BGU
Berufsgenossenschaftliche
Unfallklinik Frankfurt am Main

Akademisches Lehrkrankenhaus der
Goethe-Universität Frankfurt am Main

Ärztlicher Direktor
Prof. Dr. Dr. Reinhard Hoffmann

Leiter Wissenschaft
PD Dr. med. habil. Y. Gramlich

BG Unfallklinik Frankfurt am Main gGmbH* Postfach 60 01 60 * 60331 Frankfurt

Geschäftsstelle der AE
Deutsche Gesellschaft für Endoprothetik e.V.
Via ae-wissenschaftspreis@ae-germany.com

Ihr Zeichen:
Ihre Nachricht vom:
Unser Zeichen: M190
Ansprechpartner/in: Frau C. Diemann-Paeth
Telefon: 069/475-2116
Fax: 069/475-4826
E-Mail: wissenschaft@bgu-frankfurt.de
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Sehr geehrte Damen und Herren,

Die Diagnostik des periprothetischen Infektes ist weiterhin herausfordernd, klinikinterne Standards aber auch weltweite Empfehlungen sind häufig stark differierend und stark kontrovers diskutiert. Aufgrund der guten Verfügbarkeit und der guten Testgüte ist international die Zellzahl sowie deren Zelldifferenzierung einer der wichtigsten Parameter. Publizierte Cut-Offs der Zellzahl variieren von unter 1500 bis über 4500/µl mit einem international meist anerkannten Konsens von 3000/µl (ICM, EBJIS) als Cut-Off zur Detektion eines Infektes. Unsere Arbeitsgruppe hatte 2016 ein Test-Kit entwickelt und publiziert, das alle Untersuchungen, die in den gängigen Leitlinien (u.a. ICM, EBJIS etc) gefordert werden, inkl. *Alpha – Defensin*, beinhaltet (1). Hiermit wird jeder Patient, der sich einer Revision der Endoprothese unterzieht, auf Vorliegen eines Protheseninfektes invasiv prä-OP und intra-OP untersucht, sowie post-Op prospektiv nachverfolgt. Hieraus resultierte eine prospektive Studie an über 400 Patienten die nun in der hier vorgelegten Veröffentlichung den Aspekt der optimalen Zellzahl und Zelldifferenzierung bei der Gelenkpunktion untersucht. Es zeigt sich, dass der international gebräuchliche Grenzwert zu hoch zu sein scheint, damit zu viele Infektionen „übersehen“ werden könnten und eine Anpassung zu niedrigeren Cut-offs sinnvoll ist. Ein riskanter Bereich (Low-Grade Infektionen) ergibt sich zwischen 1500 bis 3000 Zellen /µl im Gelenkpunktat. In diesem Bereich war Alpha-Defensin als ELISA Labortest besonders hilfreich und aussagekräftig.

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Prof. Dr. Dr. med. R. Hoffmann Ärztlicher Direktor Chefarzt der Abteilung Unfallchirurgie und Orthopädische Chirurgie, BG Unfallklinik Frankfurt am Main gGmbH	**Priv.-Doz. Dr. med. habil. Y. Gramlich** Leitender Oberarzt Unfallchirurgie und Orthopädische Chirurgie Wissenschaftlicher Leiter der Klinik

Anlagen: Originalarbeit

Vorarbeiten zu dieser Studie

(1)Gramlich Y, Kremer M, Brüning C, Breuer J, Hofmann L, Klug A, Hoffmann R. Implementation of a standardized clinical test kit for diagnostics of periprosthetic infections in the clinical routine. Unfallchirurg. 2021 Dec;124(Suppl 1):247-254. English. doi: 10.1007/s00113-021-01016-4. Epub 2021 Aug 2. PMID: 34338839.

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KNEE REVISION SURGERY

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

Y. Gramlich[1] · M. Schnetz[1] · C. Ruckes[2] · M. Kemmerer[1] · M. Kremer[1] · R. Hoffmann[1] · A. Klug[1]

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Abstract

Background Various organizations have published definitions for periprosthetic joint infection (PJI) with significant differences in the cut-offs of white blood cell (WBC) count and polymorphonuclear (PMN) leukocyte cells. Herein, we aim to analyze optimal cut-offs in patients which are planned to undergo a prosthesis revision and compare them with the actual published thresholds of the International Consensus Meeting (ICM) and European Bone and Joint Infection Society (EBJIS).

Methods A test kit was compiled in a monocentric prospective study, according to the ICM criteria (2018) and 2021 EBJIS criteria. The kit was implemented using: blood samples (including leukocyte count and C-reactive protein); samples for examining the synovial fluid (WBC count, PMN cell differentiation, microbiological culture for incubation over 14 days, alpha-defensin ELISA laboratory test, and leukocyte-esterase test). The cut-offs for WBC and PMN counts were investigated using ROC analyses and Youden index. The ICM 2018 criteria were applied, using alpha-defensin in all cases. Patients which have to undergo a prosthesis revision were included, a pre-operative joint aspiration had been performed, and the patients had been followed up prospectively.

Results 405 patients were examined with the compiled test kit; 100% had a complete dataset with respect to alpha-defensin; 383 patients, according to WBC count; and 256, according to PMN cell differentiation The cut-off of 2478.89 cells/µl in the WBC count (sensitivity: 87.70%; specificity: 88.10%) and the cut-off of 66.99% in PMN differentiation showed the best accuracy (sensitivity: 86.00%; specificity: 88.80%). Other published cut-offs for WBC were tested in this cohort and showed the following accuracy: 3000/µl (EBJIS/ICM; sensitivity: 82.10%; specificity: 91.00%), 2000/µl (sensitivity: 89.60%; specificity: 83.40%), and 1500/µl (sensitivity: 91.50%; specificity: 75.00%). The published cut-offs for PMN had the following accuracy in this cohort: 80% (ICM; sensitivity: 66.3%; specificity: 96.50%), 70% (sensitivity: 82.6%; specificity: 90%), and 65% (EBJIS, sensitivity: 86%; specificity: 88.8%).

Conclusions This study aims to improve current cut-offs for PMN- and WB-Count, even though PJI diagnosis is based on the combination of all defined tests. The optimal diagnostic cut-off of WBC and PMN counts was found to be 2479/µL and 67%, respectively, whereas ICM cut-offs in this cohort seem too high, as they provide high specificity but very low sensitivity. On the other hand, a cut-off for WBC count of 1500/µl alone would be very low, leading to low specificity and very high suspicion of PJI. The current consensus guidelines could be actualized considering these results to significantly improve the diagnostic quality.

Level of evidence II.

Keywords Periprosthetic joint infection · White blood cell count · Polymorphonuclear cell · ICM consensus criteria · EBJIS criteria · Alpha-defensin · PJI test kit

Introduction

Periprosthetic joint infection (PJI) is associated with a major reduction in the quality of life, significantly increased mortality rate, and heavy treatment costs [1–3]. While early infections often present with acute clinical symptoms, the

✉ Y. Gramlich
yves.gramlich@bgu-frankfurt.de
https://www.bgu-frankfurt.de

Extended author information available on the last page of the article

diagnosis of low-grade infections continues to be a challenge. As early diagnosis and prompt treatment are essential to prevent the chronic course of disease and significantly reduce treatment costs, infection should be ruled out before revision arthroplasty in all cases [2, 4–6].

Despite the major disease burden, an international consensus on a PJI definition has yet to be established. An initial definition by the Musculoskeletal Infection Society (MSIS) in 2011 [7] was modified and served as a basis for the International Consensus on Musculoskeletal infection (ICM) definition in 2013 [8]. In 2018, the ICM proposed an updated definition which showed increased sensitivity from 86.9 to 97.7% compared with the 2013 ICM definition [9]. In response to a weak consensus with an approval rate of 68% among delegates [10], the European Bone and Joint Infection Society (EBJIS) published a three-level approach to define PJI in 2021, which has been endorsed by MSIS and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) [11]. It is well established that the diagnosis of PJI must be based on a combination of clinical, radiological, and laboratory findings. Therefore, the 2018 ICM and 2021 EBJIS definition include among others, microbiological culture results, clinical features, serum C-reactive protein (CRP), synovial white blood cell count (WBC) with the percentage of polymorphonuclear (PMN) cells, and synovial alpha-defensin. Differences are present according individual variables and cut-off values. In cases without apparent clinical manifestation, synovial fluid aspiration and analysis is a central player for accurate diagnosis. It allows a cost-effective analysis of synovial WBC and PMN as well as additional screening for established biomarkers such as alpha-defensin and enables microbiologic analysis [12]. Although the specificity of pre-operative microbiological fluid cultures is good, the sensitivity is low; incubation time can take up to 10–14 days; and negative results cannot be used to rule out PJI [13]. Biomarkers yield promising results in PJI diagnosis, but further research is needed to evaluate validity and optimize cut-off values [14]. Therefore, WBC and PMN are still regarded as the most important synovial parameters in diagnosing PJIs. In recent years, several studies were published regarding the optimal cut-off values for WBC and PMN, and the published results vary between 1500 and 4000 cells/µl and 65% and 80%, respectively [14, 15]. In the 2018 ICM criteria, the WBC cut-off is defined as > 3000 cells/µl and the PMN cut-off, as > 80% [9]. Hence, cases with a WBC count up to 3000 cells/µl are given zero points, risking a high number of false-negative diagnoses. In contrast, the 2021 EBJIS definition uses the WBC cut-off criteria of > 1500 cells/µl in combinations with other factors for *likely infections* and of > 3000 cells/µl for *confirmed infections*. The PMN cut-off is defined as > 65% for *likely infections* and as > 80% for *confirmed infections*. Implementing a three-level definition with an intermediate "*infection likely*" group, the 2021 EBJIS definition additionally includes cases with WBC and PMN between 1501 and 3000 cells/µl and 66% and 80%, respectively [11]. Although this approach includes more cases and therefore increases sensitivity, it may result in an increased number of false-positive diagnoses. Thus far, there is no international consensus on the optimal WBC and PMN cut-off criteria.

Therefore, the aim of this study was to evaluate the best WBC and PMN cut-offs to determine an accurate diagnosis of PJI. As different cut-offs are published and included in guideline-based consensus criteria, the accuracies of these published cut-offs were tested using our study collective and the accuracy was compared.

Methods

Inclusion criteria

In 2016, in a single-center prospective study, a test kit was assembled and implemented in clinical settings as previously described [16], leading to a standardized diagnostic workup for invasive PJI Diagnostic. Every patient who had to undergo a revision surgery of the arthroplasty underwent a joint aspiration prior the surgery. The test results form a database of joint aspirations from which the data were collected to outperform the current study. Patients having an unsuccessful aspiration (punctio sicca; dry tap) were not collected in the database. Eligible for this study were therefore all patients with the need for endoprosthesis revision operation due to every septic or aseptic reasons and if a successful joint aspiration result was available.

The aim of this study was to evaluate the best WBC and PMN cut-offs to determine an accurate diagnosis of PJI, using ROC analyses.

Diagnostic workup

Special conditions at the investigating hospital are a share of about 50% of revision arthroplasty in the total volume of endoprosthetic operations, as well as the presence of a special department for periprosthetic (PJI) and fracture-related infection (FRI) with more than 60 beds in the septic department. Patients presenting with painful endoprosthesis or loosening of the arthroplasty were included in this study, and joint aspiration as well as blood testing was performed prior to the operation, to rule out a PJI. Therefore, patients with the need for any septic or aseptic revision of their arthroplasty were included in this study. Joint aspiration was performed under sterile conditions in an operating room and, in case of THA, was radiographically guided. Prolonged incubation was carried out for microbial cultures and the results were given after 14 days.

In 2016, in a single-center prospective study, a test kit was assembled and implemented in clinical settings as previously described [16], including the 2014 International Consensus Meeting (ICM) criteria [17] and the Infectious Diseases Society of America (IDSA) guidelines [18]. This test kit also corresponds to the latest consensus criteria of the EBJIS 2021 [11] and the ICM 2018 [9, 19]. The test kit consisted of all recommended tests, such as blood count (standard blood count, CRP, blood cultures for bacteraemia, procalcitonin for sepsis) and joint aspiration (cell count/WBC, cell differentiation/PMN, microbiologic testing in paediatric blood culture, leukocyte-esterase test strips, and alpha-defensin ELISA). No component of the test kit is the commercially available alpha-defensin bedside test that offers immediate result reporting ("Synovasure", Zimmer, Ch), as well as synovial CRP. In case of dry tap at the hip, appropriate examination material could be obtained by a second puncture by another examiner or by CT-guided puncture. If no sufficient joint puncture could be performed, the patient was excluded from the study (Fig. 1).

Definition of infection:

After the joint aspiration, all included patients had revision operation of their arthroplasty. Therefore, intraoperative invasive diagnostics was performed in all cases in addition to the above-mentioned joint aspiration: microbiologic diagnostics including 3–6 tissue samples, histology including representative tissue samples, and sonication of implant when implant was removed [16].

The final diagnosis was then made using previously published diagnostic workups [16, 19] and the criteria of

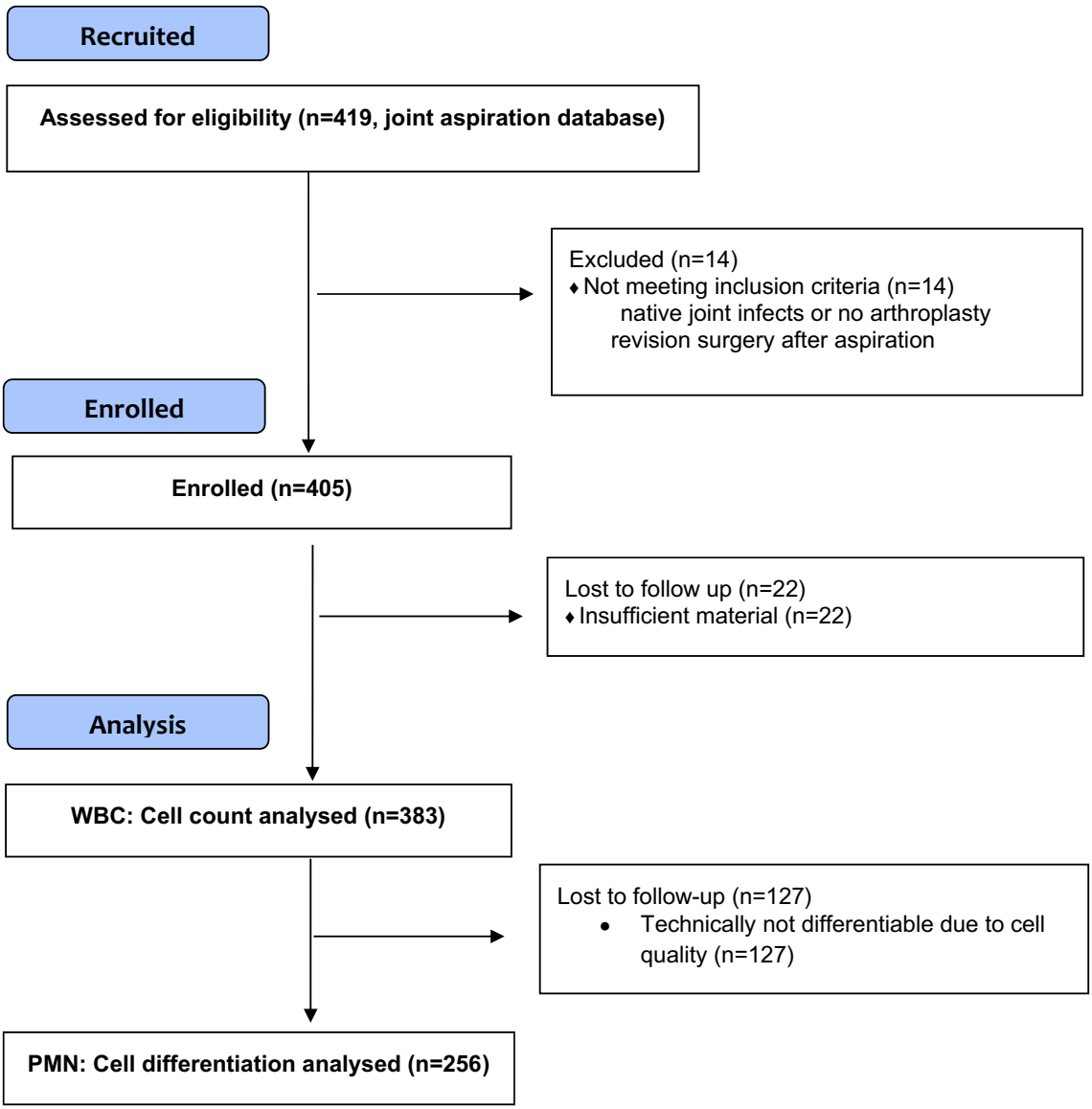

Fig. 1 The standardized flow diagram shows allocation and analyses

the international consensus meeting in latest version (ICM 2018). Therefore, pre-operative criteria (joint aspiration, major and minor criteria) and intraoperative criteria including specimen, as well as the whole patients' documentation during the follow-up were assessed. In case of lack of data according ICM 2018 criteria, the patient was excluded from the study, as the complete necessary data set of biomarkers must be available to determine the accuracy of the different tests. The cell count is the target of this study as well as the PMN cell differentiation. Alpha-defensin was therefore measured in every case to guarantee a valid PJI-diagnostic in the ICM-PJI 2018 scoring system. Patients were followed up 12 months. In this period, patients' data were screened to detect whether an infection occurred using patients records, readmission records, and interviews. This guarantees that major, minor, and invasive criteria, as well as the patients records can be taken into account to reliably decide if the patient has to be classified as infected or not according the ICM 2018 criteria.

Laboratory standard operating procedures

Cytological examination of synovial fluid by manual cell counting and cytocentrifugation with subsequent microscopic cell differentiation according to Pappenheim staining (combined May–Grünwald–Giemsa staining). After joint aspiration, the material was taken to the central laboratory for processing immediately to minimize secondary changes due to autolysis. Cell count is to be processed within 6 h after collection of the material. In manual (microscopic) cell counting, the nucleated blood cells are counted with respect to a volume (per µl; Bürker´s Method). Cytocentrifugation (Cellspin I-System; Cellspin® diagnostics GmbH, GER) is a method for the enrichment and fixation of cells for histological examination on standard slides. For this purpose, a thin-layer/monolayer preparation is prepared from a liquid matrix by centrifuging the cells contained in the liquid directly onto a slide. For the visualization and differentiation of nucleated cells, the staining characteristics of the May–Grünwald and Giemsa staining in panoptic staining according to Pappenheim was used. The diagnostic workup was previously published [16]. The laboratory tests are carried out in an accredited laboratory, and the analyses are performed by a laboratory specialist (M.D.).

Statistics and regulatory aspects

The study is based on the guidelines of Good Clinical Practices and the tenets of the Declaration of Helsinki and was approved by the institutional review board (IRB) and independent ethics committee (IEC) (ID: LAEKHFF71). Individual patients' informed consent was obtained for participation in the study as well as for joint puncture and blood collection. In sample size calculation, 90–130 joint punctures per year were identified. A Consort flow diagram shows patient recruitment and analyses (Fig. 1).

The ROC curve was calculated using a logistic regression model. Using the calculated model parameters, sensitivity and specificity were calculated for each point on the ROC curve. The value with the highest Youden index (i.e., sensitivity + specificity − 1) was selected as the optimal cut-off point. The results show sensitivity and specificity for the detection of a PJI, and in contrast, currently published cut-offs were evaluated using the dataset of our collective to show their test accuracy for PJI diagnosis.

Results

Between April 2016 and February 2020, 405 patients (186 male [45.9%] and 219 female [54.1%]) were treated using the assembled test kit and included in this prospective study. The mean age was 66.72 years (18–89 years; SD: 11.25). Of the 405 patients, 300 (74.07%) had a total knee arthroplasty (TKA), 100 (24.69%) had a total hip arthroplasty (THA), and 5 (1.23%) had a shoulder arthroplasty.

Periprosthetic joint infection was confirmed in 111 patients (27.4%) and excluded in 294 (72.6%). Of the 405 patients, 100% had a complete dataset with respect to alpha-defensin; 383 patients, according to WBC count; and 256, according to PMN cell differentiation (Fig. 1, Consort flow diagram). Owing to technical issues such as blood clotting in the test tube or technically impossible cell differentiation in the laboratory, 22 of 405 patients in the WBC group and 149 in the PMN group were not eligible. In the WBC group of the 383 patients, PJI was confirmed in 106 cases (28%) and PJI was excluded in 277 cases (72%). In the PMN cell differentiation group, 86 patients with confirmed PJI were included (34%), whereas no PJI was detected in 170 cases (66%). *Staphylococcus epidermidis* represented the most frequent detected bacteria (26%), followed by *S. aureus* (21%) and *Enterococcus faecalis* (12%) (Fig. 2).

The best accuracy to determine a PJI showed the cut-off of 2478.89 cells/µl in the WBC count (sensitivity: 87.70%; specificity: 88.10%) and the cut-off of 66.99% in PMN differentiation (sensitivity: 86.00%; specificity: 88.80%). Additionally, we performed subgroup analysis of patients with THA or TKA. An optimal cut-off of WBC count in TKA was 3085.72 cells/µl (sensitivity: 86.76%; specificity: 91.78%; false positive: 8.22%; false negative: 13.24%; positive predictive value: 76.62%, negative predictive value: 95.71%). The optimal cut-off of WBC count in THA was 2267.86 cells/µl (sensitivity: 87.50%; specificity: 82.46%; false positive: 17.54%; false negative: 12.5%; positive predictive value: 73.68%; negative predictive value: 92.16%). The optimal cut-off for PMN differentiation in THA and TKA

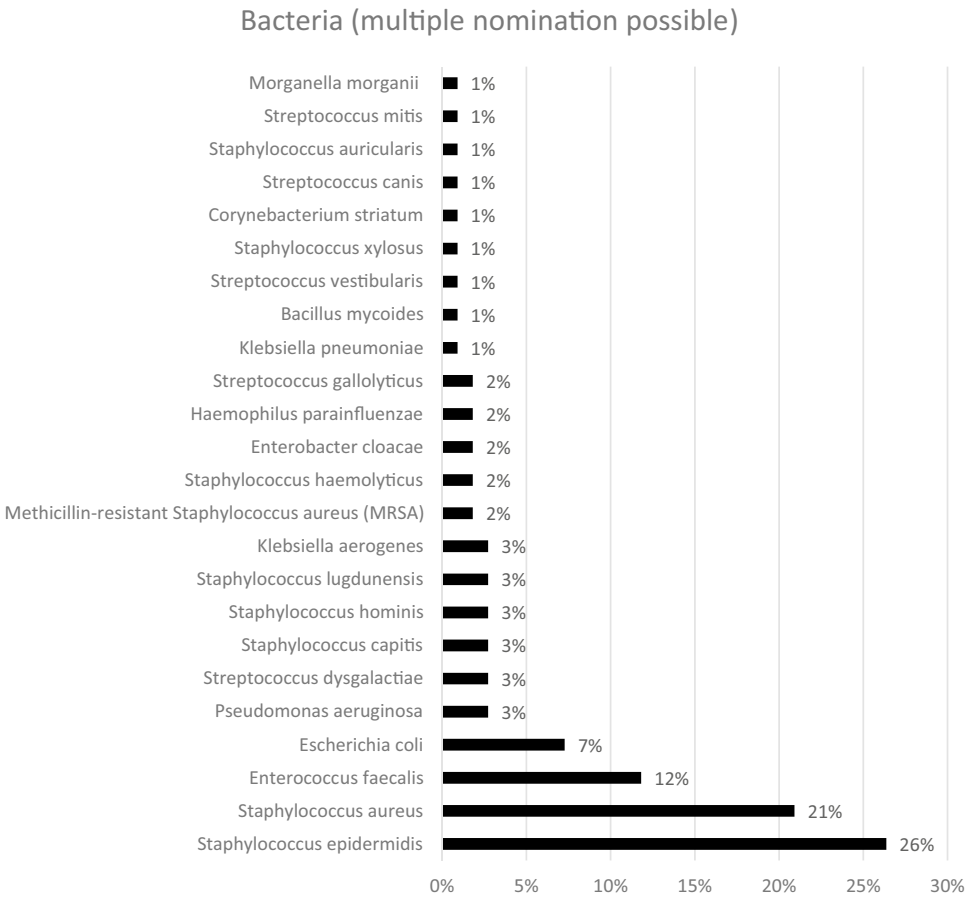

Fig. 2 Detected bacteria. Multiple nomination and combinations are possible. Overall, 111 PJI were detected, 49 (44.1%) without bacterial detection, 62 (55.9%) showing at least one bacterium

was 67.04% (sensitivity: 81.486%; specificity: 94.12%) and 67.01% (sensitivity: 87.50%; specificity: 88.15%), respectively, which was almost identical to the overall cut-off for all prosthesis of 66.99%. Previously published cut-offs were tested using our population and dataset for WBC and PMN (Table 1). Receiver-operating characteristic (ROC) curves for WBC counts and PMN cell differentiation both had an AUC of > 0.92 and were highly significant (Fig. 3). Further synovial/invasive or blood tests are listed in Table 2. We provide the data divided into three common ranges, WBC under 1500/µl, between 1500 and 3000/µl and over 3000/µl. The leukocyte-esterase test was not reliable in our cohort, as lot of tests (Table 2) were not readable due to blood debris. This was already published [16] and the data are pasted in Table 2.

Discussion

In the present prospective single-center study, we examined the sensitivity and specificity of different cut-off values for synovial WBC count and PMN cell differentiation to determine an optimal cut-off threshold for PJI definition and diagnosis algorithms in a specific cohort of patients, who have to undergo endoprosthesis revision surgery. To our knowledge, this prospective-controlled study represents one of the largest investigations thus far regarding the optimal cut-off values for WBC and PMN within the definition criteria of the 2018 ICM [9] taking into account alpha-defensin testing (ELISA) in all cases. The study also complies with recently published demands in quality of diagnostic studies for PJI [20]. Using ROC analysis, the optimal cut-offs for WBC and PMN are 2478.89 cells/µl (sensitivity: 87.70%, specificity: 88.10%) and 66.99% (sensitivity: 86.00%, specificity: 88.80%), respectively. The ICM cut-offs (WBC: 3000/µl, PMN: 80%) were too high in our collective, as they showed high specificity but very low sensitivity.

It is well established that there is no single biomarker to obtain a binary infected versus not infected result [6]. Therefore, the two major definitions use a set of features to guide clinicians in the diagnosis of PJI. The 2018 ICM definition uses a score-based classification resulting in three categories: infected, possibly infected, and not infected [9]. Likewise, the 2021 EBJIS definition uses a three-level model, which differentiates unlikely, likely, and confirmed infections [11]. Both the 2018 ICM and 2021 EBJIS definitions are valid for a diagnosis of PJI in patients with THA or TKA. Hence, our study population—wherein the majority

Table 1 Cut-off for white blood cell count (WBC) and polymorphonuclear leukocyte cell differentiation (PMN count) for diagnostics of periprosthetic joint infections

Test cut-off: WBC count	Cut-off in publication of	Sensitivity (%)	Specificity (%)	False positive (%)	False negative (%)	Positive predictive value (%)	Negative predictive value (%)
Optimal cut-off: 2478.89 cells/µl	This study	87.70	88.10	11.90	12.30	73.60	94.60
PMN > 1500 cells/µl	EBJIS 2021	91.50	75.00	24.90	8.50	58.20	95.40
PMN > 1750 cells/µl		89.60	78.70	21.30	10.40	61.70	95.20
PMN > 2000 cells/µl		89.60	83.40	16.60	10.40	67.40	95.50
PMN > 2500 cells/µl		86.80	88.10	11.90	13.20	73.60	94.60
PMN > 3000 cells/µl	ICM 2019	82.10	91.00	9.00	17.90	77.70	93.00
PMN > 10,000 cells/µl	ICM (early acute infection)*	56.60	99.30	0.70	43.40	96.80	85.70
Test cut-off: polymorphonuclear leukocyte differentiation (PMN)	Cut-off in publication of	Sensitivity (%)	Specificity (%)	False positive (%)	False negative (%)	Positive predictive value (%)	Negative predictive value (%)
Optimal cut-off: 66.99%	This study	86.00	88.80	11.20	14.00	79.60	92.60
PMN > 65%	EBJIS 2021	86.00	88.80	11.20	14.00	78.70	92.60
PMN > 70%		82.60	90.00	10.00	17.40	79.80	91.00
PMN > 80%	ICM 2019	66.30	96.50	3.50	33.70	90.10	88.10
PMN > 90%	ICM (early acute infection)*	19.80	100.00	0.00	80.20	100.00	74.20

The optimal cut-off using Youden index of "1" is shown. In comparison, the accuracy of other cut-offs is given, which were published by international consensus meeting (ICM), European Bone and Joint Infection Society (EBJIS), or various different studies. The accuracy of these cut-offs was tested versus our study population

*Not validated values in ICM consensus 2018

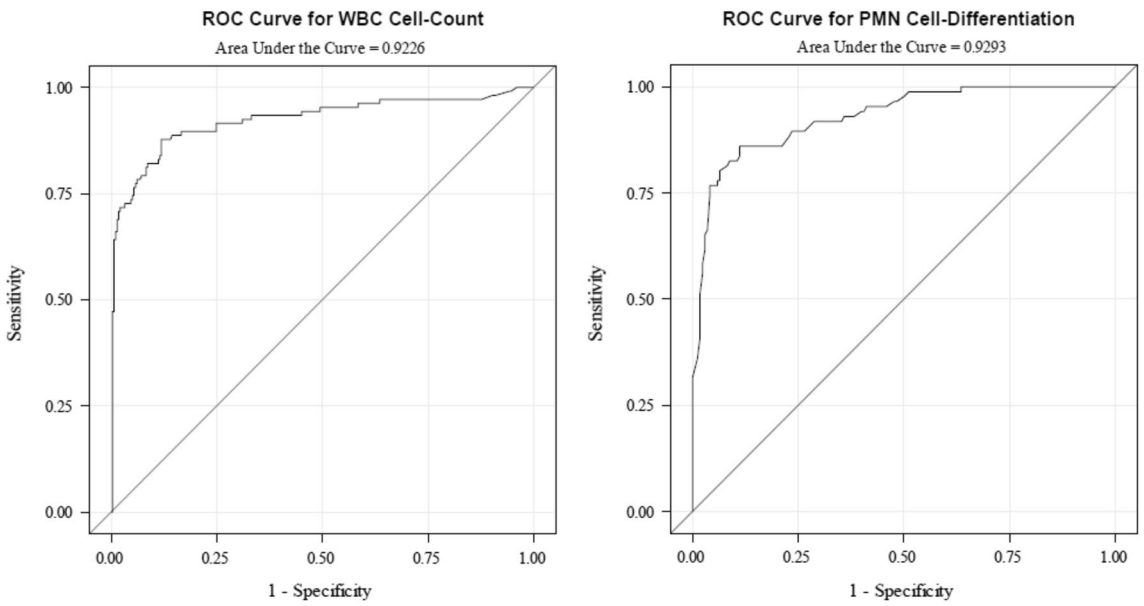

Fig. 3 Receiver-operating characteristic (ROC) curve for white blood cell count (WBC; left) and for polymorphonuclear leukocyte cell differentiation (PMN; right). Area under the curve (AUC) is over 0.92 in both ROC curves

Table 2 Diagnostic results of serum and invasive tests

	Infection confirmed				Not infected
	Total	WBC < 1500/µl	WBC 1500–3000/µl	WBC > 3000/µl	
Patients					
Age (years; mean ± SEM)	67.36 ± 1.2	65.9 ± 1.92	70.67 ± 2.76	66.72 ± 1.51	66.49 ± 0.62
Gender (*n*; f/m)	52/59	6/4	5/4	39/46	167/127
Serum parameters: mean ± SEM					
Leucocytes (1000/mm^3)	8.71 ± 0.29	8.97 ± 0.77	7.12 ± 0.45	8.85 ± 0.36	6.85 ± 0.13
CRP (mg/dl)	6.31 ± 0.76	3.41 ± 1.55	6.56 ± 3.04	6.70 ± 0.89	0.94 ± 0.14
Invasive diagnostic					
PMN % (mean ± SEM)	80.7 ± 1.8	35.5 ± 2.9	67.9 ± 8.4	84.5 ± 1.3	33.6 ± 1.9
LE strip positive	32	4	1	27	31
Not readable	39	3	4	27	84
Total	72	7	5	58	210
Alpha-defensin (mean ± SEM)	4.41 ± 0.27	0.73 ± 0.30	2.68 ± 0.52	4.98 ± 0.29	0.22 ± 0.03
Microbiologic diagnostic (positive/total)[#]	94/171	11/14	6/13	75/135	14/362

For patients with confirmed infection, patient characteristics and results are correlated with synovial white blood cell count (WBC). Serum parameters: leucocytes: normal value range: 4.500–11.000/mm3; CRP: normal value range < 0.3 mg/dl; synovial parameters: polymorphonuclear leucocytes (PMN); leukocyte esterase (LE); alpha-defensin: normal value range < 0.9; *LE* leukozyte-esterase test strip, positive if twofold positive

[#]Joint aspirations and tissue sampling, multiple nominations possible

of included patients underwent THA or TKA (98.73%)—is valid to evaluate the applied WBC and PMN cut-off values.

WBC and PMN are affected by several factors such as sampling, comorbidities, and joint site [15]. In line, we also had to exclude some cases in which analysis was not possible due to technical issues, e.g., blood clotting in test tube.

In the 2018 ICM definition, a WBC cut-off of 3,000 cells/µl is used, which showed a sensitivity of 82.10% and specificity of 91% in our study. In comparison, the 2021 EBJIS definition uses a WBC cut-off of 1500 cells/µl (in addition to another positive criteria) that resulted in a sensitivity of 91.50% and a specificity of 75% in our collective for *likely infections,* whereas a cut-off value of 3000 cells/µl was used for *confirmed infections* in THA and TKA. Naturally, a high WBC cut-off of 3000 cells/µl is particularly reliable, but according to our findings, this may result in a false-negative diagnosis in 17.90% of cases. Given the high mortality rates, underdiagnosis of PJI must be prevented. Therefore, the 2012 EBJIS definition uses a WBC count from 1501 to 3000 cells/µl for *likely infections in addition with other criteria*. Given the low sensitivity of serum CRP results, especially in PJI caused by low-virulent microorganisms, there is a risk of underdiagnosing PJI due to the preconditions that have to be met [21, 22]. False-positive rates using the EBJIS cut-off in WBC of 1500 cells/µl resulted in a false-positive rate of 24.9%, which could lead to a highly invasive treatment in nearly a quarter of all patients, which is not tolerable by clinical treatment standards. Therefore, other minor criteria are necessary to add in this cases. Interestingly, our published WBC cut-off of 2478.89 cells/µl matches a retrospective analysis of 524 joint aspirations published by Zahar et al. [23]. However, it should be noted that some published studies show a high sensitivity and low specificity of the 2018 ICM definition when including all criteria [24]. This might be because of the role of intraoperative criteria, a low sample size of PJI cases and especially a retrospective inclusion of revision THA and TKA cases. In particular, in a retrospective analysis of only revision THA and TKA, a selection bias with overestimated test sensitivity due to increased infection severity should be expected [20]. Therefore, our approach using a prospective study design of undiagnosed patients on inclusion seems to be more suitable to evaluate cut-off values for a screening test.

In a recently published meta-analysis by De Fine et al. studies were only included when THA and TKA cases were considered separately [25]. We therefore performed additional subgroup analysis which resulted in WBC cut-off values of 2267.86 cells/µl (sensitivity: 87.50%, specificity: 82.46%) for patients with THA and 3085.72 cells/µl (sensitivity: 86.76%, specificity: 91.78%) for patients with TKA. In contrast to the recently published study by Zahar et al. our WBC cut-off for TKA aspirations was higher than that for THA aspirations, whereas the overall cut-off for THA and TKA was almost identical [23]. Although this trend was discussed by two recently published reviews both of which presumed lower cut-offs in TKA than THA, the reason for the difference in the two joints was unclear [15, 23, 26]. There is a lack of studies specifically investigating TKAs and

some studies support our findings [27, 28]. Hence, given the inconsistent results in TKA, we suggest retaining an overall cut-off to keep the definitions as simple as possible for now. However, more research is required in joint-specific cut-off values for WBC and PMN, not only with respect to THA and TKA but also for total shoulder or total ankle arthroplasty to allow joint-specific alterations in future.

The additionally used PMN was also analyzed for sensitivity and specificity for different cut-off values. In the 2018 ICM definition and the 2021 EBJIS definition for confirmed infections, a PMN cut-off of 80% was used, with a single value sensitivity of only 66.30%. This results in a false-negative rate of 33.70%, which means that more than a third of patients who test negative have a PJI but would not receive necessary treatment. We suggest a PMN cut-off of 66.99% resulting in a sensitivity of 86% and specificity of 88.80%. This cut-off could be used for patients with THA and TKA, as individual cut-offs of 67.04% and 67.01% are almost identical. Our findings regarding the PMN cut-off are almost met by the *infection likely* group of the 2021 EBJIS definition that includes cases with lower PMN cut-offs between 66 and 80%. As discussed earlier regarding the WBC cut-off used in the *infection likely* group of the 2021 EBJIS definition, certain preconditions have to be met.

Obviously, single variable sensibilities and specificities for cut-off values cannot be used to adapt a cut-off in a multivariable diagnostic set. We therefore suggest a re-evaluation of the 2018 ICM definition for PJI by including our results as well as recently published results by other authors [23] to increase the pre-operative sensitivity. The primary target for the two major definitions is helping clinicians (and researchers) to decide whether a PJI might be likely or not. Hence, a low sensitivity could result in severe consequences when PJI is underdiagnosed [11]. Consistent with this, Kheir et al. recently published a study showing that certain organisms like coagulase-negative *Staphylococcus* have much lower WBC and PMN cut-off values and stress on the fact that clinicians should be aware of the low sensitivity [21]. This is in line with our patient cohort, in which *S. epidermidis* was the most frequently detected organism. Given the recently published literature including the 2021 EBJIS definition [11, 21, 23] and in line with our own findings, lower cut-offs for WBC as well as PMN could be reasonable.

Limitations

Although 405 patients could be examined and included using the compiled test kit, the full dataset for WBC was available only in 383 cases and for PMN, in only 256 cases, owing to non-differentiable cells in the sample in some cases. These laboratory issues are well known in literature, as differentiation of physiologically occurring cells is performed according to morphological criteria with adequate magnification. Counting of at least 100 nucleated cells has to be guaranteed. Drop-outs are given in Fig. 1. Different details of processing, fixation, and staining could have an impact on the cytology. The included samples/patients, however, show all quality criteria for the diagnosis of the accredited laboratory. Despite the drop-outs due to technical issues (Fig. 1; blood clotting in test tube, impossible cell differentiation), highly accurate ROC curves were generated with AUCs > 0.92. Therefore, statistical results were appropriate, even though cell differentiation in the laboratory was not possible in 22 of 405 cases in the WBC group and in 149 of 405 cases in the PMN group. Nevertheless, to our knowledge, this prospective-controlled study represents one of the largest investigations thus far regarding the optimal cut-off values for WBC and PMN within the 2018 ICM [9] and 2021 EBJIS [11] definition criteria, taking into account alpha-defensin testing (ELISA) in all cases. We believe that the study also complies with recently published demands in the quality of diagnostic studies for PJI [20]. Although we aimed to investigate an optimal cut-off for the diagnostic of PJI, it is indisputable that only the combination of all minor and major criteria together yields a reliable diagnosis; the WBC and PMN count is one essential component. Study contains five shoulder arthroplasties; it is not already known if the cut-offs for shoulders are in line with other joints and if there should be different cut-offs for upper and lower limbs [11]. Nevertheless, shoulders count only for 5 of 405 patients in our study, and so, a possible bias would be irrelevant. The study design was focussed to guarantee a reliable ROC analysis and to perform ICM 2018 criteria (using alpha-defensin) in all cases. Our institutional PJI database, according IRB approval, does not include all individual personal data of the patients, such as individual pre-operation for example, so these data are missing. The details of the used database were already published [16].

Conclusion

There is currently no single test that reliably excludes or proves an infection. White blood cell count and PMN differentiation remain easy and cost-effective tests to determine a PJI. These tests are included in all current published guidelines, but with different cut-off values. The evidence level for the cut-offs remains low. Using ROC analyses and a standardized test kit in prospective-controlled study, optimal cut-offs were evaluated for WBC count and PMN differentiation. The ICM cut-offs (WBC: 3000/µl, PMN: 80) seem to be too high, as they provide high specificity but very low sensitivity. On the other hand, the EBJIS cut-off for WBC count (suspected PJI, 1500/µl) is very low, leading to low specificity and a very high suspicion for PJI and therefore additionally minor criteria have to be taking into account in

these cases. The optimal cut-off for white blood cell count in our collective is 2478.89 cells/µl (sensitivity: 87.70%; specificity: 88.10%)**.** The optimal cut-off for PMN differentiation is 66.99% (sensitivity: 86.00%; specificity: 88.80%) in our collective. Given the recently published literature including the 2021 EBJIS definition [11, 21, 23] and in line with our own findings, lower cut-offs for WBC as well as PMN could be reasonable.

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Declarations

Conflict of interest All authors declare that they have no conflicts of interest. Study is based on institutional review board (IRB) approval.

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Authors and Affiliations

Y. Gramlich[1] · M. Schnetz[1] · C. Ruckes[2] · M. Kemmerer[1] · M. Kremer[1] · R. Hoffmann[1] · A. Klug[1]

M. Schnetz
Matthias.Schnetz@bgu-frankfurt.de

C. Ruckes
ruckes@izks-mainz.de

M. Kemmerer
Matthias.Kemmerer@bgu-frankfurt.de

M. Kremer
Michael.Kremer@bgu-frankfurt.de

R. Hoffmann
Reinhard.Hoffmann@bgu-frankfurt.de

A. Klug
Alexander.Klug@bgu-frankfurt.de

[1] Department of Trauma and Orthopedic Surgery, Berufsgenossenschaftliche Unfallklinik Frankfurt am Main, Friedberger Landstr. 430, 60389 Frankfurt am Main, Germany

[2] Interdisciplinary Center for Clinical Studies, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany