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INFECTION

Increased local bone turnover in patients with chronic periprosthetic joint infection

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Aims

The management of periprosthetic joint infection (PJI) remains a major challenge in orthopaedic surgery. In this study, we aimed to characterize the local bone microstructure and metabolism in a clinical cohort of patients with chronic PJI.

Methods

Periprosthetic femoral trabecular bone specimens were obtained from patients suffering from chronic PJI of the hip and knee (n = 20). Microbiological analysis was performed on preoperative joint aspirates and tissue specimens obtained during revision surgery. Microstructural and cellular bone parameters were analyzed in bone specimens by histomorphometry on undecalcified sections complemented by tartrate-resistant acid phosphatase immunohistochemistry. Data were compared with control specimens obtained during primary arthroplasty (n = 20) and aseptic revision (n = 20).

Results

PJI specimens exhibited a higher bone volume, thickened trabeculae, and increased osteoid parameters compared to both control groups, suggesting an accelerated bone turnover with sclerotic microstructure. On the cellular level, osteoblast and osteoclast parameters were markedly increased in the PJI cohort. Furthermore, a positive association between serum (CRP) but not synovial (white blood cell (WBC) count) inflammatory markers and osteoclast indices could be detected. Comparison between different pathogens revealed increased osteoclastic bone resorption parameters without a concomitant increase in osteoblasts in bone specimens from patients with *Staphylococcus aureus* infection, compared to those with detection of *Staphylococcus epidermidis* and *Cutibacterium* spp.

Conclusion

This study provides insights into the local bone metabolism in chronic PJI, demonstrating osteosclerosis with high bone turnover. The fact that *Staphylococcus aureus* was associated with distinctly increased osteoclast indices strongly suggests early surgical treatment to prevent periprosthetic bone alterations.

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Keywords: Periprosthetic joint infection, Osteoclast, Bone remodelling

Article focus

- Histomorphometric assessment of periprosthetic bone specimens in patients with chronic periprosthetic joint infection (PJI).
- Correlation with clinical parameters and comparison with specimens obtained during primary arthroplasty and aseptic revision.

Key messages

- PJI specimens showed markedly increased bone microstructure and turnover parameters compared to both control groups.

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- *Staphylococcus aureus* infections were associated with the highest osteoclastic bone resorption indices.

Strengths and limitations

- This is the first study to demonstrate alterations of local bone metabolism in chronic PJI comparing different pathogens.
- The small sample size in subgroup analysis, and potential influence of other factors on bone turnover outcomes, present limitations to this study.

Introduction

Periprosthetic joint infection (PJI) is a serious complication in orthopaedic surgery, requiring extensive and repeated revision surgery and prolonged systemic antibiotic treatment.[1,2] The incidence of PJI after primary total hip or knee arthroplasty (THA or TKA) ranges from 1% to 2%.[3,4] A clinical hallmark of chronic PJI is a persistent, often low-inflammatory clinical course. Failure to control infection even caused by low-virulent microorganisms is thought to be associated with establishment of surface-adherent, multicellular architectures referred to as biofilms.[5] In fact, the most commonly encountered pathogens, i.e. *Staphylococcus aureus* (*S. aureus*), coagulase-negative staphylococci (CoNS; e.g. *Staphylococcus epidermidis*), *Streptococcus* spp., aerobic Gram-negative bacteria, *Cutibacterium* spp., and *Enterococcus* spp., exhibit a well-documented ability to form biofilms, which has been shown to be of pathogenic relevance in animal models of infection.[6-8] Biofilm formation not only protects bacteria from innate immune effector mechanism,[9-13] but also has a severe impact on bacterial antimicrobial susceptibility.[14] Consequently, PJI commonly demands aggressive surgical treatment procedures.

In fact, chronic PJI generally necessitates a two-stage surgical approach in which prosthesis explantation is followed by targeted antimicrobial treatment and subsequent reimplantation of a new prosthesis.[4,15] This approach is, however, associated with substantial morbidity and mortality, resulting in an enormous economic burden worldwide.[3,4,16,17] Obviously, an in-depth understanding of PJI pathogenesis is required to enable development of optimal prevention and treatment approaches. PJI is often accompanied by implant-associated osteomyelitis, leading to severe skeletal alterations.[18] The acute phase of osteomyelitis is characterized by rapid bacterial replication, infiltration of neutrophils, suppurative inflammation, and necrosis of the bone.[19] If the infection progresses to a chronic phase of osteomyelitis, perturbations of bone remodelling come to the fore. While physiological bone remodelling is characterized by a balanced bone resorption by osteoclasts and new bone formation by osteoblasts,[20] chronic implant-associated osteomyelitis may lead to perturbated bone remodelling with a high rate of bone resorption via activation of osteoclasts, as well as an altered bone formation by osteoblasts.[19] Consequently, chronic osteomyelitis results in a combination of osteolytic bone destruction and sclerotic bone thickening with a high bone fragility and rigidity, as evidenced in mouse models.[19,21,22] Clinically, enhanced bone resorption and osteolysis can lead to prosthesis loosening and periprosthetic fracture.[23,24]

Recent studies have suggested that bacterial species cause distinct alterations in bone remodelling.[25-27] However, knowledge about specific effects of defined pathogens on bone microstructure, bone remodelling, and cellular turnover parameters originates only from in vitro or mouse experiments, and human data are scarce. One study previously examined human specimens from the acetabulum of patients affected by PJI and found increased osteoclast numbers,[28] but did not differentiate into pathogens, did not compare PJI with aseptic revision cases, and did not correlate the results with clinical data. Therefore, the aim of this clinical biopsy study was to investigate bone microstructural and cellular bone parameters of peri-implant bone specimens obtained from patients with chronic PJI compared with control specimens, obtained during primary implantation as well as aseptic revision surgery. At the same time, we also intended to compare the local bone homeostasis between PJI subtypes with detection of different bacteria.

Methods

Study design, patient cohorts, and specimen collection. A total of 60 patients undergoing revision surgery or primary implantation of knee or hip arthroplasty were included. For the PJI cohort (n = 20), the main inclusion criterion was septic revision after THA or TKA with explantation of the prosthesis due to chronic PJI. The surgical therapeutic approach consisted of a two-stage procedure with explantation of the prosthesis and debridement of infected tissue, followed by a pathogen-directed systemic antibiotic therapy and re-implantation of the prosthesis after six weeks. Representative trabecular bone specimens were obtained at the implant-bone interface in the metaphyseal femur during prosthesis explantation and surgical debridement using a standardized approach. Patients undergoing primary THA or TKA due to osteoarthritis (n = 20), and patients undergoing aseptic revision surgery due to aseptic prosthesis loosening, recurrent dislocations, implant malposition, or arthrofibrosis (n = 20), were included as two control groups. In these groups, trabecular bone specimens were collected in the same standardized manner, i.e. from the endosteal femoral bone surface during primary implantation or from the implant-bone interface during aseptic revision. Height, weight, BMI, prosthesis survival (time between primary implantation and revision surgery), and use of cement during primary implantation were extracted from the patients' medical records. To determine the areal bone mineral density (aBMD, g/cm^2) and corresponding T-scores, dual energy X-ray absorptiometry (DXA) was performed at the proximal femur and lumbar spine (L1 to L4) using a Lunar Prodigy enCore 2007 system (GE Healthcare, USA). Measurements were performed at the corresponding skeletal sites only in the absence of implants or

degenerative disease. The lowest T-score of the measurement sites was used for analysis. Patients with conditions potentially influencing bone health, e.g. previous fragility fractures, cancer or myeloproliferative diseases, rheumatic, renal, liver, or gastrointestinal diseases, other metabolic or endocrine diseases, glucocorticoid treatment, or prolonged immobilization > three months, were excluded. All procedures performed in this study were approved by the local ethics committee (Hamburg Medical Chamber; 2022-300213-WF) and complied with the ethical standards of the Declaration of Helsinki.[29]

Diagnosis of PJI. The presence of a PJI was determined according to the International Consensus Meeting (ICM) 2018 criteria.[30] Serum CRP and synovial white blood cell (WBC) count, measured preoperatively, were collected from medical records in patients who underwent septic or aseptic revision. Microbiological analysis and pathogen differentiation were performed on preoperative joint aspirates and five tissue samples obtained during revision surgery, as described previously.[31] Two positive cultures with identical pathogen were considered clinically relevant. Chronic PJI was defined as an infection ≥ four weeks after primary implantation or duration of clinical symptoms ≥ three weeks.[32]

Sample preparation, histology, and histomorphometry. After fixation in 4% formaldehyde for at least three days, dehydration, and embedding in methyl methacrylate, bone specimens were cut into 4 µm thick sections using a Microtec rotation microtome (TechnoMed, Germany) before being stained with toluidine blue and von Kossa. Bone microstructural and cellular parameters were evaluated on stained undecalcified sections under a light microscope and using the software OsteoMeasure (OsteoMetrics, USA). Analysis of the following parameters was carried out according to the American Society for Bone and Mineral Research (ASBMR) guidelines: bone volume per tissue volume (BV/TV, %), trabecular number (Tb.N, mm^{-1}), trabecular thickness (Tb.Th, µm), trabecular separation (Tb.Sp, µm), osteoid volume per bone volume (OV/BV, %), osteoid surface per bone surface (OS/BS, %), osteoid thickness (O.Th, µm), number of osteoblasts per bone perimeter (N.Ob/B.Pm, mm^{-1}), number of osteoclasts per bone perimeter (N.Oc/B.Pm, mm^{-1}), osteoblast surface per bone surface (Ob.S/BS, %), and osteoclast surface per bone surface (Oc.S/BS, %).[33] Microstructural and osteoid parameters were analyzed using the von Kossa staining with a magnification of 4× and six coherent fields of view. For analysis of cellular parameters (N.Ob/B.Pm, N.Oc/B.Pm, Ob.S/BS, Oc.S/BS), toluidine blue staining with a magnification of 20× and ten coherent fields of view were used.

TRAP immunohistochemistry. For immunohistochemistry, a representative part of each biopsy was decalcified in ethylenediaminetetraacetic acid (EDTA), dehydrated, and embedded in paraffin. Tartrate-resistant acid phosphatase (TRAP) activity staining was performed on 4 µm sections using naphthol AS-MX phosphate and Fast Red Violet LB salt (both MilliporeSigma, USA) in 40 mM acetate buffer (pH 5) as described previously.[34] Osteoclast indices were verified in these sections.

Statistical analysis. Statistical analysis and data visualization were performed using GraphPad Prism 9 (GraphPad Software, USA). Normal distribution of the data was tested using the Shapiro-Wilk test. For comparison of two groups, an independent-samples *t*-test and Mann-Whitney U test were used for parametric and non-parametric data, respectively. If more than two groups were compared, one-way analysis of variance (ANOVA) with Tukey's multiple comparison test was carried out for parametric and the Kruskal-Wallis test with Dunn's multiple comparison test for non-parametric data. Comparison of categorical data was performed using the chi-squared test. For the analysis of potential associations between clinical data and bone parameters in the PJI cohort, linear regression analysis was performed and the coefficient of determination R^2 was calculated. Data are presented as mean (standard deviation (SD)) unless otherwise stated. Exact p-values are reported unless $p < 0.001$, and p-values ≤ 0.05 were considered statistically significant.

Results

High bone turnover osteosclerosis in patients with PJI. The three study groups were similar in age, sex, height, weight, BMI, and ratio of hip versus knee surgery (Table I). Furthermore, the aseptic and PJI cohorts were not different regarding the use of cement during primary implantation as well as prosthesis survival. Assessment of bone mineral density by DXA showed mean T-scores in the range of osteopenia for the control and aseptic cohort, and in the normal range for the PJI cohort. As expected, the PJI cohort was characterized by higher serum CRP levels and synovial WBC counts than the aseptic cohort. Of the 20 PJI patients, *S. aureus* could be detected in six patients (30%), *S. epidermidis* in six patients (30%), *Cutibacterium* spp. (i.e. *Cutibacterium acnes* or *Cutibacterium avidum*) in three patients (15%), *Streptococcus dysgalactiae* in one patient (5%), and *Enterococcus faecalis* in one patient (5%) (Table II). In two patients no causative pathogen was identified, and diagnosis of culture-negative PJI was based on presence of minor criteria. One patient had a polymicrobial infection with *S. epidermidis*, *Escherichia coli*, *Proteus mirabilis*, and *Candida albicans*.

In the histological sections stained with von Kossa, the periprosthetic bone tissue of the PJI cohort showed a combination of osteosclerosis and osteoid accumulation (Figure 1a). Histomorphometry revealed a higher bone volume and trabecular thickness, as well as lower trabecular separation but equal trabecular number, in the PJI group compared to the control group (Figure 1b). Trabecular thickness of the PJI group was also higher than that of the aseptic group. Osteoid indices were higher in the PJI group compared to the control and aseptic groups (Figure 1c). Comparison of cellular bone turnover parameters in the three groups revealed higher osteoblast and osteoclast indices in the PJI group compared to the control and aseptic groups (Figures 2a and 2b).

Table I. Overview of clinical, densitometric, and laboratory data in the study cohort.

Parameter	Control (n = 20)	Aseptic (n = 20)	PJI (n = 20)	p-value
Sex, n (%)				
Male	10 (50)	7 (35)	7 (35)	0.535*
Female	10 (50)	13 (65)	13 (65)	
Mean age, yrs (SD)	70.3 (9.3)	74.6 (9.2)	69.9 (12.5)	0.299†
Mean height, cm (SD)	172.4 (10.3)	170.1 (11.0)	170.8 (8.6)	0.768†
Mean weight, kg (SD)	88.1 (17.4)	85.5 (24.0)	98.4 (26.5)	0.137‡
Mean BMI, kg/m^2 (SD)	29.7 (5.3)	29.3 (6.0)	34.1 (10.5)	0.232‡
Location, n (%)				
Hip	10 (50)	6 (30)	11 (55)	0.243*
Knee	10 (50)	14 (70)	9 (45)	
Cemented fixation, n (%)	N/A	17 (85)	13 (65)	0.144*
Mean prosthesis survival, mths (SD)	N/A	95.2 (107.9)	54.9 (54.2)	0.465§
Mean DXA T-score$_{lowest}$ (SD)	-1.7 (1.2)	-1.4 (1.4)	-0.5 (1.8)	0.147†
Mean serum CRP, mg/l (SD)	N/A	5.7 (13.5)	56.2 (64.1)	< 0.001§
Mean synovial WBC count, µl^{-1} (SD)	N/A	1,961 (1,841)	27,385 (24,240)	< 0.001§

*Chi-squared test.
†One-way analysis of variance (ANOVA) with Tukey's multiple comparison test.
‡Kruskal–Wallis test with Dunn's multiple comparison test.
§Mann-Whitney U test.
DXA, dual energy X-ray absorptiometry; N/A, not applicable; PJI, periprosthetic joint infection; SD, standard deviation; WBC, white blood cell.

Table II. Distribution of detected bacteria in the periprosthetic joint infection group.

Bacteria	n (%)
Staphylococcus aureus	6 (30)
Staphylococcus epidermidis	6 (30)
Cutibacterium spp.	3 (15)
Streptococcus dysgalactiae	1 (5)
Enterococcus faecalis	1 (5)
Polymicrobial/culture-negative	3 (15)

There was no difference between the control group and the aseptic group for the cellular bone parameters. Closer examination of the histological toluidine blue and TRAP-stained sections confirmed high abundance of osteoclasts and resorption activity, respectively, especially in *S. aureus* and *Enterococcus faecalis* infections (Figure 2c).

Associations between clinical and biopsy parameters in the PJI cohort. We detected a weak negative association between prosthesis survival and periprosthetic bone volume fraction (Figure 3). However, prosthesis survival was not associated with osteoid or cellular bone parameters. Assessment of possible associations between serum CRP or synovial WBC count and bone parameters revealed a positive association between serum CRP and the number of osteoclasts, but not number of osteoblasts or structural parameters (Figure 4a). Synovial WBC count was not associated with structural, osteoid, or cellular bone properties (Figure 4b).

Comparison of microstructural and cellular bone parameters between different pathogens in PJI. Microstructural and cellular bone parameters of the PJI group were compared between *S. aureus*, *S. epidermidis*, and *Cutibacterium* spp. Local microstructure, osteoid parameters, and osteoblast indices did not differ between the three pathogens (Figures 5a and 5b). Importantly however, PJI patients with *S. aureus* specifically showed higher osteoclast indices compared to *S. epidermidis* and *Cutibacterium* spp. (Figure 5c). An additional presentation of microstructural and cellular bone parameters of infrequently detected pathogens is provided in Supplementary Figure a. In this analysis (although being limited to one specimen), it was noticeable that *Enterococcus faecalis* and polymicrobial infections were associated with an increase in osteoclasts that was similar to the increase seen in the *S. aureus* group.

Discussion

Although it is known that osteomyelitis in PJI considerably affects local bone metabolism,[3] clinical investigations have not yet systematically examined periprosthetic bone properties in affected patients. In the present study, we have demonstrated that the bone tissue in PJI is affected by an unfavourable combination of osteosclerosis and increased bone turnover. Since *S. aureus* infections were associated with the strongest increase in osteoclasts without concomitant increase in osteoblasts, treatment concepts in these infections may need to focus on addressing periprosthetic bone loss. Based on our results, it should be investigated whether drugs that inhibit bone resorption could be used supportively in chronic PJI.

The observed increase in osteoid formation accompanied by increased osteoblast and osteoclast indices suggests an accelerated bone metabolism in chronic PJI. An elevated number of osteoclasts has also been previously detected in acetabular biopsies from PJI patients.[28] These collective findings provide an explanation for the clinical phenomenon of osteolytic bone destruction in PJI

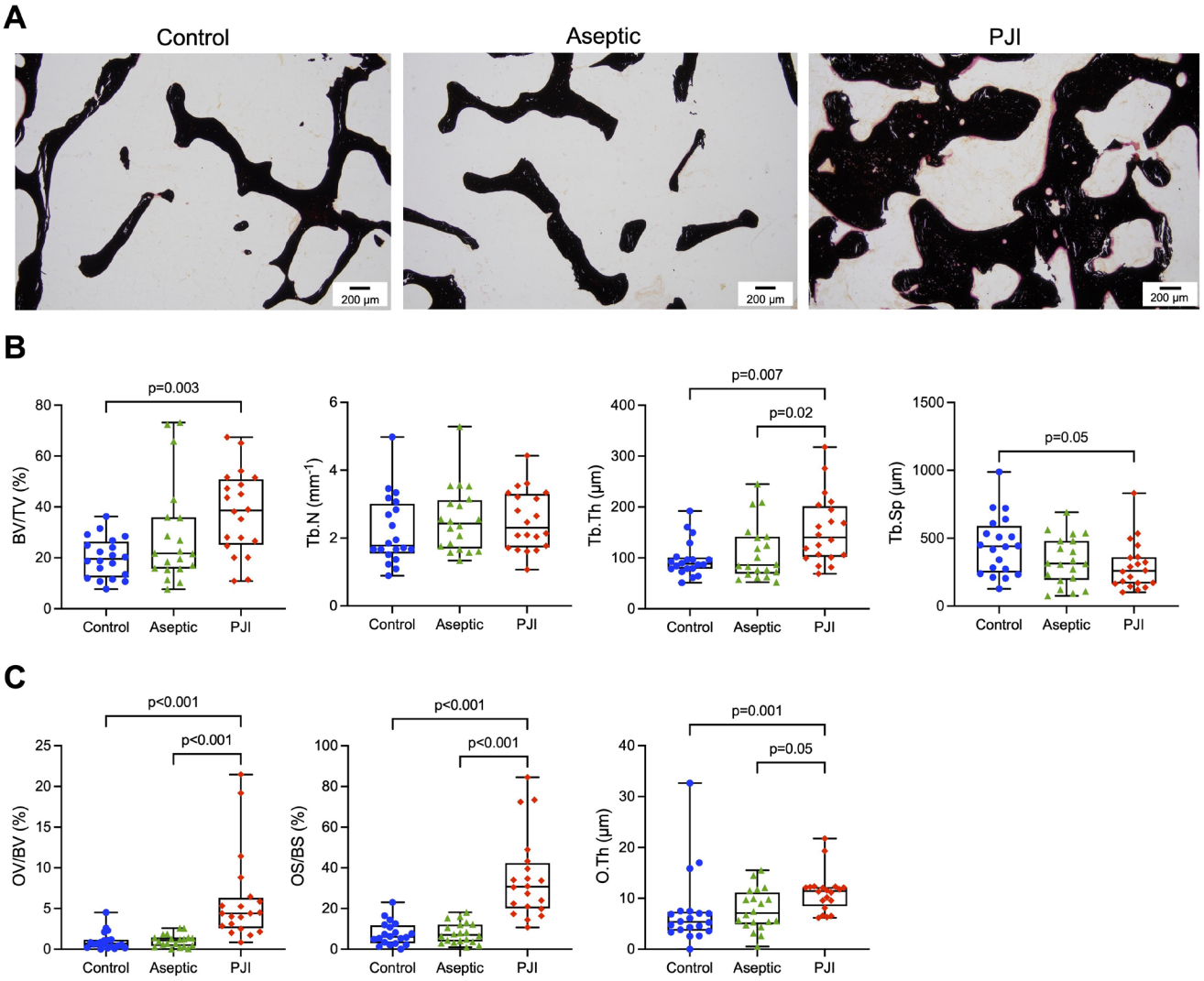

Fig. 1

Periprosthetic bone microstructure in periprosthetic joint infection (PJI) compared to controls and aseptic revisions. a) Representative histological images (von Kossa staining) from the control, aseptic, and PJI groups. b) Quantification of bone volume per tissue volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp). c) Quantification of osteoid volume per bone volume (OV/BV), osteoid surface per bone surface (OS/BS), and osteoid thickness (O.Th). Microstructural bone parameters and osteoid parameters were compared between the control, aseptic, and PJI groups. Boxes represent 25% and 75% percentiles, lines represent median, whiskers represent range, and coloured symbols represent individual values. One-way analysis of variance with Tukey's multiple comparison test was used for normal distributed data and Kruskal–Wallis test with Dunn's multiple comparison test was used for non-parametric data. Exact p-values are reported unless $p < 0.001$.

associated with implant loosening.[23,24] We also detected a local increase in bone microstructure (i.e. osteosclerosis). In line with this result, previous mouse studies have also demonstrated not only increased osteoclast indices but an overall accelerated bone turnover with simultaneous osteolytic and sclerotic processes.[19,21] In our clinical experience, the combination of osteolysis and osteosclerosis in PJI presents a surgical challenge, as fixation of the new prosthesis after healed infection can be problematic. While osteolysis requires advanced fixation systems involving longer stems and cemented techniques, osteosclerosis, for example, can make cement penetration difficult, which is supported by the previously reported high rates of aseptic loosening after revision total knee arthroplasty for PJI.[35]

Our results showed a positive association between serum, but not synovial, inflammatory markers and osteoclast indices in the PJI cohort. This result suggests that a systemic rather than local inflammatory response may be crucial for osteoclast activation. Prompted by these results, we compared microstructural and cellular bone parameters of different pathogens in chronic PJI. We found that specimens from patients with *S. aureus* PJI exhibited higher osteoclast indices compared to patients with *S. epidermidis* and *Cutibacterium* spp. infections. Although analysis of the other specimens from patients

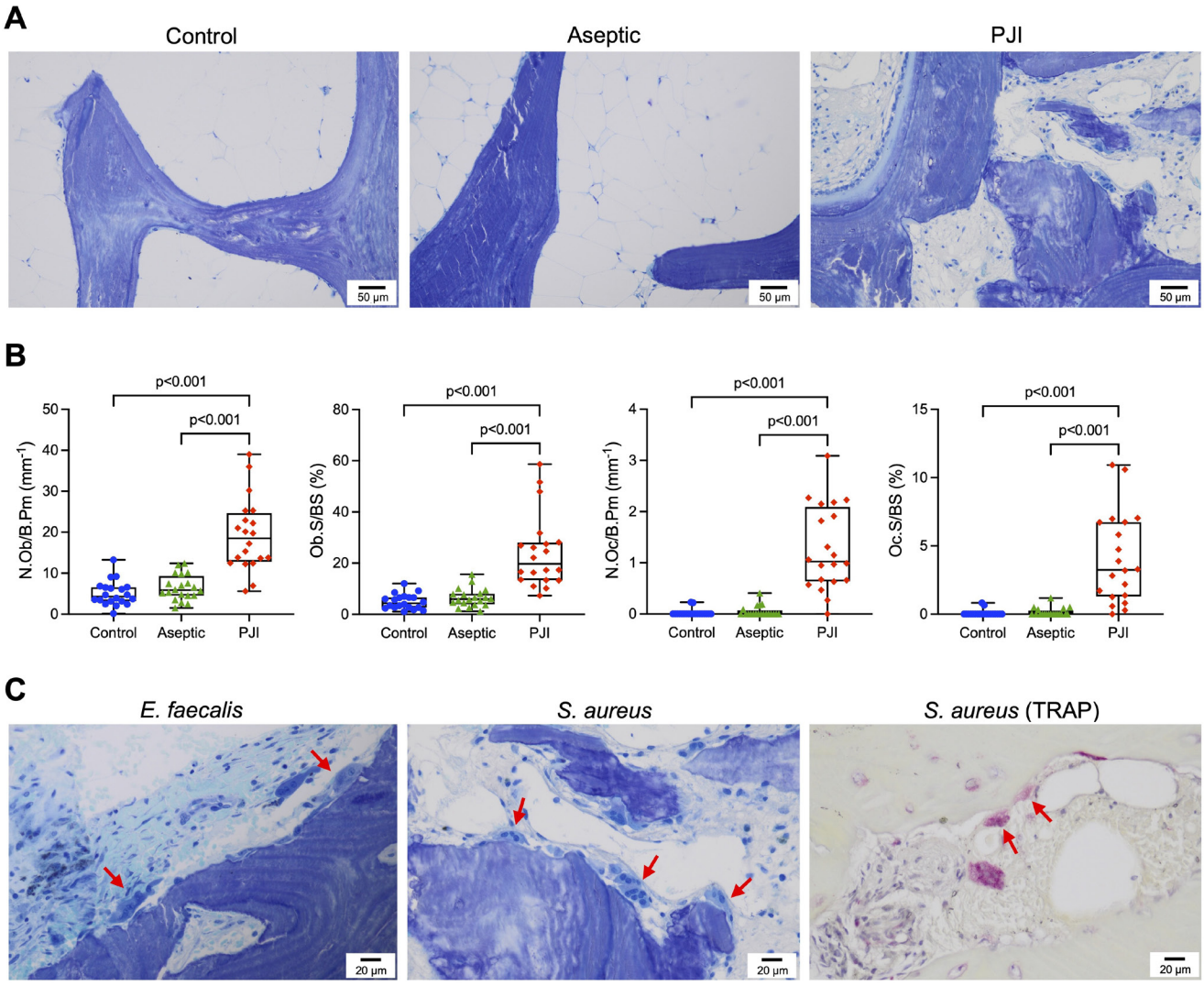

Fig. 2

Cellular bone turnover parameters of periprosthetic joint infection (PJI) compared to controls and aseptic revisions. a) Representative histological images (toluidine blue staining) from the control, aseptic, and PJI groups. b) Quantification and comparison of cellular bone turnover parameters (number of osteoblasts per bone perimeter (N.Ob/B.Pm), osteoblast surface per bone surface (Ob.S/BS), number of osteoclasts per bone perimeter (N.Oc/B.Pm), osteoclast surface per bone surface (Oc.S/BS)) between the control, aseptic, and PJI groups. Boxes represent 25% and 75% percentiles, lines represent median, whiskers represent range, and coloured symbols represent individual values. c) Detailed histological images of toluidine blue staining and tartrate-resistant acid phosphatase (TRAP) staining from PJI patients. Red arrows indicate osteoclasts. One-way analysis of variance with Tukey's multiple comparison test was used for normal distributed data and Kruskal–Wallis test with Dunn's multiple comparison test was used for non-parametric data. Exact p-values are reported unless $p < 0.001$.

with detection of other pathogens was limited to one specimen each, *Enterococcus faecalis* and polymicrobial infections also showed a marked increase in osteoclast indices. Together, it is possible that individual pathogens have differential effects on osteoclast activation, which should be further investigated in future studies focusing on pathogen-specific effects.

Previous studies have identified mechanisms by which *S. aureus* promotes the recruitment and activation of osteoclasts in the context of osteomyelitis. For instance, cell surface associated staphylococcal protein A was found to increase secretion of receptor activator of nuclear factor kappa-B ligand (RANKL) in osteoblasts, thereby promoting osteoclastogenesis.[36,37] *S. aureus* biofilm formation per se was demonstrated to result in osteoclastogenesis and osteolysis by increasing RANKL secretion in osteoblasts.[38] Additionally, *S. aureus* derived lipoproteins promote osteoclastogenesis via secretion of the proinflammatory proteins tumour necrosis factor alpha (TNFα), interleukin-6 (IL-6), and RANKL by osteoblasts.[39–41] The presence of *S. aureus* in osteocytes has been demonstrated in mouse models and clinical specimens from single PJI patients.[42,43] It has also been shown that *S. aureus* is able to directly infect osteoclasts and replicates intracellularly,[44] promoting fusion and bone resorbing activity.[45] In this way, *S. aureus* could escape

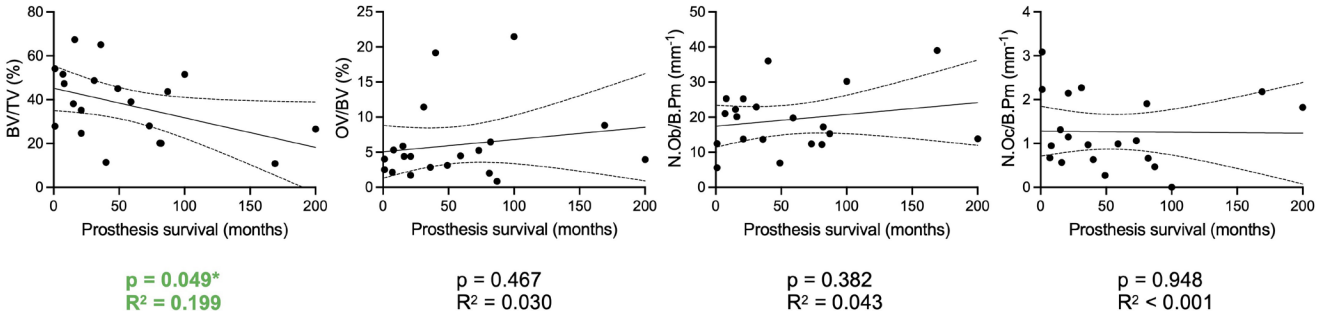

Fig. 3

Associations between prosthesis survival and histological, periprosthetic bone tissue parameters in the periprosthetic joint infection group. Linear regression analysis between prosthesis survival in months and skeletal parameters (bone volume per tissue volume (BV/TV), osteoid volume per bone volume (OV/BV), number of osteoblasts per bone perimeter (N.Ob/B.Pm), number of osteoclasts per bone perimeter (N.Oc/B.Pm)) (n = 20). Exact p-values and R^2 values are displayed.

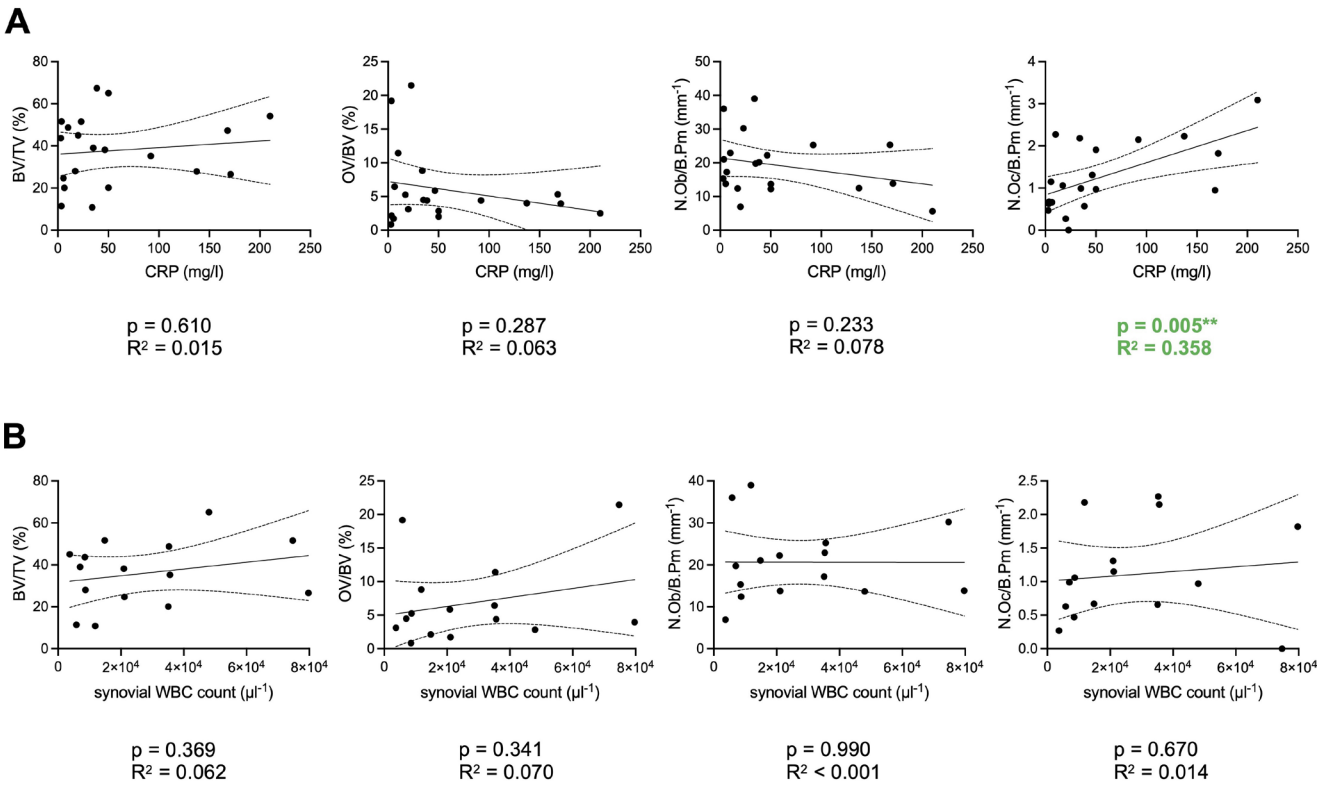

Fig. 4

Associations between laboratory inflammation markers and histological bone parameters in the periprosthetic joint infection group. a) Linear regression analysis between serum CRP and bone parameters (bone volume per tissue volume (BV/TV), osteoid volume per bone volume (OV/BV), number of osteoblasts per bone perimeter (N.Ob/B.Pm), number of osteoclasts per bone perimeter (N.Oc/B.Pm)) (n = 20). b) Linear regression analysis between synovial white blood cell (WBC) count and the same bone parameters (n = 15). Exact p-values (unless < 0.001) and R^2 values are depicted. **p < 0.05.

the immune system, leading to the chronicity of infection. In contrast, only scarce data exist about the interaction of *S. epidermidis* and *Cutibacterium* spp. with bone cells. These species are members of the physiological human skin microbiome, but can also frequently be found in skeletal and implant-associated infections. They have a relatively low virulence with an indolent course of infection, but are both able to form biofilms on the implant surface.[15,18] In contrast to *S. aureus*, *S. epidermidis* and *Cutibacterium* spp. are opportunistic microorganisms less well equipped with virulence factors.[3,18] Consequently, CoNS and *Cutibacterium* spp. have a milder inflammatory response in PJI, and thus the secretion of proinflammatory and osteoclast-stimulating factors may be lower.[15,18,26]

Treatment of PJI is still a major challenge in orthopaedic surgery. In particular, the high rate of bacteria persistence and PJI recurrence limits the success of PJI therapies.[4,15] *S.*

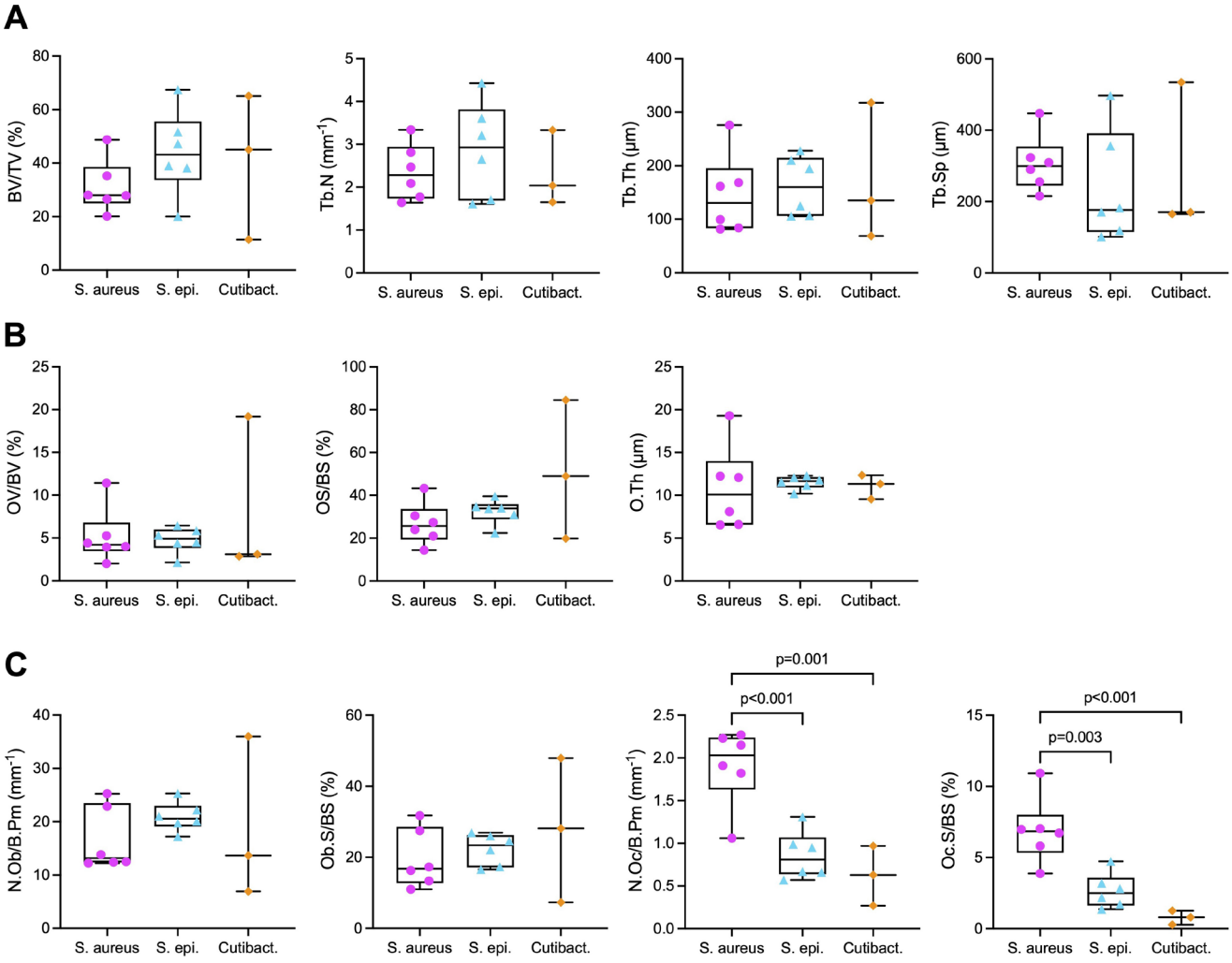

Fig. 5

Comparison of bone metabolism parameters between patients with different periprosthetic joint infection (PJI) pathogens. Quantification and comparison of a) microstructural, b) osteoid, and c) cellular bone parameters between PJI specimens obtained from patients with *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Cutibacterium* spp. infections. Boxes represent 25% and 75% percentiles, lines represent median, whiskers represent range, and coloured symbols represent individual values. One-way analysis of variance with Tukey's multiple comparison test was used for normal distributed data and Kruskal–Wallis test with Dunn's multiple comparison test was used for non-parametric data. Exact p-values are reported unless $p < 0.001$. BV/TV, bone volume per tissue volume; N.Ob/B.Pm, number of osteoblasts per bone perimeter; N.Oc/B.Pm, number of osteoclasts per bone perimeter; Ob.S/BS, osteoblast surface per bone surface; Oc.S/BS, osteoclast surface per bone surface; OS/BS, osteoid surface per bone surface; O.Th, osteoid thickness; OV/BV, osteoid volume per bone volume; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness.

aureus infection has been shown to be associated with failure of surgical implant-retaining debridement and antibiotics procedure.[3,15] The higher recruitment of osteoclasts in chronic PJI with *S. aureus* demonstrated here, and the well-known ability of *S. aureus* to persist intracellularly in osteoblasts and osteoclasts, may contribute to the higher failure rate in *S. aureus* PJI.[3] The cure rate of a two-staged surgical procedure ranges from 60% to 90% in chronic PJI, with especially worse outcomes in *S. aureus* infections,[18] underlining the need for further improvements in the therapeutic approach. A previous study tested the effect of an anti-RANKL antibody in a mouse model of *S. aureus* osteomyelitis, and found a reduction of infectious osteolysis without an increase of necrotic bone areas.[46] Thus, anti-RANKL antibodies could exert an osteoprotective effect on vital bone without affecting the removal of necrotic bone.

Some limitations of this study must be noted. Aseptic loosening due to wear particles usually causes periprosthetic inflammatory osteolysis by several mechanisms, such as activation of osteoclasts or suppression of osteoblastic bone formation.[40,47] The aseptic control group used in this study consisted of several other aseptic complications requiring revision surgery, such as recurrent dislocations, implant malposition, or arthrofibrosis. Thus, this group does not serve as an aseptic loosening control with wear particle induced inflammatory osteolysis as in other studies.[26] Although this is the first clinical study assessing microstructural and cellular bone parameters in chronic PJI

comparing different pathogens, one limitation of our study is the small number of included specimens, which prevented further conclusions about pathogen-specific effects, especially for subgroup analysis of infrequently detected pathogens. Another limitation of our study is that the molecular mechanisms of activation of bone metabolism by specific pathogens could not be addressed. Thus, further studies are necessary to confirm pathogen-specific effects on bone turnover in PJI and to investigate underlying mechanisms.

In conclusion, we have demonstrated that the periprosthetic bone matrix in patients with chronic PJI is defined by an unfavourable combination of osteosclerosis and high bone turnover. While *S. aureus* infection was associated with the strongest increase in osteoclast indices, it is now important to find mechanistic explanations for how different pathogens directly (e.g. by infecting bone cells) or indirectly (e.g. by triggering an inflammatory response) affect bone metabolism. Progress in understanding the pathogen-specific disruption of bone turnover in PJI appears essential to develop novel therapeutic approaches.

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Supplementary material

Figure showing histomorphometric quantification of bone metabolism parameters in infrequently detected pathogens.

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