, as a positive test result corresponds mostly with resistance and may confirm a difficult to treat infection (i.e., caused by a pathogen resistant to biofilm-active treatment) which requires implant removal. In addition, it justifies the addition of toxic vancomycin in case of infection caused by oxacillin-resistant staphylococci.

4. Summary and outlook

The prospective evaluation of alpha defensin and D-lactate in synovial fluid, and multiplex PCR in synovial and sonication fluid in patients with prosthetic failure showed no novel test to be superior compared to standard diagnostic tests (Table 2). Based on our results, we do not recommend using the novel biomarker alpha defensin as a routine screening test, as recently confirmed by other authors (102). Considering its poorer performance and the higher costs, the leukocyte count analysis in synovial fluid

cannot be replaced by this test. However, in specific situations, e.g., if the high leukocyte count is not interpretable due to underlying rheumatologic diseases or the healing process in the early postoperative setting, it can be used as a confirmatory test. We did not find a difference regarding the test accuracy when comparing the bedside lateral flow test and the laboratory ELISA test. The choice should be made taking expenses, turnaround time and feasibility/infrastructure into account.

Table 2. Summary of all studies showing results of conventional (white) and novel (grey) diagnostic tests using institutional criteria and other definition criteria (MSIS and IDSA). If results of multiple cohorts are available, mean (and range) are shown.

		Institutional criteria		**Other criteria**	
Specimen	**Diagnostic test**	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Synovial fluid	Absolute leukocyte count	**80** (73-88)	**95** (91-98)	**85**	**93**
	% Granulocytes	**71** (57-83)	**97** (92-100)	**81**	**95**
	Leukocyte count (combined)	**84** (80-86)	**92** (89-96)	-	-
	Alpha defensin ELISA	**50**	**98**	**79** (73-85)	
	Alpha defensin ADLF	**50** (46-54)	**99** (98-99)	**76** (67-84)	**96**
	D-lactate	**89** (86-92)	**86** (82-89)	**94**	**78**
	Culture	**56** (46-69)	**98** (97-100)	-	**-**
	Multiplex PCR	**60**	**89**	-	**-**
Periprosth. tissue	Culture	**46** (42-51)	**99** (96-100)	**79**	**100**
	Histopathology	**76** (72-80)	**100**	**88**	
Sonication	Culture	**59** (44-71)	**97** (91-100)	-	-
	Multiplex PCR	**51**	**94**	-	-

D-lactate seems to be a promising and accurate diagnostic test for the diagnosis of PJI. Its performance is comparable to the one of leukocyte count in synovial fluid. The short turnaround time, the low amount of required synovial fluid and the low cost are advantages of this novel biomarker. The causes for the low specificity should be further investigated and based on new findings the assay should be further improved. Alternative determination methods such as chromatographic or electrochemical processes may improve the discrimination. Due to its high sensitivity but low specificity the test may be used in combination with a highly specific test.

Multiplex PCR in sonication fluid and synovial fluid showed a comparable performance to conventional cultures, having the advantage of being fully automated and providing results within 5 h. It demonstrated a superior detection rate for low-virulent pathogens in synovial fluid. Improvement of the primer setup, especially for additional species of oral streptococci and anaerobes, and detection threshold for low-virulent pathogens may increase the performance of PCR. The overall sensitivity for detection of antimicrobial resistance by mPCR directly from clinical samples was low. False-negative mPCR results occurred mainly in coagulase-negative staphylococci, especially for oxacillin and rifampin. However, the specificity of mPCR was high and a positive result reliably predicts resistance and for some pathogens and antibiotics full agreement was observed. Including universal primers in the PCR test assay may improve the detection rate but requires additional sequencing step.

Based on our results, multiplex PCR cannot replace conventional culture due to low sensitivity and the need of reliable susceptibility testing.

Novel innovations focus on other culture-independent technologies such as next-generation sequencing, which has shown great promise in the diagnosis of PJI and might be used as an effective tool for the identification of pathogens (64, 67, 103). With the advantage of massively parallel DNA sequencing technologies, it has become possible to rapidly and comprehensively sequence all the microbial genetic material within a clinical sample (metagenomics). Pooled sensitivity and specificity of a recent meta-analysis were reported at 81% and 94%, respectively (103). However, its value for detection of pathogens of low-grade infections and its drawbacks are yet to be determined.

Indeed, the use of highly sensitive definition criteria to confirm PJI in our institution for clinical practice and studies has a considerable impact on the performance of diagnostic tests. By using less sensitive criteria, i.e. MSIS or IDSA criteria, the sensitivity of tests increased at cost of a lower specificity (see Table 2). This fact is probably the main reason for discrepant results of our analyses compared to previous studies. However, the impact of this observation is highly relevant in light of our goal to focus on low-grade infections. Based on our results, the test accuracy of every new test should be interpreted with regard to the used definition criteria. In our analyses, none of the novel tests outperformed the current standard tests.

5. References

1. Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. The Journal of bone and joint surgery American volume. 2007;89(4):780-5.

2. Dale H, Fenstad AM, Hallan G, Havelin LI, Furnes O, Overgaard S, et al. Increasing risk of prosthetic joint infection after total hip arthroplasty. Acta Orthop. 2012;83(5):449-58.

3. Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J. Economic burden of periprosthetic joint infection in the United States. The Journal of arthroplasty. 2012;27(8 Suppl):61-5.e1.

4. Gundtoft PH, Overgaard S, Schonheyder HC, Moller JK, Kjaersgaard-Andersen P, Pedersen AB. The "true" incidence of surgically treated deep prosthetic joint infection after 32,896 primary total hip arthroplasties: a prospective cohort study. Acta Orthop. 2015;86(3):326-34.

5. Kapadia BH, Berg RA, Daley JA, Fritz J, Bhave A, Mont MA. Periprosthetic joint infection. The Lancet. 2015.

6. Tande AJ, Patel R. Prosthetic joint infection. Clin Microbiol Rev. 2014;27(2):302-45.

7. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med. 2004;351(16):1645-54.

8. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, et al. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis. 2013;56(1):e1-e25.

9. Parvizi J, Zmistowski B, Berbari EF, Bauer TW, Springer BD, Della Valle CJ, et al. New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society. Clin Orthop Relat Res. 2011;469(11):2992-4.

10. Parvizi J, Tan TL, Goswami K, Higuera C, Della Valle C, Chen AF, et al. The 2018 definition of periprosthetic hip and knee infection: An evidence-based and validated criteria. J Arthroplasty. 2018;33(5):1309-14 e2.

11. Parvizi J, Gehrke T, International Consensus Group on Periprosthetic Joint I. Definition of periprosthetic joint infection. The Journal of arthroplasty. 2014;29(7):1331.

12. Renz N, Yermak K, Perka C, Trampuz A. Alpha Defensin Lateral Flow Test for Diagnosis of Periprosthetic Joint Infection: Not a Screening but a Confirmatory Test. The Journal of bone and joint surgery American volume. 2018;100(9):742-50.

13. Shohat N, Bauer T, Buttaro M, Budhiparama N, Cashman J, Della Valle CJ, et al. Hip and Knee Section, What is the Definition of a Periprosthetic Joint Infection (PJI) of the Knee and the Hip? Can the Same Criteria be Used for Both Joints?: Proceedings of International Consensus on Orthopedic Infections. The Journal of arthroplasty. 2019;34(2s):S325-s7.

14. McNally M, Sousa R, Wouthuyzen-Bakker M, Chen AF, Soriano A, Vogely HC, et al. The EBJIS definition of periprosthetic joint infection. Bone Joint J. 2021;103-b(1):18-25.

15. Parvizi J, Gehrke T, Chen AF. Proceedings of the International Consensus on Periprosthetic Joint Infection. Bone Joint J. 2013;95-B(11):1450-2.

16. Izakovicova P, Borens O, Trampuz A. Periprosthetic joint infection: current concepts and outlook. EFORT Open Reviews. 2019;4(7):482-94.

17. Rakow A, Perka C, Trampuz A, Renz N. Origin and characteristics of haematogenous periprosthetic joint infection. Clin Microbiol Infect. 2019;25(7):845-50.

18. Zeller V, Kerroumi Y, Meyssonnier V, Heym B, Metten MA, Desplaces N, et al. Analysis of postoperative and hematogenous prosthetic joint-infection microbiological patterns in a large cohort. J Infect. 2018;76(4):328-34.

19. Corvec S, Portillo ME, Pasticci BM, Borens O, Trampuz A. Epidemiology and new developments in the diagnosis of prosthetic joint infection. The International journal of artificial organs. 2012;35(10):923-34.

20. Vasoo S, Chan M, Sendi P, Berbari E. The Value of Ortho-ID Teams in Treating Bone and Joint Infections. J Bone Jt Infect. 2019;4(6):295-9.

21. Karczewski D, Winkler T, Renz N, Trampuz A, Lieb E, Perka C, et al. A standardized interdisciplinary algorithm for the treatment of prosthetic joint infections. The bone & joint journal. 2019;101-b(2):132-9.

22. Li C, Renz N, Trampuz A, Ojeda-Thies C. Twenty common errors in the diagnosis and treatment of periprosthetic joint infection. Int Orthop. 2020;44(1):3-14.

23. Renz N, Trampuz A. Pocket Guide to Diagnosis & Treatment of Periprosthetic Joint Infection (PJI) 2019 [Available from: https://pro-implant.org/tools/pocket-guide/1] accessed 20 July 2021

24. Perez-Prieto D, Portillo ME, Puig-Verdie L, Alier A, Martinez S, Sorli L, et al. C-reactive protein may misdiagnose prosthetic joint infections, particularly chronic and low-grade infections. Int Orthop. 2017;41(7):1315-9.

25. Piper KE, Fernandez-Sampedro M, Steckelberg KE, Mandrekar JN, Karau MJ, Steckelberg JM, et al. C-reactive protein, erythrocyte sedimentation rate and orthopedic implant infection. PLoS One. 2010;5(2):e9358.

26. Berbari E, Mabry T, Tsaras G, Spangehl M, Erwin PJ, Murad MH, et al. Inflammatory blood laboratory levels as markers of prosthetic joint infection: a systematic review and meta-analysis. The Journal of bone and joint surgery American volume. 2010;92(11):2102-9.

27. Akgun D, Muller M, Perka C, Winkler T. The serum level of C-reactive protein alone cannot be used for the diagnosis of prosthetic joint infections, especially in those caused by organisms of low virulence. The bone & joint journal. 2018;100-b(11):1482-6.

28. Schulz P, Dlaska CE, Perka C, Trampuz A, Renz N. Preoperative synovial fluid culture poorly predicts the pathogen causing periprosthetic joint infection. Infection. 2020.

29. Qu X, Zhai Z, Wu C, Jin F, Li H, Wang L, et al. Preoperative aspiration culture for preoperative diagnosis of infection in total hip or knee arthroplasty. Journal of clinical microbiology. 2013;51(11):3830-4.

30. Renz N, Mudrovcic S, Perka C, Trampuz A. Orthopedic implant-associated infections caused by Cutibacterium spp. - A remaining diagnostic challenge. PLoS One. 2018;13(8):e0202639.

31. Morgenstern C, Cabric S, Perka C, Trampuz A, Renz N. Synovial fluid multiplex PCR is superior to culture for detection of low-virulent pathogens causing periprosthetic joint infection. Diagnostic microbiology and infectious disease. 2017;(in press).

32. Ottink KD, Strahm C, Muller-Kobold A, Sendi P, Wouthuyzen-Bakker M. Factors to Consider When Assessing the Diagnostic Accuracy of Synovial Leukocyte Count in Periprosthetic Joint Infection. J Bone Jt Infect. 2019;4(4):167-73.

33. Trampuz A, Hanssen AD, Osmon DR, Mandrekar J, Steckelberg JM, Patel R. Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. Am J Med. 2004;117(8):556-62.

34. Schinsky MF, Della Valle CJ, Sporer SM, Paprosky WG. Perioperative testing for joint infection in patients undergoing revision total hip arthroplasty. The Journal of bone and joint surgery American volume. 2008;90(9):1869-75.

35. Ghanem E, Parvizi J, Burnett RS, Sharkey PF, Keshavarzi N, Aggarwal A, et al. Cell count and differential of aspirated fluid in the diagnosis of infection at the site of total knee arthroplasty. The Journal of bone and joint surgery American volume. 2008;90(8):1637-43.

36. Dinneen A, Guyot A, Clements J, Bradley N. Synovial fluid white cell and differential count in the diagnosis or exclusion of prosthetic joint infection. The bone & joint journal. 2013;95-b(4):554-7.

37. Krenn V, Morawietz L, Perino G, Kienapfel H, Ascherl R, Hassenpflug GJ, et al. Revised histopathological consensus classification of joint implant related pathology. Pathol Res Pract. 2014;210(12):779-86.

38. Kolbel B, Wienert S, Dimitriadis J, Kendoff D, Gehrke T, Huber M, et al. [CD15 focus score for diagnostics of periprosthetic joint infections : Neutrophilic granulocytes quantification mode and the development of morphometric software (CD15 quantifier)]. Zeitschrift fur Rheumatologie. 2015;74(7):622-30.

39. Krenn VT, Liebisch M, Kolbel B, Renz N, Gehrke T, Huber M, et al. CD15 focus score: Infection diagnosis and stratification into low-virulence and high-virulence microbial pathogens in periprosthetic joint infection. Pathol Res Pract. 2017;213(5):541-7.

40. Dudareva M, Barrett L, Figtree M, Scarborough M, Watanabe M, Newnham R, et al. Sonication versus Tissue Sampling for Diagnosis of Prosthetic Joint and Other Orthopedic Device-Related Infections. Journal of clinical microbiology. 2018;56(12).

41. 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. The Journal of bone and joint surgery American volume. 1999;81(5):672-83.

42. Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med. 2007;357(7):654-63.

43. Portillo ME, Salvado M, Alier A, Martinez S, Sorli L, Horcajada JP, et al. Advantages of sonication fluid culture for the diagnosis of prosthetic joint infection. The Journal of infection. 2014;69(1):35-41.

44. Portillo ME, Salvado M, Trampuz A, Siverio A, Alier A, Sorli L, et al. Improved diagnosis of orthopedic implant-associated infection by inoculation of sonication fluid into blood culture bottles. J Clin Microbiol. 2015;53(5):1622-7.

45. Karbysheva S, Grigoricheva L, Golnik V, Popov S, Renz N, Trampuz A. Influence of retrieved hip- and knee-prosthesis biomaterials on microbial detection by sonication. European cells & materials. 2019;37:16-22.

46. Lenski M, Scherer MA. Diagnostic potential of inflammatory markers in septic arthritis and periprosthetic joint infections: a clinical study with 719 patients. Infectious diseases (London, England). 2015;47(6):399-409.

47. Xie K, Dai K, Qu X, Yan M. Serum and Synovial Fluid Interleukin-6 for the Diagnosis of Periprosthetic Joint Infection. Sci Rep. 2017;7(1):1496.

48. Wouthuyzen-Bakker M, Ploegmakers JJW, Ottink K, Kampinga GA, Wagenmakers-Huizenga L, Jutte PC, et al. Synovial Calprotectin: An Inexpensive Biomarker to Exclude a Chronic Prosthetic Joint Infection. The Journal of arthroplasty. 2018;33(4):1149-53.

49. Wouthuyzen-Bakker M, Ploegmakers JJW, Kampinga GA, Wagenmakers-Huizenga L, Jutte PC, Muller Kobold AC. Synovial calprotectin: a potential biomarker to exclude a prosthetic joint infection. The bone & joint journal. 2017;99-b(5):660-5.

50. Parvizi J, McKenzie JC, Cashman JP. Diagnosis of periprosthetic joint infection using synovial C-reactive protein. The Journal of arthroplasty. 2012;27(8 Suppl):12-6.

51. Plate A, Anagnostopoulos A, Glanzmann J, Stadler L, Weigelt L, Sutter R, et al. Synovial C-reactive protein features high negative predictive value but is not useful as a single diagnostic parameter in suspected periprosthetic joint infection (PJI). J Infect. 2019;78(6):439-44.

52. Deirmengian C, Kardos K, Kilmartin P, Gulati S, Citrano P, Booth RE, Jr. The Alpha-defensin Test for Periprosthetic Joint Infection Responds to a Wide Spectrum of Organisms. Clinical orthopaedics and related research. 2015;473(7):2229-35.

53. Kasparek MF, Kasparek M, Boettner F, Faschingbauer M, Hahne J, Dominkus M. Intraoperative Diagnosis of Periprosthetic Joint Infection Using a Novel Alpha-Defensin Lateral Flow Assay. The Journal of arthroplasty. 2016;31(12):2871-4.

54. Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Booth RE, Jr., et al. The alpha-defensin test for periprosthetic joint infection outperforms the leukocyte esterase test strip. Clinical orthopaedics and related research. 2015;473(1):198-203.

55. Shahi A, Parvizi J, Kazarian GS, Higuera C, Frangiamore S, Bingham J, et al. The Alpha-defensin Test for Periprosthetic Joint Infections Is Not Affected by Prior

Antibiotic Administration. Clinical orthopaedics and related research. 2016;474(7):1610-5.

56. Frangiamore SJ, Gajewski ND, Saleh A, Farias-Kovac M, Barsoum WK, Higuera CA. alpha-Defensin Accuracy to Diagnose Periprosthetic Joint Infection-Best Available Test? The Journal of arthroplasty. 2016;31(2):456-60.

57. Bingham J, Clarke H, Spangehl M, Schwartz A, Beauchamp C, Goldberg B. The alpha defensin-1 biomarker assay can be used to evaluate the potentially infected total joint arthroplasty. Clinical orthopaedics and related research. 2014;472(12):4006-9.

58. Pupaibool J, Fulnecky EJ, Swords RL, Jr., Sistrunk WW, Haddow AD. Alpha-defensin-novel synovial fluid biomarker for the diagnosis of periprosthetic joint infection. Int Orthop. 2016;40(12):2447-52.

59. Suda AJ, Tinelli M, Beisemann ND, Weil Y, Khoury A, Bischel OE. Diagnosis of periprosthetic joint infection using alpha-defensin test or multiplex-PCR: ideal diagnostic test still not found. Int Orthop. 2017;41(7):1307-13.

60. Wetters NG, Berend KR, Lombardi AV, Morris MJ, Tucker TL, Della Valle CJ. Leukocyte esterase reagent strips for the rapid diagnosis of periprosthetic joint infection. The Journal of arthroplasty. 2012;27(8 Suppl):8-11.

61. Tischler EH, Cavanaugh PK, Parvizi J. Leukocyte esterase strip test: matched for musculoskeletal infection society criteria. The Journal of bone and joint surgery American volume. 2014;96(22):1917-20.

62. Colvin OC, Kransdorf MJ, Roberts CC, Chivers FS, Lorans R, Beauchamp CP, et al. Leukocyte esterase analysis in the diagnosis of joint infection: can we make a diagnosis using a simple urine dipstick? Skeletal Radiol. 2015;44(5):673-7.

63. Shafafy R, McClatchie W, Chettiar K, Gill K, Hargrove R, Sturridge S, et al. Use of leucocyte esterase reagent strips in the diagnosis or exclusion of prosthetic joint infection. The bone & joint journal. 2015;97-b(9):1232-6.

64. Wang C, Huang Z, Li W, Fang X, Zhang W. Can metagenomic next-generation sequencing identify the pathogens responsible for culture-negative prosthetic joint infection? BMC Infect Dis. 2020;20(1):253.

65. Goswami K, Parvizi J, Maxwell Courtney P. Current Recommendations for the Diagnosis of Acute and Chronic PJI for Hip and Knee-Cell Counts, Alpha-Defensin, Leukocyte Esterase, Next-generation Sequencing. Curr Rev Musculoskelet Med. 2018;11(3):428-38.

66. Torchia MT, Austin DC, Kunkel ST, Dwyer KW, Moschetti WE. Next-Generation Sequencing vs Culture-Based Methods for Diagnosing Periprosthetic Joint Infection After Total Knee Arthroplasty: A Cost-Effectiveness Analysis. The Journal of arthroplasty. 2019;34(7):1333-41.

67. Wang CX, Huang Z, Fang X, Li W, Yang B, Zhang W. Comparison of broad-range polymerase chain reaction and metagenomic next-generation sequencing for the diagnosis of prosthetic joint infection. Int J Infect Dis. 2020;95:8-12.

68. Melendez DP, Greenwood-Quaintance KE, Berbari EF, Osmon DR, Mandrekar JN, Hanssen AD, et al. Evaluation of a Genus- and Group-Specific Rapid PCR Assay Panel on Synovial Fluid for Diagnosis of Prosthetic Knee Infection. Journal of clinical microbiology. 2016;54(1):120-6.

69. Portillo ME, Salvado M, Sorli L, Alier A, Martinez S, Trampuz A, et al. Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint infection and aseptic failure. The Journal of infection. 2012;65(6):541-8.

70. Achermann Y, Vogt M, Leunig M, Wust J, Trampuz A. Improved diagnosis of periprosthetic joint infection by multiplex PCR of sonication fluid from removed implants. J Clin Microbiol. 2010;48(4):1208-14.

71. Prieto-Borja L, Rodriguez-Sevilla G, Aunon A, Perez-Jorge C, Sandoval E, Garcia-Canete J, et al. Evaluation of a commercial multiplex PCR (Unyvero i60(R)) designed for the diagnosis of bone and joint infections using prosthetic-joint sonication. Enfermedades infecciosas y microbiologia clinica. 2016.

72. Suarez-Carmona M, Hubert P, Delvenne P, Herfs M. Defensins: "Simple" antimicrobial peptides or broad-spectrum molecules? Cytokine & growth factor reviews. 2015;26(3):361-70.

73. Bonanzinga T, Zahar A, Dütsch M, Lausmann C, Kendoff D, Gehrke T. How Reliable Is the Alpha-defensin Immunoassay Test for Diagnosing Periprosthetic Joint Infection? A Prospective Study. Clinical orthopaedics and related research. 2017;475(2):408-15.

74. Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J. Diagnosing periprosthetic joint infection: has the era of the biomarker arrived? Clinical orthopaedics and related research. 2014;472(11):3254-62.

75. Sigmund IK, Holinka J, Gamper J, Staats K, Bohler C, Kubista B, et al. Qualitative alpha-defensin test (Synovasure) for the diagnosis of periprosthetic infection in revision total joint arthroplasty. The bone & joint journal. 2017;99-b(1):66-72.

76. Ewaschuk JB, Naylor JM, Zello GA. D-lactate in human and ruminant metabolism. The Journal of nutrition. 2005;135(7):1619-25.

77. Kortekangas P, Peltola O, Toivanen A, Aro HT. Synovial-fluid D-lactic acid in bacterial and other acute joint effusions. Scandinavian journal of rheumatology. 1994;23(4):203-5.

78. Marcos MA, Vila J, Gratacos J, Brancos MA, Jimenez de Anta MT. Determination of D-lactate concentration for rapid diagnosis of bacterial infections of body fluids. Eur J Clin Microbiol Infect Dis. 1991;10(11):966-9.

79. Gratacos J, Vila J, Moya F, Marcos MA, Collado A, Sanmarti R, et al. D-lactic acid in synovial fluid. A rapid diagnostic test for bacterial synovitis. The Journal of rheumatology. 1995;22(8):1504-8.

80. Chen Z, Wang Y, Zeng A, Chen L, Wu R, Chen B, et al. The clinical diagnostic significance of cerebrospinal fluid D-lactate for bacterial meningitis. Clinica chimica acta; international journal of clinical chemistry. 2012;413(19-20):1512-5.

81. Jun Y, Jianghua L. Diagnosis of Periprosthetic Joint Infection Using Polymerase Chain Reaction: An Updated Systematic Review and Meta-Analysis. Surgical infections. 2018;19(6):555-65.

82. Qu X, Zhai Z, Li H, Li H, Liu X, Zhu Z, et al. PCR-based diagnosis of prosthetic joint infection. Journal of clinical microbiology. 2013;51(8):2742-6.

83. Cazanave C, Greenwood-Quaintance KE, Hanssen AD, Karau MJ, Schmidt SM, Gomez Urena EO, et al. Rapid molecular microbiologic diagnosis of prosthetic joint infection. J Clin Microbiol. 2013;51(7):2280-7.

84. Bemer P, Plouzeau C, Tande D, Leger J, Giraudeau B, Valentin AS, et al. Evaluation of 16S rRNA gene PCR sensitivity and specificity for diagnosis of prosthetic joint infection: a prospective multicenter cross-sectional study. J Clin Microbiol. 2014;52(10):3583-9.

85. Dempsey KE, Riggio MP, Lennon A, Hannah VE, Ramage G, Allan D, et al. Identification of bacteria on the surface of clinically infected and non-infected prosthetic hip joints removed during revision arthroplasties by 16S rRNA gene sequencing and by microbiological culture. Arthritis research & therapy. 2007;9(3):R46.

86. Gomez E, Cazanave C, Cunningham SA, Greenwood-Quaintance KE, Steckelberg JM, Uhl JR, et al. Prosthetic joint infection diagnosis using broad-range PCR of biofilms dislodged from knee and hip arthroplasty surfaces using sonication. Journal of clinical microbiology. 2012;50(11):3501-8.

87. Malandain D, Bemer P, Leroy AG, Leger J, Plouzeau C, Valentin AS, et al. Assessment of the automated multiplex-PCR Unyvero i60 ITI(R) cartridge system to diagnose prosthetic joint infection: a multicentre study. Clin Microbiol Infect. 2017.

88. Morgenstern C, Renz N, Cabric S, Perka C, Trampuz A. Multiplex polymerase chain reaction and microcalorimetry in synovial fluid: can pathogen-based detection assays improve the diagnosis of septic arthritis? Journal of Rheumatology. 2018.

89. Renz N, Feihl S, Cabric S, Trampuz A. Performance of automated multiplex PCR using sonication fluid for diagnosis of periprosthetic joint infection: a prospective cohort. Infection. 2017.

90. Renz N, Cabric S, Morgenstern C, Schuetz MA, Trampuz A. Value of PCR in sonication fluid for the diagnosis of orthopedic hardware-associated infections: Has the molecular era arrived? Injury. 2018;49(4):806-11.

91. Kuiper JW, Pander P, Vos SJ. Good accuracy of the alpha-defensin lateral flow test for hip periprosthetic joint infection: A pilot study in a retrospective cohort of 52 patients. World J Orthop. 2020;11(1):36-46.

92. Scheijen JL, Hanssen NM, van de Waarenburg MP, Jonkers DM, Stehouwer CD, Schalkwijk CG. L(+) and D(-) lactate are increased in plasma and urine samples of type 2 diabetes as measured by a simultaneous quantification of L(+) and D(-) lactate by reversed-phase liquid chromatography tandem mass spectrometry. Exp Diabetes Res. 2012;2012:234812.

93. Smith SM, Eng RH, Campos JM, Chmel H. D-lactic acid measurements in the diagnosis of bacterial infections. Journal of clinical microbiology. 1989;27(3):385-8.

94. Mayeur C, Gratadoux JJ, Bridonneau C, Chegdani F, Larroque B, Kapel N, et al. Faecal D/L lactate ratio is a metabolic signature of microbiota imbalance in patients with short bowel syndrome. PLoS One. 2013;8(1):e54335.

95. Kaliterna J, Weusthuis RA, Castrillo JI, Van Dijken JP, Pronk JT. Transient responses of Candida utilis to oxygen limitation: regulation of the Kluyver effect for maltose. Yeast (Chichester, England). 1995;11(4):317-25.

96. Rieger UM, Mesina J, Kalbermatten DF, Haug M, Frey HP, Pico R, et al. Bacterial biofilms and capsular contracture in patients with breast implants. Br J Surg. 2013;100(6):768-74.

97. Rohacek M, Weisser M, Kobza R, Schoenenberger AW, Pfyffer GE, Frei R, et al. Bacterial colonization and infection of electrophysiological cardiac devices detected with sonication and swab culture. Circulation. 2010;121(15):1691-7.

98. Hischebeth GT, Randau TM, Buhr JK, Wimmer MD, Hoerauf A, Molitor E, et al. Unyvero i60 implant and tissue infection (ITI) multiplex PCR system in diagnosing periprosthetic joint infection. J Microbiol Methods. 2016;121:27-32.

99. Zong Z, Peng C, Lü X. Diversity of SCCmec elements in methicillin-resistant coagulase-negative staphylococci clinical isolates. PLoS One. 2011;6(5):e20191.

100. Sigmund IK, Renz N, Feihl S, Morgenstern C, Cabric S, Trampuz A. Value of multiplex PCR for detection of antimicrobial resistance in samples retrieved from patients with orthopaedic infections. BMC Microbiology. 2020;20(1).

101. Widmer AF, Wiestner A, Frei R, Zimmerli W. Killing of nongrowing and adherent Escherichia coli determines drug efficacy in device-related infections. Antimicrob Agents Chemother. 1991;35(4):741-6.

102. Amanatullah DF, Cheng RZ, Huddleston Iii JI, Maloney WJ, Finlay AK, Kappagoda S, et al. The routine use of synovial alpha-defensin is not necessary. The bone & joint journal. 2020;102-b(5):593-9.

103. Li M, Zeng Y, Wu Y, Si H, Bao X, Shen B. Performance of Sequencing Assays in Diagnosis of Prosthetic Joint Infection: A Systematic Review and Meta-Analysis. The Journal of arthroplasty. 2019;34(7):1514-22.e4.

6. Acknowledgements

I would like to thank Univ.-Prof. Dr. Dr. h.c. N.P. Haas, the former managing director of the Center for Musculoskeletal Surgery (CMSC) and Univ.-Prof. Dr. C. Perka, the current medical director of the Center for Musculoskeletal Surgery (CMSC) for giving me the opportunity to gain clinical experience and to foster research projects in this exclusive field of septic surgery. Only a visionary environment enables to produce such a work with high clinical relevance. Both directors gave me the initiative to enter into the scientific field.

Furthermore, I thank the companies Zimmer Biomet and Curetis GmbH for providing diagnostic test kits and reagents for the respective studies. In addition, Zimmer Biomet and Pro-Implant Foundation provided unrestricted educational grants.

I would like to express my sincere thanks to my clinical and research fellows at Charité – Universitätsmedizin Berlin, which enabled me to conduct the studies. Special thanks go to Irene Katharina Sigmund, Katsiaryna Yermak, Svetlana Karbysheva, Christian Morgenstern, Sabrina Cabric, Philipp Schulz, Anastasia Rakow and Susanne Feihl, who were all actively involved in data collection, performance of tests, interpretation of results and writing of the manuscripts, which represent the basis for this work. Furthermore, I thank the clinical team including Donara Margaryan, Wiebke Jansen, Luise Martin and the entire orthopedic and trauma surgery team of the CMSC, who did the clinical work while I benefitted of the time dedicated to do research.

Moreover, I offer my sincere gratitude to my family, my parents Mario and Christine Renz and my sister Lia Bischof, and friends in Switzerland, who supported me during my stay in Berlin and throughout life.

Finally, I would like to express my sincere thanks and gratitude to my mentor PD Dr. med. Andrej Trampuz, who shared all his knowledge in the field of infectious diseases, with a special focus on implant-associated infections, with me and supported me during my stay at the Charité - Universitätsmedizin Berlin and even further on. His impressive dedication and endless interest for this field and the focus on the patients' well-being inspired and shaped me considerably. Without his input and optimism, this work would have never been accomplished.

7. Statutory declaration

Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,
- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und mich zur Einhaltung dieser Satzung verpflichte.

Datum Unterschrift

16.08.2021