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

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SUMMARY

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.

Introduction

Periprosthetic joint infection (PJI) represents a serious complication after arthroplasty, which is associated with considerable morbidity and mortality. An accurate diagnosis of infection is crucial to plan adequate treatment. Several attempts were made to investigate different biomarkers, such as alpha-2-macroglobulin, adenosine deaminase, procalcitonin, IL-1, IL-6, IL1β and alpha defensin, which can be helpful in distinction of PJI from aseptic pathology.[1–3] Currently used diagnostic tests of synovial fluid lack sensitivity or specificity for PJI.[4] For example, synovial fluid alpha defensin 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 rather than screening test.[5,6] Synovial fluid culture requires time and has limited sensitivity and specificity in chronic low-grade PJI.[4,7,8] The low level of inflammation and subtle clinical symptoms may impede the diagnosis of low-grade PJI, which usually occur several months to years after arthroplasty. The diagnosis is also difficult in the early postoperative period where leukocyte count, C-reactive protein and clinical signs hamper a reliable diagnosis due to local tissue inflammation.[9–11]

D-lactate is the predominant form of lactate produced by different bacterial species. It is present in the blood of mammals only in nanomolar concentrations accounting for 1–5% of total lactate concentration and produced mainly by intestinal microbiota.[12,13] In eukaryotic cells, endogenous D-lactate is produced through metabolism of methylglyoxal in a concentration, which does not contribute to acidemia.[14] Several studies were carried

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Table 1
According to working definition of the European Bone and Joint Infection Society (EBJIS), periprosthetic joint infection is defined if ≥1 criterion is fulfilled.

Test	Criteria
Clinical features	Sinus tract (fistula) *or* visible purulence around prosthesis
Histopathology	Acute inflammation in periprosthetic tissue[a]
Cell count in joint aspirate[b]	>2000/µg leukocytes *or* >70% granulocytes
Microbiology	Microbial growth in: • Synovial fluid *or* • ≥2 tissue samples[c] *or* • Sonication fluid (≥50 CFU/ml)[d]

[a] Acute inflammation is defined as ≥23 granulocytes per high/power field, corresponding to type II or III according to Krenn and Morawietz.[33]
[b] Leukocyte cut-offs are not considered diagnostic within 6 weeks after surgery, in active rheumatic joint disease, periprosthetic fracture, joint trauma or dislocation.
[c] Periprosthetic tissue culture was considered positive if a high-virulent organism grew in ≥1 specimen (*Staphylococcus aureus*, Enterobacteriaceae, *Streptococcus* spp., *Candida* spp.) or a low-virulent organism grew in ≥2 specimen (coagulase-negative staphylococci, enterococci, *Cutibacterium* [formerly known as *Propionibacterium*] spp., and other bacteria of the skin microbiome).
[d] 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.[22]

out to measure the D-lactate concentration in primary sterile body fluids already back in the 1990s in order to discriminate infection from aseptic inflammation.[15–17] D-lactate was shown to be a promising marker for the diagnosis of bacterial infection in patients with meningitis and arthritis,[16,18] including in patients receiving antimicrobial therapy.[15]

The aim of this study is to evaluate the performance of D-lactate in synovial fluid as independent diagnostic marker for diagnosis of early and delayed/late PJI using sensitive diagnostic criteria and to compare it with synovial fluid leukocyte count. We hypothesized that D-lactate is a reliable marker for the diagnosis of PJI, independent of the inflammatory reaction.

Patients and methods

Study design and population

This prospective diagnostic cohort study 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 before revision arthroplasty for evaluation of infection between May 2016 and March 2017. Only one (the first collected) synovial fluid sample per patient was considered.

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

Diagnosis of periprosthetic joint infection

PJI was defined according to the previously published criteria,[19] which became the working criteria of the European Bone and Joint Infection Society (EBJIS)[6] and 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 symptoms 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.[20]

Retrieval and investigation of synovial fluid, periprosthetic tissue and implants

Synovial fluid was aspirated under sterile conditions preoperatively in the outpatient department or during revision surgery before opening the joint capsule. One ml of 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.) or a medium or low-virulent organism grew in ≥2 specimen (coagulase-negative staphylococci, enterococci, *Cutibacterium* [formerly known as *Propionibacterium*] spp., and other bacteria of the skin microbiome).

The retrieved prosthetic components were sent for sonication, as previously described.[21] 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.[22]

Determination of synovial fluid leukocyte count, percentage of granulocytes and erythrocyte count

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 haematology analyzer (XE-2100, Sysmex, Norderstedt, Germany). Clotted specimens were treated with 10 µl hyaluronidase (Sigma-Aldrich Chemie, Taufkirchen, Germany) for 10 min at room temperature.

Determination of synovial fluid D-lactate

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 h after aspiration. The tests were performed according to the manufacturerś 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

Table 2
Characteristics of patients.

	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
Male sex, no. (%)	81 (55)	30 (68)	51 (49)	0.032
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)	

Table 3
Performance of non-microbiological and microbiological tests according to working criteria of EBJIS.

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[a]	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[b]	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%[b]	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 [c]	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[d]	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[d]	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 was 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, significantly positive microbiology.

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

[b] 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 ($n = 1$).

[c] 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.

[d] Growth of low-virulent microorganism in only one specimen was not sufficient for the diagnosis of PJI.

reacts with the fluorescent substrate to yield coloration of the mixture.[23]

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, incubated at 37 °C for 30 min and then read at 570 nm by Microplate Absorbance Reader (DYNEX Technologies MRX, Chantilly, VA, USA).

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 3). 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 in Fig. 3, bootstrap resampling with 10,000 replicates was performed. The correlation between erythrocyte and D-lactate concentration was estimated using Pearson coefficient (ρ). For all statistical analyses IBM SPSS 22.0 (Statistical

Table 4
Isolated microorganisms in 23 patients with culture-positive PJI.

Pathogen	No. (%)
Coagulase-negative staphylococci[a]	11 (48)
Staphylococcus aureus	5 (22)
Streptococcus spp.[b]	3 (13)
Gram-negative rods[c]	3 (13)
Enterococcus spp.	1 (4)
Bacteroides fragilis	1 (4)

Note: The sum exceeds 100% as there was one mixed infection.
[a] Including *S. epidermidis* ($n=8$), *S. lugdunensis* ($n=2$), *S. haemolyticus* ($n=1$).
[b] Including *S. gallolyticus* ($n=1$), *S. agalactiae* ($n=2$).
[c] Including *E. coli* ($n=1$), *Campylobacter coli* ($n=1$) and one patient with a mixed infection (*E. coli, K. oxytoca, P. mirabilis, and Bacteroides fragilis).*

package for the Social Sciences Corporation, Chicago, IL, USA) was used. ROC and other plots were produced by R Computing environment.[24]

Results

Patient demographic data

Table 2 summarizes characteristics of 148 patients, including 103 (70%) 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.

Performance of conventional tests and microbiology

Performance of diagnostic tests is shown in Table 3. The synovial fluid leukocyte count showed a sensitivity of 80%. There were 21 cases (48%) of culture negative PJI. Significant microbial growth was documented in 23 patients with PJI (52%), whereas formal contamination (i.e. insignificant growth) was detected in 8 cases with PJI and in 19 cases with aseptic failure. Table 4 summarizes the causative pathogens of PJI. The total of 23 culture-positive PJI were caused by low-virulent pathogens in 10 episodes (43%) and by high-virulent pathogens in 13 episodes (57%).

Performance of synovial fluid D-lactate

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 3). 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 a contamination with 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 ($p < 0.001$). For the commercial D-lactate test kit, 50 µl of synovial fluid is required. The turn-around time of both tests was 30–45 min.

Comparison of synovial fluid D-lactate and leukocyte count

No significant differences were observed between any pairwise comparisons of AUCs between investigated synovial fluid biomarkers ($AUC_{D\text{-lactate}}$ vs. AUC_{WBC} $p=0.8$; Fig. 1). The distribution of D-lactate and leukocyte count in PJI and aseptic failures is depicted in Fig. 2. 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. Of these 12 patients, 11 underwent revision surgery and eventually in 6 of 12 cases the full diagnostic evaluation was performed confirming the aseptic pathology.

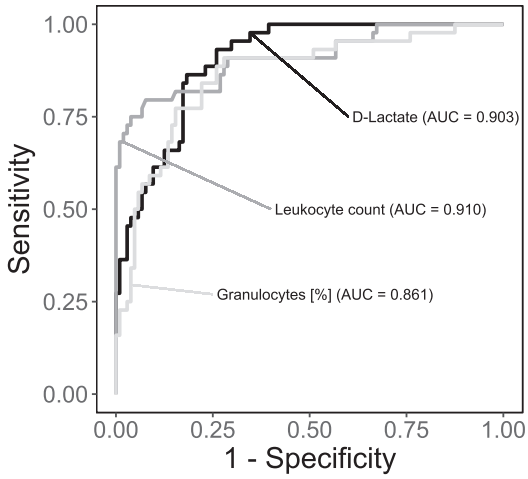

Fig. 1. The ROC curve of synovial fluid biomarkers for PJI. The AUC of D-lactate, leukocyte count and percentage of granulocytes are 0.903, 0.910 and 0.861, respectively.

Table 5
Positive and negative likelihood ratios (LR+ and LR−) of D-lactate and leukocyte count in all, early and delayed/late PJI.

Type of PJI	Test	LR+	LR−
All PJI	D-lactate test	4.72	0.17
	Leukocyte count	9.14	0.22
Early PJI	D-lactate test	2.38	0.00
	Leukocyte count	2.61	0.21
Delayed/late PJI	D-lactate test	5.19	0.20
	Leukocyte count	13.00	0.23

In acute PJI, D-lactate and leukocyte count showed both a sensitivity of 100%, whereas in chronic PJI the sensitivity decreased to 81% and 72%, respectively ($p=0.268$). The performance of D-lactate and leukocyte count in early and delayed/late infections is shown in Fig. 3. 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 (67% vs. 58%; $p = 0.572$) and sensitivity (86% vs. 100%; $p = 0.364$) due to smaller sample size (n = 19). In delayed/late situations leukocyte count D- was more specific (94% vs. 84%; $p=0.027$).

The likelihood ratio (LR) of D-lactate and leukocyte count is summarized in Table 5. The overall positive and negative LR showed moderate influence on the posttest probability of the disease for both tests. In case of negative test, D-lactate is superior over leukocyte count and effects posttest probability more, especially for early PJI. In case of positive test result, leukocyte count has more impact on posttest probability, however both struggle for early PJI.

Synovial fluid D-lactate concentration and microbiology

In culture-negative PJI, the mean concentration of D-lactate was significantly lower than in culture-positive PJI (0.915 mmol/l

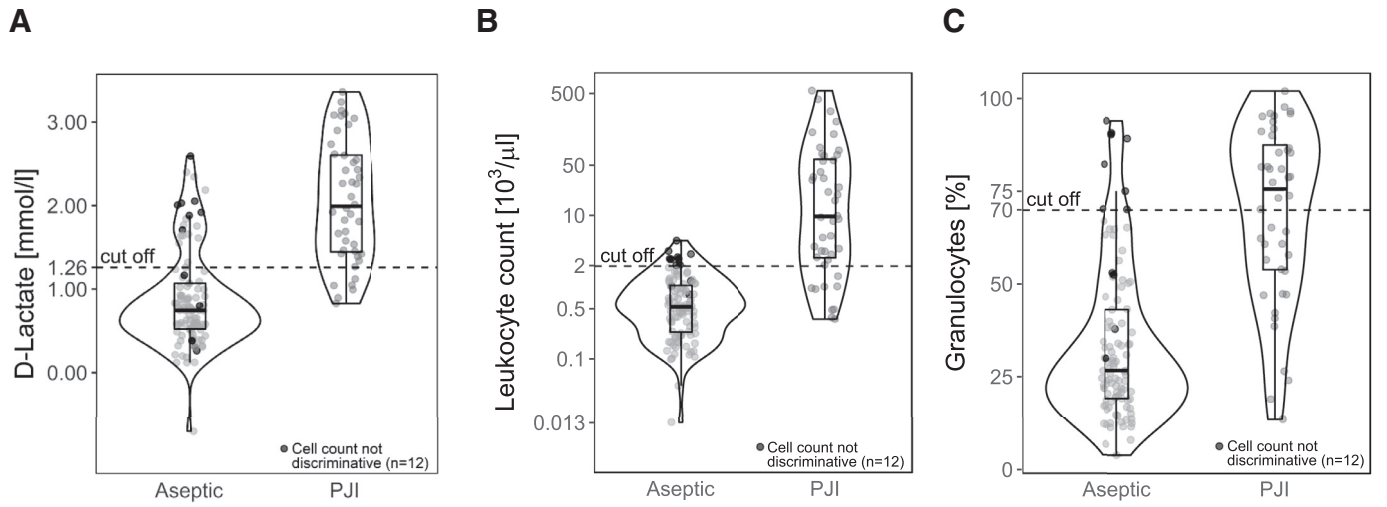

Fig. 2. Distribution of D-lactate (A) and leukocyte count (B) and percentage of granulocytes (C) in patients with aseptic failure and PJI. Twelve cases with underlying inflammatory conditions and elevated leukocyte count or percentage of granulocytes above the threshold are presented with dark grey dots.

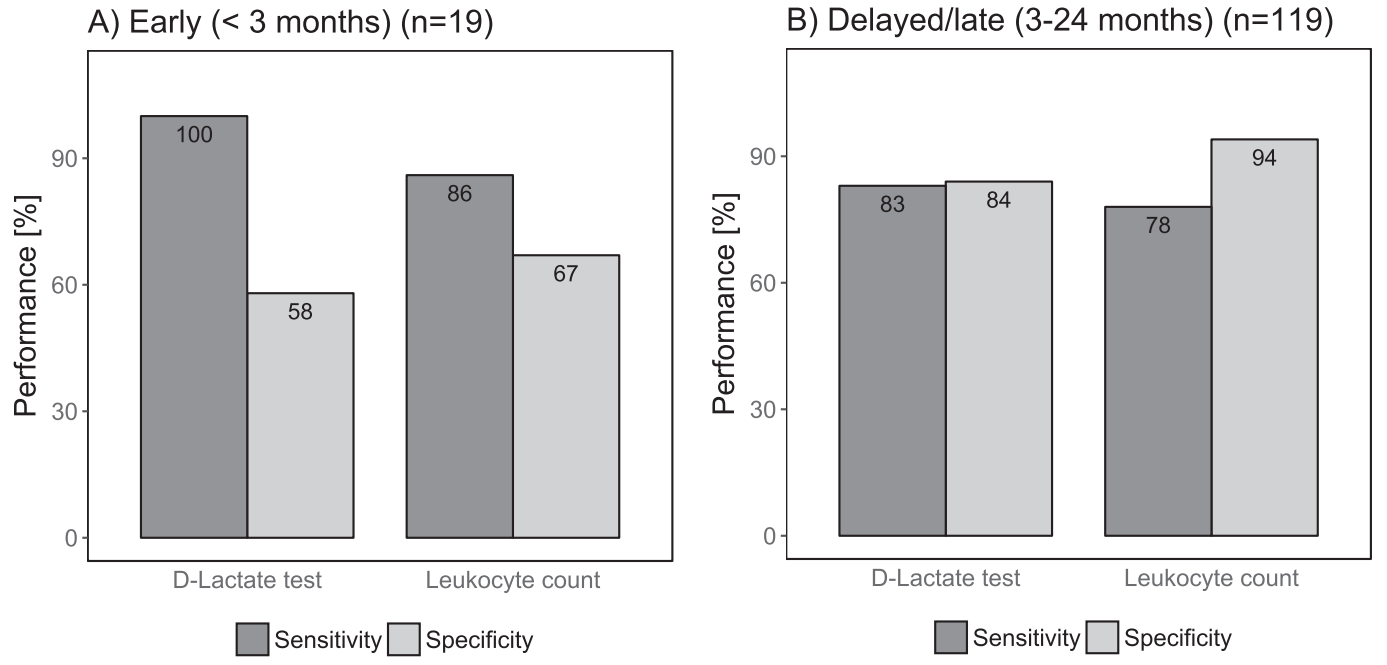

Fig. 3. Performance of synovial fluid D-lactate test and leukocyte count in early postoperative PJI (A) and delayed or late PJI (B). Difference in specificity in delayed/late PJI was significant ($p = 0.027$), whereas in early PJI not ($p = 0.572$).

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; $p = 0.196$).

Correlation between synovial fluid erythrocyte and D-lactate concentration

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, however, it did not reach significance ($\rho = -0.199$, $p = 0.195$) (Fig. 4). The difference between the aseptic and PJI subgroups was significant ($p < 0.01$).

Discussion

Several biomarkers have been investigated as diagnostic test for PJI in recent years.[1,2,25] However, none was exclusively assessed regarding their ability to detect low-grade infections and early postoperative infections, both of which are challenging to differentiate from aseptic conditions. The performance of diagnostic tests strongly depends on the applied definition criteria for infection. Most studies used MSIS definition criteria,[26] which miss several low-grade infections due to the high threshold to confirm infection.[6] In this study, we used criteria with lower threshold for diagnosing PJI, detecting also low-grade PJI.[6,27] In contrast to MSIS criteria, CRP and ESR are not considered as diagnostic criterion for PJI as they are of little benefit in low-grade infections and are not specific for PJI.[11] Furthermore, the leukocyte esterase is not included, as it provides reliable results only in samples not contaminated with blood.[25]

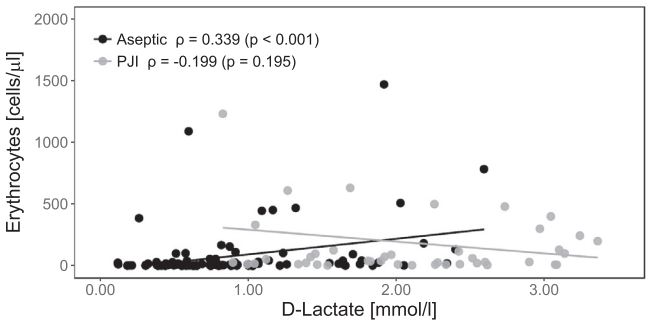

Fig. 4. Correlation between synovial fluid erythrocyte and D-lactate concentration in patients with aseptic failure and PJI. *Note:* ρ = Pearson's correlation.

Delayed infections are 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, still detectable amounts of D-lactate are produced. There was a statistically significant difference of D-lactate concentration in culture negative PJI and aseptic cases, corroborating the septic aetiology in samples with negative culture. In addition, the D-lactate concentration seems to depend on the number of bacteria, as concentration of D-lactate was higher in culture-positive than in culture-negative PJI.

In our study, 6 patients with chronic PJI had a false-negative synovial fluid D-lactate test, two of which were culture-positive (1 polymicrobial infection with sinus tract and coagulase-negative staphylococci in synovial fluid). In four of them, the synovial fluid leukocyte count was also normal and in 3 of them, infection was only confirmed by positive periprosthetic tissue histopathology (i.e. type II or III according to Krenn and Morawietz[33]). It remains unclear whether these cases are really PJI or they represent overdiagnosed cases of PJI by using working criteria of EBJIS. Against this background, assuming, these four cases were aseptic failures, the sensitivity and specificity of D-lactate would be 95% and 82%, respectively.

In one case, sinus tract was present, which was previously described to alter the diagnostic markers in synovial fluid due to the constant drainage of the inflammation. Whereas production of D-lactate was described for several bacterial species including *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli, Klebsiella pneumoniae* and *Bacteroides fragilis* as well as for Lactobacillales and gut microbiota,[15,17,28] data on D-lactate production by other bacteria in body fluids is limited. No influence of bacterial virulence on D-lactate concentration could be estimated according to our data and data in the literature.[16]

D-lactate concentration was increased above the cut-off in 19 patients with aseptic failure. Based on the positive correlation between erythrocytes and D-lactate in the aseptic group, 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.[29] In patients with PJI, the slightly negative correlation 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.

We acknowledge several limitations to this initial work. First, the lack of patient follow-up limits the value of this study. However, as the used definition criteria are rather prone to overdiagnose than underdiagnose PJI, the number of missed PJI are rather negligible. Furthermore, we only included a small number of patients with early postoperative PJI, as their joints are not routinely aspirated but rather immediately surgically revised. The information about prior antibiotic use is not complete therefore, the effect of antimicrobial therapy on D-lactate performance could not be assessed. Finally, the main limitation of this method is that D-lactate dehydrogenase may cross-react with many endogenous substances, including fructose 1,6-bisphosphate, 3-phosphoglyceric acid, pyruvic acid, L-lactate, and S-lactoyl-glutathione,[12,30] leading to poor accuracy. Alternatively the high-performance liquid chromatography showed better and reliable measurements and should be considered for further analysis.[31,32]

In conclusion, synovial fluid D-lactate is an accurate diagnostic test for the diagnosis of PJI, comparable to the synovial fluid leukocyte count. It requires only 50 µl of synovial fluid, has a short turnaround time and is inexpensive. Modifications of the test may potentially improve its specificity or may be combined with a confirmatory test with higher specificity.

Conflict of interests

The authors declare that there is no conflicts of interests with respect to the research, authorship, and/or publication of this article. The funding organization, the PRO-IMPLANT Foundation, had no influence on the study design, collection, analysis, and interpretation of data and in the publication process.

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