Performance of established test methods for diagnosing chronic periprosthetic joint infections caused by low-virulence pathogens

From Medical University of Vienna, Vienna, Austria

M. Luger,[1] L. Rabitsch,[1] M. McNally,[2] M. Rupp,[3] C. Böhler,[1] K. Staats,[1] R. Windhager,[1] I. K. Sigmund[1]

[1]Department of Orthopaedics and Trauma Surgery, Medical University of Vienna, Vienna, Austria
[2]Nuffield Orthopaedic Centre, Oxford University Hospitals, Oxford, UK
[3]Department of Trauma, Hand and Reconstructive Surgery, University Hospital Giessen, Giessen, Germany

Cite this article:
Bone Joint Res 2025;14(12):
1135–1144.

DOI: 10.1302/2046-3758.
1412.BJR-2025-0236.R1

Correspondence should be sent to Irene Katharina Sigmund irene.sigmund@meduniwien.ac.at

Aims

Chronic periprosthetic joint infections (PJIs) caused by low-virulence pathogens can be challenging to differentiate from aseptic failure. The aim of this study was to assess the diagnostic accuracy of the most commonly used tests for diagnosing these PJIs.

Methods

A consecutive series of 420 patients undergoing revision total hip or knee arthroplasty were studied. Coagulase-negative staphylococci, *Cutibacterium spp.*, enterococci, *Actinomyces spp.*, and fungi were classified as low-virulence pathogens. Of the 207 PJIs defined by the European Bone and Joint Infection Society (EBJIS) criteria, 60 were chronic infections caused by low-virulence pathogens. A total of 213 cases were classified as aseptic, resulting in a total of 273 cases included in the analysis. The performance of established test methods was assessed using receiver operating characteristic (ROC) curves.

Results

The calculated synovial fluid percentage of polymorphonuclear neutrophils (SF-%PMN) cut-off of > 67% demonstrated the best preoperative performance with a sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) of 97.1% (95% CI 83.9 to 100), 97.2% (95% CI 91.9 to 100), 97.1% (95% CI 91.6 to 100), 97.2% (95% CI 91.9 to 100), and 0.972 (95% CI 0.933 to 1.000), followed by SF white blood cell count (SF-WBC) > 2,403 cells/µl with estimates of 85.4% (95% CI 71.1 to 93.4), 100% (95% CI 94.6 to 100), 100% (100), 93.3% (95% CI 88.0 to 98.5), and 0.927 (95% CI 0.872 to 0.982). While a %PMN cut-off of > 67% outperformed the established threshold of > 80% (p = 0.010), the calculated SF-WBC cut-off of > 2,403 G/l did not perform significantly better than the established threshold of > 3,000 G/l (p = 0.266). Intraoperatively, permanent paraffin-fixed sections showed the best accuracy with performance values of 89.3% (95% CI 78.1 to 95.3), 100% (95% CI 97.7 to 100), 100% (100), 97.1% (95% CI 94.8 to 99.4), and 0.946 (95% CI 0.906 to 0.987), followed by frozen sections (85.7% (95% CI 67.7 to 94.6), 99.2% (95% CI 95.1 to 100), 95.8% (95% CI 87.8 to 100), 96.9% (95% CI 94.0 to 99.9), and 0.922 (95% CI 0.853 to 0.991)).

Conclusion

Synovial fluid %PMN and WBC, and permanent histological sections demonstrated the highest diagnostic performance for diagnosing PJI caused by low-virulence pathogens. Intraoperative frozen sections proved to be a valuable intraoperative tool, particularly in cases with unclear preoperative diagnosis. A lower cut-off for SF-%PMN may be considered to more accurately diagnose these infections.

Bone & Joint Research

Article focus

- This study investigated the diagnosis of chronic periprosthetic joint infections (PJIs) specifically caused by low-virulence pathogens.
- The diagnostic accuracy of various established test methods (serum CRP, synovial fluid white blood cell count (SF-WBC), percentage of polymorphonuclear neutrophils in synovial fluid (SF-%PMN), conventional culture (synovial fluid, tissue, sonication fluid), alpha-defensin, and histological analysis) using the European Bone and Joint Infection Society (EBJIS) definition as the reference standard.

Key messages

- Preoperatively, SF-WBC and SF-%PMN demonstrated the highest diagnostic accuracy.
- Lower cut-off values (SF-WBC > 2,403 cells/µl, SF-%PMN > 67%) are strongly indicative of chronic PJI due to low-virulence organisms.
- Histology provided the best diagnostic performance among intraoperative methods.

Strengths and limitations

- This is the first study to comprehensively assess multiple diagnostic tests for low-virulence chronic PJI within a single cohort using the EBJIS definition.
- This study includes one of the largest cohorts focused specifically on low-virulence pathogens.
- Limitations include its retrospective design and the lack of a standardized definition for 'low-virulence pathogens' in current literature.

Introduction

Periprosthetic joint infection (PJI) represents one of the most severe complications after total hip or knee arthroplasty and places a considerable burden on healthcare systems.[1] Accurate diagnosis is essential to determine an appropriate treatment strategy, minimize morbidity and mortality, and preserve joint function and quality of life.[2,3] With the introduction of standardized infection definitions, substantial improvements in diagnosis have been achieved.[4-6] However, infections caused by low-virulence pathogens (e.g. coagulase-negative staphylococci and *Cutibacterium spp.*) continue to pose a major challenge for orthopaedic surgeons and clinicians.[7] While patients with PJI caused by high-virulence pathogens often present with characteristic local and systemic signs of infection (e.g. fever, pain, erythema, swelling, impaired joint function, and wound dehiscence or effusion), patients with infections caused by low-virulence pathogens often have only unspecific and/or mild symptoms (e.g. chronic pain and early loosening), which are difficult to distinguish from aseptic complications. Due to the indolent clinical presentation without the characteristic signs of an acute infection, some surgeons may not perform pre- and/or intraoperative tests to confirm or exclude PJI at all. However, if performed, diagnostic tests may remain inconclusive,[8] potentially misinterpreting these PJIs as aseptic failure.

Low-virulence organisms are capable of forming biofilms on implants or necrotic bone and tissue.[8,9] Mature biofilms protect bacteria from the host immune system reducing and inhibiting the production and release of inflammatory proteins and, hence, the overall immune response. Concentrations of inflammatory parameters (e.g. CRP, interleukin-6, white blood cell count) in the blood, synovial fluid, and tissue of the affected joint may be completely normal or minimally elevated, making the diagnosis difficult.

The lack of diagnosis or the inconclusive results may lead to insufficient therapy of the hidden infection, significantly increasing the risk of persistent infection or reinfection.[10] Therefore, in all painful arthroplasties, PJI should be considered during preoperative workup and planning.[10,11]

To date, little is known about the exact diagnostic value of commonly used diagnostic tests in chronic PJIs caused by low-virulence organisms, and only very few studies have specifically considered these infections when calculating diagnostic accuracies.[12] Most studies do not differentiate between specific causative microorganisms in their analyses.[13,14]

The aim of this study was to assess the diagnostic accuracies of established serum and synovial fluid parameters, cultures, and histology for diagnosing chronic PJI caused by low-virulence pathogens.

Methods

Study design

This study was conducted in accordance with the World Medical Association's Declaration of Helsinki and the guideline for diagnostic studies at a single tertiary hospital specialized in treating PJI.[15,16] Ethical approval by the Medical University of Vienna institutional ethical review board was obtained (EK 2172/2023). Between January 2015 and December 2023, a consecutive series of patients who underwent revision surgery following total hip (THA) and knee arthroplasty (TKA) was retrospectively included. Patients who had undergone surgery within the last six weeks or at the second stage of a two-stage exchange procedure, an antibiotic-loaded cement spacer in place, and periprosthetic fractures were excluded. PJI was diagnosed according to the European Bone and Joint Infection Society 2021 definition.[4] Prior to revision surgery, a standardized diagnostic workup was initiated at our institution. Accordingly, demographic data, comorbidities, clinical features (e.g. sinus tract, pus, fever, local signs of infection), blood samples, synovial fluid analysis, microbiology, histology, and radiographs were documented. For cases treated before 2021, the diagnosis was retrospectively reassessed using the EBJIS criteria to ensure consistency across the entire cohort.

A chronic PJI was defined as symptom duration of more than four weeks. Coagulase-negative staphylococci, *Cutibacterium spp.*, enterococci, *Actinomyces*, and fungi (e.g. *Candida spp.*) were classified as low-virulence pathogens.[9] The presence of at least two positive samples with the same pathogen was required for diagnosis.

Diagnostic tests

Clinical features (sinus tract, visible purulence surrounding the prosthesis) were documented upon admission. Blood samples were collected one to three days preoperatively, and CRP was quantified using lithium-heparin plasma and automated particle-enhanced immunoturbidimetric analysis (cobas8000 c702 module; Roche Diagnostics International,

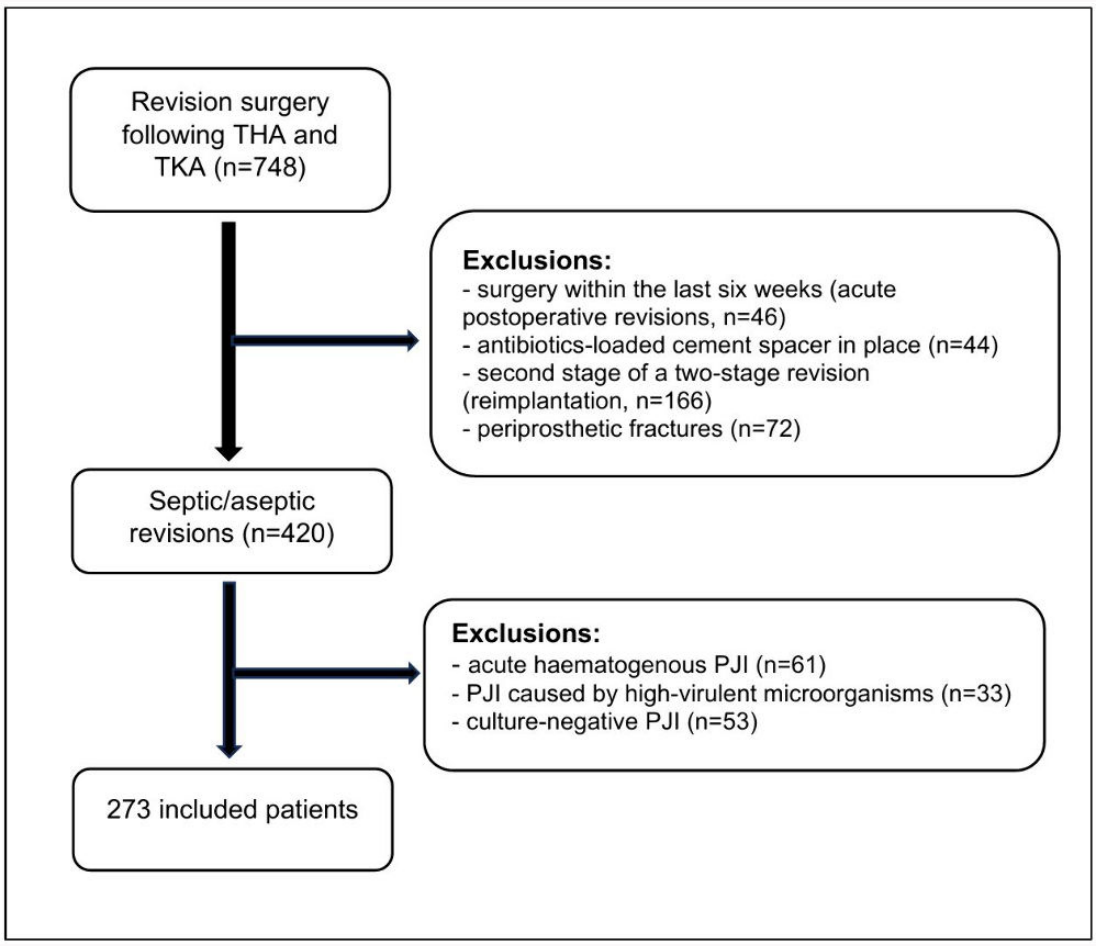

Fig. 1
Flowchart illustrating patient selection. PJI, periprosthetic joint infection; THA, total hip arthroplasty; TKA, total knee arthroplasty.

Switzerland). Under sterile conditions, synovial fluid samples were obtained preoperatively. Furthermore, synovial fluid white blood cell count (SF-WBC) and percentage of polymorphonuclear neutrophils (SF-%PMN) were quantified by flow-cytometry (XN-9100; Sysmex K. K., Japan). Intraoperatively, an alpha-defensin lateral flow test (Synovasure; Zimmer Biomet, USA) was conducted using synovial fluid obtained prior to capsulotomy. At least three tissue samples were collected intraoperatively for cultures and histopathological analysis. Explanted prosthesis components were sent for sonication. Cultures of synovial fluid, tissue samples, and sonication fluid were incubated on aerobic and anaerobic culture media for at least 14 days using a pre-described procedure.[17] Histopathological analysis (frozen and permanent sections) was performed by a specialized pathologist (see Acknowledgements). At least five high-power fields (HPFs) were assessed in each sample.[18] Frozen section results were communicated to the orthopaedic surgeon 15 to 30 minutes after dispatch, during the revision surgery.

Demographic data

The patient selection process is illustrated in Figure 1. Of the 273 included patients, 60 were classified as chronic PJI caused by low-virulence pathogens, and 213 as aseptic. Demographic details of the entire study cohort are given in Table I.

Statistical analysis

Continuous variables are given as median and IQR, categorical variables are described as absolute and relative frequencies (percentages). Chi-squared test, independent-samples *t*-test, and Fisher's exact test were used wherever statistically suitable. Calculation of diagnostic accuracies was based on the receiver operating characteristic (ROC) analysis using the EBJIS definition as the gold-standard reference. A two-sided z-test was used to compare AUCs of all included parameters. Furthermore, sensitivity, specificity, accuracy, positive (PPV) and negative predictive value (NPV), and positive (LR+) and negative likelihood ratio (LR-) were determined for all parameters. When calculating diagnostic performance, the respective variable was excluded from the definition to avoid incorporation bias. For metric test methods, the performance of the recommended cut-offs in both the EBJIS confirmatory and likely categories was analyzed. Additionally, the optimal cut-off was determined using the Youden Index. For all tests, a p-value of < 0.05 was regarded as significant. Statistical analysis was performed using XLSTAT version 2023.1.2.1406 (Lumivero, USA).

Results

All analyzed diagnostic tests showed significant differences between the septic and aseptic groups, with higher concentrations or a higher number of positive cases in the septic group.

Table I. Demographics of all included patients.

Demographics	Chronic PJIs caused by low-virulence pathogens (n = 60)	Aseptic cases (n = 213)	p-value	Total (n = 273)
Median age, yrs (IQR)	74.0 (60.8 to 78.3)	71.0 (61.0 to 78.0)	0.353*	72.0 (61.0 to 78.0)
Female sex, n (%)	32 (53.3)	135 (63.4)	0.158†	167 (61.2)
Median BMI, kg/m² (IQR)	28.6 (24.3 to 32.0)	27.6 (24.0 to 31.9)	0.584*	27.7 (24.1 to 32.0)
ASA grade ≥ III, n (%)	35 (58.3)	101 (47.4)	0.135†	136 (49.8)
Localization, n (%)			0.025†	
Hip	38 (63.3)	100 (46.9)		138 (50.5)
Knee	22 (36.7)	113 (53.1)		135 (49.5)
Preoperative antibiotics, n (%)	11 (18.3)	2 (0.9)	< 0.001‡	13 (4.8)
Rheumatoid arthritis, n (%)	3 (5.0)	13 (6.1)	> 0.999†	16 (5.9)
Sinus tract, n (%)	13 (21.7)	0 (0.0)	< 0.001‡	13 (4.8)
Intraoperative purulence, n (%)	7 (13.5)	0 (0.0)	< 0.001‡	7 (3.4)
Median CRP, mg/l (IQR)	28.1 (10.9 to 60.7)	2.8 (1.2 to 6.3)	< 0.001*	3.7 (1.5 to 12.1)
Median SF-WBC, cells/µl (IQR)§	21,831 (6,216 to 39,140)	< 1,000 (N/A)	< 0.001*	1,000 (1,000 to 5,095)
Median SF-%PMN, % (IQR)	90.0 (81.5 to 94.5)	26.0 (17.0 to 48.0)	< 0.001*	85.0 (62.3 to 94.0)
Positive SF culture, n (%)	29 (55.8)	3 (2.1)	< 0.001‡	32 (16.6)
Positive α-defensin, n (%)	11 (57.9)	0 (0.0)	< 0.001‡	11 (9.6)
Positive tissue culture, n (%)	45 (78.9)	11 (6.4)	< 0.001†	56 (24.5)
Positive sonication fluid culture, n (%)	49 (86.0)	21 (10.6)	< 0.001†	70 (27.3)
≥ 1 culture(s) positive, n (%)	58 (96.7)	22 (10.6)	< 0.001†	80 (29.9)
Positive histology – permanent sections, n (%)	49 (89.1)	0 (0.0)	< 0.001‡	49 (19.1)
Positive histology – frozen sections, n (%)	22 (84.6)	1 (0.8)	< 0.001‡	23 (15.0)

*Independent-samples *t*-test.
†Chi-squared test.
‡Fisher's exact test.
§The lowest value given by our laboratory is 1,000 cells/µl, lower values are given as < 1,000 cells/µl.
ASA, American Society of Anesthesiologists; N/A, not available; %PMN, percentage of polymorphonuclear neutrophils; SF, synovial fluid; WBC, white blood cell count.

Table II. Distribution of microorganisms in revision total hip and knee arthroplasty.

Microorganism	Hips (n = 38)	Knees (n = 22)	p-value	Total (n = 60)
Coagulase-negative staphylococci, n (%)	17 (44.7)	10 (45.5)	0.957*	27 (45.0)
Cutibacterium spp., n (%)	8 (21.1)	3 (13.6)	0.731†	11 (18.3)
Enterococcus spp., n (%)	4 (10.5)	3 (13.6)	0.700†	7 (11.7)
Actinomyces neuii, n (%)	1 (2.6)	0 (0.0)	> 0.999†	1 (1.7)
Candida spp., n (%)	2 (5.3)	1 (4.5)	> 0.999†	3 (5.0)
Polymicrobial, n (%)	6 (15.8)	5 (22.7)	0.503*	11 (18.3)

*Chi-squared test.
†Fisher's exact test.

Of the 60 PJI cases, coagulase-negative staphylococci (CoNS) were most frequently isolated (n = 27; 45%). *Cutibacterium spp.* were found in 11 cases (18.3%); seven infections (11.7%) were caused by *Enterococcus spp.*, three (5.0%) by *Candida albicans*, and one by *Actinomyces neuii* (Table II). Overall, 11 cases were polymicrobial infections, but only low-virulence organisms were cultured in these cases (one case each of: *Enterococcus faecalis/Candida albicans*, CoNS/*Actinomyces neuii*; and two cases each of: *Enterococcus faecalis*/CoNS, CoNS/*Candida albicans*, CoNS/*Aspergillus fumigatus*, and three cases of CoNS/*Cutibacterium acnes*).

Diagnostic accuracies

Performance metrics are presented in Table III, and the corresponding ROC curves are illustrated in Figure 2. All confirmatory criteria of the EBJIS definition showed ≥ 99% specificity.

Table III. Diagnostic accuracy of different test methods for diagnosing chronic periprosthetic joint infections caused by low-virulence pathogens using the established cut-off values.

Parameter	Sensitivity (95% CI)	Specificity (95% CI)	Youden's index	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)	AUC (95% CI)
Sinus tract	21.7 (13.1 to 33.8)	100 (97.8 to 100)	0.217	1.000 (1.000 to 1.000)	0.819 (0.772 to 0.866)	- (-)	0.783 (0.686 to 0.895)	0.608 (0.556 to 0.661)
Pus	13.5 (6.5 to 26.1)	100 (97.0 to 100)	0.135	1.000 (1.000 to 1.000)	0.776 (0.718 to 0.834)	- (-)	0.865 (0.777 to 0.963)	0.567 (0.520 to 0.614)
CRP (> 10 mg/l)	75.0 (62.6 to 84.3)	84.1 (78.5 to 88.5)	0.591	0.577 (0.467 to 0.687)	0.921 (0.883 to 0.959)	4.727 (3.347 to 6.677)	0.297 (0.191 to 0.462)	0.796 (0.735 to 0.856)
SF-WBC (> 1,500 cells/µl)	90.2 (76.7 to 96.6)	86.7 (77.6 to 92.5)	0.770	0.771 (0.652 to 0.890)	0.947 (0.897 to 0.998)	6.809 (3.891 to 11.915)	0.112 (0.044 to 0.286)	0.885 (0.826 to 0.944)
SF-WBC (> 3,000 cells/µl)	80.5 (65.6 to 89.9)	100 (94.6 to 100)	0.805	1.000 (1.000 to 1.000)	0.912 (0.854 to 0.970)	- (-)	0.195 (0.105 to 0.363)	0.902 (0.841 to 0.964)
SF-%PMN (> 65)	97.1 (83.9 to 100)	94.4 (80.7 to 99.3)	0.916	0.944 (0.870 to 1.000)	0.971 (0.916 to 1.000)	17.486 (4.542 to 67.319)	0.030 (0.004 to 0.209)	0.958 (0.911 to 1.000)
SF-%PMN (> 80)	80.0 (63.7 to 90.1)	100 (88.2 to 100)	0.800	1.000 (1.000 to 1.000)	0.837 (0.727 to 0.948)	- (-)	0.200 (0.103 to 0.388)	0.900 (0.833 to 0.967)
SF-culture	55.8 (42.3 to 68.4)	97.9 (93.6 to 99.5)	0.536	0.906 (0.805 to 1.000)	0.857 (0.803 to 0.911)	26.212 (8.338 to 82.397)	0.452 (0.333 to 0.614)	0.768 (0.699 to 0.837)
Alpha-defensin	57.9 (36.3 to 76.8)	99.0 (93.7 to 100)	0.569	0.917 (0.760 to 1.000)	0.922 (0.871 to 0.974)	55.579 (7.620 to 405.404)	0.425 (0.251 to 0.721)	0.784 (0.670 to 0.899)
Tissue-culture (single)	78.9 (66.5 to 87.6)	93.6 (88.7 to 96.5)	0.726	0.804 (0.700 to 0.908)	0.931 (0.893 to 0.968)	12.344 (6.862 to 22.208)	0.225 (0.136 to 0.372)	0.863 (0.806 to 0.919)
Tissue-culture (two concordant)	57.1 (44.1 to 69.2)	100 (97.3 to 100)	0.571	1.000 (1.000 to 1.000)	0.877 (0.831 to 0.923)	- (-)	0.429 (0.317 to 0.580)	0.786 (0.720 to 0.851)
Sonication (> 1 CFU)	86.0 (74.3 to 92.9)	89.4 (84.3 to 93.0)	0.754	0.700 (0.593 to 0.807)	0.957 (0.928 to 0.986)	8.146 (5.364 to 12.372)	0.157 (0.082 to 0.299)	0.877 (0.827 to 0.927)
Sonication (> 50 CFU)	57.9 (45.0 to 69.8)	100 (97.7 to 100)	0.579	1.000 (1.000 to 1.000)	0.892 (0.852 to 0.933)	- (-)	0.421 (0.311 to 0.571)	0.789 (0.725 to 0.854)
≥ 1 positive culture*	100 (92.6 to 100)	83.2 (77.4 to 87.7)	0.832	0.632 (0.535 to 0.729)	1.000 (1.000 to 1.000)	5.943 (4.393 to 8.093)	0.0 (-)	0.916 (0.890 to 0.941)
≥ 2 positive cultures†	78.0 (65.7 to 86.7)	100 (97.6 to 100)	0.780	1.000 (1.000 to 1.000)	0.936 (0.903 to 0.970)	- (-)	0.220 (0.136 to 0.356)	0.890 (0.836 to 0.943)
Histology: permanent sections	89.3 (78.1 to 95.3)	100 (97.7 to 100)	0.893	1.000 (1.000 to 1.000)	0.971 (0.948 to 0.994)	- (-)	0.107 (0.050 to 0.228)	0.946 (0.906 to 0.987)
Histology: frozen sections	85.7 (67.7 to 94.6)	99.2 (95.1 to 100)	0.844	0.958 (0.878 to 1.000)	0.969 (0.940 to 0.999)	108.185 (15.261 to 766.936)	0.149 (0.060 to 0.369)	0.922 (0.853 to 0.991)

*At least one positive culture in one of the samples (synovial fluid, tissue culture, sonication fluid culture) with a low-virulence microorganism.
†At least two positive samples with the phenotypically identical low-virulence microorganism in all samples (synovial fluid, tissue culture, sonication fluid culture).
AUC, area under the curve; CFU, colony-forming units; LR-, negative likelihood ratio; LR+, positive likelihood ratio; NPV, negative predictive value; %PMN, percentage of polymorphonuclear neutrophils; PPV, positive predictive value; SF, synovial fluid; WBC, white blood cell count.

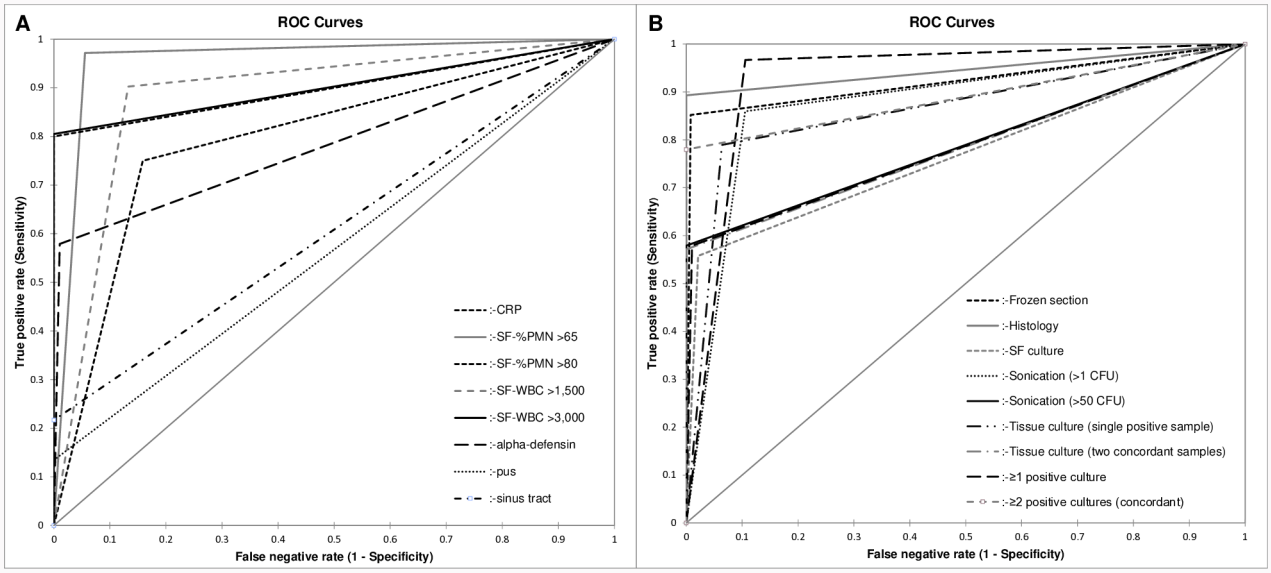

Fig. 2

a) Receiver operating characteristic (ROC) curves for accuracy of CRP, clinical features (sinus tract, pus), alpha-defensin, synovial fluid (SF) cell count (white blood cell count (WBC), percentage of polymorphonuclear neutrophils (%PMN)), and b) cultures (synovial fluid, tissue culture (single positive sample, two concordant samples), cultures overall (single positive sample, two concordant samples)), sonication, and histology (definitive histology, frozen section) in diagnosing chronic periprosthetic joint infection caused by low-virulence pathogens. CFU, colony-forming units.

Table IV. Diagnostic accuracy of metric variables at their calculated ideal cut-off values for diagnosing chronic periprosthetic joint infections caused by low-virulence pathogens.

Parameter	Sensitivity (95% CI)	Specificity (95% CI)	Youden's index	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)	AUC (95% CI)
CRP (> 9.8 mg/l)	76.7 (64.4 to 85.6)	84.1 (78.5 to 88.5)	0.608	0.582 (0.474 to 0.691)	0.926 (0.889 to 0.963)	4.832 (3.430 to 6.807)	0.277 (0.175 to 0.440)	0.804 (0.745 to 0.863)
SF-WBC (> 2,403 cells/µl)	85.4 (71.1 to 93.4)	100 (94.6 to 100)	0.854	1.000 (1.000 to 1.000)	0.933 (0.880 to 0.985)	- (-)	0.146 (0.070 to 0.307)	0.927 (0.872 to 0.982)
SF-%PMN (> 67)	97.1 (83.9 to 100)	97.2 (91.9 to 100)	0.944	0.971 (0.916 to 1.000)	0.972 (0.919 to 1.000)	34.971 (5.059 to 241.753)	0.029 (0.004 to 0.203)	0.972 (0.933 to 1.000)
Sonication (> 4 CFU)	78.8 (65.7 to 87.8)	96.9 (93.3 to 98.7)	0.758	0.872 (0.777 to 0.968)	0.945 (0.914 to 0.977)	25.756 (11.570 to 57.339)	0.218 (0.129 to 0.369)	0.879 (0.822 to 0.936)

AUC, area under the curve; CFU, colony-forming units; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NPV, negative predictive value; %PMN, percentage of polymorphonuclear neutrophils; PPV, positive predictive value; SF, synovial fluid; WBC, white blood cell count.

Using the established cut-off values, the best preoperative test method was SF-%PMN > 65% with a sensitivity, specificity, PPV, NPV, and AUC of 97.1% (95% CI 83.9 to 100), 94.4% (95% CI 80.7 to 99.3), 94.4% (95% CI 87.0 to 100), 97.1% (95% CI 91.6 to 100), and 0.958 (95% CI 0.911 to 1.000), followed by SF-WBC > 3,000 cells/µl with performance estimates of 80.5% (95% CI 65.6 to 89.9), 100% (95% CI 94.6 to 100), 100% (100), 91.2% (95% CI 85.4 to 97.0), and 0.902 (95% CI 0.841 to 0.964). The best intra- or postoperative test method was permanent section of deep tissue samples showing estimate values of 89.3% (95% CI 78.1 to 95.3), 100% (95% CI 97.7 to 100), 100% (100), 97.1% (95% CI 94.8 to 99.4), and 0.946 (95% CI 0.906 to 0.987).

Frozen section showed a similarly high performance with accuracy estimates of 85.7% (95% CI 67.7 to 94.6), 99.2% (95% CI 95.1 to 100), 95.8% (95% CI 87.8 to 100), 96.9% (95% CI 94.0 to 99.9), and 0.922 (95% CI 0.853 to 0.991). When frozen sections were performed, in eight out of nine cases (88.9%) with an unclear diagnosis before surgery but definitive postoperative infection diagnosis, infection diagnosis could be made with the results of frozen sections. In 109 aseptic cases, only one patient had positive frozen sections (0.9%), demonstrating high negative agreement.

Ideal cut-off values were calculated for all metric variables (CRP, SF-WBC, SF-%PMN, and sonication) and are shown in Table IV. ROC curves are given in Figure 3. Notably, for all parameters, lower cut-off values yielded higher diagnostic accuracies. CRP at 9.8 mg/l showed the best AUC but did not perform significantly better compared to the established cut-off of 10 mg/l (p = 0.582). When assessing

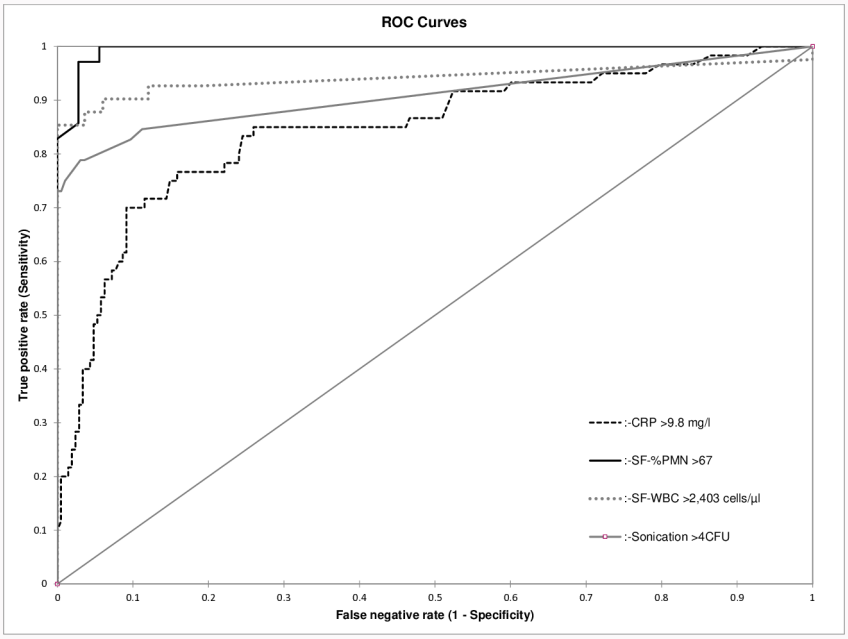

Fig. 3
Receiver operating characteristic (ROC) curves for accuracy of metric variables (CRP, synovial fluid white blood cell count (SF-WBC), synovial fluid percentage of polymorphonuclear neutrophils (SF-%PMN), sonication fluid culture) in diagnosing chronic periprosthetic joint infection caused by low-virulence pathogens, calculating ideal cut-off values. CFU, colony forming units.

SF-WBC, 2,403 cells/µl yielded the highest AUC (0.927 (95% CI 0.872 to 0.982)), however no significant differences between the three values were observed (1,500 cells/µl vs 2,403 cells/µl, p = 0.097; 1,500 vs 3,000, p = 0.433, 2,403 vs 3,000, p = 0.266). In SF-%PMN, the lower cut-offs of > 65% and 67% showed a significantly higher AUC than the established cut-off of > 80% (> 65% vs > 67%, p = 0.578; > 67% vs > 80%, p = 0.010; > 65% vs > 80%, p = 0.045). In sonication fluid culture, the calculated ideal cut-off of > 4 CFU showed the highest AUC 0.879 (95% CI 0.822 to 0.936); both the > 1 CFU and the > 4 CFU cut-off significantly outperformed the 50 CFU cut-off (p < 0.001). Of the 38 patients with a sonication fluid culture result between 1 and 50 CFU, ten patients (26%) showed identical microbial growth in tissue culture(s) and/or synovial fluid culture(s).

Two concordant positive samples of any culture (synovial fluid, tissue, sonication fluid) demonstrated a higher AUC than one single positive tissue culture (0.890 vs 0.863, p = 0.078, two-sided z-test) and two concordant tissue cultures (0.890 vs 0.786, p < 0.001, two-sided z-test).

Discussion

Preoperatively, SF-%PMN demonstrated the highest diagnostic accuracy for detecting chronic PJI caused by low-virulence pathogens in our cohort. A cut-off of > 67% demonstrated high sensitivity (97%), specificity (97%), PPV (97%), and NPV (97%), providing excellent discriminatory value in our study. Furthermore, this cut-off outperformed the established cut-off of > 80% (AUC 0.972 vs 0.900, p = 0.010). Similar results were observed in the literature. Ghanem et al[19] investigated the value of %PMN in chronic knee infections (n = 161/429 revision surgeries), showing a high sensitivity (95%) and specificity (95%) when applying the cut-off of > 64%. Trampuz et al[20] observed equal performance values (sensitivity: 97%, specificity: 98%) in a cohort of 133 revisions (34 chronic PJIs) at a cut-off of > 65%. In a study of 405 revision cases with 111 chronic PJIs, the cut-off of > 67% demonstrated the highest sensitivity (86%) and specificity (89%).[21] Kheir et al[22] calculated in their study of 1,202 revision cases (549 chronic PJIs) a sensitivity of 91% and specificity of 86% when a cut-off of > 65% PMN was used. Therefore, it seems that the percentage of PMN is lower in PJI caused by low-virulence microorganisms. Due to their low virulence characteristic, these microorganisms induce a reduced immunological response compared to high-virulence organisms, such as *Staphyloccocus aureus*, *Streptococcus spp.*, and Gram-negatives, potentially limiting diagnostic value of several synovial biomarkers.[13] Based on our results and those in the literature, a lower cut-off value (> 67%) should be strongly considered as indicative of chronic PJI caused by low-virulence pathogens. The EBJIS definition of PJI proposes that a cut-off of > 65% indicates a likely infection.[4] Our results support this threshold and suggest that such values are frequently associated with infections caused by low-virulence organisms.

The SF-WBC demonstrated a high accuracy in the preoperative diagnosis of PJI caused by low-virulence microorganisms as well. The established cut-off of > 3,000 cells/µl yielded high specificity (100%) along with good sensitivity (81%) in our cohort, which is in line with the literature. In a study evaluating the SF-WBC in diagnosing chronic PJI (n = 567/1,220 revisions), this cut-off showed a sensitivity of 82% and specificity of 95%.[23] Kheir et al[22] calculated similar performance values (sensitivity: 83%, specificity: 94%) when applying the same cut-off, while Baker et al[24] showed a higher sensitivity (sensitivity: 92%, specificity: 99%). However, other studies assessed the optimal SF-WBC cut-off for accurate diagnosis of chronic PJIs and calculated lower cut-off values. In a study by Shohat et al,[23] the optimal

cut-off was > 2,533 cells/µl. In another study by Zahar et al,[25] it was > 2,582 cells/µl, while it was > 2,479 cells/µl in a cohort of 405 revision surgeries including 111 chronic PJIs.[21] In our cohort, a similar lower optimal cut-off value of > 2,403 cells/µl was observed. At this SF-WBC count, sensitivity was 85% and specificity 100%. However, this optimal cut-off value (> 2,403 cells/µl) did not outperform the established cut-off of > 3,000 cells/µl (AUC 0.927 vs 0.902; p = 0.266). Therefore, we recommend continuing to apply the previously established cut-off, but lower values need to be interpreted with caution and in conjunction with other diagnostic test results.

In general, preoperative synovial fluid analysis can be recommended in all revision arthroplasty cases. In patients presenting with indolent symptoms and lacking characteristic signs of acute infection, where the clinical presentation may suggest an aseptic failure, SF-WBC > 2,403 cells/µl and SF-%PMN > 67% should be considered indicative of possible chronic PJI caused by low-virulence pathogens. These findings should prompt the surgeon to pursue further diagnostic evaluation to confirm or rule out infection.

Histology has shown high accuracies in the diagnosis of PJI.[26,27] Although some concerns exist within the scientific community regarding its sensitivity in CoNS and *Cutibacterium* spp.,[4,28] histological analysis proved to be the most reliable intra-/postoperative method for diagnosing chronic periprosthetic joint infections caused by low-virulence microorganisms in our cohort. A threshold of ≥ 5 PMN in ≥ 5 high-power-fields, permanent sections showed an excellent performance (sensitivity: 89%, specificity: 100%, AUC 0.946). With similar performance values (sensitivity: 86%, specificity: 99%, AUC 0.922), frozen sections demonstrated an additional advantage in preoperative unclear cases in our study. In 89% of these cases, the diagnosis of infection could be accurately established based on frozen section results alone, highlighting its intraoperative utility. Due to the excellent accuracy of frozen and permanent sections, we highly recommend collecting deep tissue samples in all revision cases for histological examination, especially in cases where the diagnosis (septic vs aseptic) is inconclusive.

Sonication fluid culture has become a valuable adjunct-tool to other traditional culture methods, especially in low-virulence microorganisms capable of forming biofilm.[29,30] Overall, sensitivities and specificities of tissue culture and sonication were comparable in our study, and no significant differences were found between the 'likely' and 'confirmatory' criteria for each test (single positive tissue sample vs > 1 CFU in sonication and two concordant positive tissue samples vs > 50 CFU in sonication; p > 0.05). The calculated ideal, lower cut-off value of > 4 CFU performed significantly better than the established cut-off of > 50 CFU (AUC 0.879 (95% CI 0.822 to 0.936) and 0.789 (95% CI 0.725 to 0.854); p < 0.001). Sensitivity was markedly higher at 79% vs 58%, while specificity remained high (97% vs 100%). These results are comparable to Trampuz et al[30] and Holinka et al.[28] Notably, in 26% of our patients with a sonication fluid culture between 1 and 50 CFU (n = 10/38), the identical microorganism was cultured in tissue samples and/or synovial fluid culture. Therefore, a positive sonication culture with 1 to 50 CFU should be interpreted with caution, as this positive result may represent the true causative microorganism.

Furthermore, ≥ two positive cultures of any sample (synovial fluid, tissue, and sonication fluid) increased accuracy compared to using individual culture methods alone (p < 0.001). This combined evaluation led to a sensitivity of 78% (SF-culture: 56%; two positive tissue samples (concordant): 57%; > 50 CFU in sonication: 58%), without compromising specificity (100%). A total of 35 cases (16.4%, 35/213) categorized as 'likely' infected based on the EBJIS definition showed a single microbiological growth without leading to a definitive diagnosis of PJI. In 16 of those cases, CoNS were cultivated, *Cutibacterium spp*. in seven cases, *Corynebacterium spp*. in four, *Aspergillus spp*. in three, *Bacillus spp*. in two, and *Acinetobacter baumanii*, *Paenibacillus lucanolyticus,* and *Micrococcus luteus* in one case, respectively. Therefore, one positive culture sample should always be interpreted in conjunction with other diagnostic test methods within the criteria, as recommended in the EBJIS definition.[4]

CRP demonstrated only moderate accuracy (sensitivity: 75%, specificity: 84%, AUC 0.796) in our cohort, consistent with findings from Akgün et al[31] and Pérez-Prieto et al.[32] Both groups reported high false-negative rates in cases involving low-virulence microorganisms (36% and 32%). In those false-negative cases, *Cutibacterium spp*. and CoNS accounted for 86% and 70% of cultivated microorganisms. Due to the inadequate immune response in PJIs caused by these low-virulence microorganisms, a normal CRP level cannot reliably exclude an infection.[12,33] Hence, CRP is not a suitable screening tool in these infections. Grzelecki et al[34] demonstrated that CRP cut-off values vary by pathogen virulence, with 9 mg/l optimal for low-virulence and 13.8 mg/l for high-virulence organisms. Our data support a similar trend, the optimal CRP cut-off of 9.8 mg/l in our cohort yielded a higher AUC but did not perform significantly better than the widely used threshold of 10 mg/l (p = 0.582), supporting the continued clinical utility of the established cut-off.

The EBJIS definition itself proved to be highly reliable for diagnosing PJI caused by low-virulence pathogens in our study. All confirmatory criteria specified in the EBJIS definition demonstrated a specificity of ≥ 99%, indicating excellent accuracy for confirming infection. Therefore, this PJI definition can be strongly recommended for clinical practice in diagnosing these challenging cases. It should be noted, however, that different diagnostic standards (e.g. ICM 2018,[35] MSIS 2013,[36] IDSA 2013[5]) may influence sensitivity and specificity outcomes.

This study has several limitations. First, its retrospective nature. Additionally, not all parameters were available for every patient, a common challenge in clinical practice. The definition of 'low-virulence pathogens' also remains inconsistently addressed in the literature. In this study, we adopted the classification proposed by Boyle et al,[9] who define low-virulence organisms based on their indolent clinical course, biofilm-forming ability, and frequent association with chronic PJI. Other studies may use slightly different classifications, which can affect comparability. Furthermore, the relatively small sample size may limit the generalizability of our findings. Nevertheless, to our knowledge, this is the first study to evaluate the diagnostic performance of multiple tests for chronic PJI caused by low-virulence pathogens within a single cohort, and the first to apply the EBJIS definition in this context. While a previous study has examined

low-grade infections, it did not clearly distinguish between low- and high-virulence organisms.[37] Most existing studies and diagnostic criteria are based on mixed cohorts and frequently overlook these differences. By focusing exclusively on PJIs caused by low-virulence pathogens, our study addresses this important gap and may help to refine diagnostic approaches for this particularly challenging subgroup. Our findings suggest that lower cut-off values, especially for SF-%PMN, are required to accurately diagnose these cases. Notably, our study includes one of the largest cohorts of PJI cases attributed specifically to low-virulence pathogens.

Preoperatively, SF-%PMN and SF-WBC are the most accurate test methods to diagnose chronic PJIs caused by low-virulence pathogens. Cut-offs of > 67% and > 3,000 cells/µl can be recommended to accurately identify this challenging group of patients. Intra- and postoperatively, definitive histology (permanent sections) and frozen sections at ≥ 5 PMN in ≥ 5 HPFs showed the highest accuracies in our study. Additionally, frozen sections proved to be a reliable intraoperative tool, particularly in cases with unclear preoperative diagnosis.

References

1. **Szymski D**, **Walter N**, **Hierl K**, **Rupp M**, **Alt V**. Direct hospital costs per case of periprosthetic hip and knee joint infections in Europe - a systematic review. *J Arthroplasty*. 2024;39(7):1876–1881.
2. **Tande AJ**, **Patel R**. Prosthetic joint infection. *Clin Microbiol Rev*. 2014;27(2):302–345.
3. **Kapadia BH**, **Berg RA**, **Daley JA**, **Fritz J**, **Bhave A**, **Mont MA**. Periprosthetic joint infection. *Lancet*. 2016;387(10016):386–394.
4. **McNally M**, **Sousa R**, **Wouthuyzen-Bakker M**, **et al**. The EBJIS definition of periprosthetic joint infection. *Bone Joint J*. 2021;103-B(1):18–25.
5. **Osmon DR**, **Berbari EF**, **Berendt AR**, **et al**. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis*. 2013;56(1):e1–e25.
6. **Parvizi J**, **Tan TL**, **Goswami K**, **et al**. The 2018 definition of periprosthetic hip and knee infection: an evidence-based and validated criteria. *J Arthroplasty*. 2018;33(5):1309–1314.
7. **Kayani B**, **Mancino F**, **Baawa-Ameyaw J**, **Roussot MA**, **Haddad FS**. Unexpected positive cultures in aseptic revision hip and knee arthroplasty. *Bone Jt Open*. 2024;5(10):832–836.
8. **Izakovicova P**, **Borens O**, **Trampuz A**. Periprosthetic joint infection: current concepts and outlook. *EFORT Open Rev*. 2019;4(7):482–494.
9. **Boyle KK**, **Wood S**, **Tarity TD**. Low-virulence organisms and periprosthetic joint infection-biofilm considerations of these organisms. *Curr Rev Musculoskelet Med*. 2018;11(3):409–419.
10. **Trebse R**, **Roskar S**. Evaluation and interpretation of prosthetic joint infection diagnostic investigations. *Int Orthop*. 2021;45(4):847–855.
11. **Staphorst F**, **Jutte PC**, **Boerboom AL**, **Kampinga GA**, **Ploegmakers JJW**, **Wouthuyzen-Bakker M**. Should all hip and knee prosthetic joints be aspirated prior to revision surgery? *Arch Orthop Trauma Surg*. 2021;141(3):461–468.
12. **Sigmund IK**, **Puchner SE**, **Windhager R**. Serum inflammatory biomarkers in the diagnosis of periprosthetic joint infections. *Biomedicines*. 2021;9(9):1128.
13. **Ottink KD**, **Strahm C**, **Muller-Kobold A**, **Sendi P**, **Wouthuyzen-Bakker M**. Factors to consider when assessing the diagnostic accuracy of synovial leukocyte count in periprosthetic joint infection. *J Bone Jt Infect*. 2019;4(4):167–173.
14. **Hipfl C**, **Mooij W**, **Perka C**, **Hardt S**, **Wassilew GI**. Unexpected low-grade infections in revision hip arthroplasty for aseptic loosening: a single-institution experience of 274 hips. *Bone Joint J*. 2021;103-B(6):1070–1077.
15. **World Medical Association**. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191–2194.
16. **Cohen JF**, **Korevaar DA**, **Altman DG**, **et al**. STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. *BMJ Open*. 2016;6(11):e012799.
17. **Sigmund IK**, **Holinka J**, **Gamper J**, **et al**. Qualitative α-defensin test (Synovasure) for the diagnosis of periprosthetic infection in revision total joint arthroplasty. *Bone Joint J*. 2017;99-B(1):66–72.
18. **Sigmund IK**, **Holinka J**, **Lang S**, **et al**. A comparative study of intraoperative frozen section and alpha defensin lateral flow test in the diagnosis of periprosthetic joint infection. *Acta Orthop*. 2019;90(2):105–110.
19. **Ghanem E**, **Parvizi J**, **Burnett RSJ**, **et al**. Cell count and differential of aspirated fluid in the diagnosis of infection at the site of total knee arthroplasty. *J Bone Joint Surg Am*. 2008;90-A(8):1637–1643.
20. **Trampuz A**, **Hanssen AD**, **Osmon DR**, **Mandrekar J**, **Steckelberg JM**, **Patel R**. Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. *Am J Med*. 2004;117(8):556–562.
21. **Gramlich Y**, **Schnetz M**, **Ruckes C**, **et al**. The optimal diagnostic cut-off of WBC and PMN counts for joint aspiration in periprosthetic joint infection is 2479/µL and 67%, respectively: ICM criteria thresholds are too high. *Arch Orthop Trauma Surg*. 2023;143(8):5229–5238.
22. **Kheir MM**, **Tan TL**, **Shohat N**, **Foltz C**, **Parvizi J**. Routine diagnostic tests for periprosthetic joint infection demonstrate a high false-negative rate and are influenced by the infecting organism. *J Bone Joint Surg Am*. 2018;100-A(23):2057–2065.
23. **Shohat N**, **Goswami K**, **Fillingham Y**, **et al**. Diagnosing periprosthetic joint infection in inflammatory arthritis: assumption is the enemy of true understanding. *J Arthroplasty*. 2018;33(11):3561–3566.
24. **Baker CM**, **Goh GS**, **Tarabichi S**, **Shohat N**, **Parvizi J**. Synovial C-Reactive protein is a useful adjunct for diagnosis of periprosthetic joint infection. *J Arthroplasty*. 2022;37(12):2437–2443.
25. **Zahar A**, **Lausmann C**, **Cavalheiro C**, **et al**. How reliable is the cell count analysis in the diagnosis of prosthetic joint infection? *J Arthroplasty*. 2018;33(10):3257–3262.
26. **Sigmund IK**, **McNally MA**, **Luger M**, **Böhler C**, **Windhager R**, **Sulzbacher I**. Diagnostic accuracy of neutrophil counts in histopathological tissue analysis in periprosthetic joint infection using the ICM, IDSA, and EBJIS criteria. *Bone Joint Res*. 2021;10(8):536–547.
27. **Mazzella FM**, **Zhang Y**, **Bauer TW**. Update on the role of pathology and laboratory medicine in diagnosing periprosthetic infection. *Hum Pathol*. 2024;147:5–14.
28. **Holinka J**, **Bauer L**, **Hirschl AM**, **Graninger W**, **Windhager R**, **Presterl E**. Sonication cultures of explanted components as an add-on test to routinely conducted microbiological diagnostics improve pathogen detection. *J Orthop Res*. 2011;29(4):617–622.
29. **Dudareva M**, **Barrett L**, **Figtree M**, **et al**. Sonication versus tissue sampling for diagnosis of prosthetic joint and other orthopedic device-related infections. *J Clin Microbiol*. 2018;56(12):e00688-18.
30. **Trampuz A**, **Piper KE**, **Jacobson MJ**, **et al**. Sonication of removed hip and knee prostheses for diagnosis of infection. *N Engl J Med*. 2007;357(7):654–663.
31. **Akgün D**, **Müller M**, **Perka C**, **Winkler T**. High cure rate of periprosthetic hip joint infection with multidisciplinary team approach using standardized two-stage exchange. *J Orthop Surg Res*. 2019;14(1):78.
32. **Pérez-Prieto D**, **Portillo ME**, **Puig-Verdié L**, **et al**. C-reactive protein may misdiagnose prosthetic joint infections, particularly chronic and low-grade infections. *Int Orthop*. 2017;41(7):1315–1319.
33. **Luger M**, **Böhler C**, **Puchner SE**, **et al**. Serum albumin-to-globulin ratio and CRP-to-albumin ratio did not outperform serum CRP in diagnosing periprosthetic joint infections. *Bone Joint Res*. 2024;13(8):372–382.
34. **Grzelecki D**, **Kocon M**, **Mazur R**, **Grajek A**, **Kowalczewski J**. The diagnostic accuracy of blood C-reactive protein and erythrocyte sedimentation rate in periprosthetic joint infections - a 10-year analysis of 1510 revision hip and knee arthroplasties from a single orthopaedic center. *J Orthop Surg Res*. 2025;20(1):276.
35. **Shohat N**, **Bauer T**, **Buttaro M**, **et al**. Hip and knee section, what is the definition of a Periprosthetic Joint Infection (PJI) of the knee and the hip? Can the same criteria be used for both joints?: Proceedings of International Consensus on Orthopedic Infections. *J Arthroplasty*. 2019;34(2S):S325–S327.
36. **Parvizi J**, **Gehrke T**. Definition of periprosthetic joint infection. *J Arthroplasty*. 2014;29(7):1331.
37. **Choe H**, **Kobayashi N**, **Abe K**, **Hieda Y**, **Tezuka T**, **Inaba Y**. Evaluation of serum albumin and globulin in combination with C-reactive protein

improves serum diagnostic accuracy for low-grade periprosthetic joint infection. *J Arthroplasty*. 2023;38(3):555–561.

Author information

M. Luger, MD, Orthopaedic Resident
L. Rabitsch, MD, Orthopaedic Resident
C. Böhler, MD, PD, ap Prof., Orthopaedic Consultant
K. Staats, MD, PD, PhD, Orthopaedic Consultant
R. Windhager, Univ Prof, MD, Head of Department
I. K. Sigmund, ap Prof, PD, MD, Orthopaedic Consultant
Department of Orthopaedics and Trauma Surgery, Medical University of Vienna, Vienna, Austria.

M. McNally, MD, FRCSEd, FRCS (Orth), Professor, Nuffield Orthopaedic Centre, Oxford University Hospitals, Oxford, UK.

M. Rupp, MD, PD, Orthopaedic Consultant, Department of Trauma, Hand and Reconstructive Surgery, University Hospital Giessen, Giessen, Germany.

Author contributions

M. Luger: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Conceptualization, Data curation.
L. Rabitsch: Writing – review & editing, Data curation.
M. McNally: Methodology, Writing – review & editing, Conceptualization.
M. Rupp: Methodology, Writing – review & editing, Conceptualization.
C. Böhler: Writing – review & editing.
K. Staats: Investigation, Writing – review & editing.
R. Windhager: Writing – review & editing.
I. K. Sigmund: Supervision, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Conceptualization, Data curation.

Funding statement

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

ICMJE COI statement

M. McNally reports royalties from Oxford University Press, consulting fees from Bonesupport and Peptilogics, payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Bonesupport, and support for attending meetings and/or travel from the EBJIS, MSIS, Bonesupport, and Peptilogics, all of which are unrelated to this study. I. K. Sigmund is Treasurer for the EBJIS. R. Windhager reports consulting fees from Johnson & Johnson and Stryker, a product development agreement with DePuy Synthes, and a life case observation agreement and educational agreement with Johnson & Johnson, all of which are unrelated to this study.

Data sharing

All data generated or analyzed during this study are included in the published article.

Acknowledgements

We would like to thank our whole clinical team (orthopaedic surgeons, ID physicians, microbiologist, pathologists, and laboratory team) who helped us in our clinical routine.

Ethical review statement

Ethical approval was obtained from the institutional ethical review board (EK 2172/2023).

Open access funding

The open access fee for this article was funded by the Medical University of Vienna.