BJR

INFECTION

Diagnostic accuracy of neutrophil counts in histopathological tissue analysis in periprosthetic joint infection using the ICM, IDSA, and EBJIS criteria

I. K. Sigmund,
M. A. McNally,
M. Luger,
C. Böhler,
R. Windhager,
I. Sulzbacher

From Medical University of Vienna, Vienna, Austria

Aims

Histology is an established tool in diagnosing periprosthetic joint infections (PJIs). Different thresholds, using various infection definitions and histopathological criteria, have been described. This study determined the performance of different thresholds of polymorphonuclear neutrophils (≥ 5 PMN/HPF, ≥ 10 PMN/HPF, ≥ 23 PMN/10 HPF) , when using the European Bone and Joint Infection Society (EBJIS), Infectious Diseases Society of America (IDSA), and the International Consensus Meeting (ICM) 2018 criteria for PJI.

Methods

A total of 119 patients undergoing revision total hip (rTHA) or knee arthroplasty (rTKA) were included. Permanent histology sections of periprosthetic tissue were evaluated under high power (400× magnification) and neutrophils were counted per HPF. The mean neutrophil count in ten HPFs was calculated (PMN/HPF). Based on receiver operating characteristic (ROC) curve analysis and the z-test, thresholds were compared.

Results

Using the EBJIS criteria, a cut-off of ≥ five PMN/HPF showed a sensitivity of 93% (95% confidence interval (CI) 81 to 98) and specificity of 84% (95% CI 74 to 91). The optimal threshold when applying the IDSA and ICM criteria was ≥ ten PMN/HPF with sensitivities of 94% (95% CI 79 to 99) and 90% (95% CI 76 to 97), and specificities of 86% (95% CI 77 to 92) and 92% (95% CI 84 to 97), respectively. In rTKA, a better performance of histopathological analysis was observed in comparison with rTHA when using the IDSA criteria ($p < 0.001$).

Conclusion

With high accuracy, histopathological analysis can be supported as a confirmatory criterion in diagnosing periprosthetic joint infections. A threshold of ≥ five PMN/HPF can be recommended to distinguish between septic and aseptic loosening, with an increased possibility of detecting more infections caused by low-virulence organisms. However, neutrophil counts between one and five should be considered suggestive of infection and interpreted carefully in conjunction with other diagnostic test methods.

Cite this article: *Bone Joint Res* 2021;10(8):536–547.

Keywords: Periprosthetic joint infection, Histology, Histopathology, EBJIS, Arthroplasty

Article focus

- This article evaluated the performance of histopathology. We assessed the best thresholds of polymorphonuclear neutrophils in ten high-powered fields (HPFs) for diagnosing periprosthetic joint infection (PJI) when using the new European Bone and Joint Infection Society (EBJIS), Infectious Diseases Society of America (IDSA), and 2018 International Consensus Meeting (ICM) criteria.

Key messages

- Histopathological analysis can be used as a confirmatory criterion in diagnosing PJI.
- A threshold of ≥ five N/HPF can be recommended to distinguish between septic and aseptic loosening.

Correspondence should be sent to Irene K. Sigmund; email: irene.sigmund@meduniwien.ac.at

doi: 10.1302/2046-3758.108.BJR-2021-0058.R1

Bone Joint Res 2021;10(8):536–547.

- Our analysis suggests that the new EBJIS definition may be more sensitive in comparison to the IDSA and 2018 ICM criteria.

Strengths and limitations

- Previous studies did not use uniform infection definitions.
- This is the first study to evaluate the performance of histology when using various infection definitions.
- Retrospective study design.

Introduction

Histopathological analysis has become firmly embedded in the clinical work-up to aid in diagnosing periprosthetic joint infection (PJI). According to the clinical practice guidelines from the American Academy of Orthopedic Surgeons (AAOS), strong evidence supports the use of histopathology to diagnose PJI.[1] Additionally, the presence of acute inflammation in histopathological examination was defined as a confirmatory criterion for PJI by the Infectious Diseases Society of America (IDSA)[2] and the European Bone and Joint Infection Society (EBJIS) in their infection criteria,[3] while the International Consensus Meeting (ICM) criteria (reconvened in 2018) includes a positive histology only as a minor criterion.[4] Sensitivities between 67% and 100%, and specificities between 93% and 100% for histology, have been reported.[5-7]

Neutrophil count was first proposed for PJI diagnosis by Mirra et al[8] in permanent stained histology sections. This was subsequently confirmed in frozen sections.[9] Pandey et al[6] showed that the presence of polymorphonuclear neutrophils (PMNs) in periprosthetic tissue (pseudomembrane or pseudocapsule) correlates strongly with septic failure.[6] However, due to various testing thresholds reported in different studies, the perfect cut-off remains unclear. In a meta-analysis, Zhao et al[10] concluded that a threshold of ten PMNs per high-powered field (HPF) is better than five PMNs for diagnosing PJI, although no statistically significant difference was demonstrated between these thresholds.[10] Tsaras et al[11] also found no difference between ten PMN/HPF and five PMN/HPF in their systematic review.[11] In addition, Morawietz et al[12] reported a different threshold of an average of 23 PMN per ten HPFs for distinguishing between septic and aseptic loosening.[12]

Previous studies did not use uniform infection definitions. A comparison between histological and microbiological results or clinical features was usually performed. Therefore, the aim of this study was to assess and compare the performances of the different thresholds of PMNs in ten HPFs when using the EBJIS,[3] the IDSA,[2] and the 2018 ICM criteria.[4]

Methods

Study design. Patients having revision surgery after total hip (rTHA) or knee (rTKA) arthroplasty between January 2015 and June 2018 were recruited, at a single tertiary healthcare centre providing advanced specialist treatment of PJI. Permanent sections of tissue samples, which were intraoperatively collected during revision surgery, were examined. Demographic data and results of serum and synovial fluid analysis, histology, and microbiology were recorded. Patients without histopathological assessment, patients with a cement spacer in place, surgery within the last six weeks, or a second stage of two-stage revision were excluded. A PJI was defined according to EBJIS,[3] IDSA,[2] and the 2018 updated ICM criteria.[4]

Local institutional ethical review board approval was obtained (EK 1455/2019) and the study was conducted in accordance with the Declaration of Helsinki.[13]

Examination of diagnostic test methods. In each patient having revision surgery, a standardized work-up was performed to aid in PJI diagnosis. This work-up was directed towards identifying the components of the EBJIS, IDSA, and ICM diagnostic criteria. The presence of a sinus tract or visible pus during the operation or aspiration was recorded. Preoperatively, blood samples were taken to assess the serum CRP level. Under sterile conditions, a joint aspiration was done preoperatively. Synovial fluid was analyzed for white blood cell count (WBC), percentage of polymorphonuclear neutrophils (%PMN), and sent for microbiological investigations as previously described.[14]

For microbiological analysis, at least one synovial fluid sample, at least three tissue samples, and at least one sonication fluid sample were processed per standard laboratory protocol with cultures held for 14 days.[15] The qualitative alpha-defensin lateral flow test was performed as previously described.[14]

Histopathological analysis. During revision surgery, at least two samples of periprosthetic membrane and the pseudocapsule were collected (mean 4.5 samples; 2 to 11) and histopathologically processed. The samples were fixed in 4.5% formaldehyde for 12 hours, paraffin embedded, and sections of 3 µm thickness were cut and stained with haematoxylin and eosin.

For this study, all permanent sections were evaluated under high power (400× magnification). A conventional light microscope (Olympus BX53, Olympus GesmbH, Austria) with a diameter of the visual field of 0.5 mm was used; hence the visual field was 0.196 mm^2. In the study by Morawietz et al,[12] a diameter of 0.625 mm was used (visual field 0.307 mm^2), so the neutrophil count was multiplied by (0.307/0.196) to give a true comparison.

For each section, the areas of inflammation containing the highest number of PMNs were viewed. At least ten 400× magnification HPFs were analyzed in detail. Only PMNs within the tissue were counted; PMNs within blood vessels or within haemorrhagic areas were ignored. A maximum of ten PMNs were counted per HPF. The number of PMNs in each of the ten HPFs was counted. The ten counts were added and divided by ten to give the mean PMN/HPF (number of PMN/HPF of ten HPFs divided by ten). For each patient, the section with the

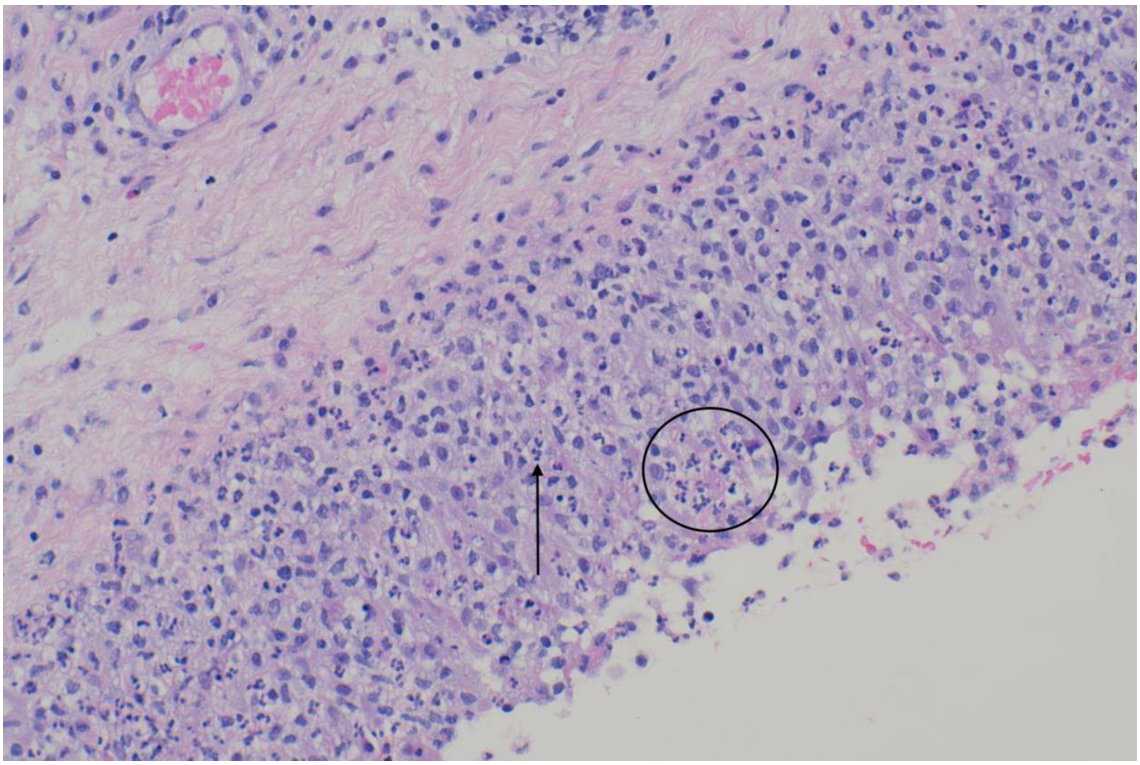

Fig. 1

Histopathological picture of a periprosthetic membrane with ≥ ten polymorphonuclear neutrophils (PMNs) per high-powered field (HPF) (400×) in a 76-year-old woman with a periprosthetic knee infection according to the European Bone and Joint Infection society (EBJIS) criteria, the Infectious Diseases Society of America (IDSA) criteria, and International Consensus Meeting (ICM 2018) criteria. Serum CRP was 261 mg/l, synovial fluid white blood cell count was 4,786 cells/l, and the percentage of PMNs was 93%. β-hemolytic Streptococcus Group B were detected in 2/2 aspirates, 4/4 tissue samples, and in the sonication fluid. Intraoperatively, pus was visible and the alpha-defensin lateral flow test was positive. The circle shows several PMNs and the arrow a single PMN.

highest mean PMN/HPF count was taken as the definitive number for diagnosis.

Each tissue sample was interpreted blindly without knowledge of the other clinical, biochemical, or microbiological results (Figure 1).

Statistical analysis. Continuous variables were described as mean and range, and categorical variables were expressed as absolute and relative frequencies (percentage). Independent-samples *t*-test and Fisher's exact test were used to compare metric and binary variables between groups. Using receiver operating characteristic (ROC) curve analysis, the best neutrophil cut-off for diagnosing PJI was assessed using the section with the highest mean PMN/HPF count. Three previously published cut-offs (≥ 23 PMN/10 HPFs, ≥ 5 PMN/HPF, and ≥ 10 PMN/HPF) were evaluated against each of the three diagnostic criteria (EBJIS, IDSA, and ICM). Sensitivity, specificity, accuracy, positive (PPV) and negative (NPV) predictive value, positive (LR+) and negative likelihood ratio (LR-), area under the ROC-curve (AUC), and their 95% confidence intervals (CIs) were calculated. The *z*-test was used to compare AUCs. To avoid incorporation bias, histology was excluded from the infection definitions. The significance level for the tests was $p < 0.05$. Statistical analysis was done in XLSTAT statistical and data analysis solution (Version 2019.3.2.; Addinsoft 2020, USA).

Results

Demographic data according to the EBJIS criteria. A total of 119 patients having revision surgery after a total hip (n = 59, 50%) or knee (n = 60, 50%) arthroplasty were included. The mean age of the whole study cohort was 71 years (24 to 93); 76 patients (64%) were female (Table I). Using the EBJIS criteria, 56 patients (47%) were defined as septic; applying the IDSA criteria, 54 patients (45%) were classified as septic; and using the ICM criteria, 44 patients (37%) were diagnosed as having a PJI.

In 29 of the 56 septic patients (n = 29/56; 52%), microbiological culture was positive. The most common causative microorganism was *Staphylococcus aureus* (n = 9), followed by coagulase-negative staphylococci (n = 6) and *Streptococcus* spp (n = 3). In cases with an infection caused by high-virulence microorganisms (n = 21), two showed ≤ one PMN/HPF and the remainder (n = 19) ≥ ten N/HPF. In all septic patients with low-virulence microorganisms (n = 8), ≥ ten PMN/HPF were observed. There was no statistical difference between the groups ($p = 0.384$, independent-samples *t*-test).

Performance of histopathological analysis. The mean PMN/HPF was 1.1 (0 to 4.1) in the aseptic group and 8.9 (0 to 10.0) in the septic group when applying the EBJIS criteria ($p < 0.001$, independent-samples *t*-test). Table II

Table I. Demographic details of the whole study cohort when using the European Bone and Joint Infection Society criteria.

Characteristic	Aseptic group (n = 55)	Septic group (n = 64)	p-value	Total (n = 119)
Mean patient age, yrs (range)	71 (28 to 90)	71 (24 to 93)	0.893*	71 (24 to 93)
Female sex, n (%)	37 (67)	39 (61)	0.567†	76 (64)
Mean BMI (range)	29 (17 to 43)	29 (18 to 51)	0.952*	29 (17 to 51)
ASA grade				
Type 1, n (%)	5 (9)	5 (8)	1.000*	10 (8)
Type 2, n (%)	31 (56)	25 (39)	0.068*	56 (47)
Type 3, n (%)	19 (35)	33 (52)	0.067*	52 (44)
Type 4, n (%)	0 (0)	1 (2)	1.000*	1 (1)
Joint				
Hip, n (%)	26 (47)	33 (52)	0.714†	59 (50)
Knee, n (%)	29 (53)	31 (48)	0.714†	60 (50)
Antibiotics, n (%)	2 (4)	9 (14)	0.061†	11 (9)
Mean serum CRP, mg/dl (range)	0.8 (0.0 to 3.8)	6.87 (0.1 to 54.0)	< 0.001*	4.1 (0.0 to 54.0)

*Independent-samples *t*-test.
†Fisher's exact test.
ASA, American Society of Anesthesiologists; EBJIS, European Bone and Joint Infection Society.

shows the performance of histopathology depending on the chosen threshold and applied infection definition.

Comparison with EBJIS criteria. Based on receiver operating characteristics, a cut-off of ≥ five PMN/HPF (p = 0.008) and ≥ ten PMN/HPF (p = 0.012, z-test) showed a better accuracy in comparison to 23 PMN/10 HPFs when using the EBJIS criteria (Figure 2). The comparison analysis between ≥ five PMN/HPF and ≥ ten PMN/HPF showed no statistically significant difference (p = 0.916, z-test). When using the EBJIS criteria, sensitivity, specificity, and AUC of histopathological analysis were 91.3% (95% CI 79.0 to 97.0), 86.3% (95% CI 76.3 to 92.5), and 0.89 (95% CI 0.83 to 0.95) at an optimal cut-off level of ≥ five PMN/HPF (Figure 2).

Comparison with IDSA criteria. The optimal cut-off level was ≥ ten PMN/HPF with a sensitivity, specificity, and AUC of 93.8% (95% CI 78.6 to 99.2), 86.2% (95% CI 77.2 to 92.0), and 0.90 (95% CI 0.84 to 0.96), respectively, against IDSA criteria (Figure 3). Based on ROC curve analysis, a threshold of ≥ ten PMN/HPF performed better compared with ≥ five PMN/HPF (p = 0.011) and 23 PMN/10 HPFs (p < 0.001). In addition, ≥ five PMN/HPF showed a statistically significanty better accuracy in comparison to the cut-off of ≥ 23 PMN/10 HPFs (p = 0.029).

Comparison with 2018 ICM criteria. Using the ICM criteria, sensitivity, specificity, and AUC were 90.0 (95% CI 76.2 to 96.5), 92.4% (95% CI 84.0 to 96.7), and 0.91 (95% CI 0.86 to 0.97) at an optimal cut-off level of ≥ ten PMN/HPF determined by ROC curve analysis. A threshold of ≥ ten PMN/HPF showed a better accuracy compared with ≥ five PMN/HPF (p = 0.047) and ≥ 23 PMN/10 HPFs (p < 0.001); and ≥ five PMN/HPF performed better than ≥ 23 PMN/10 HPFs (p = 0.015).

Revision total hip arthroplasty. When using the EBJIS criteria, a trend towards a better performance of ≥ five PMN/HPF (p = 0.082) and ≥ ten PMN/HPF (p = 0.160) in comparison with ≥ 23 PMN/10 HPFs was observed in patients with revision surgery after a THA, but not at a statistically significant level. No difference was shown between ≥ five PMN/HPF and ten PMN/HPF (p = 0.744). In THA patients, sensitivity, specificity, and AUC of histopathological analysis were 87.5% (95% CI 68.0 to 96.3), 85.7% (95% CI 70.0 to 94.1), and 0.87 (95% CI 0.78 to 0.96) at a cut-off level of ≥ five PMN/HPF (Figure 4).

When applying the IDSA criteria, a threshold of ≥ ten PMN/HPFs performed better in comparison with ≥ 23 PMN/10 HPFs (p = 0.022, z-test) in rTHA. Between ≥ five PMN/HPF and ≥ ten PMN/HPF, no difference was found (p = 0.299). However, specificity (79.5% (95% CI 65.2 to 89.0)) of ≥ ten PMN/HPF was higher (≥ 5 PMN/HPFs: 70.5% (95% CI 55.6 to 81.9); ≥ 23 PMN/10 HPF: 59.1% (95% CI 44.4 to 72.3)) with identical sensitivity (≥ 23 PMN/10 HPFs, ≥ 5 PMN/HPF, ≥ 10 PMN/HPF: all 86.7% (95% CI 60.6 to 97.3)).

Using the ICM criteria, the accuracy of ≥ ten PMN/HPF was higher compared with ≥ 23 PMN/10 HPFs (p = 0.007, z-test) and similar to ≥ five PMN/HPF (p = 0.213, z-test). Specificity (87.5% (95% CI 73.3 to 94.9)) was highest at a cut-off level of ≥ ten PMN/HPF (≥ 23 PMN/10 HPF: 65.0% (95% CI 49.4 to 77.9), ≥ 5 PMN/HPF: 77.5% (95% CI 62.2 to 87.8)), with identical sensitivities (≥ 23 PMN/10 HPFs, ≥ 5 PMN/HPF, ≥ 10 PMN/HPF: 89.5% (95% CI 67.1 to 98.1)).

Revision total knee arthroplasty. When using the EBJIS criteria, a trend towards a better performance of ≥ five PMN/HPF (p = 0.146, z-test) and ≥ ten PMN/HPF (p = 0.107, z-test) in comparison with ≥ 23 PMN/10 HPFs was observed in patients with rTKA, but not at a statistically significant level. Comparative analysis between ≥ five PMN/HPF and ≥ ten PMN/HPF showed no difference (p = 0.840). Sensitivity of ≥ ten PMN/HPF (86.4% (95% CI 65.6 to 95.9)) was lower compared with ≥ 23 PMN/10 HPF and ≥ five PMN/HPF (both 95.5% (95% CI 76.2 to 100)), but specificity (97.4% (95% CI 85.1 to 100)) was highest at a cut-off level of ≥ ten PMN/HPF (≥ 23 PMN/10

Table II. Performance of histopathology when using the European Bone and Joint Infection Society, the Infectious Diseases Society of America, and the Musculoskeletal Infection Society criteria depending on the applied threshold. Values in brackets are 95% confidence intervals.

Performance	Histopathology (cut-off 23 PMN/10 HPFs)	Histopathology (cut-off 5 PMN/HPF)	Histopathology (cut-off 10 PMN/HPF)
EBJIS			
Sensitivity	93.0 (80.5 to 98.2)	93.0 (80.5 to 98.2)	86.0 (72.2 to 93.7)
Specificity	72.4 (61.3 to 81.2)	84.2 (74.2 to 90.8)	93.4 (85.1 to 97.4)
Accuracy	79.8 (72.6 to 87.0)	87.4 (81.4 to 93.4)	90.8 (85.6 to 96.0)
PPV	65.6 (53.7 to 77.5)	76.9 (65.5 to 88.4)	88.1 (78.3 to 97.9)
NPV	94.8 (89.1 to 100)	95.5 (90.6 to 100)	92.2 (86.2 to 98.2)
LR+	3.37 (2.32 to 4.89)	5.89 (3.48 to 9.97)	13.08 (5.56 to 30.78)
LR-	0.10 (0.03 to 0.29)	0.08 (0.03 to 0.25)	0.15 (0.07 to 0.32)
AUC	0.83 (0.76 to 0.89)	0.89 (0.83 to 0.94)	0.90 (0.84 to 0.96)
IDSA			
Sensitivity	93.8 (78.6 to 99.2)	93.8 (78.6 to 99.2)	93.8 (78.6 to 99.2)
Specificity	64.4 (53.9 to 73.6)	74.7 (64.6 to 82.7)	86.2 (77.2 to 92.0)
Accuracy	72.3 (64.2 to 80.3)	79.8 (72.6 to 87.0)	88.2 (82.4 to 94.0)
PPV	49.2 (36.6 to 61.7)	57.7 (44.3 to 71.1)	71.4 (57.8 to 85.1)
NPV	96.6 (91.9 to 100)	97.0 (92.9 to 100)	97.4 (93.8 to 100)
LR+	2.63 (1.96 to 3.54)	3.71 (2.56 to 5.38)	6.80 (3.99 to 11.58)
LR-	0.10 (0.03 to 0.38)	0.08 (0.02 to 0.32)	0.07 (0.02 to 0.28)
AUC	0.79 (0.72 to 0.86)	0.84 (0.78 to 0.91)	0.90 (0.84 to 0.96)
MSIS			
Sensitivity	92.5 (79.3 to 98.0)	92.5 (79.3 to 98.0)	90.0 (76.2 to 96.5)
Specificity	69.6 (58.7 to 78.8)	81.0 (70.8 to 88.2)	92.4 (84.0 to 96.7)
Accuracy	77.3 (69.8 to 84.8)	84.9 (78.4 to 91.3)	91.6 (86.6 to 96.6)
PPV	60.7 (48.4 to 72.9)	71.2 (58.8 to 83.5)	85.7 (75.1 to 96.3)
NPV	94.8 (89.1 to 100)	95.5 (90.6 to 100)	94.8 (89.8 to 99.8)
LR+	3.05 (2.16 to 4.30)	4.87 (3.06 to 7.75)	11.85 (5.45 to 25.75)
LR-	0.11 (0.04 to 0.32)	0.09 (0.03 to 0.28)	0.12 (0.04 to 0.28)
AUC	0.81 (0.75 to 0.88)	0.87 (0.81 to 0.93)	0.91 (0.86 to 0.97)

AUC, area under the curve; EBJIS, European Bone and Joint Infection Society; IDSA, Infectious Diseases Society of America; LR-, negative likelihood ratio; LR+, positive likelihood ratio; MSIS, Musculoskeletal Infection Society; NPV, negative predictive value; PMN/HPF, polymorphonuclear neutrophils/high-powered field; PPV, positive predictive value.

HPFs: 76.3% (95% CI 60.5 to 87.1), ≥ 5 PMN/HPF: 86.8% (72.1 to 94.6)) (Figure 5).

Applying the IDSA criteria, a cut-off of ≥ ten PMN/HPF showed a higher accuracy in comparison with ≥ 23 PMN/10 HPFs ($p < 0.001$) and ≥ five PMN/HPF ($p = 0.018$, z-test). Sensitivities of the different thresholds were identical (100% (95% CI 77.9 to 100)), but specificity (93.0% (95% CI 80.5 to 98.2)) was higher at a cut-off level of ≥ ten PMN/HPF (≥ 23 PMN/10 HPF: 69.8% (95% CI 54.8 to 81.4), ≥ five PMN/HPF: 79.1% (95% CI 64.5 to 88.7]).

Using the ICM criteria, a better accuracy was observed at a threshold of ≥ ten PMN/HPF compared to ≥ 23 PMN/10 HPFs ($p = 0.012$, z-test). No difference was seen between ≥ 23 PMN/10 HPFs and ≥ five PMN/HPF ($p = 0.164$, z-test), or between ≥ five PMN/HPF and ≥ ten PMN/HPF ($p = 0.250$).

Comparison between rTKA and rTHA. In rTKA, a better performance of histopathological analysis was observed in comparison with rTHA when using the IDSA criteria ($p < 0.001$, z-test; cut-off ≥ ten PMN/HPF). When applying the ICM criteria, a trend towards a better performance of histology in total knee revision surgery was shown, but not at a statistically significant level ($p = 0.139$, z-test; cut-off ≥ ten PMN/HPF). No difference was calculated when the EBJIS criteria were used ($p = 0.223$, z-test; cut-off ≥ five PMN/HPF).

Of the 29 septic patients with bacterial growth, 13 patients with a septic knee and 16 patients with a septic hip had a positive microbiology. Three septic knee patients and three septic hip patients showed microbial growth of low-virulence organisms. There was no statistically significant difference between low- and high-virulence organisms between the septic hip and knee patients ($p = 1.000$, Fisher's exact test).

Discussion

This study demonstrated that histopathology has high accuracy when compared with the EBJIS, IDSA, and ICM criteria. Therefore, histopathology can be recommended as a confirmatory criterion in diagnosing PJI. High sensitivities and specificities were observed regardless of the chosen infection definition. However, depending on the

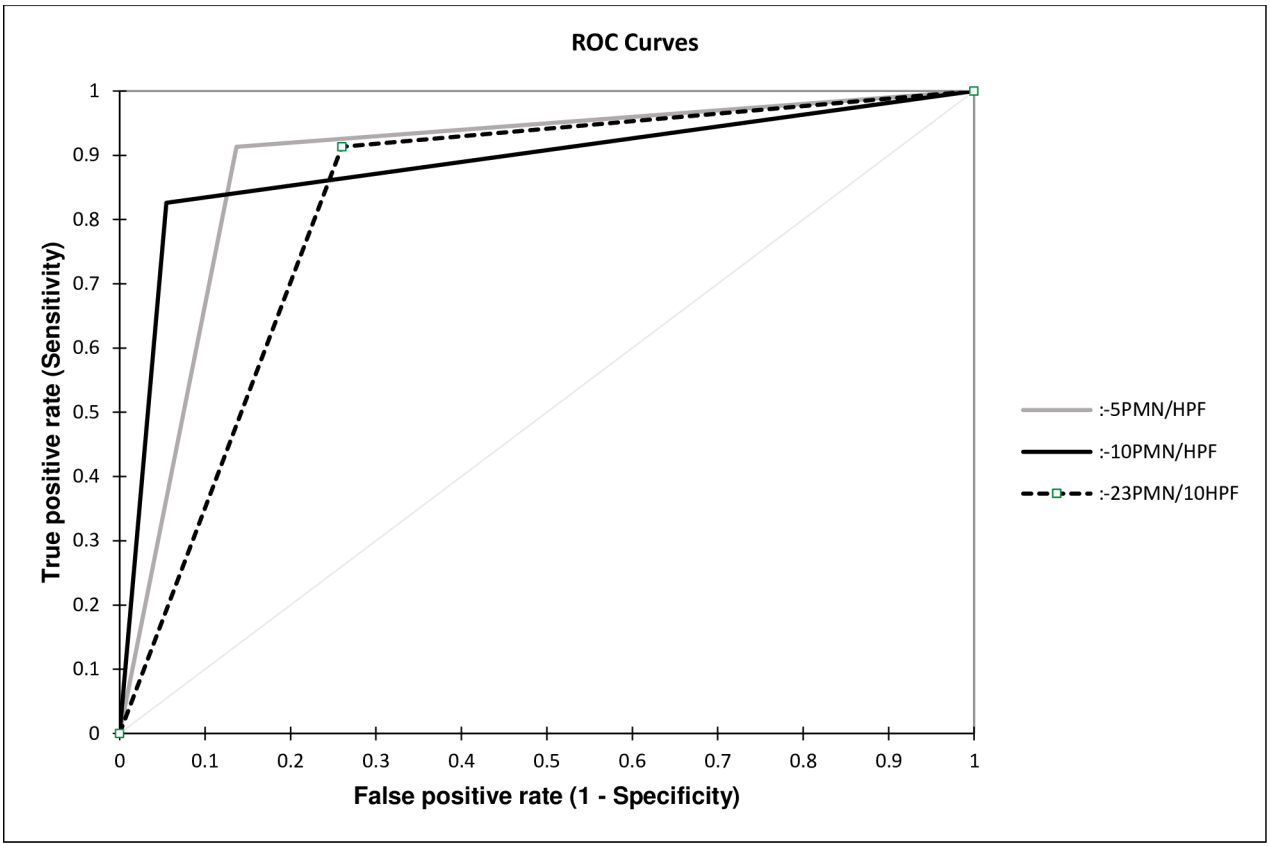

Fig. 2

Receiver operating characteristic (ROC) curves for diagnostic accuracy of periprosthetic joint infection (PJI) based on the different cut-off levels of polymorphonuclear neutrophils/high-powered field (400× magnification) in tissue samples retrieved from patients with indicated revision surgery after total hip or knee arthroplasty when using the European Bone and Joint Infection Society criteria.

chosen cut-off, the values varied considerably. The best cut-off for PMNs per HPF in tissue samples evaluated by histopathological analysis was ≥ ten PMN/HPF when using the IDSA and ICM criteria with sensitivities of 94% and 90%, and specificities of 86% and 92%, respectively. However, it should be noted that the recommended cut-off for ICM was only ≥ five PMN/HPF. This was first proposed in the 2011 MSIS definition and maintained in the 2013 and 2018 updates.[16–18] If this lower cut-off was applied, sensitivities remained similar, but specificities were significantly reduced. The IDSA recommended that 'the presence of acute inflammation' was diagnostic but no cut-off was identified.[2]

When EBJIS criteria were applied, both thresholds (≥ 5 PMN/HPF, ≥ 10 PMN/HPF) yielded acceptable results for diagnosing infection. No differences between these two cut-offs (p = 0.916) were observed. A threshold of ≥ five PMN/HPF can be recommended as diagnostic of infection with a sensitivity of 91% and specificity of 86%. A threshold of ≥ ten PMN/HPF showed a higher specificity (95%) but at the cost of sensitivity (83%). In all analyses, a cut-off of 23 PMN/10 HPFs had significantly poorer accuracy compared to five or ten PMN/HPF.

These findings highlight the differences between the infection definitions. The combination of criteria in the EBJIS definition identified more infections, compared to both the IDSA and ICM definitions (EBJIS (n = 56/119), IDSA (n = 54/119), ICM (n = 44/119)). While the EBJIS criteria may be more sensitive to detection of low-grade infection, the ICM criteria can misdiagnose septic failures as aseptic, especially when caused by low-virulence microorganisms.[19] This could be a possible explanation of the lower cut-off of PMN/HPF when using the EBJIS criteria.

All studies recorded in Table III concluded that the presence of numerous PMNs in periprosthetic tissue samples correlates strongly with a PJI.[5–7,12,20–26] However, histology was mostly compared with clinical features and/or positive cultures,[5–7,12,20–24] rather than published infection definitions. In 1999, Pandey et al,[6] using similar histological criteria, concluded that ≥ five PMN/HPFs with a sensitivity of 72% and 100% specificity are diagnostic of infection, but histology was only compared to culture-proven infection; no definition of a PJI was available at that time. In addition, the microbiology samples were only incubated for five days. In recent years, it is recommended that

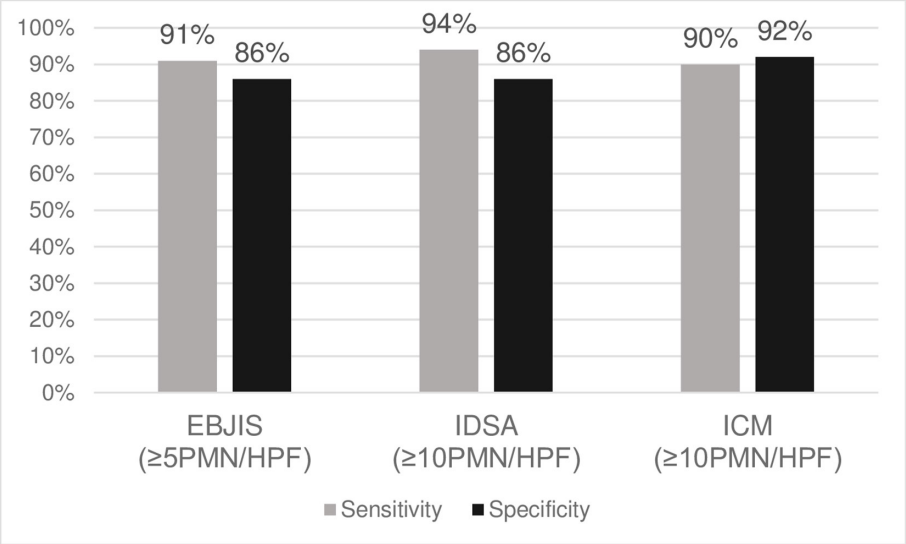

Fig. 3

Sensitivities and specificities of histopathology at the optimal cut-off level according to the European Bone and Joint Infection Society (EBJIS; ≥ 5 polymorphonuclear neutrophil (PMN)/high-powered field (HPF)), the Infectious Diseases Society of America (IDSA; ≥ 10 PMN/HPF), and the International Consensus Meeting (ICM; ≥ 10 PMN/HPF) criteria.

cultures should be retained for 14 days to increase the detection rate of low-virulence microorganisms, such as *Cutibacterium* spp. or coagulase-negative staphylococci.[17] Feldman et al[21] published a series of 33 patients (23 hips, 10 knees) comparing histopathology with intraoperative culture. When using a cut-off of ≥ five PMN/HPF, sensitivity and specificity of 100% and 96% were described. Pons et al[7] also reported a very good sensitivity of 100% and specificity of 98% at a cut-off of ≥ five PMN/HPF in 83 patients, but an infection was present if acute inflammation in the histopathological samples was found in at least three specimens together with positive cultures in more than half the specimens collected. With this infection definition, an incorporation bias is likely. The incubation time was again only five days in this study (Table III). Other studies included sinus tract and/or clinical and/or radiological features and/or laboratory findings,[12,20,22–24] but previously only Buttaro et al[26] and Kwiecien et al[27] used the Musculoskeletal Infection Society criteria published in 2011.[28]

In all infections caused by low-virulence and 90% of high-virulence microorganisms, ≥ ten PMN/HPF were found. In two cases, the PMN/HPF was lower than five PMN/HPF. In these two cases, the PMN count was lower than one PMN/HPF and so would not have been diagnosed by the lower threshold of Morawietz et al[12] at ≥ 23 PMN/10 HPFs. This may represent sampling error during surgery, when a piece of tissue at the margin of the inflamed zone may be harvested and may not demonstrate many polymorphs. This problem has also been identified in histological analysis of infected fractures.[29] However, ≥ five PMN/HPF can be regarded as diagnostic of a periprosthetic infection, as there was no improvement in accuracy with a cut-off of ≥ ten PMN/HPF. Fewer than five neutrophils should be considered suggestive and should be interpreted carefully in conjunction with other diagnostic tests as described by different societies.[2-4]

In rTHA, published sensitivities of histological analysis ranged between 45% and 100%, and specificities between 92% and 100%;[5–7,20,22,26] and sensitivities in rTKA were described between 68% and 100%, and specificities between 95% and 99%,[5,23,24,27] which are in line with our results although different infection and different histological criteria were used.

In our study, we showed that the EBJIS (cut-off ≥ 5 PMN/HPF) and ICM criteria (cut-off ≥ 10 PMN/HPF) were equally accurate in hip and knee revision. IDSA (cut-off ≥ 10 PMN/HPF) was significantly less accurate in hip revision ($p < 0.001$, z-test). Banit et al[5] observed similar results in a cohort of 55 rTKAs and 63 rTHAs.[5] While specificities were nearly the same (rTKA 96%, rTHA 92%), sensitivities varied markedly (rTKA 100%, rTHA 45%). A possible explanation of this difference in rTKA and rTHA could be the better soft-tissue coverage in hips having the ability to suppress infection and leading to more indolent infections. However, Kwiecien et al[27] described a trend for sensitivity of histological analysis to be higher in rTKA compared with rTHA ($p = 0.32$). While again specificities were the same (99%), sensitivities were 68% in rTKA and 79% in rTHA. Several studies have shown variable white cell counts in the synovial fluid of hips, knees, or other prosthetic joints.[30] There may be important differences in the ability of each joint to produce an inflammatory response. Another explanation could be a different spectrum of microorganisms

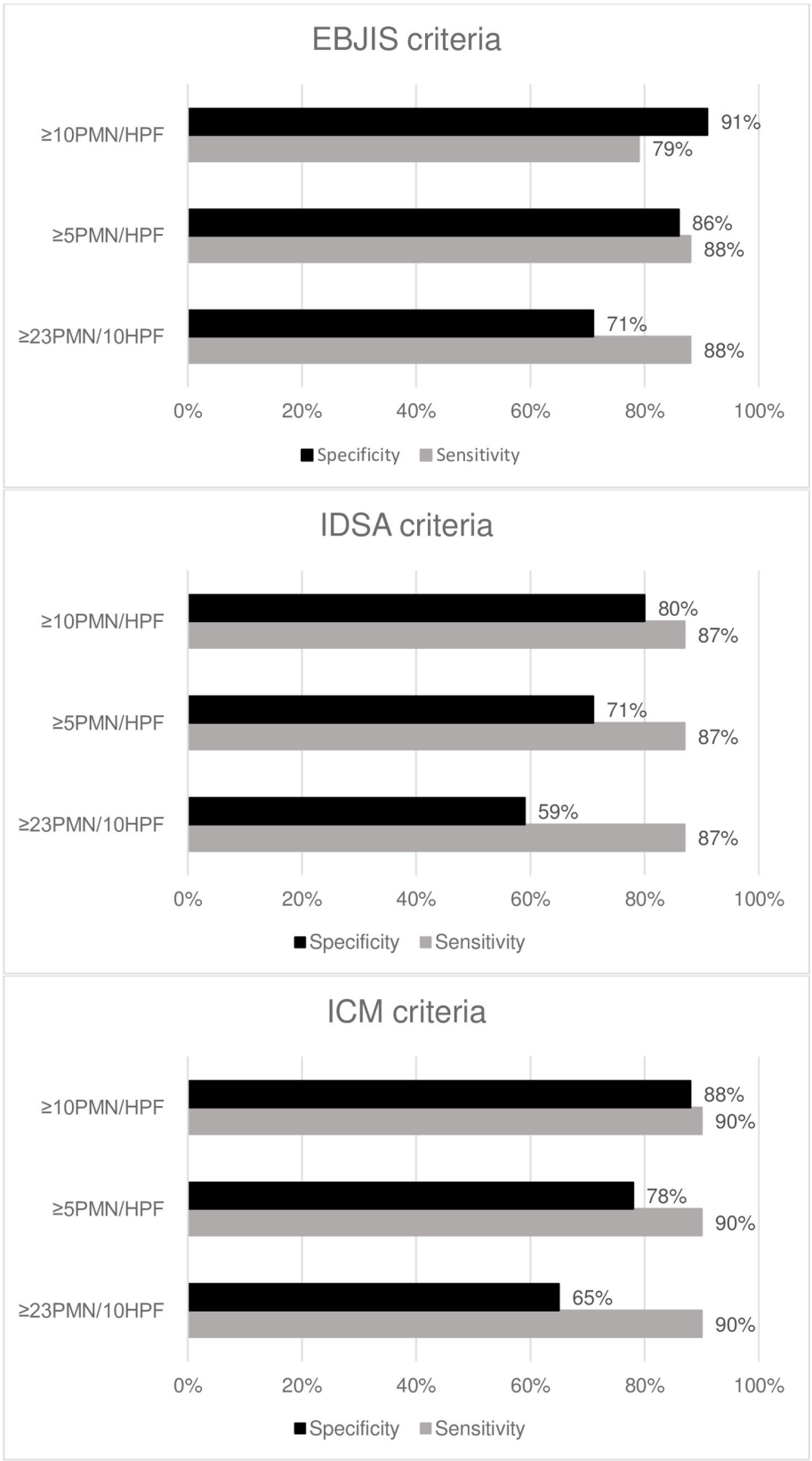

Fig. 4

Sensitivities and specificities of histology depending on different polymorphonuclear neutrophils per high-powered field (PMN/HPF) and the used infection definition (European Bone and Joint Infection Society (EBJIS), Infectious Diseases Society of America (IDSA), and International Consensus Meeting (ICM)) in patients who had revision surgery after total hip arthroplasty.

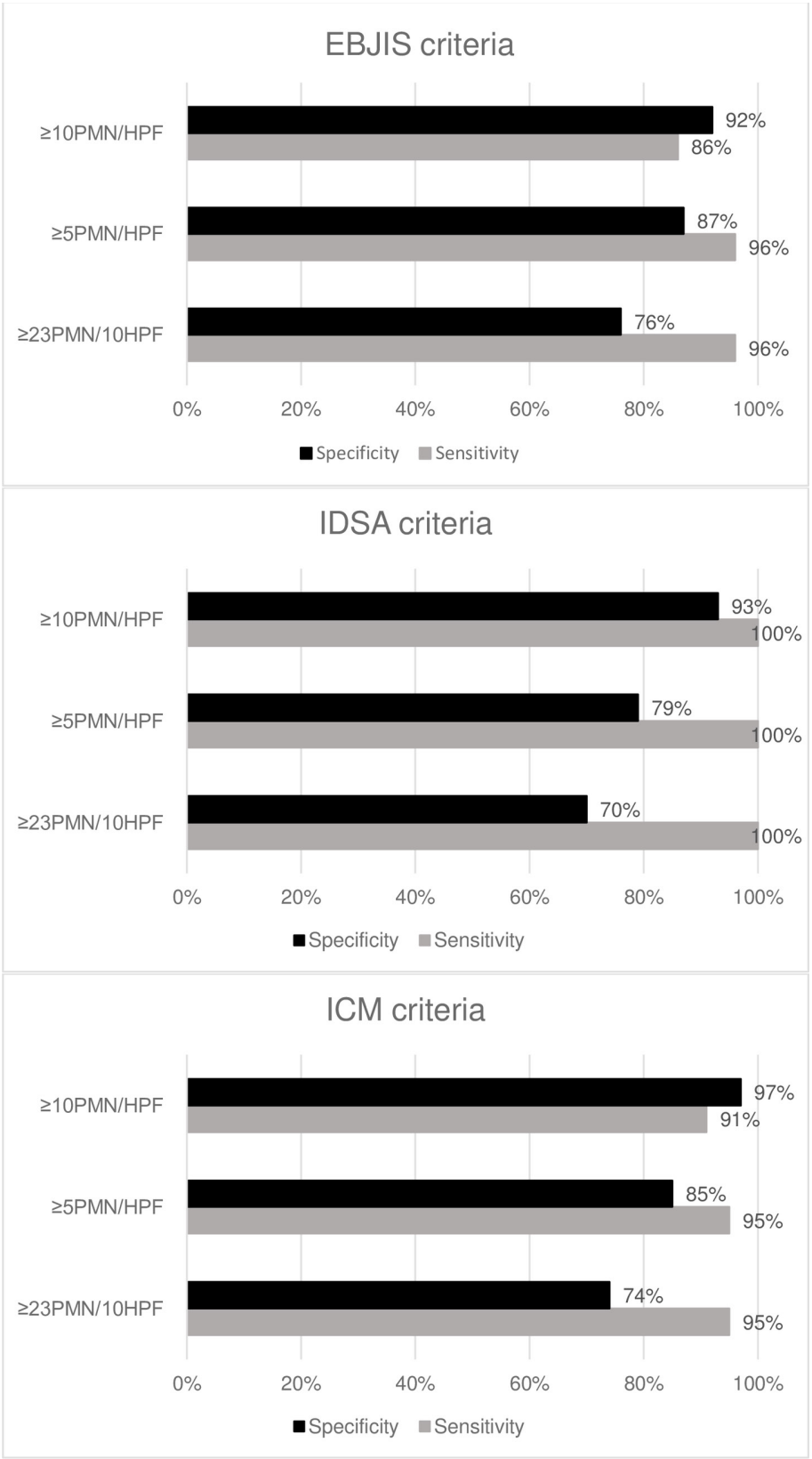

Fig. 5

Sensitivities and specificities of histology depending on different polymorphonuclear neutrophils per high-powered field (PMN/HPF) and the used infection definition (European Bone and Joint Infection Society (EBJIS), Infectious Diseases Society of America (IDSA), and International Consensus Meeting (ICM)) in patients who had revision surgery after total knee arthroplasty.

Table III. Comparison of the literature.

Literature	Included patients, n	Localization	Threshold (≥ PMN/HPF)	Examined HPFs	Tissue	Infection definition	Sensitivity	Specificity
Morawietz et al[12]	147	118 hips, 29 knees	23 PMN/10HPF*	10	Periprosthetic membrane	Positive aspiration and/or clear clinical or radiological findings and/ or persistence of elevated haematological parameters	73%	95%
Tohtz et al[20]	64	Hip	23 PMN/10HPF*	10	Periprosthetic membrane	Present sinus tract and/or positive histopathology, at least two tissue samples with same bacterial organism	87%	100%
Pandey et al[6]	602	Hip	5	10	Pseudomembrane, pseudocapsule	Positive culture	72%	100%
Feldman et al[21]	33	23 hip, 10 knee	5	5	Periprosthetic membrane, pseudocapsule	Intraoperative culture	100%	96%
Pons et al[7]	83	Hip	5	Not mentioned	Joint synovial samples of the hip capsule	Positive histopathological samples in minimum three samples together with positive cultures in more than half the samples taken	100%	98%
Nilsdotter-Augustinsson[22]	47	Hip	5	10	Tissue samples around the prostheses	Based on clinical symptoms, laboratory and radiological findings, preoperative cultures of synovial fluid aspirations, or occurrence of fistulas or significant bacterial growth	81%	100%
Fink et al[23]	144	Knee	5	10	Synovium and periprosthetic connective tissue	Demonstration of the same microorganism in at least two of the cultures; or demonstration of a microorganism in at least one sample and at least five neutrophilic polymorph leucocytes per HPF (400×) in the associated histological preparation	90%	95%
Buttaro et al[26]	76	Hip	5	10	Pseudocapsule, the cement-bone interface, and any other tissue involved according to the surgeon's judgement	MSIS (2011)	90%	94%
Kwiecien et al[27]	200	100 hip, 100 knee	5	3	Tissue samples from multiple sites	Modified MSIS (2011)	74%	99%
DellaValle et al[24]	94	Knee	10	5	Synovial tissue adjacent to the implant	If an organism grew on the solid media from at least two cultures or if two of the following three criteria were met: at least one culture was positive, the final histopathology was consistent with infection, or gross purulence was present	88%	96%
Banit et al[5]	121	3 shoulder, 55 knee, 63 hip	10	5	Samples from any area suspicious for infection	Positive intraoperative culture	67%	93%
Lonner et al[25]	175	142 hip, 33 knee	10	5	Pseudocapsule, areas considered suspicious by the surgeon, and tissue that was identified at the various host-implant interfaces	A positive intraoperative culture	84%	99%
			5	5			84%	96%

Continued

Table III. Continued

Literature	Included patients, n	Localization	Threshold (≥ PMN/HPF)	Examined HPFs	Tissue	Infection definition	Sensitivity	Specificity
Present study	119	59 hips, 60 knees	5	10	Periprosthetic membrane, pseudocapsule	EBJIS	93%	84%
			10	10	Periprosthetic membrane, pseudocapsule	IDSA	94%	86%
			10	10	Periprosthetic membrane, pseudocapsule	MSIS (2018)	90%	92%

*These studies reported a threshold of a mean of 23 neutrophils in ten high-powered fields.
EBJIS, European Bone and Joint Infection Society; HPF, high-powered field; IDSA, Infectious Diseases Society of America; MSIS, Musculoskeletal Infection Society; N, neutrophil.

between these two joints. However, in our study, no difference was shown regarding microorganism spectrum. More studies are needed to explore this topic fully.

Our study is limited by its retrospective design, and the fact that the detection of neutrophils was only based on cell morphology. No immunohistochemistry was used. In a cohort of 147 patients also using immunohistochemistry (identification of CD15 by anti-CD15 antibodies) for neutrophil identification, Morawietz et al[12] found a lower threshold of 23 PMN/10 HPFs diagnostic for PJI with a sensitivity of 73% and specificity of 95% when compared only with microbiological results. A different cut-off may be possible if immunohistochemistry would have been used. Nevertheless, further studies are needed to elucidate this topic more precisely.

Due to the differently applied infection definitions and histological criteria (applied threshold, number of evaluated HPFs; Table III), a comparison of the literature is difficult. Attention should also be paid to the type of microscope used in diagnosing PJI. In most previous studies, the used diameter of the visual field was not mentioned (Table III). Our threshold applies to microscopes with a visual field diameter of 0.625 mm (0.307 mm^2). If a microscope with a diameter of 0.5 mm (0.196 mm^2) is used, the cut-off needs to be divided with (0.307/0.196) (EBJIS mean cut-off ≥ 5 PMN/HPF = ≥ 3.2 PMN/HPF; IDSA and ICM mean cut-off ≥ 10 PMN/HPF = ≥ 6.4 PMN/HPF).

In conclusion, histopathological analysis can be recommended as a confirmatory criterion as it is accurate and comparatively inexpensive. The effective threshold of PMN/HPF depends on the infection definition chosen. In our cohort, more PJI cases were identified when applying the EBJIS criteria, suggesting that it is a more sensitive definition. Hence, a lower cut-off of ≥ five PMN/HPF can be recommended for both hip and knee revision. If the ICM or IDSA criteria are used, a cut-off of ≥ ten PMN/HPF is recommended, but with the possibility to miss some PJIs, particularly in the hip with the IDSA criteria. However, a lower mean PMN/HPF count between 1 to 5 does not exclude PJI and should be interpreted carefully in conjunction with other tests within the diagnostic criteria.

References

1. **AAOS**. Diagnosis and prevention of periprosthetic joint infections: clinical practice guideline. 2019. https://aaos.org/globalassets/quality-and-practice-resources/pji/pji-clinical-practice-guideline-final-2-17-21.pdf (date last accessed 28 July 2021).
2. **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.
3. **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.
4. **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.
5. **Banit DM**, **Kaufer H**, **Hartford JM**. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res*. 2002;401:230–238.
6. **Pandey R**, **Drakoulakis E**, **Athanasou NA**. An assessment of the histological criteria used to diagnose infection in hip revision arthroplasty tissues. *J Clin Pathol*. 1999;52(2):118–123.
7. **Pons M**, **Anglés F**, **Sánchez C**, **et al**. Infected total hip arthroplasty-the value of intraoperative histology. *Int Orthop*. 1999;23(1):34–36.
8. **Mirra JM**, **Amstutz HC**, **Matos M**, **Gold R**. The pathology of the joint tissues and its clinical relevance in prosthesis failure. *Clin Orthop Relat Res*. 1976;117:221–240.
9. **Athanasou NA**, **Pandey R**, **de Steiger R**, **McLardy Smith P**. The role of intraoperative frozen sections in revision total joint arthroplasty. *J Bone Joint Surg Am*. 1997;79-A(9):1433–1434.
10. **Zhao X**, **Guo C**, **Zhao GS**, **Lin T**, **Shi ZL**, **Yan SG**. Ten versus five polymorphonuclear leukocytes as threshold in frozen section tests for periprosthetic infection: a meta-analysis. *J Arthroplasty*. 2013;28(6):913–917.
11. **Tsaras G**, **Maduka-Ezeh A**, **Inwards CY**, **et al**. Utility of intraoperative frozen section histopathology in the diagnosis of periprosthetic joint infection: a systematic review and meta-analysis. *J Bone Joint Surg Am*. 2012;94-A(18):1700–1711.
12. **Morawietz L**, **Tiddens O**, **Mueller M**, **et al**. Twenty-Three neutrophil granulocytes in 10 high-power fields is the best histopathological threshold to differentiate between aseptic and septic endoprosthesis loosening. *Histopathology*. 2009;54(7):847–853.
13. **World Medical Association**. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191–2194.
14. **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.
15. **Butler-Wu SM**, **Burns EM**, **Pottinger PS**, **et al**. Optimization of periprosthetic culture for diagnosis of Propionibacterium acnes prosthetic joint infection. *J Clin Microbiol*. 2011;49(7):2490–2495.
16. **Parvizi J**. New definition for periprosthetic joint infection. *Am J Orthop*. 2011;40(12):614–615.
17. **Parvizi J**, **Gehrke T**, **Chen AF**. Proceedings of the International consensus on periprosthetic joint infection. *Bone Joint J*. 2013;95-B(11):1450–1452.
18. **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.
19. **Renz N**, **Yermak K**, **Perka C**, **Trampuz A**. Alpha defensin lateral flow test for diagnosis of periprosthetic joint infection: not a screening but a confirmatory test. *J Bone Joint Surg Am*. 2018;100-A(9):742–750.

20. **Tohtz SW**, **Müller M**, **Morawietz L**, **Winkler T**, **Perka C**. Validity of frozen sections for analysis of periprosthetic loosening membranes. *Clin Orthop Relat Res.* 2010;468(3):762–768.
21. **Feldman DS**, **Lonner JH**, **Desai P**, **Zuckerman JD**. The role of intraoperative frozen sections in revision total joint arthroplasty. *J Bone Joint Surg Am.* 1995;77-A(12):1807–1813.
22. **Nilsdotter-Augustinsson A**, **Briheim G**, **Herder A**, **Ljunghusen O**, **Wahlström O**, **Ohman L**. Inflammatory response in 85 patients with loosened hip prostheses: a prospective study comparing inflammatory markers in patients with aseptic and septic prosthetic loosening. *Acta Orthop.* 2007;78(5):629–639.
23. **Fink B**, **Makowiak C**, **Fuerst M**, **Berger I**, **Schäfer P**, **Frommelt L**. The value of synovial biopsy, joint aspiration and C-reactive protein in the diagnosis of late peri-prosthetic infection of total knee replacements. *J Bone Joint Surg Br.* 2008;90-B(7):874–878.
24. **Della Valle CJ**, **Sporer SM**, **Jacobs JJ**, **Berger RA**, **Rosenberg AG**, **Paprosky WG**. Preoperative testing for sepsis before revision total knee arthroplasty. *J Arthroplasty.* 2007;22(6 Suppl 2):90–93.
25. **Lonner JH**, **Desai P**, **Dicesare PE**, **Steiner G**, **Zuckerman JD**. The reliability of analysis of intraoperative frozen sections for identifying active infection during revision hip or knee arthroplasty. *J Bone Joint Surg Am.* 1996;78-A(10): 1553–1558.
26. **Buttaro MA**, **Martorell G**, **Quinteros M**, **Comba F**, **Zanotti G**, **Piccaluga F**. Intraoperative synovial C-reactive protein is as useful as frozen section to detect periprosthetic hip infection. *Clin Orthop Relat Res.* 2015;473(12):3876–3881.
27. **Kwiecien G**, **George J**, **Klika AK**, **Zhang Y**, **Bauer TW**, **Rueda CA**. Intraoperative frozen section histology: matched for musculoskeletal infection Society criteria. *J Arthroplasty.* 2017;32(1):223–227.
28. **Parvizi J**, **Zmistowski B**, **Berbari EF**, **et al**. New definition for periprosthetic joint infection: from the Workgroup of the musculoskeletal infection Society. *Clin Orthop Relat Res.* 2011;469(11):2992–2994.
29. **Morgenstern M**, **Athanasou NA**, **Ferguson JY**, **Metsemakers WJ**, **Atkins BL**, **McNally MA**. The value of quantitative histology in the diagnosis of fracture-related infection. *Bone Joint J.* 2018;100-B(7):966–972.
30. **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.

Author information:
- I. K. Sigmund, PD, MD, Orthopaedic Surgeon
- M. Luger, Cand.Med, Medical Student
- C. Böhler, PD, MD, Orthopaedic Surgeon
- R. Windhager, o. Univ.Prof, MD, Professor
  Department of Orthopaedics and Trauma Surgery, Medical University of Vienna, Vienna, Austria.
- M. A. McNally, MD, FRCSEd, FRCS(Orth), King James IV Professor, Oxford University Hospitals NHS Foundation Trust, Oxford, UK.
- I. Sulzbacher, Univ.Prof, MD, Professor, Department of Pathology, Medical University of Vienna, Vienna, Austria.

Author contributions:
- I. K. Sigmund: Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing.
- M. A. McNally: Methodology, Writing - original draft.
- M. Luger: Investigation.
- C. Böhler: Investigation.
- R. Windhager: Investigation.
- I. Sulzbacher: Methodology, Investigation, Writing - original draft.

Funding statement:
- No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Open access funding:
- For the open access funding, the funding institutions were the Department of Pathology and the Department of Orthopaedics and Trauma Surgery, Medical University of Vienna (Funding number: APC 600204315).

Ethical review statement:
- Ethical approval was granted by the Medical University of Vienna (EK 1455/2019).