Aus dem Centrum für Muskuloskeletale Chirurgie
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DISSERTATION

Evaluation and development of a new diagnostic tool for early and reliable diagnostic of prosthetic joint infection

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I. Abstract

A. Abstract (English)

Background: Periprosthetic joint infection (PJI) results in high morbidity, prolonged treatment and considerable healthcare expenses. Preoperative diagnosis of PJI is critical for optimal treatment. Determination of leukocyte count in synovial fluid is currently used as an independent criterion for the diagnosis of PJI in definition criteria. Nevertheless, leukocyte count lacks either sensitivity or specificity for diagnosing PJI in specific cases. Due to subtle inflammation of the periprosthetic tissue and stationary-growth of microorganisms in biofilm, low-grade PJI may be missed. On the other hand, in the early postoperative period, the physiologic healing process cannot be distinguished by synovial fluid leukocyte count from PJI.
D-lactate is a product of bacterial metabolism and can be used as pathogen-specific biomarker for diagnosis of PJI. The aim of this study is to evaluate the performance of D-lactate in synovial fluid as an independent diagnostic marker for diagnosis of early and delayed / late PJI using standard diagnostic criteria and to compare it with synovial fluid leukocyte count.

Methods: Patients with suspected infection of prosthetic hip, knee or shoulder were included in the prospective analysis. The joint aspiration was performed either before or during the revision surgery. 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% and the specificity was 80.8%. The AUCs of D-lactate concentration and leukocyte count were 90.3% and 91.0%, 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: In this prospective study synovial fluid D-lactate showed similar performance to the leukocyte count for diagnosis of PJI. For the D-lactate calculation only 50 µl synovial fluid is required. The test has short turnaround time and low cost. Due to its positive correlation in aseptic failures, the interference of the erythrocytes with D-lactate cannot be excluded. The overall diagnostic performance of D-lactate test is promising. For its use in the clinical environment, the specific role in low-grade and early postoperative infection should be examined.

B. Abstract (Deutsch)

Hintergrund: Periprothetische Gelenkinfektionen (PPI) führen zu einer hohen Morbidität, einer längeren Behandlungsdauer und erheblichen Gesundheitsausgaben. Die präoperative Diagnose von PPI ist entscheidend für eine optimale Behandlung. Die Bestimmung der Leukozytenzahl in der Synovialflüssigkeit wird aktuell als ein unabhängiges Kriterium für die Diagnose von PPI in den Definitionskriterien verwendet. Trotzdem zeigt die Leukozytenzahl eine begrenzte Sensitivität oder Spezifität in gewissen Situationen. Durch subtile Entzündung des periprothetischen Gewebes und der stationären Wachstumsphase der Mikroorganismen im Biofilm können „low-grade" PPI verpasst werden. Andererseits kann in der frühen postoperativen Phase die Leukozytose in der Synovialflüßigkeit von der frühen postoperativen Infektion aufgrund des physiologischen Heilungsprozess nicht sicher differenziert werden.
D-Laktat ist ein Produkt des bakteriellen Stoffwechsels und kann als ein Pathogen-spezifischer Biomarker für die Diagnose von PPI verwendet werden. Das Ziel dieser Arbeit ist das Leistungspotential von D-Laktat in der Synovialflüssigkeit als unabhängiger diagnostischer Marker für die Diagnose einer frühen und verzögerten / späten PPI zu bewerten. Die Leistung von D-Laktat und Leukozytenzahl in der Synovialflüssigkeit wurden mittels standardisierten diagnostischen Kriterien untersucht und miteinander verglichen.

Methoden: Patienten mit Verdacht auf eine Infektion der Hüft-, Knie- oder Schulterprothese wurden in die prospektive Analyse eingeschlossen. Die Aspiration der Synovialflüssigkeit wurde entweder vor oder während der Revision durchgeführt. PPI wurde gemäß den Arbeitskriterien der European Bone and Joint Infection Society (EBJIS) definiert. Das D-Laktat aus der Synovialflüssigkeit wurde spektrophotometrisch bei 570 nm bestimmt, die Leukozyten der Synovialflüssigkeit wurden durchflusszytometrisch gezählt. Die diagnostische Leistung der untersuchten Parameter wurde mittels der ROC-Analyse (Receiver Operating Characteristic) durchgeführt.

Ergebnisse: Von 148 Patienten wurde bei 44 (30%) eine PPI und bei 104 (70%) eine aseptische Ursache diagnostiziert. Die Sensitivität des D-Laktats für die Diagnose einer PPI (bei einem Grenzwert von 1,263 mmol/l) betrug 86,4% und die Spezifität 80,8%. Die AUCs der D-Laktat-Konzentration und der Leukozytenzahl betrugen 90,3% bzw. 91,0% (p=0,8). Die Virulenz des Erregers hatte keinen Einfluss auf die D-Lactat-Konzentration ($p = 0{,}123$). Die Erythrozytenkonzentration in der Synovialflüssigkeit korrelierte mit dem D-Laktat bei Patienten mit aseptischem Versagen ($\rho = 0{,}339$, $p < 0{,}01$).

Schlussfolgerung: In dieser prospektiven Studie zeigte das D-Laktat in der Synovialflüssigkeit eine ähnliche Leistung wie die Leukozytenzahl für die Diagnose der PPI. Für die Bestimmung des D-Laktats sind nur 50 µl Synovialflüssigkeit notwendig, der Test hat eine kurze Durchlaufzeit und ist kostengünstig. Wegen einer positiven Korrelation in aseptischem Fällen kann eine Interferenz zwischen den Erythrozyten und dem D-Laktat nicht ausgeschlossen werden. D-Laktat ist ein vielversprechender Biomarker für die Diagnostik von PPI. Für die Etablierung des Tests im

klinischen Alltag wäre seine spezifische Rolle bei ”low-grade” und früh postoperativen Infektionen zu untersuchen.

II. Synopsis

C. Background

C.1. Epidemiology

Prosthetic joint replacement relieves pain, induces patient mobility and tremendously improves the quality of life. This already frequent procedure is expected to rise with the aging of the population. A minority of the patients experience revision operation due to prosthesis failure. The complications are divided in aseptic mechanical complications such as loosening, periprosthetic fractures, dislocation or wear. On the other hand periprosthetic joint infection (PJI) occurs in 0.5% to 1% of patients after primary implantation and in 3% to 5% after revision surgery, resulting in high morbidity, prolonged treatment and considerable health-care expenses (1).

C.2. Pathophysiology of PJI

The diagnosis of PJI is challenging due to specific pathophysiological conditions, particularly because of the bacterial biofilm formation in the prosthetic joint. Preoperative diagnosis of PJI is critical for optimal surgical and antimicrobial treatment (2, 3).

Biofilm is an efficient evolutionary means of bacterial survival under suboptimal living conditions, such as lack of nutrients. It cause resistance of bacteria to the immune system and to the antibiotic treatment after bacterial colonization of implanted prosthetic material. Bacteria exists in two main forms: planktonic form, where bacteria replicate fast and are metabolically active, and as biofilm – where metabolic activity of bacteria is low or dormant. Biofilms are up to 1000x more resistant against antibiotic treatment because of low replication rates. Only a few antibiotics are able to eradicate biofilm in its early stage within the first 3 weeks. Rifampin is active against staphylococcal infection and quinolones against gram-negative rods (4).

The implant-associated infection occurs in 3 ways:

- Perioperative contamination of the implant during insertion or during the early phase of wound healing in case of persistent wound secretion
- Trough haematogenous or lymphatic dissemination from a distant focus e.g. from skin, lung, urogenital, gastrointestinal, oral cavity infection or infectious endocarditis any time after implantation
- Colonization through direct contact with infectious surface e.g. preexisting osteomyelitis or septic arthritis

C.3. Classification of PJI

PJI is defined as infection involving the joint prosthesis and surrounding tissue. Prosthetic joint infection can be classified in regard to time manifestation after implantation on early postoperative, delayed postoperative and late infections. Early-onset PJI occurs < 3 months after the last surgery (in most cases within 4 weeks after surgery). These infections initiate in intraoperative contamination of the surgical site / implant or during the early phase of wound healing and are usually caused by highly virulent microorganisms. Delayed-onset (also called low-grade) PJI typically occurs between 3 and 24 months. These infections are acquired at the time of surgery but are caused by less virulent microorganisms such that the overt presentation of infection occurs after 3 months postoperatively.

Late-onset PJI, occurring >24 months after surgery, is frequently caused due to haematogenous spread but may also be due to an extremely indolent infection initiated at the time of surgery (5).

Based on clinical manifestation we can divide PJI into acute and chronic incidents. In acute infection, the symptoms usually occur within 4 weeks after implantation in early postoperative infection, or there is a new symptom onset any time after implantation that lasts no longer than 4 weeks in hematogenous infections. Symptoms that last more than 4 weeks are defined as chronic infection (5).

Timing and symptoms manifestation indicate the virulence of the organism and the mode of initiation of infection. A hematogenous PJI has to be suspected if the symptoms are accompanied with systemic inflammation signs such as fever and chills. High virulent bacteria mostly cause acute symptom onset any time after implantation presents with pain, swelling and erythema. More indolent manifestations such as chronic pain and effusion, or presence of sinus tract are mostly due to low virulent microorganisms or to a compromised host immune system. The patients can experience symptoms of low-grade infection over several months and years.

C.4. Laboratory diagnostic of PJI

There are several diagnostic tests, which, apart from clinical signs and symptoms, help to diagnose PJI. Materials such as synovial fluid, periprosthetic tissue and the prosthetic surface are used for diagnostic purposes.

Today's best practices with the highest sensitivity and specificity among the tests is detection of leukocyte count and percentage of granulocytes in synovial fluid. Synovial fluid surrounds an infected prosthetic joint and it is easily collected through joint puncture. Several novel biomarkers are currently being investigated such as alpha defensin, calprotectin, interleukin (IL)-1, IL-6, and D-lactate, which could further improve diagnostic of PJI. Moreover, synovial fluid is valuable media for bacterial cultivation. The periprosthetic tissue is used for microbiological testing and for the histopathological analysis.

The biofilm with dormant bacteria covers the prosthetic surface. With mechanical methods such as sonication or vortexing of the explanted prosthesis we detach the biofilm from the implant

surface and cultivate it just like periprosthetic tissue (6). Chemical methods of biofilm removal, e.g. with dithiothreitol (DTT) have also been studied recently (7).

Moreover, serum parameters such as C-reactive protein (CRP), procalcitonin, and erythrocyte sedimentation rate are used for the diagnosis of PJI. They are useful in haematogenous infection and are relevant in the early postoperative period. The prolonged or unexpected elevation of the systemic inflammation parameters with relevant symptoms, such as prolonged wound drainage and pain may indicate an infection process. An important point is that PJI is mostly a local process and especially in the chronic infection the serum parameters will be in normal range.

D. Current issues in the diagnostic of PJI

There is a lack of validated, defined criteria to uniformly diagnose PJI in scientific and clinical practice. Among the three currently available classification systems, the Musculoskeletal Infection Society (MSIS) criteria (8) and the Infectious Diseases Society of America (IDSA) criteria (9) are widely used in the United States, whereas the proposed European Bone and Joint Infection Society (EBJIS) criteria are increasingly used across Europe (10-12) – Table 1. In contrast to the MSIS and IDSA criteria, the proposed EBJIS criteria also consider sonication of the removed implant in the diagnosis and uses lower cut-off values for synovial fluid leukocyte count, allowing better detection of low-grade PJI (11). The application of an accurate classification system is not only of paramount relevance in clinical practice, but also for the evaluation of the performance of any novel diagnostic test.

Several attempts were made to investigate different biomarkers, such as alpha-2-macroglobulin, adenosine deaminase, procalcitonin, IL-1, IL-6, and alpha defensin, which can be helpful in distinction of PJI from aseptic pathology (13, 14). Currently used diagnostic tests of synovial fluid lack sensitivity or specificity for PJI (15).

For example, alpha defensin, an antimicrobial peptide released by neutrophils in response to bacterial pathogens, was recently proposed as a novel diagnostic biomarker in synovial fluid. The studies showed high sensitivity in the subgroup of early postoperative PJI, whereas its sensitivity in low-grade infections was limited. Therefore, alpha defensin was proposed as confirmatory instead of a screening test (11, 12).

Synovial fluid culture requires time and has limited sensitivity and specificity in chronic low-grade PJI (3, 15, 16). The low level of inflammation and subtle clinical symptoms may impede the diagnosis of low-grade PJI, which usually occurs several months to years after arthroplasty.

The diagnosis is also difficult in the early postoperative period where leukocyte count, CRP and clinical signs hamper a reliable diagnosis due to local tissue inflammation (17). Moreover, the leukocyte count cannot be used in the early postoperative period, in crystal-induced inflammation, and after periprosthetic fracture or dislocation since these conditions induce aseptic inflammation. Nevertheless, measuring the leukocyte count and differential in synovial fluid are the most accurate and widely used diagnostic tests, included in all three definition criteria (18).

Table 1 Main diagnostic criteria for PJI - working EBJIS criteria, MSIS and IDSA.

Working definition of the European Bone and Joint Infection Society (EBJIS) – **(at least one of the following criteria)**	**Musculoskeletal Infection Society (MSIS)** **(at least one MAJOR criteria OR at least three MINOR criteria)**	**Infectious Diseases Society of America (IDSA) (at least one of the following criteria)**
• Purulence around the prosthesis or sinus tract **• Increased synovial fluid leukocyte count[1]** **• Positive histopathology[2]** **• Significant microbial growth in synovial fluid, periprosthetic tissue[3] or sonication culture[4]**	MAJOR criteria: • Two positive periprosthetic cultures • Sinus tract communicating with the prosthesis MINOR criteria: • Elevated CRP[1] and ESR (> 30 mm/hour) • Elevated synovial fluid leukocyte count[2] or positive leukocyte esterase strip test (++ or +++) • Elevated synovial fluid percentage of granulocytes[3] • A single positive culture • Positive histologic analysis of periprosthetic tissue[4]	• Sinus tract communicating with the prosthesis • Purulence without other etiology surrounding the prosthesis • Acute inflammation seen on histopathologic examination of the periprosthetic tissue • Two or more intraoperative cultures or a combination of preoperative aspiration and intraoperative cultures yielding an indistinguishable organism[1]
1) Leukocytes > 2000/µL or > 70% granulocytes; not interpretable within 6 weeks of surgery, in rheumatic joint	1) > 10 mg/L in chronic infections or > 100 mg/L in acute infections	1) Growth of a virulent microorganism (e.g. *Staphylococcus aureus*) in a single

disease, after periprosthetic fracture or dislocation **2) Defined as a mean of > 23 granulocytes per 10 high-power fields (type II or type III of periprosthetic membrane)** **3) Periprosthetic tissue culture was considered positive if ≥ one specimen was positive with highly virulent organisms or ≥ two specimens showed microbial growth of a low virulent pathogen** **4) Sonication was considered positive if > 50 CFU/mL sonication fluid grew**	2) > 3000 leukocytes/µL in chronic infections or > 10,000 leukocytes/µL in acute infections 3) > 80% in chronic infections or > 90% in acute infections 4) Defined as > 5 neutrophils per high-power field in five high-power fields observed on periprosthetic tissue at 400 x magnification	specimen of a tissue biopsy or synovial fluid may also represent PJI

Abbreviations: CFU – colony-forming units; CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; PJI – prosthetic joint infection;

E. Previous studies on D-lactate

Lactic acid was discovered in 1780 by Swedish chemist Carl Wilhelm Scheele who isolated the lactic acid from sour milk. Lactic acid has two optical forms L (+) and D (-). L-lactic acid is a biological isomer present in mammalian cells in 1-2 mmol/l. A clinical condition L-lactate acidosis occurs because of tissue hypoxia.

On the other hand, bacterial metabolism produces D-lactate and microbial fermentation in the colon contributes to the serum D-lactate (19). In humans the methylglyoxal pathway is an additional source of D-lactate in serum, which is present only in nanomolar concentrations, accounting for 1–5% of total lactate concentration.

D-lactate acidosis is a rare metabolic condition in humans, which is occasionally observed as a consequence of short bowel syndrome or following jejuno-ileal bypass surgery (20). D-lactate acidosis has been defined as ≥ 2.5 - 3 mmol/l of D-lactate in serum. The condition is caused by excessive gastrointestinal fermentation of carbohydrate by lactobacilli and was first described in 1979. The predominant organ system affected by D-lactate is the central nervous system. Presenting symptoms may include slurred speech, ataxia, altered mental status or even coma (21). Because of this interaction, D-lactate could play an important role in the microbiota–gut–brain interaction (22).

The use of D-lactate as a marker for infection was proposed in 1986 (23). D-lactate was shown to be a promising marker for the diagnosis of infection in primarily sterile body fluids including in patients receiving antimicrobial therapy (24, 25). A recent study showed high diagnostic value of D-lactate in evaluation of bacterial meningitis (26).

Several studies were carried out to measure the D-lactate concentration in primarily sterile body fluids already back in the 1990s in order to discriminate infection from aseptic inflammation (23, 24, 27). Gratacos et al. showed in 1995 that D-lactate could be used as a rapid diagnostic test to distinguish between bacterial and aseptic synovitis with sensitivity and specificity of 85% and 96% respectively (28). In literature D-lactate was elevated regardless of microbe type with the exception of *Pasteurella multocida* where D-lactate was not elevated (24).

The first study on D-lactate for diagnosing PJI was published by Yermak et al. in 2019 (29). More recent evidence on the performance of D-lactate in PJI was conducted by Karbysheva et al. in 2020 (30). In the study, a larger population size of 224 samples was included and showed similar results on sensitivity and specificity of D-lactate of 92.4% and 88.6% respectively.

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

Current diagnostic criteria are still not optimal for PJI especially in specific conditions such as presence of aseptic inflammation in early postoperative period and in the presence of chronic infection with low-grade pathogens.

D-lactate is not a novel marker of the infection but it has never been tested in PJI up to now. We hypothesized that D-lactate is a reliable marker for the diagnosis of PJI and began a prospective study. The aim of the study "Performance of synovial fluid D-lactate for the diagnosis of periprosthetic joint infection: A prospective observational study" (29) was to evaluate the performance of D-lactate in synovial fluid for diagnosis of early and delayed/late PJI using proposed EBJIS criteria and to compare it with synovial fluid leukocyte count.

F.1. Study design and population

In the prospective diagnostic cohort study we included consecutive patients aged 18 years or older who were evaluated for a painful prosthetic hip, knee or shoulder joint and underwent a diagnostic joint aspiration. In order to evaluate the infection we performed the diagnostic joint aspiration prior to the revision arthroplasty or during revision arthroplasty but before the joint capsule was opened. We collected the samples over 10 months. Only one (the first collected) synovial fluid sample per patient was considered.

Excluded were patients with diluted synovial fluid after joint instillation, insufficient synovial fluid volume (<3 ml) or in whom the synovial fluid analysis was performed more than 48 hours after aspiration. A standardized case-report form was used to collect patient history, demographic, clinical, radiological, microbiological, histopathological and laboratory data. An interdisciplinary team consisting of orthopedic surgeons, infectious diseases specialists and internal medicine specialists evaluated patients. The synovial fluid D-lactate test results were not communicated to the treating orthopedic surgeons. The study was performed in accordance with the Declaration of Helsinki.

F.2. Definition of PJI

PJI was defined according to proposed European Bone and Joint Infection Society (EBJIS) criteria when at least one of the following criteria was present: (i) macroscopic purulence of synovial fluid or surrounding the prosthesis, (ii) presence of sinus tract, (iii) increased synovial fluid leukocyte count (>2000 leukocytes/µl or >70% granulocytes), (iv) histopathological evidence of inflammation in periprosthetic tissue defined as a mean of >23 granulocytes per high-power field, corresponding to type II or type III periprosthetic membrane, (v) significant microbial growth in synovial fluid, periprosthetic tissue or sonication culture. The criteria are summarized in Table 1.

Acute infection was diagnosed if the infection occurred within 4 weeks after surgery or if the patient reported new onset of symptoms any time after implantation lasting not longer than 4 weeks. Infections that occurred more than 4 weeks after the last surgery and were symptomatic for more than 4 weeks were defined as chronic infections. Furthermore, based on the interval between last revision surgery or primary implantation and time of aspiration, all infections were classified into early (i.e. < 3 months) and delayed or late (i.e. >3 months) infections.

F.3. Methods

Synovial fluid was aspirated under sterile conditions preoperatively in the outpatient department or during revision surgery before opening the joint capsule. One ml of aspirated synovial fluid was inoculated into a pediatric blood culture bottle (BacTec PedsPlus/F, Beckton Dickinson and Co), one ml was introduced in a native vial for aerobic and anaerobic culture (0.1 ml each) and the remaining fluid was inoculated in thioglycolate broth for enrichment. The pediatric blood culture bottle was incubated at 36 ± 1°C for 14 days or until growth was detected. The aerobic cultures were incubated at 37°C and inspected daily for 7 days, and the anaerobic ones were incubated for 14 days. The colonies of microorganism morphology were identified by standard microbiological methods using automated system VITEK 2 (bioMérieux, Marcy L'Etoile, France). For detection of urate and pyrophosphate crystals, a 1 ml-aliquot was sent to the pathologist for examination of the synovial fluid with polarization microscopy.

In addition, 3-5 periprosthetic tissue samples were collected during surgery from the implant-bone or cement-bone interface for microbiological and histopathological analysis, if revision surgery was performed. Periprosthetic tissue culture was considered positive if a high-virulent organism grew in ≥1 specimen of synovial fluid, periprosthetic tissue or sonication (*Staphylococcus aureus*, Enterobacteriaceae, *Streptococcus* spp., *Candida* spp.). In case of medium or low-virulent microorganisms (coagulase-negative staphylococci, enterococci, *Cutibacterium acnes* [formerly known as *Propionibacterium acnes*] an isolation of ≥2 specimen was considered as positive.

The retrieved prosthetic components were sent for sonication, as previously described. Sonication was considered positive if ≥1 CFU/ml of a high-virulent organism or >50 CFU/ml of a low-virulent organism grew in sonication fluid (31).

Histological tissue analysis demonstrates acute inflammation evidenced by neutrophilic infiltrate in the periprosthetic area. Histopathological evidence of inflammation in periprosthetic tissue was defined as a mean of >23 granulocytes per high-power field, corresponding to type II or type III periprosthetic membrane. Type I is induced trough wear-particles while type IV is indeterminate (32).

In order to determine synovial fluid leukocyte count, erythrocyte count and percentage of granulocytes one ml of synovial fluid was transferred into a vial containing ethylenediaminetetraacetic acid (EDTA). The leukocyte and erythrocyte count was determined by flow cytometry using an automated hematology analyzer (XE-2100, Sysmex, Norderstedt,

Germany). Clotted specimens were treated with 10 µl hyaluronidase (Sigma-Aldrich Chemie, Taufkirchen, Germany) for 10 minutes at room temperature.

D-lactate was determined spectrophotometrically from the optical density of the prepared sample. One 1 ml-aliquot was transferred to a native vial for determination of D-lactate using a commercial kit (D-lactam Kit; VL-Diagnostics, Leipzig, Germany). Aliquots for D-lactate determination were stored at 4°C± 1°C and analyzed within 48 hours after aspiration. The tests were performed according to the manufacturer's instructions. The determination is based on spectrophotometric method with a standard microplate absorbance reader at 570 nm, requiring 50 µl of synovial fluid. In the assay D-lactate dehydrogenase (D-LDH) catalyzes the oxidation of D-lactic acid to pyruvate, along with the concomitant reduction of nicotinamide adenine dinucleotide (NAD+) to NADH. NADH reacts with the fluorescent substrate to yield coloration of the mixture (33). The reaction is presented in Figure 1.

Figure 1 D-lactate dehydrogenase (D-LDH) catalyzes the oxidation of D-lactic acid to pyruvate, along with the concomitant reduction of nicotinamide adenine dinucleotide (NAD+) to NADH.

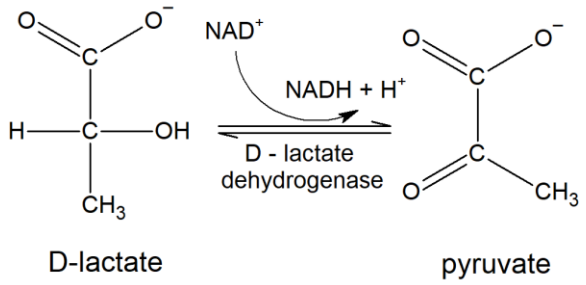
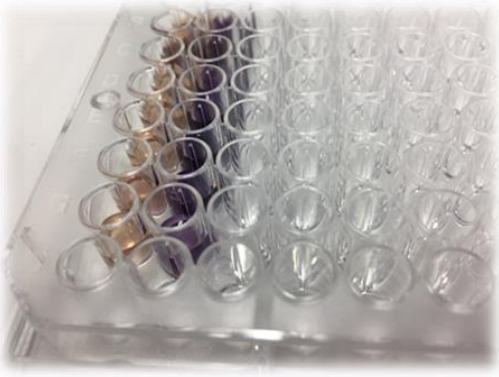


Figure 2 Reagents applied to a flat-bottom 96-well plate in turbidity control (first row) and reaction (second row) after the incubation. D-lactate amount is higher in the darker samples NADH reacts with the fluorescent substrate to yield coloration of the mixture.



The D-lactam assay contains lithium D-lactate standard for preparation of a calibration curve, which was processed for each batch. The reaction mixture contained 0.025 ml of synovial fluid

sample, 0.08 ml of substrate mix and 0.045 ml of enzymatic mix. The turbidity control mixture contained 0.025 ml of synovial fluid sample, 0.08 ml of substrate mix and 0.045 ml of purified water. The reagents were applied to a flat-bottom 96-well plate (Figure 2), incubated at 37°C for 30 min and then read at 570 nm by Microplate Absorbance Reader (DYNEX Technologies MRX, Chantilly, VA, USA). For the commercial D-lactate test kit, 50 µl of synovial fluid is required. The turn-around time of both tests was 30-45 minutes.

F.3.1. Statistical analysis

Youden's J statistic was used for determining D-lactate cut-off point on the ROC curve. The area under the ROC curve (AUC) was used to assess the diagnostic performance of D-lactate test, leukocyte count and percentage of granulocytes. Two-sided independent samples Student´s t-test was applied to assess statistical significance in the mean concentration of D-lactate between groups. The sample size calculation was based on the assumption that the sensitivity of D-lactate is 90% compared to 80% for conventional diagnostic tests, including leukocyte count, periprosthetic tissue histopathology and culture, i.e. difference of 10% (power 80%). DeLong's test for two correlated ROC curves was used to determine if the difference between AUCs is statistically significant. The significance level α of 0.05 was selected for all performed statistical tests. A 95% confidence interval (CI) for AUCs was estimated with DeLong's method and 95% CI for other performance measures was estimated using bootstrap resampling with 10,000 replicates (Table 5). Test for two independent medians, χ^2-test and Fischer's exact test were used for estimating p-values in Table 2. To estimate p-values between sensitivities of D-lactate and leukocyte count in acute and delayed/late infection, bootstrap resampling with 10,000 replicates was performed. The correlation between erythrocyte and D-lactate concentration in the Figure 5 was estimated using Pearson coefficient (ρ). For all statistical analyses IBM SPSS 22.0 (Statistical package for the Social Sciences Corporation, Chicago, IL, USA) was used. ROC and other plots were produced by R Computing environment.

F.4. Results

There were 148 patients included in the study. Synovial fluid was collected from 103 (70%) prosthetic knee, 43 (29%) hip and 2 (1%) shoulder prosthesis. Forty-four patients (30%) were diagnosed with PJI and 104 (70%) with aseptic prosthetic failure. Most patients (n=102, 69%) underwent revision surgery, 62 of these with aseptic failures and 40 with PJI. The patient characteristics are summarized in Table 2. The present sample size is larger compared to recently published similar prospective diagnostic studies (13, 14).

Table 2 Patients characteristics in the study group.

	All patients (n=148)	Patients with PJI (n=44)	Patients with aseptic failure (n=104)	p-value
Median (range) patient age (years)	69.5 (29-93)	69.0 (41-89)	69.5 (29-93)	0.857
Sex, no. (%)				0.032
Male	81 (55)	30 (68)	51 (49)	
Joint, no. (%)				0.006
Knee	103 (70)	24 (55)	79 (76)	
Hip	43 (29)	18 (41)	25 (24)	
Shoulder	2 (1)	2 (4)	0 (0)	
Patients undergoing revision surgery, no. (%)	102 (69)	40 (91)	62 (60)	<0.001
Timing of joint aspiration after primary surgery, no. (%)				0.765
Early (<3 months)	19/138 (14)	7/43 (16)	12/95 (13)	
Delayed (3-24 months)	55/138 (40)	16/43 (37)	39/95 (41)	
Late (>24 months)	64/138 (46)	20/43 (47)	44/95 (46)	

In total 102 (69.9%) patients received a revision surgery, among them 40 patients with PJI and 62 patients without PJI. In 4 non-operated PJI patients: one had acute, delayed shoulder infection and was operated in external clinic; in three, late chronic knee infections with positive cell count were not operated due to team decision. In one of them, only granulocyte percentage was slightly elevated over the cut-off. In all 4 cases D-lactate was elevated and in only one sample a *Corynebacterium* sp. was defined as contamination. The full diagnostic was performed in 63 (42.6%) patients.

Table 3 In acute PJI D-lactate and leukocyte count showed a sensitivity of 100%, in chronic PJI there were 6 and 9 false negative results, respectively.

Patients with PJI (n=44)	**Synovial fluid** **D-lactate >1.263 mmol/l**	**Synovial fluid leukocyte count >2000/µl**
Acute PJI n = 12 (27%)	12 / 12	12 / 12
Chronic PJI n = 32 (73%)	26 / 32 (6 false negative)	23 / 32 (9 false negative)

F.4.1. Performance of conventional tests and microbiology

PJI was defined according to proposed European Bone and Joint Infection Society (EBJIS) criteria (Table 1) and described in methods. The performance of diagnostic tests in our study is summarized in Table 5.

There were 21 cases (48%) of culture negative PJI. Significant microbiological growth was documented in 23 patients (52%) with PJI, whereas formal contamination (i.e. insignificant growth) was detected in 8 cases with actual PJI and in 19 cases with aseptic failure. The most frequent causative organism were coagulase-negative staphylococci with 11 cases (48%), with most frequent pathogen *S. epidermidis*. There were 5 cases of infection with *S. aureus* (22%) and 3 cases of *Streptococcus* spp. In other specimens gram-negative rods grew (n=3), *Enterococcus* spp. (n=1) 4% and *Bacteroides fragilis* 1% (n=1). In our study a significant microbial growth occurred in 23 patients with PJI (52% of PJI), whereas contamination was observed in 8 cases with PJI (insignificant growth) and in 19 cases with aseptic failure (contamination). In general, infection with pathogens from the skin flora, mostly with *S. aureus* and coagulase-negative staphylococci, contribute to between 50-60% of PJI, while streptococci and enterococci together account for only 10% according to the large studies including more than 1800 joints (34).

Microbial contamination most frequently occurs in tissue cultures (8%), which are collected in 70% of patients. In contrast, sonication fluid showed lowest contamination rates. Sample manipulation could explain the higher contamination rates in tissue cultures. Table 4 shows microbiological contamination rate and microbial flora in the group without PJI. In 17 cases there was a delayed/late presentation and in 2 cases an early presentation of symptoms after a prosthetic joint implantation. In Table 4 shows the contamination rates in different samples.

Table 4 Microbial contamination of different patient specimens collected for diagnostic culture, stratified by the type of sample (Synovial fluid, periprosthetic tissue and sonication fluid) in patients without PJI.

Pathogen	Synovial fluid (n=148; 100%)	Periprosthetic tissue (n=103; 70%)	Sonication fluid (n=88; 60%)
Mixed flora: *S. caprae, S. epidermidis, ,C. acnes*		2	
Staphylococcus capitis	2	-	-
Staphylococcus epidermidis	3	-	-
Staphylococcus hominis	-	2	-
Staphylococcus saccharolyticus	-	-	1
Streptococcus sanguinis	-	1	-
Bacillus cereus	1	1	-
Cutibacterium acnes	2	2	2
Total: 19 (% of total collected samples)	**8 (6%)**	**8 (8%)**	**3 (3%)**

Synovial fluid culture, periprosthetic tissue culture and sonication fluid culture showed overall low rates of sensitivity, 45.5%, 41.5% and 43.6%, respectively. The combination of all three cultures' specimens increased the sensitivity up to 52.3%, underlining the importance of application of different culture methods in the clinical practice.

The synovial fluid leukocyte count showed a sensitivity of 80% and specificity of 91%. Among the non-microbiological tests leukocyte count had the highest AUC of 91% and an accuracy of 87.8%. Granulocyte count had a sensitivity of 57%, specificity of 92% and AUC of 86%. The ROC curve for non-microbiological tests is shown in figure 3.

Table 5 Performance of non-microbiological and microbiological tests according to proposed EBJIS criteria.

Positive Findings	**Aseptic failure (n=104)**	**PJI* (n=44)**	**AUC (%) (95% CI)**	**Sensitivity (%) (95% CI)**	**Specificity (%) (95% CI)**	**PPV (%) (95% CI)**	**NPV (%) (95% CI)**	**Accuracy (%) (95% CI)**
Non-microbiological tests								
Clinical features[1]	0	19	-	43.2 (29.5-56.8)	100	100	80.6 (77.0-84.6)	83.1 (79.1-87.2)
Synovial fluid D-lactate >1.263 mmol/l	19	38	90.3 (85.7-95.0)	86.4 (75.0-95.5)	81.7 (74.0-88.5)	66.7 (57.8-76.6)	93.5 (88.7-97.5)	83.1 (77.0-89.1)
Synovial fluid leukocyte count >2000/µl[2]	9	35	91.0 (85.1-96.8)	79.5 (68.2-90.9)	91.3 (85.6-96.2)	80.0 (69.4-90.2)	91.4 (86.8-96.0)	87.8 (82.4-92.6)
Synovial fluid granulocyte percentage >70%[2]	8	25	86.1 (79.4-92.9)	56.8 (40.9-70.5)	92.3 (86.5-97.1)	75.9 (62.9-88.9)	83.5 (78.8-88.3)	81.8 (75.7-87.2)
Leukocyte count or percentage of granulocytes[3]	9	35	-	79.5 (68.2-90.9)	89.4 (83.7-95.2)	76.2 (66.0-87.2)	91.3 (86.5-95.9)	86.5 (81.1-91.9)
Histopathology of periprosthetic tissue	0/43	25/34	-	73.5 (58.8-88.2)	100	100	82.7 (75.4-91.5)	88.3 (81.8-94.8)

Microbiological tests								
Synovial fluid culture	8	20	-	45.5 (31.8-61.4)	100	100	81.2 (77.6-86.0)	83.8 (79.7-85.5)
Periprosthetic tissue culture[4]	7/63	17/41	-	41.5 (26.8-56.1)	100	100	72.4 (68.8-77.8)	76.9 (71.2-82.7)
Sonication fluid culture[4]	5/49	17/39	-	43.6 (28.2-59.0)	100	100	69.0 (63.6-75.4)	75.0 (68.2-81.8)
Any culture specimen	19	23	-	52.3 (38.6-65.9)	100	100	83.2 (79.4-87.4)	85.8 (81.8-89.9)

NOTE: If denominator is shown, the test was not performed in all patients. * PJI was confirmed when at least one of the following criteria were present: clinical features (i.e. macroscopic purulence of synovial fluid or surrounding the prosthesis or presence of sinus tract) increased synovial fluid leukocyte count (>2000 leukocytes/µl or >70% granulocytes), histopathological evidence of inflammation in periprosthetic tissue or significantly positive microbiology.

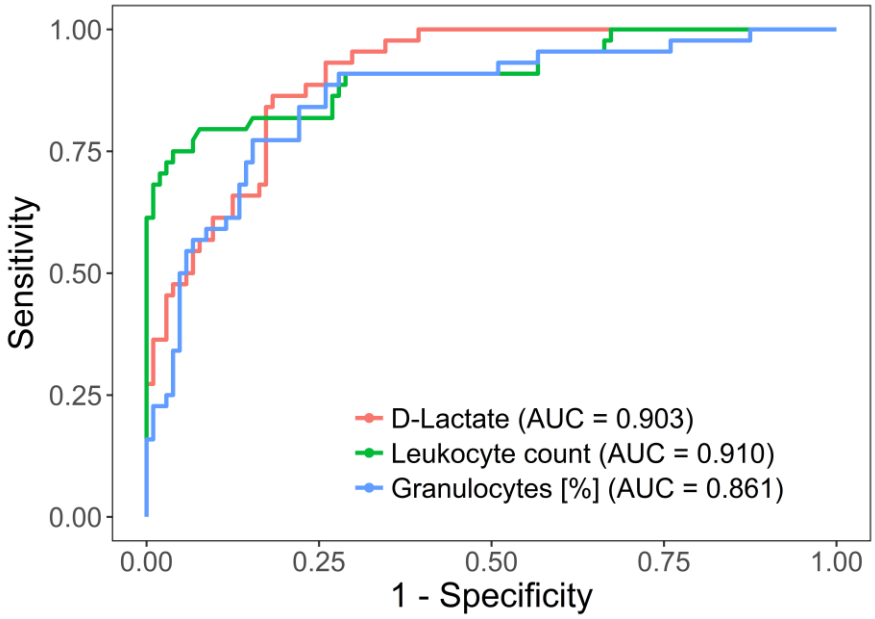
1 Eleven patients had visible purulence of the synovial fluid, 1 patient had sinus tract and 7 patients had both.

2 In 12 of 148 patients, the leukocyte count (n=9) or granulocyte percentage (n=8) were increased but were not diagnostic for PJI because of concomitant crystal arthropathy (n=1), recurrent dislocation (n=2), rheumatologic joint disease (n=3), early postoperative status (n=2), trauma (n=2), periprosthetic fracture (n=1) or metallosis with crystals (n=1).

3 The false positive results were interpreted as positive for assessing performance. In 3 cases, leukocyte count and percentage of granulocytes were not elevated above the cut-off although defined as not interpretable.

4 Growth of low-virulent microorganism in only one specimen was not sufficient for the diagnosis of PJI

Figure 3 The ROC curve of synovial fluid biomarkers for PJI.

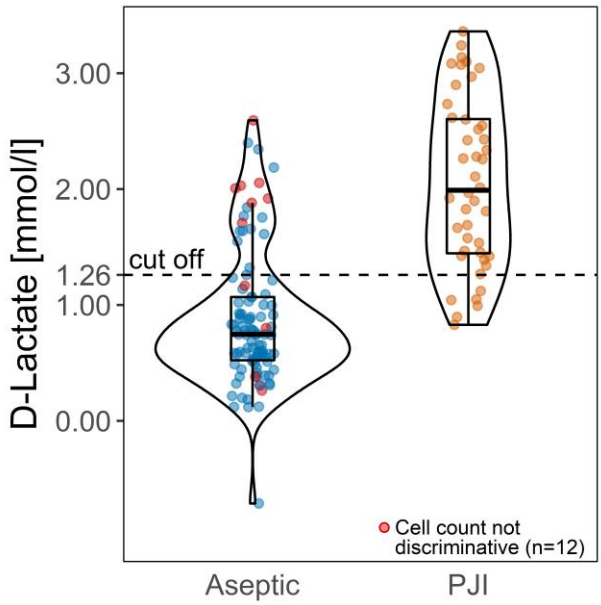


F.4.2. Performance of D-lactate in PJI

The optimal D-lactate cut-off value was calculated at 1.263 mmol/l. The sensitivity and specificity of the D-lactate test were 86.4% and 81.7%, respectively (Table 5). In 19 cases of aseptic failure D-lactate concentration was increased above the cut-off, including 12 aseptic cases with leukocyte count and differential under the threshold and 7 cases with non-interpretable cell count due to underling inflammatory condition. In 2 cases of false positive D-lactate samples contamination with a pathogen of skin flora was documented. D-lactate showed a negative result in 6 patients diagnosed with PJI according to applied definition criteria. Of these, in 2 cases the diagnosis of PJI was based on only one present criterion (increased synovial fluid leukocyte count or positive histopathology); in the remaining 4 cases, the diagnosis of PJI was based on multiple fulfilled criteria, including one case with sinus tract. The mean D-lactate concentration was significantly lower in aseptic failures than in PJI cases ($p < 0.001$).

No significant differences were observed between any pairwise comparisons of AUCs between investigated synovial fluid biomarkers ($AUC_{D\text{-}lactate}$ vs AUC_{WBC} $p=0.8$; Figure 3). The distribution of D-lactate in PJI and aseptic failures is depicted in Figure 4. In the 12 aseptic cases with non-diagnostic elevated leukocyte count due to underlying inflammatory conditions, 7 cases had positive D-lactate result and in 5 cases D-lactate was negative. In 6 of 12 cases the full diagnostic evaluation was performed confirming the aseptic pathology.

Figure 4 Distribution of D-lactate in patients with aseptic failure and PJI. Twelve cases with underlying inflammatory conditions and elevated leukocyte count are presented with red dots.

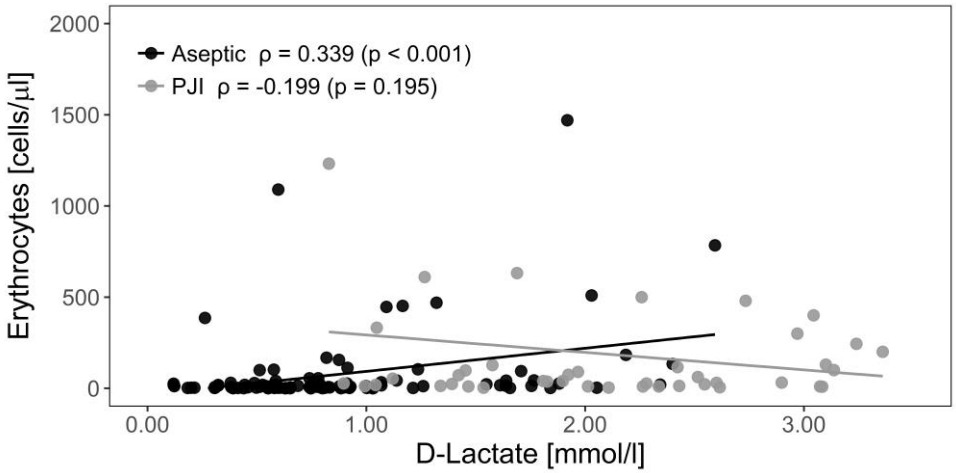


In acute PJI, D-lactate and leukocyte count showed a sensitivity of 100%, whereas in chronic PJI the sensitivity decreased to 81% and 72%, respectively (p=0.268) (Table 3). The performance of D-lactate and leukocyte count in early and delayed/late infections was compared. Whereas D-lactate showed a higher sensitivity compared to leukocyte count, leukocyte count was more specific for both groups. In patients presenting early after surgery, the tests showed a similar specificity (leukocyte count 67% vs D-lactate 58%; p= 0.572), whereas in delayed/late situations, leukocyte count was clearly more specific (leukocyte count 94% vs. D-lactate 84%; p=0.027). The sensitivity of leukocyte count and D-lactate in early infection was 86% and 100% respectively. In delayed/late infection D-lactate showed a sensitivity of 83% and leukocyte count was 78%. Among 36 delayed/late confirmed PJI there were 9 PJI with an acute presentation, suggesting hematological origin of the infection.

In culture-negative PJI, the mean concentration of D-lactate was significantly lower than in culture-positive PJI (0.915 mmol/l vs. 2.421 mmol/l; p=0.004). The mean D-lactate concentration of culture-negative PJI was significantly higher than in aseptic contaminated cases (0.915 mmol/l vs. 1.40 mmol/l; p <0.001). No significant difference in D-lactate concentration was observed comparing PJI caused by low-virulent and high-virulent microorganisms (2.047 mmol/l vs. 2.586 mmol/l; p=0.074) or early and delayed or late infections (1.459 vs. 1.217; p=0.196).

A positive correlation between erythrocytes and D-lactate overall ($\rho = 0.185$, p=0.02), as well as in the subgroup with aseptic failures ($\rho = 0.339$, p <0.01) was observed. In the subgroup with PJI a negative correlation was found, but it did not reach significance ($\rho = -0.199$, p= 0.195). The difference between the aseptic and PJI subgroups was significant (p <0.01). These results are presented in the Figure 5.

Figure 5 Correlation between synovial fluid erythrocyte and D-lactate concentration in patients with aseptic failure and PJI. Note: ρ= Pearson`s correlation.



G. Discussion

The investigation of synovial fluid with the determination of leukocyte count and the percentage of granulocytes was first used for native joints to distinguish osteoarthritis from rheumatic joint diseases and septic arthritis (2, 35). Later the synovial fluid analysis was extended to prosthetic joints, in which the optimal cut-off value to discriminate PJI from aseptic failure has remained a matter of debate. Leukocytes ranging from >1,100/µl to >4,200/µl and granulocyte percentages ranging from 65% to 80% have been proposed as cut-off values by different investigators (2, 8, 36). Our results for synovial fluid leukocyte count and percentage of granulocytes are slightly lower than in other studies (37). This could be explained through different cut-offs and study population difference as several studies exclude patients with inflammatory joint conditions. The synovial fluid leukocyte count showed a sensitivity of 80% in our study. However, in 12 patients the absolute or relative leukocyte count was elevated due to aseptic conditions, including rheumatologic joint disease (n=3), recurrent dislocation (n=2), early postoperative status (n=2), trauma (n=2), crystal arthropathy (n=1), periprosthetic fracture (n=1), and metallosis with crystals (n=1).

It is possible, that the sensitivity and specificity for leukocyte count and probably for D-lactate were lower than in other studies, as we have applied more sensitive definition criteria for PJI. In fact, most other studies on PJI are using MSIS definition criteria, which were shown to miss low-grade infections due to higher threshold to define PJI (12).

High sensitivity in previous studies and bacterial metabolism as the predominant source of D-lactate make the test attractive for further research. There are no peer-reviewed articles in D-lactate performance in synovial fluid of prosthetic joint. It seems that D-lactate could be beneficial in cases where cell count cannot be interpreted. We hypothesized that D-lactate is a reliable marker for the diagnosis of PJI, independent of the inflammatory reaction.

In the study, we included all consecutive patients with a suspicion of PJI. This prospective cohort is valuable as it shows performance of the test in the clinical environment. At the calculated cut-off value of 1.263 mmol/l the sensitivity and specificity of the D-lactate test were 86.4% and 81.7%, respectively. No significant differences were observed between any pairwise comparisons of AUCs of D-lactate, leukocytes and granulocytes ($AUC_{D\text{-}lactate}$ vs AUC_{WBC} p=0.8).

The total of 23 culture-positive PJI were caused by low-virulent pathogens in 10 episodes (43%) and by highly virulent pathogens in 13 episodes (57%). There were 21 cases (48%) of culture-negative PJI, which is higher than described in the literature. There the frequency varies from 5-35%. Antecedent antimicrobial therapy is the most common reason for negative cultures. In this study, the information about preoperative antibiotic treatment is lacking. Another explanation for higher amount of low-grade infection is greater sensitivity of EBJIS criteria. A history of previous PJI, chronic infection with low-grade pathogens and biofilm formation, inadequate use of available microbiological methods, and postoperative wound drainage or vascular insufficiency are also associated with culture-negative PJI (6, 38, 39).

In our study no influence of bacterial virulence on D-lactate concentration could be estimated (low-virulent 2.047 mmol/l vs. high-virulent 2.586 mmol/l; p=0.074). Also, time manifestation of infection in early and delayed or late infections (1.459 vs. 1.217; p=0.196) showed no influence. This data shows us that vivid bacteria produce D-lactate regardless of time period after implantation. On the other hand, it seems that D-lactate concentration depends on the amount of metabolic active bacteria, as in culture-negative PJI, where the mean concentration of D-lactate was significantly lower than in culture-positive PJI (0.915 mmol/l vs. 2.421 mmol/l; p=0.004). There was a difference of D-lactate concentration in culture-negative PJI and aseptic cases, corroborating the septic etiology in samples with negative culture.

There were 19 patients with positive clinical signs of PJI. In this PJI subgroup, D-lactate was elevated in 18 cases (94.7%). Surprisingly, D-lactate was clearly negative in one chronic, polymicrobial infected knee prosthesis with a sinus tract. We observed that all synovial fluid tests failed to diagnose an infection in this case. Only microbiological tissue samples and tissue histology showed the PJI, underlying the importance of tissue sampling during the revision procedure. At least 4 tissue samples were collected in order to increase the sensitivity of tissue cultures. Synovial fluid culture and periprosthetic tissue culture are valuable diagnostic tools for identification of the infecting pathogens and determination of antimicrobial susceptibility. Sensitivity of aspirated fluid varies in recent studies from 50-87%. Acute infections have higher sensitivity than chronic infection, due to dormant bacterial metabolic state and biofilm formation in the latter. The cultivation of sonication fluid and multiple sampling of periprosthetic tissue improved sensitivity (6, 40).

Delayed infection is known to evoke only subtle clinical signs and symptoms most likely due to the low microbial burden. As the bacterial metabolism decreases with maturation of the biofilm, detectable amounts of D-lactate are still produced. Further investigation requires studies in specific

condition as low-grade infection or infection in acute postoperative period to make a further breakthrough in diagnostic of PJI.

D-lactate showed a negative result in 6 patients diagnosed with PJI according to applied definition criteria. Of these, in 2 cases the diagnosis of PJI was based on only one present criterion (increased synovial fluid leukocyte count or positive histopathology); in the remaining 4 cases, the diagnosis of PJI was based on multiple fulfilled criteria, including one case with sinus tract. It remains unclear whether these cases are actually PJI cases or they represent over-diagnosed cases.

Nevertheless, classification of doubtful cases, regardless of the D-lactate results, was for the clinical purposes discussed in the interdisciplinary team and the therapy concept was developed by consensus.

On the other hand, D-lactate was falsely increased in 19 patients with aseptic failure, which lowered the specificity of the test. There were 12 aseptic cases with leukocyte count and differential under the threshold and 7 cases with non-interpretable cell count due to underlying inflammatory condition. Further studies are needed to investigate D-lactate trends in inflammatory joint diseases.

In the search for an explanation of falsely elevated D-lactate values, we assumed possible interaction during the spectrophotometric process. Due to the positive correlation between erythrocytes and D-lactate in the aseptic group ($\rho = 0.339$, $p < 0.01$), we hypothesize that hemoglobin may cause the false-positive D-lactate test due to similar absorbance wavelengths, i.e. 540 nm for hemoglobin and 570 nm for D-lactate (41).

In patients with PJI, the slightly negative correlation ($\rho = -0.199$, $p = 0.195$) can be explained by a significant source of D-lactate from bacterial metabolism, where other factors cannot influence concentration. We have not evaluated whether centrifugation of the synovial fluid sample may potentially improve the specificity of the D-lactate test. Moreover, a modification of spectrophotometric D-lactate dehydrogenase enzymatic assay or development of other assays such as electrochemical detection principle may further improve the reliability of PJI diagnostic. The clinical use of D-lactate assay as a routine diagnostic tool will depend on the access to reliable and quick D-lactate assay, such as a bedside, point-of-care test.

H. Conclusion

For the first time a synovial fluid D-lactate was investigated for the purpose of PJI. It is an accurate diagnostic test and shows comparable results to the synovial fluid leukocyte count. The results so far have been very promising and because D-lactate is a quantitative test rather than a dichotomous yes/no test, the use of different cutoff values allows the physician to decide whether the test is more sensitive (screening rule out) or more specific (confirmatory rule in). Moreover, it requires only 50 µl of synovial fluid, has a short turnaround time and is inexpensive. These promising data suggest that adding D-lactate to diagnostic algorithm could improve the diagnostic of PJI.

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III. Statutory Declaration

“I, Katsiaryna Yermak, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic “Evaluation and development of a new diagnostic tool for early and reliable diagnostic of prosthetic joint infection”, independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

Date Signature

IV. Declaration of share in publication

Katsiaryna Yermak had the following share in the following publication:

Co-first-authorship (marked in the authors byline with *):

Katsiaryna Yermak*, Svetlana Karbysheva*, Carsten Perka, Andrej Trampuz, Nora Renz. "Performance of synovial fluid D -lactate for the diagnosis of periprosthetic joint infection: A prospective observational study"

Journal of Infection. 2019; 0163-4453

doi: 10.1016/j.jinf.2019.05.015. [Epub ahead of print]

Contribution in detail:

Overview

The scientific publication selected for the present doctoral thesis was developed under the supervision of PD Dr. Andrej Trampuz, Head of the Septic surgery unit in the Center for musculoskeletal surgery at Charité - Universitätsmedizin Berlin. In this study we recruited prospectively all patients who underwent the routine diagnostic synovial fluid aspiration due to a suspicion of infection of the prosthetic joint. D-lactate was measured in the synovial fluid for the purpose of infection-identification. The study design and timeline was supervised by Dr. Trampuz and Dr. Renz.

Author Katsiaryna Yermak (Yermak) was involved in each phase of the research project. Her contribution is described in the following text:

Contribution, development of the research idea and literature research

Yermak conducted a detailed research on current challenges and issues in the diagnosis of prosthetic joint infection, studies involving novel biomarkers and current D-lactate application in the research and clinical practice. Moreover, Katsiaryna Yermak elaborated the possible D-lactate interaction with the spectrophotometric method.

Recruitment of the patients and joint puncture

Yermak recruited patients in the ambulant setting if a diagnostic joint puncture was indicated. Orthopedic surgeons in both clinic locations Charité – Campus Mitte and Campus Virchow-Clinic, performed the synovial fluid aspiration.

Laboratory work (sample preparation, D-lactate measurement)

After obtaining the study samples, Yermak was responsible for proper transfer, storage and measurement of D-lactate using the spectrophotometric method. Each of both authors conducted around 50% of measurements.

Database and statistical analysis

Yermak was responsible for the data collection and its organization. The data analysis plan was optimized with Dr. Renz in weekly study meetings, in order to transparently and clearly present the research data.

Preparation of the manuscript and publication

Initially, Yermak wrote the draft of the manuscript, whose single sections were discussed with other co-authors. Afterwards Yermak produced several revised versions which were intensively discussed with Dr. Renz an instantly supervised by PD Dr. Trampuz, Univ. Prof. Dr. Perka provided a valuable review on the paper.
Dr. Trampuz submitted the article to the Journal of Infection. As the article was initially accepted with minor revision, Yermak prepared the new version and accurately replied to reviewers´ comments.

Presentation of the results in the scientific meetings and conferences

The total duration of the study till publication was 4 years. During this time, preliminary results were presented in several international conferences and workshops. Yermak presented first preliminary data on D-lactate in form of the e-poster in the ECCMID in Vienna 2017.

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Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

V. The extract from the Journal Summary List (ISi Web of Knowledge^{SM})

JOURNAL OF INFECTION

Rank	14
Total cites	6,636
Journal impact factor	4.603
Eigenfactor Score	0.014730

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE,SSCI
Selected Categories: **"INFECTIOUS DISEASES"** Selected Category Scheme: WoS

Gesamtanzahl: 88 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	LANCET INFECTIOUS DISEASES	20,494	25.148	0.067280
2	Lancet HIV	1,476	11.355	0.007950
3	CLINICAL INFECTIOUS DISEASES	61,618	9.117	0.120010
4	EMERGING INFECTIOUS DISEASES	29,657	7.422	0.057980
5	Eurosurveillance	8,482	7.127	0.031200
6	INFECTIOUS DISEASE CLINICS OF NORTH AMERICA	2,503	5.449	0.005170
7	CLINICAL MICROBIOLOGY AND INFECTION	15,983	5.394	0.039650
8	JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY	29,292	5.217	0.050730
9	JOURNAL OF INFECTIOUS DISEASES	45,662	5.186	0.075270
10	Journal of the International AIDS Society	3,638	5.131	0.013920
11	AIDS	20,578	4.914	0.038030
12	INTERNATIONAL JOURNAL OF HYGIENE AND ENVIRONMENTAL HEALTH	4,282	4.848	0.006360
13	Current HIV/AIDS Reports	1,490	4.710	0.004890
14	JOURNAL OF INFECTION	6,636	4.603	0.014730
15	Travel Medicine and Infectious Disease	1,230	4.450	0.003610
16	Current Opinion in HIV and AIDS	2,266	4.409	0.008060
17	ACS Infectious Diseases	749	4.325	0.003090
18	INTERNATIONAL JOURNAL OF ANTIMICROBIAL AGENTS	10,395	4.253	0.016630

Selected JCR Year: 2017; Selected Categories: "INFECTIOUS DISEASES"

VI. The selected publication

Authors:
Katsiaryna Yermak, Svetlana Karbysheva, Carsten Perka, Andrej Trampuz, Nora Renz

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

Journal:
Journal of Infection

Publication History:
Published online: 21 May 2019
Accepted: 17 May 2019

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

Impact Factor (JCR Year 2017):
4.603

VII. Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

VIII. List of publications

1. **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. *Journal of Infection*. 2019. https://doi.org/10.1016/j.jinf.2019.05.015. *impact factor: 4.603*

2. Renz N, **Yermak K**, Perka C, Trampuz A. Alpha defensin lateral flow test for diag-nosis of periprosthetic joint infection: not a screening but a confirmatory test. *The Journal of Bone & Joint Surgery*. 2018. https://doi.org/10.2106/JBJS.17.01005. *impact factor: 4.840*

3. 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? *Clinical Orthopaedics and Related Research* 2018. https://doi.org/10.1097/CORR.0000000000000336. *impact factor: 4.091*

4. Hrovat G., Fister Jr, I., **Yermak K**., Stiglic G., Fister I. (2015). Interestingness measure for mining sequential patterns in sports. *Journal of Intelligent & Fuzzy Systems*. 2015. https://doi.org/10.3233/IFS-151676. *impact factor: 1.812*

IX. Acknowledgment

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