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Diagnostic value of the CD 15 focus score in two-stage revision arthroplasty of periprosthetic joint infections

High specificity in diagnosing infect eradication

Introduction

Periprosthetic joint infections (PJI) represent a major medical challenge to the various surgical and diagnostic disciplines [4, 5, 17, 20, 24–26, 29]. Currently, two-stage revision arthroplasty, where the infected implant is replaced by an antibiotic-loaded cement spacer until there are no further signs of infection and a new implant can be applied, is regarded as one of the most important therapeutic procedures and remains useful in cases where treatment success is difficult to predict and the infection persistence rates are high [2, 8, 12, 13, 16, 27]. The histopathological criteria for PJI are defined adequately, although some deviations remain [5, 7, 24, 29]. For the eradication of infection, which is defined as absence of infection [8] in the context of two-stage revision arthroplasty, mainly data on cryosection diagnosis [4, 7, 12, 13] are available, in some cases with low sensitivities and without a precise definition of the field area [6]. There is a single systematic histopathological analysis of the perispacer tissue which was performed on a small collective of patients, also without definition of the field area [14]. To date, there is no defined histopathological classification for diagnosing infection eradication.

Doctoral thesis by Caroline Liewen
These data represent a part of Caroline Liewen's doctoral thesis at the Charité University Medicine, Berlin (supervisor of the thesis: Prof. Dr. med. Veit Krenn).

Aim of this analysis

In total, the sample comprises 112 cases that were subjected to revision due to the presence of infection upon two-stage revision arthroplasty. The data were gathered from a retrospective, correlative, histopathologically based multicenter analysis (27 orthopedic clinics from 9 German federal states) during the period from 2017 to 2020. The histopathological data were collected through histopathological diagnosis according to the SLIM consensus classification (SLIM = synovial-like interface membrane) and the CD15 focus score (CD15 FS) [18, 19], and correlated with clinical and microbiological data. The quantifying evaluation by means of the CD15 FS (single and tenfold) was not performed with knowledge of the microbiological data and was therefore blinded. Correlation with the microbiological data as the gold standard was only performed after a 14-day cultivation period. The aim was to determine the limit value of the CD15 FS (single and tenfold) for the eradication of infection, especially the sensitivity, specificity, NPV, and PPV.

Materials and methods

Semi-automatic and automatic procedures were performed under accredited conditions (DIN EN ISO/IEC 17020: 2012, registration number: D-IS-21311-01-00). These procedures included drainage and paraffinization of the tissue samples (Leica PELORIS®, Xpress 120 SACURA®, Germany) and semi-automatic (Leica HM325 and Zeiss®, Germany) as well as fully automatic microtomization (AutoTEC a120, SACURA®, Germany) with the implementation of laboratory tracking that makes use of a barcode system (VENTANA, VANTAGE workflow solution®, ROCHE). HE staining and PAS staining were performed using SACURA (Prisma staining module®). The Berlin blue reaction was performed with ST-5020 LEICA®. The Oil Red O staining was not carried out automati-

cally [15]. CD15 was detected by indirect immunohistochemistry (Roche, Product number: 05266904001, clone: MMA). The procedure was performed by a fully automated staining system (BenchmarkXT®, ICH Slide Stainer®, Roche, Basel, Switzerland).

Histopathological diagnostics

Histopathological diagnosis of SLIM was performed under accredited conditions (DIN EN ISO/IEC 17020:2012, registration number: D-IS-21311-01-00) on the basis of routine histopathological diagnosis in a histopathological diagnostic center that is active throughout Germany and that focuses on the musculoskeletal system (MVZ-HZMD-Trier-GmbH, Germany). The histopathological diagnosis was performed according to the revised SLIM consensus classification [18] and the infection diagnosis was based on the CD15 FS [19]. These were conducted as definitive diagnostic evaluations by three experienced medical specialists in the field of pathology (years active as a pathology specialist: 11, 13, and 17) with specialization in musculoskeletal medicine (C.D., M.O., and V.K.). Discussions took place between the specialists in isolated cases with inconclusive typing, with subsequent compromise-based determination of the diagnosis as interobserver validation.

SLIM classification

In all cases, the tissue samples were transmitted with an inquiry concerning bacterial infection and tissue classification, particularly with the inquiry as to histopathological infection eradication (i.e., histopathological diagnosis of complete eradication without proof of bacterial pathogens). Histopathological analysis and typing was performed according to the criteria of the SLIM consensus classification [18], and particle characterization according to the particle algorithm [23]. The Polymetylmethacrylate (PMMA) particle quantification was carried out semi-quantitatively into low-grade PMMA depositions (1–2 particles per field area: 1.2 mm^2) and high-grade PMMA depositions (more than 2 particles per field area: 1.2 mm^2).

CD15 FS for a defined field area

The evaluation was carried out according to CD15 FS [19], which indicates a sensitivity of 0.91 and a specificity of 0.92 in the diagnosis of primary infection for PJI. This principle was applied once (1 × CD15 FS) and, on the basis of published data [14, 21], ten times (10 × CD15 FS). The field area that was evaluated using an intermediate-power field (objective magnification 20×) was determined with morphometric software. Determination of the visual field diameter as the basis for area calculation was carried out by means of a computer-aided interactive morphometric analysis (Leica application suite, version 4.5.0, May 2014). In the case of 1 × CD15 FS the field area was 1.2 mm^2 and in the case of 10 × CD15 FS it was 12 mm^2. As a result, a maximum amount of tissue area was evaluated in order to adequately capture the heterogeneous expression of infections.

Clinical and microbiological diagnosis of infections

Diagnosis of the PJI was performed on the basis of the proposed criteria [20, 24, 29]. Incubation of the samples was carried out according to standardized criteria in a microbiological reference laboratory through 14-day anaerobic and aerobic cultivation and was conducted with an extended pathogen spectrum (e.g., mycotic infections). The microbiological findings were considered to be positive for two-stage prosthesis revisions if one pathogen or the pathogen from the microbiological preliminary diagnosis could be detected in a sample.

Statistical methods

Statistical analysis of the collected data was performed using SPSS Statistics (IBM SPSS Statistics, version 25.0.0.0, 64-bit). Prism (GraphPad Prism, version 8.4.0 for Mac, GraphPad Software, La Jolla, CA, USA) was used to generate the graphics. Frequencies, mean values, minimum values, maximum values, and standard deviations were determined for the purpose of descriptive statistics. Comparative statistical analyses were carried out with the help of the R programming language (The R Foundation for Statistical Computing; version 3.5.1, General Public License; [The R Project]; www.r-project.org). Variance analyses (ANOVA) were specifically performed, and in the case of statistically significant differences in the data that were generated in this way, a post-hoc test (Tukey HSD test) was also conducted. In this study, p-values were weighted according to the usual significance levels ($p < 0.05$ significant, $p < 0.01$ highly significant). The Mann–Whitney U test was used to address the question of whether CD15 FS for microbiological detection differs as well as to achieve more precise stratification in high- and low-grade infections. Then followed the Shapiro–Wilk test to examine normal distribution. The distributional form of the groups was investigated using the Kolmogorov–Smirnov test. The question of whether the eradication of infections was successful with regard to microbiological pathogen detection in relation to intracellular PMMA particles containing antibiotics was verified for independence by means of the chi-square test.

Results

Collective of patients

A total of 112 cases were found involving patients who underwent two-stage revision arthroplasty surgery because of an infection. Based on the histopathological findings, the material was classified according to the clinical question using CD15 FS, typed according to the revised SLIM consensus classification and evaluated as perispacer SLIM (type 9), and the detection of PMMA depositions was additionally commented. The tissue samples were obtained from 27 orthopedic clinics located in 9 German federal states: Berlin ($n = 1$), Schleswig-Holstein ($n = 1$), Baden-Wuerttemberg ($n = 7$), Hamburg ($n = 7$), Rhineland-Palatinate ($n = 2$), North Rhine-Westphalia ($n = 9$), Lower Saxony ($n = 4$), Saxony-Anhalt ($n = 1$), and Bavaria ($n = 4$).

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Diagnostic value of the CD 15 focus score in two-stage revision arthroplasty of periprosthetic joint infections. High specificity in diagnosing infect eradication

Abstract

Introduction. The purpose of this study is to use the CD15 focus score (FS) to determine the sensitivity and specificity of bacterial infection persistence in spacer-based two-stage revision arthroplasty.
Methods. The analysis comprises 112 cases that were subjected to revision due to the presence of infection upon replacement of a joint endoprosthesis. The histopathological data were collected in accordance with the synovial-like interface membrane (SLIM) classification and the CD15-FS and correlated with the microbiological data (MD). The quantifying evaluation of the CD15-FS was performed without knowledge regarding the microbiological data (MD). Correlation with the MD was performed after a 14-day cultivation period.
Results. With a single evaluation (1 focus, field area: 1.2 mm2) with a score value of 42, the CD15-FS showed a sensitivity for the eradication of infections of 0.64 and a specificity of 0.79 (PPV = 0.5; NPV = 0.87). With tenfold evaluation (10 foci, field area: 12 mm2) with a score value of 220, the sensitivity for the eradication was 0.68, the specificity 0.91 (PPV = 0.7; NPV = 0.89). No statistically significant correlation between the score values and the different infectious species could be detected. Based on the MD in 112 cases the rate of infection eradication was 75%. Polymethylmethacrylate-particles (PMMA) were detected in the perispacertissue in 64 cases (58%). No significant correlation could be established between microbiological pathogen detection and the presence of PMMA.
Conclusion. In all cases (n = 112), periimplant synovial tissue (SLIM) with variable fibroblastic cellularity, capillary proliferation, leukocytic infiltration, fibrin deposition, new formation of woven bone and detection of PMMA particles was observed. These cases were classified as type IX perispacer synovialis/SLIM: type IX-A with histopathological infection eradication and type IX-B with histopathological infection persistence.

Keywords

Periprosthetic joint infection · CD15 focus score · Eradication of infection · Two-stage revision arthroplasty · SLIM-Classification

Diagnostische Bedeutung des CD15-Fokus-Score bei zweizeitigem Gelenkendoprothesenwechsel. Hohe Spezifität für die Diagnose einer Infekteradikation

Zusammenfassung

Hintergrund. Ziel der Arbeit war es, mittels des CD15-Fokus-Scores (FS) eine Aussage zu Sensitivität und Spezifität der bakteriellen Infektpersistenz bei Spacer-basiertem zweizeitigem Gelenkendoprothesenwechsel zu ermitteln.
Methoden. Die Stichprobe umfasste 112 Fälle in denen aufgrund einer Infektion bei einem Gelenkendoprothesenwechsel eine Revision erfolgte. Die histopathologischen Daten wurden gemäß der SLIM-Klassifikation („synovial-like interface membrane“) und des CD15-FS erhoben und mit den mikrobiologischen Daten korreliert. Die quantifizierende Bewertung durch den CD15-FS erfolgte ohne Kenntnis der mikrobiologischen Daten(MD). Die Korrelation erfolgte erst nach Einhaltung einer 14-tägigen Kultivierung.
Ergebnisse. Der CD15-FS zeigte bei einfacher Auswertung eine Sensitivität für die Infekteradikation von 0,64 und eine Spezifität von 0,79 (PPV = 0,5; NPV = 0,87). Bei 10-facher Auswertung ergab sich eine Sensitivität von 0,68 und eine Spezifität von 0,91 (PPV = 0,7; NPV = 0,89). Es ließ sich kein signifikanter Zusammenhang mit den infektiösen Spezies nachweisen. Den MD zufolge betrug die Rate der Infekteradikation 75 %. In 64 Fällen bestand ein Partikelnachweis (Polymethylmethacrylat [PMMA]). Es konnte kein signifikanter Zusammenhang zwischen Erregernachweis und Präsenz von PMMA gezeigt werden.
Schlussfolgerung. In sämtlichen Fällen zeigte sich periimplantäres Gewebe mit variabler fibroblastischer Zellularität, Kapillarproliferation, Leukozyteninfiltration, Fibrinablagerung, Neubildung von Geflechtknochen und Nachweis von PMMA. Die Klassifikation erfolgte als Synovialis vom Perispacertyp – Typ 9, entsprechend Typ 9A mit histopathologischer Infekteradikation oder Typ 9B mit histopathologischer Infektpersistenz.

Schlüsselwörter

Periprothetische Gelenkinfektion · CD15-Fokus-Score · Eradikation einer Infektion · Zweizeitige Revisionsarthroplastik · SLIM-Klassifiaktion

Age and gender of the patient collective

The median age of the collective was 71.6 years (range = 36–94; SD = 11.1). Of these, 61 (54%) were female (range = 50–94; M = 72.7; SD = 10.1) and 51 (46%) male (range = 36–89; M = 68.2; SD = 11.9).

Joint spacer locations

The locations of the joint spacers are as follows: the knee joint in 54 cases (48%); the hip joint in 56 cases (50%); the ankle joint in one case (about 1%); and the shoulder joint in one case (about 1%). All PMMA spacers contained antibiotics. There were no data available on the service life of the joint spacers.

Sample size and number of samples

The average sample size (maximum diameter) was 41 mm. The number of samples obtained from different locations (and thus separate transmissions) varied between 1 ($n = 37$), 2 ($n = 25$), 3 ($n = 20$), 4 ($n = 10$), 5 ($n = 11$), 6 ($n = 4$), 7 ($n = 3$), and 9 ($n = 2$).

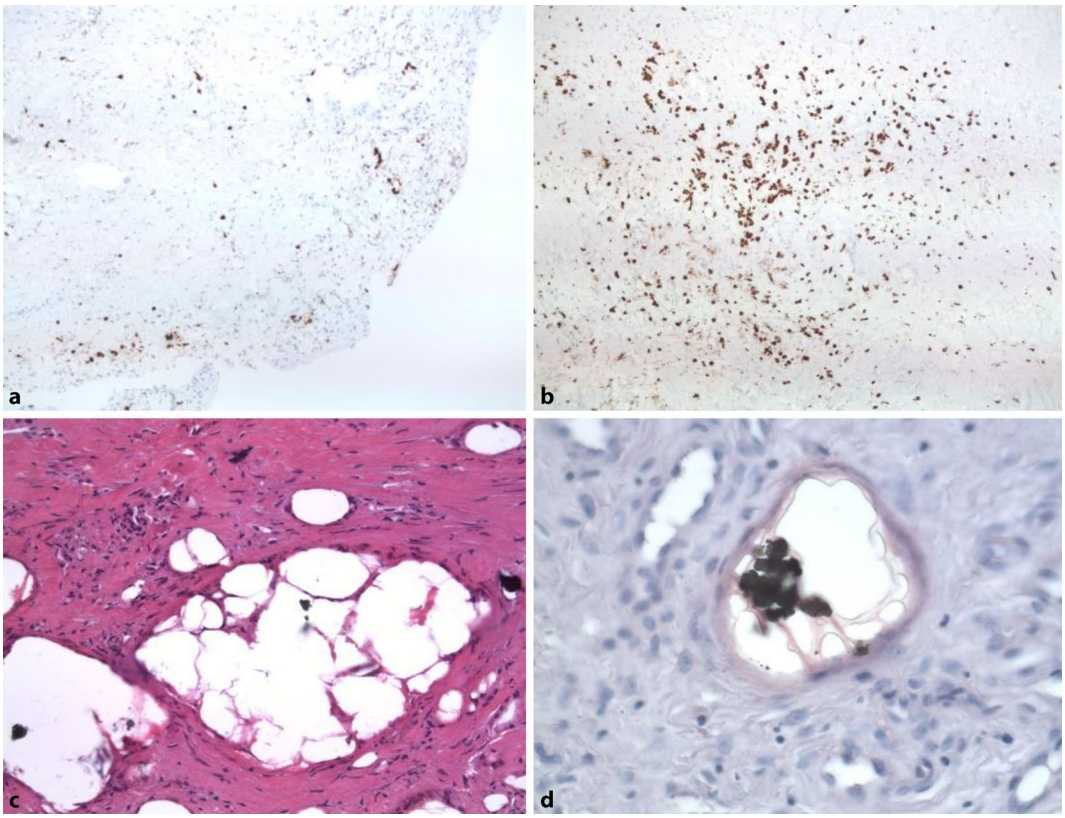

Fig. 1 ◀ Histopathological findings. **a** Eradication of infection, type IX-A: CD15 FS 1×= 25, 10×= 108. Microbiological findings: negative (sex: male, location: knee) CD15 immunohistochemistry, original magnification: about 125×. **b** Persistence of infection, type IX-B: CD15 FS 1×= 350, 10×= 600. Microbiological findings: *Staphylococcus caprae* (sex: female, location: hip) CD15 immunohistochemistry, original magnification: about 125×. **c** PMMA zirconium dioxide depositions: (sex: female, location: knee). Microbiological findings: negative. HE staining, original magnification: about 250×. **d** PMMA zirconium dioxide depositions: (sex: female, location: knee), Microbiological findings: negative. Oil red O staining, original magnification: about 500×

Histopathological results

SLIM classification and perispacer synovialis/perispacer SLIM

The diagnostic classification principles of the SLIM classification were applied to the tissue in cases of two-stage revision of the joint endoprosthesis. Periimplant fibrous, synovialis-like or synovial tissue with variable fibroblastic cellularity, capillary proliferation, fibrin deposition, detection of fractured bone tissue (fractured bone trabeculae), and also focal reactive formation of new woven bone as well as multifocal accumulations of macrophages with focal PMMA particle detection were found in all cases ($n = 112$). There was variable inflammatory infiltration, predominantly by segmented cells as well as neutrophil granulocytes, in certain cases also eosinophilic granulocytes. There was no evidence of granulomatous inflammatory infiltration in any of the cases. The neutrophilic granulocyte infiltrates were evaluated according to CD15 FS, while eosinophilic granulocytes were not immunohistochemically represented. These peri-implant changes were classified in the histopathological findings as changes comparable to two-stage prosthesis revision, specifically as perispacer SLIM (type IX; **Figs. 1a–d and 2**).

PMMA particle classification according to the particle algorithm

There was PMMA detection in 64 cases (58%) with additional detection of PMMA additives (zirconium dioxide). The majority of these PMMA depositions were detectable as extracellular depositions, mostly in the form of detached, vacuole-like structures (**Fig. 1c**). The PMMA residues in the vacuoles and in the macrophage cytoplasm were characterized by a weak Oil red O positivity in the internal structures of irregular vacuoles (**Fig. 1d**). The detection was focal in all cases, with the localization of PMMA mainly in the superficial tissue sections and in tissue particles containing fibrin depositions. In the semi-quantitative evaluation, the PMMA positive cases included 47 cases with low and 17 cases with high PMMA deposition. The zirconium dioxide depositions appeared as dark granular, black-colored particle aggregates with localization in the centers of the PMMA vacuoles and also in PMMA vacuoles with a peripheral layer (**Fig. 1c**). Abrasive particle depositions in the form of polyethylene (PE), metal, and ceramic particles as residuals of the joint endoprosthesis that had previously been implanted were only detectable focally and in individual cases (less than 10%).

PMMA depositions and microbiological findings

In order to investigate a possible influence of the presence of intracellular PMMA spacer particles impregnated with antibiotics on the eradication of infections, it was examined whether there are differences in the microbiological detection of bacteria in relation to the intracellular PMMA particle detection. Since the mode of data collection did not allow for an exact quantification of the concentration of antibiotics added, it is only possible to differentiate between PMMA particles being present and not being present. A chi-square test was carried out statistically. The condition that the expected

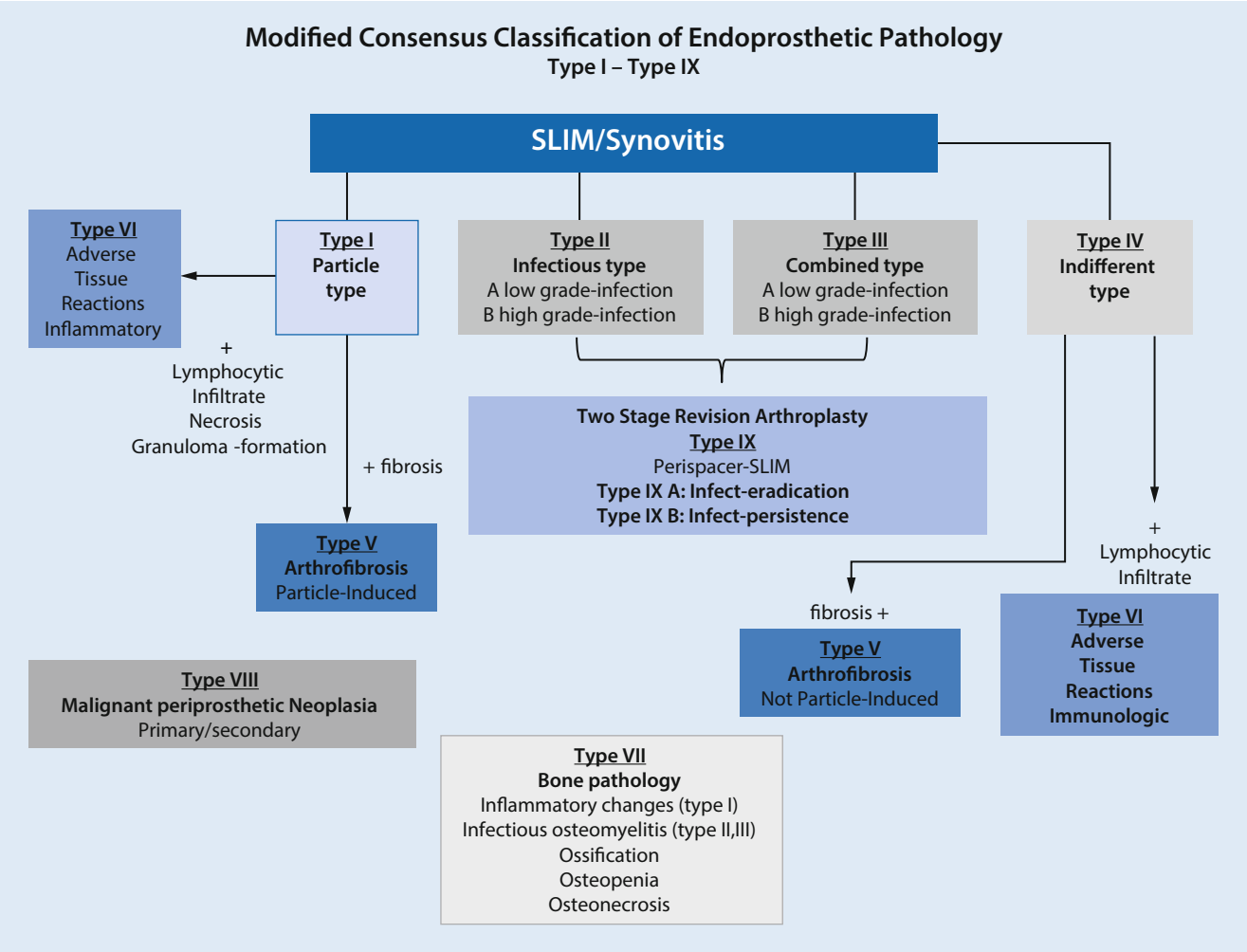

Fig. 2 ▲ Proposal of the revised SLIM/synovitis classification

frequency of each cell may not be less than five was satisfied. However, no significant correlation could be established between microbiological pathogen detection and the presence of PMMA particles in the perispacer tissue, $\chi^2(1) = 0.78$, $p = 0.378$.

CD15 FS (in single and tenfold application)

Following an exploratory inspection of the data, two cases were identified as extreme values and were excluded from the collective. In this way it is ensured that the present sample reflects an average population and that the statistical test procedures are not distorted.

After examining the Shapiro–Wilk test for 10 foci for positive, $W(28) = 0.72$, $p < 0.001$, and negative findings, $W(84) = 0.84$, $p < 0.001$, as well as with one focus for positive, $W(28) = 0.70$, $p < 0.001$, and negative findings, $W(84) = 0.67$, $p < 0.001$, the available data (**Table 1**) do not show a normal distribution. It was also examined whether the location (knee or hip) showed a normal distribution with regard to microbiological detection (positive or negative). For the hip location, there was no normal distribution for 10 HPF in positive, $W(18) = 0.73$, $p < 0.001$, or in negative findings, $W(38) = 0.78$, $p < 0.001$. For the knee location, there was no normal distribution for 10 HPF with a positive result, $W(10) = 0.73$, $p < 0.01$, but there was for a negative result, $W(44) = 0.95$, $p > 0.05$. Additionally, for the hip location for 1 HPF in positive, $W(18) = 0.76$, $p < 0.001$, and in negative findings, $W(38) = 0.88$, $p = 0.001$, there was no normal distribution. In the knee location, no normal distribution was found for 1 HPF in positive, $W(10) = 0.56$, $p < 0.001$, or negative findings, $W(44) = 0.62$, $p < 0.001$. For this reason, the Mann–Whitney U test with exact sample distribution of U according to Dinneen & Blakesley (9) is used for verification of the issue. The condition is that the data for both groups (microbiological findings) must be approximately equally distributed. Following standardization of the dependent variable, this condition was reviewed using the Kolmogorov–Smirnov test and showed a similar distribution form for both groups for 10 HPF, $p = 0.19$, as well as for 1 HPF, $p = 0.15$. Furthermore, this condition concerning the location was reviewed in relation to the respective HPF for microbiological detection. The hip location demonstrated a similar dis-

Table 1 Descriptive statistics for microbiologically positive and negative findings according to location with single and tenfold CD15 focus score quantification (1 and 10 foci)

Positive/negative finding	Localization		*n*	Min	Max	M	SD
Positive	Knee	Quantification of granulocytes per 1 FS	10	12	551	104.9	160.2
		Quantification of granulocytes per 10 FS	10	55	1712	452.1	494.7
	Hip	Quantification of granulocytes per 1 FS	18	19	686	205.0	235.2
		Quantification of granulocytes per 10 FS	18	50	2620	598.1	707.4
Negative	Knee	Quantification of granulocytes per 1 FS	44	1	225	34.0	42.0
		Quantification of granulocytes per 10 FS	44	1	310	107.5	71.8
	Hip	Quantification of granulocytes per 1 FS	38	4	95	31.4	21.8
		Quantification of granulocytes per 10 FS	38	7	600	127.1	108.9
	Shoulder	Quantification of granulocytes per 1 FS	1	7	7	7.0	–
		Quantification of granulocytes per 10 FS	1	40	40	40.0	–
	UAJ	Quantification of granulocytes per 1 FS	1	1	1	1.0	–
		Quantification of granulocytes per 10 FS	1	4	4	4.0	–

FS focus score

tribution for both groups with 10 HPF, $p = 0.34$, and with 1 HPF, $p = 0.45$. For the knee, a homogeneous distribution was also found for both groups with 10 HPF, $p = 0.60$, and with 1 HPF, $p = 0.87$. With regard to the question of whether CD15 FS per 10 HPF differs for microbiological detection, a significant difference was found in the medians for positive cases (Mdn = 248) and negative cases (Mdn = 95), U = 353.50, Z = –5.528, $p < 0.001$, r = –0.522. This difference could also be observed with 1 HPF for positive cases (Mdn = 55) and for negative cases (Mdn = 26.5), U = 475.50, Z = –4.708, $p < 0.001$, r = –0.445. It was also examined whether the hip location has an effect regarding microbiological detection. Once again, there was a difference in the medians per 10 HPF for positive cases (Mdn = 247.5) and negative cases (Mdn = 95), U = 115.00, Z = –3.983, $p < 0.001$, r = –0.532. This effect can also be observed for the medians per 1 HPF for positive cases (Mdn = 55) and negative cases (Mdn = 31), U = 871.00, Z = –3.722, $p < 0.001$, r = –0.497. Likewise, significant differences for the knee location were investigated. There was a difference in the medians per 10 HPF for positive cases (Mdn = 258) and negative cases (Mdn = 107), U = 60.50, Z = –3.552, $p < 0.001$, r = –0.483. In the case of 1 HPF, there was also a difference in the medians for positive cases (Mdn = 60) and negative cases (Mdn = 23), U = 106.50, Z = –2.529, $p < 0.001$, r = –0.344.

It was additionally examined whether there are differences depending on the location (hip or knee) in the case of positive detection. Here too, the data does not exhibit a normal distribution for 10 HPF, W(28) = 0.72, $p < 0.001$, or for 1 HPF, W(28) = 0.70, $p < 0.001$ (Shapiro–Wilk test). The Kolmogorov–Smirnov test was significant for 1 HPF, $p < 0.05$, but not for 10 HPF, $p = 0.56$. As before, this allows for an interpretation of the medians for 10 HPF. The distributions for 1 HPF differ from one another depending on location, whereby only statements regarding the average ranks can be made. Using the Mann–Whitney U test, no significant difference could be found with 10 HPF and the hip location (Mdn = 247.5) or knee location (Mdn = 258), U = 80.00, Z = –0.480, $p = 0.65$. With 1 HPF and the hip location ($M_{Rang} = 13.06$) and knee location ($M_{Rang} = 17.10$), a difference was similarly insignificant, U = 64.00, Z = –1.247, $p = 0.22$ (**Fig. 3**; **Table 1**).

CD15 focus score for positive and negative microbiological findings in the case of one CD15 focus

With respect to the cut-off values when applying a focus, the values 36 and 42 resulted in an identical sum of sensitivity and specificity. Because the focus is on identifying healthy patients (i.e., no persistence of infection), the cut-off of 42 (sensitivity = 0.64, specificity = 0.79) was considered clinically superior to the cut-off of 36 (sensitivity = 0.75, specificity = 0.68; **Fig. 3**; **Table 1**).

The CD15 FS in cases of the group with a positive microbiological finding ($n = 28$) with 545.9 cells per average was greater than the mean of the SLIM cases with negative microbiological findings ($n = 84$) with 114.3 cells per average. The difference in the cell count per focus between the two groups is 431.6. The SLIM cases of the group with a positive microbiological finding ($n = 28$) have a significantly higher CD 15 FS ($p < 0.001$ Mann–Whitney U test) than the cases with negative microbiological findings ($n = 84$; **Fig. 3**; **Table 1**).

With the microbiological findings functioning as the gold standard, the sensitivity amounts to 0.68, the specificity to 0.91, and the sum of the two 1.59 (positive predictive value [PPV]: 0.7; negative predictive value [NPV]: 0.89; accuracy: 0.85; area under the curve [AUC]: 0.85). The numerical value of 220 was specified as the limiting value between a positive and negative microbiological finding (**Fig. 4**).

CD15 focus score and specification of the pathogens

Concerning the question whether 10 × CD15 FS for stratification varies, there was no significant difference in the medians for a high-grade infection (Mdn = 235.5) and a low-grade infec-

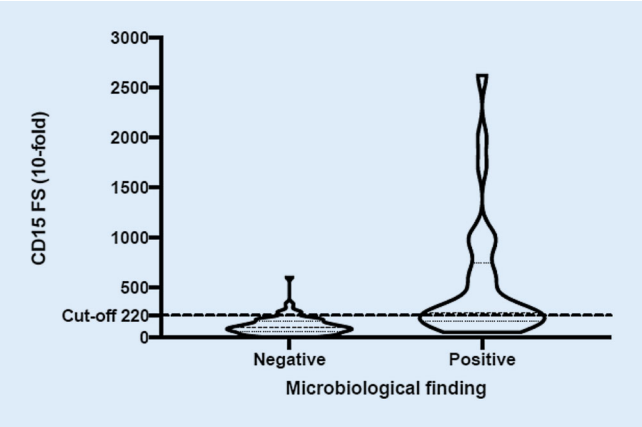

Fig. 3 ◀ Violin plot for microbiologically positive and negative findings with tenfold CD15 focus score quantification (10 foci)

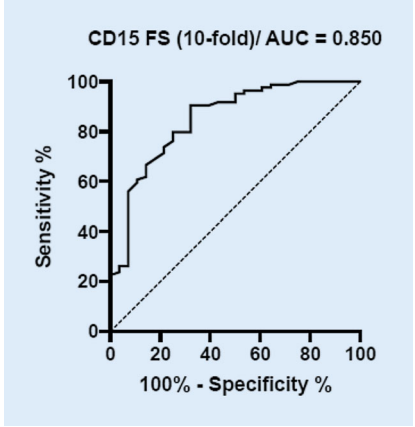

Fig. 4 ▲ ROC curve for microbiologically positive or negative findings with tenfold CD15 focus score quantification (10 foci)

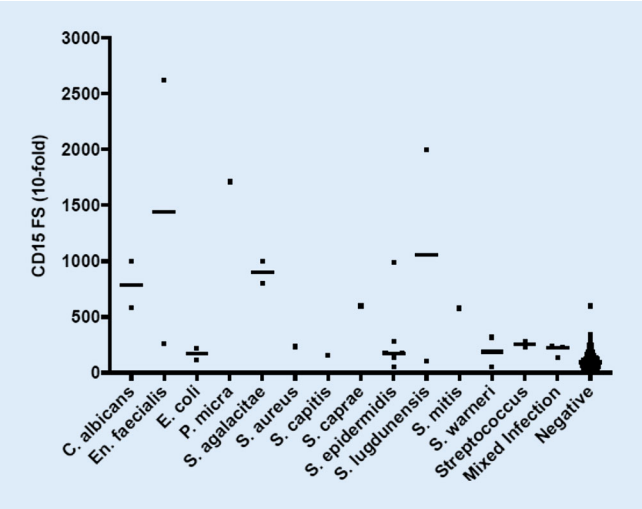

Fig. 5 ◀ Scatter plot for the individual microbiological pathogens with tenfold CD15 focus score quantification (10 foci). The median is shown as the measure of central tendency (*horizontal line*)

tion (Mdn = 300), U = 76, Z = –0.929, $p = 0.37$. Similarly, 1 HPF demonstrated no difference for a high-grade infection (Mdn = 55) and for a low-grade infection (Mdn = 60), U = 72, Z = –1.114, $p = 0.28$. A scatter plot of these results for the individual microbiological pathogens with tenfold CD15 FS quantification (10 foci) demonstrates no significance between CD15 FS quantification and microbiological pathogen species (**Fig. 5**).

Discussion

Two-stage revision arthroplasty

Implantations of joint endoprostheses are one of the most important surgical therapies in the world of medicine for improving quality of life and restoring mobility, and an immense growth in implant procedures is recognized worldwide [10].

PJI pose a serious complication and represent a major medical challenge in various surgical and diagnostic disciplines [3–5, 17, 20, 24–26, 29].

Currently, two-stage revision arthroplasty is one of the most important therapeutic procedures, whereby the success of the treatment is difficult to predict and there is a high persistence of infection [2, 8, 12, 13, 16, 27]. While there is no consensus on the exact therapeutic procedures and diagnostic principles, consensus meetings have laid the foundations for standardized and reproducible infection diagnostics for all diagnostic areas [1, 5].

Limitations

The dataset used is from a selective sample of patients undergoing two-stage revision arthroplasty due to clinical signs and microbiologically confirmed infectious inflammation. This selectivity is caused by the clinical procedure and is therefore unavoidable. A comparison with patients undergoing the same procedure after non-infectious inflammation is impossible to achieve because only the infectious type of the SLIM consensus classification can be administered.

The moderate specificity at 1 focus (specificity = 0.79) as well as the very high specificity at 10 foci (specificity = 0.91) allow a reliable statement with regards to successful bacterial infection eradication in two-stage revision arthroplasty. Especially for the use of CD 15 FS with 10 foci, the value might be of great importance for clinical application. To detect unsuccessful eradication (persistence of the pathogen) in the event of a two-stage revision is not possible due to the high false-negative rate.

Histopathological criteria for the eradication of infections

Alongside clinical microbiological diagnostics, histopathological diagnostics have a central task in the diagnosis of infections [3–5, 24]. The latter can be described as an indirect diagnosis of infection, since the quantification of segmented neutrophil granulocytes by means of various methods provides a diagnostic statement regarding a bacterial infection or the exclusion of an infection.

All the various differential diagnostic infectious and non-infectious typing techniques are embodied [18] in the

SLIM consensus classification, which, as a classification with good resonance, makes a significant contribution to the pathology of joint endoprosthetics. The first version of the classification [22] currently features a totality of "Cited by 131 PubMed Central articles."

On the occasion of an international consensus meeting, the different quantification systems of segmented neutrophil granulocytes for the diagnosis of infections related to two-stage revision arthroplasty were analyzed and evaluated on the basis of a systematic literature review. Thus, a recommendation was made on the basis of consensus, which called for more than five segmented neutrophil granulocytes in at least three HPF [1]. The advantage of this quantification is the simple HE-based morphological analysis. A disadvantage is the fact that neutrophil granulocytes are exclusively morphologically defined by HE and, in particular, that the exact field area is not specified, which can lead to diagnostic ambiguity [28].

CD15 focus score

The CD15 FS provides a valid method for histopathological diagnosis of primary PJI and was established as the gold standard through correlation with microbiological diagnoses [19]. This permits the histopathological diagnosis of an infection or the ruling out of an infection with high sensitivity and specificity and also allows an indicative typing of the bacterial species: this is based on a specific attribute of segmented neutrophil granulocytes, whereby the quantification takes place in a defined field area [19]. In this correlative microbiological research, CD15 FS was applied to the issue of the persistence or the eradication of infection in the case of two-stage revision arthroplasty, and was broadened to permit not only a single quantification but also a tenfold quantification. The latter principle is essentially based on published data from a small collective, also without precise specification of the field area [6]. Since bacterial infections generally exhibit a heterogeneous and multifocal distribution in the tissue, an analysis of a large area of the peri-implant tissue would therefore appear to be useful and necessary. For these reasons, a minimum number of tissue samples from different locations has been proposed for microbiological diagnostics [17, 29], so as to achieve an efficient diagnosis of infections as the diagnostic standard.

For a defined field area, CD15 FS showed a sensitivity for the eradication of infections of 0.64 and a specificity of 0.79 (PPV = 0.5; NPV = 0.87) for a single evaluation (1 focus) with a score value of 42. For tenfold evaluation (10 foci) with a score value of 220, the sensitivity for the eradication of infections was 0.68, the specificity 0.91 (PPV = 0.7; NPV = 0.89). It is interesting to note that a comparatively similar value was obtained in a tenfold evaluation, which was recorded using similar quantification modalities [14]. With regard to specificity, these values are comparable to other quantification systems for neutrophil granulocytes, although much smaller areas were analyzed in these analyses [6] and no precise area definition of the HPF (field area) was made. Nevertheless, a definitive area determination of the field area is necessary for cellular diagnostic quantification and is generally recommended by the WHO in order to achieve a reproducible histopathological diagnosis [11].

Bacterial infections exhibit focal dispersion. Therefore, it is more likely to truly detect the pathogen if more samples of the infected area are taken. The quantity of tissue samples is subject to a minimum requirement for microbiological diagnostics and can therefore also be applied to histopathological diagnostics.

In contrast to primary infection diagnostics [19], there was no statistically significant correlation between the CD15 FS and the diverse bacterial species. This may be due to the fact that the peri-implant tissue presents an inflammatory infiltration upon two-stage revision, which is caused by the mechanical load of the spacer and by PMMA particle depositions, which is not or not only bacterially induced.

PMMA spacer particles

There are no systematic studies regarding PMMA particles in two-stage revision arthroplasty. Based on the analysis of a small collective, it was possible to detect PMMA particles [20]. No percentages were collected and no possible correlation between intrasynovial PMMA particles and their influence on the eradication of infections was investigated. In this analysis, 58% of the patients were found to have PMMA particles in the peri-implant perispacer tissue. Since the PMMA particles could be detected in the superficial fibrous tissue sections and were not integrated into the osseous tissue, these particles are to be evaluated as components of the PMMA spacer and not as possible components of a cemented primary prosthesis. No significant correlation could be established between microbiological pathogen detection and the presence of PMMA particles impregnated with antibiotics in the tissue. This could be interpreted to mean that the eradication of bacterial infections brought about by antibiotics does not result from intrasynovial PMMA particles, but from direct antibiotic diffusion from the spacer or from submicroscopic PMMA particles into the synovial tissue.

Infection eradication rate based on microbiological findings

The rate of infection eradication in 112 cases from 27 different hospitals was 75%. A limiting factor to be noted is that the definition of eradication was based exclusively on the microbiological and clinical findings, and this after a very short time interval following spacer explantation, which can naturally entail a limitation of the significance.

SLIM classification: extension and definition of a SLIM type for two-stage revision arthroplasty (type 9: 9a with infection eradication and 9b with infection persistence)

In all cases, peri-implant synovial tissue (SLIM) with variable fibroblastic cellularity, capillary proliferation, leukocytic infiltration, fibrin deposition, detection

of fractured bone (lamellar bone, woven bone), and variable detection of PMMA particles of the spacer were observed. These data are in close agreement with previously published data [14].

Suggested classification: eradication of infection: type 9A and persistence of infection: type 9B

Based on the consensus classification [18], it is proposed to designate a perispacer synovialis/SLIM type 9 without histopathological signs of a bacterial infection, infection eradication: type 9A (**Figs. 1a and 2**) and with histopathological signs of a bacterial infection, infection persistence: type 9B (**Figs. 1b and 2**). The histopathological findings should also include the value of the granulocyte quantification, in particular also CD15 FS, so as to provide a quantified and thus more objective indication with regard to the degree of probability concerning the histopathological diagnosis of infection. As a matter of course, the microbiological findings are the obligatory and ultimately decisive findings with regard to therapy, since definition of the species and determination of the antibiotic sensitivity determine the rationale for therapeutic medicinal intervention.

This formulaic presentation is intended to facilitate the histopathological interpretation of the findings and make a direct contribution to the diagnosis of infections in the differential diagnostic context. The integration of microbiological, laboratory, and clinical findings constitutes an obligatory component for the final diagnosis of the eradication or the persistence of infection in cases of two-stage revision arthroplasty [1]. Should the available data be unclear or contradictory, a standardized histological finding based on defined criteria can provide additional and crucial assistance. Based on the findings, a standardized and reproducible classification is proposed, which should contribute to the clarity of the histopathological findings by means of a simple type designation, in order to provide a rationale for subsequent therapies.

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Compliance with ethical guidelines

Conflict of interest. C. Liewen, V.T. Krenn, R. Dieckmann, L. Bause, M. Liebisch, A. Niemeier, A. Trampuz, and V. Krenn declare that there is no conflict of interest with regard to the contents of this publication. Regardless of a possible conflict of interest, this scientific contribution is independent and product neutral.

This article does not include any studies performed on humans or animals. The Ethics Committee of the State Medical Association of Rhineland-Palatinate, Mainz, Germany, has issued a positive vote under the reference number 837.400.15 (10168), "*CD 15 Quantifizierung (CD 15 Focus Score) als Basis der periprothetischen Infektionsdiagnostik: Subspezifizierung der bakteriellen Infektion in niedrigpathogene und hochpathogenen Bakterien*" (English: "CD 15 quantification (CD 15 focus score) as the basis of periprosthetic infection diagnosis: sub-specification of bacterial infection in low-pathogenic and highly pathogenic bacteria"), in which it is stated that there are no ethical or legal concerns.

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