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Original Contributions

# Histopathologic findings in culture-positive secondary osteomyelitis

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ABSTRACT

As peripheral vascular disease and diabetes mellitus are increasingly common, chronic wounds are often seen. Bone biopsies, with imaging and microbial cultures, are often obtained to evaluate for osteomyelitis. Because much of the historical literature describing the histology of osteomyelitis pertains to primary osteomyelitis, this study characterizes the histologic findings and provides correlation with culture results in secondary osteomyelitis.

The histologic features of bone biopsies were assessed over a 5 year period. Concurrent laboratory and radiographic data were obtained and these data were compared with culture results.

This study included 163 cases, of which 104 were culture-positive osteomyelitis. All culture-positive cases had been present longer than 28 days and had at least one of the following histologic features: neutrophilic inflammation, plasmacytic inflammation, or eosinophilic fibrosis. However, none of these findings were restricted to culture-positive cases. Overall, plasmacytic and neutrophilic inflammation provided similar specificity, and positive predictive values for osteomyelitis. Medullary fibrosis gave a sensitivity of 95%, the highest for any single feature, and the combination of fibrosis and neutrophilic inflammation had the greatest specificity of 96%. Additionally, neutrophilic inflammation correlated often with isolation of *Staphylococcus aureus*, while plasma cell predominance was found more frequently with other infectious agents.

This study describes histologic features in secondary osteomyelitis, which may challenge the widespread inclination to equate a neutrophilic inflammation with 'acute osteomyelitis' and 'chronic osteomyelitis' with one rich in plasma cells. We report an early correlation between common histopathologic findings and specific culture isolates, which can be further refined with additional research.

## 1. Introduction

As longevity increases so does the prevalence of chronic disease, including peripheral vascular disease and diabetes mellitus [1,2]. One consequence of this is an increase in the frequency of complicated wounds, particularly on extremities and at pressure points. Pathologists have opportunities to assist in the evaluation of bone biopsy samples for osteomyelitis (OM). In this context it is necessary to parse a variety of histopathologic changes, some of which are seen in non-infectious processes, and others that may be specific for osteomyelitis.

A distinction is made between primary OM, in which bacteria enter the bone hematogenously, and secondary OM in which bacteria gain entry via direct invasion [3,4]. In decades past, primary OM was the dominant form of the disease, whereas secondary OM was an uncommon sequel to trauma. OM is further classified according to time course. Acute OM refers to those cases presenting within 2 weeks of onset, and chronic OM is variably defined but generally indicating an illness of over 6 weeks' duration, and a subacute phase in the interim [3-5]. Lastly, some authors delineate an 'inoculation' type of secondary OM, in which pathogens are introduced by puncture or penetrating injury, in contrast to the more common scenario in which secondary OM results from adjacent soft tissue infection [6].

Bone biopsy is considered the "gold standard" for the diagnosis of OM, and while there is an unsettled debate in the clinical literature about its role, it is nevertheless undertaken in specific circumstances [3-6]. First, in cases where there is conflicting clinical data, the biopsy is performed to establish the diagnosis of OM, while simultaneously obtaining material for microbiologic studies. Second, biopsy may be performed if there is suspicion of a neoplastic condition. Third, when the clinical diagnosis of OM is considered certain, biopsy may be performed purely for the sake of microbiologic studies the results of which will inform antibiotic selection. Lastly, bone biopsy is often performed at the time of digital amputation to evaluate the bone 'margin'; significant therapeutic choices are based upon the margin bone biopsy, including

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the selection, route, and duration of antibiotic therapy, the timing of wound closure, and the need for surgical and/or hyperbaric intervention [3,4,6-9].

Much of what we know about the histopathology of OM derives from observations, in autopsy or amputation specimens, made during the pre-antibiotic heyday of primary osteomyelitis [10-14]. The process described under these circumstances is rooted in the medullary space, with inconsistent secondary involvement of the cortex. It is characterized initially by suppuration and, in patients who survive without surgical intervention, eventually organizes itself into a distinct focus of repair (Brodie's abscess). Later on, when antibiotics rendered the disease no longer surgical or lethal, and thereby less frequently the subject of anatomic pathologists, relevant entries in standard textbooks remained substantially unchanged as secondary OM became the dominant form of the disease [11,15-17].

Pathologists have adapted what they know of primary OM to the evaluation of secondary osteomyelitis, in some cases asserting that the two pathologic processes are the same [11]. However, the two conditions differ in important ways. First, the pathogenesis of primary OM as it is currently understood [18,19] posits a key role for raised intramedullary pressure in the face of an intact cortex, a mechanism that seems unlikely in secondary OM in which a breached cortex is thought to be an early event. Primary OM is usually mono-microbial and, though children with certain underlying conditions are predisposed to it, is found within relatively healthy young hosts [12,13,20-24]. The typical patient with secondary OM has comorbidities that often include vascular insufficiency. The evaluation of primary OM, both clinically and pathologically, is free of the confounding effects of a surrounding soft tissue infection; whereas, when secondary OM is considered, the bone in question is exposed to the same microenvironment, mechanical trauma, pressure, and manipulation bearing upon the adjacent soft tissue. The osseous histologic effects of these factors can only be guessed. We know, for example, that intramedullary inflammation closely resembling OM is frequent in such non-infectious settings as degenerative joint disease and osteonecrosis (Fig. 1) [25].

In accordance with available texts, pathologists have come to equate acute osteomyelitis with a neutrophil-rich inflammatory infiltrate and chronic osteomyelitis with mononuclear inflammation [18-24,26-28]. This paradigm does not appear to be derived from primary OM, wherein descriptions stress neutrophils in early stages and a localized sequestrum-involucrum organizational late stage. Additionally, various authors have proposed 'plasma cell osteomyelitis' as an independent entity, distinct from evolving acute OM, sclerosing osteomyelitis of Garre, or Brodie's abscess [29,30]. Evidence concerning the application of these histologic principles in the diagnosis of secondary OM is limited and discouraging [24,26-28,31]. In many studies, the primary limitation was a lack of standardized criteria for the histopathologic diagnosis of OM [26,28]. There are only a few studies in which histopathologic criteria were rigorously defined [26,28]. In view of the weight given pathologic interpretations in this setting, we undertook a preliminary study to assess the diagnostic performance of set of objective histopathologic criteria.

## 2. Methods

The appropriate institution review board evaluated the proposed study and deemed it exempt from full board review. Consecutive bone biopsy specimens for a 5-year period (2011 through 2015) were identified in the anatomic pathology information system using keyword search. Only core biopsies were included as they were the most common specimen and were predominantly procured by interventional radiology. Curettage specimens were essentially non-existent at this institution. Cases were excluded if no bone tissue was obtained or if concurrent cultures were not performed; the latter situation was commonly encountered with the majority of resection specimens. The remaining cases were de-identified and reviewed; multiple specimens

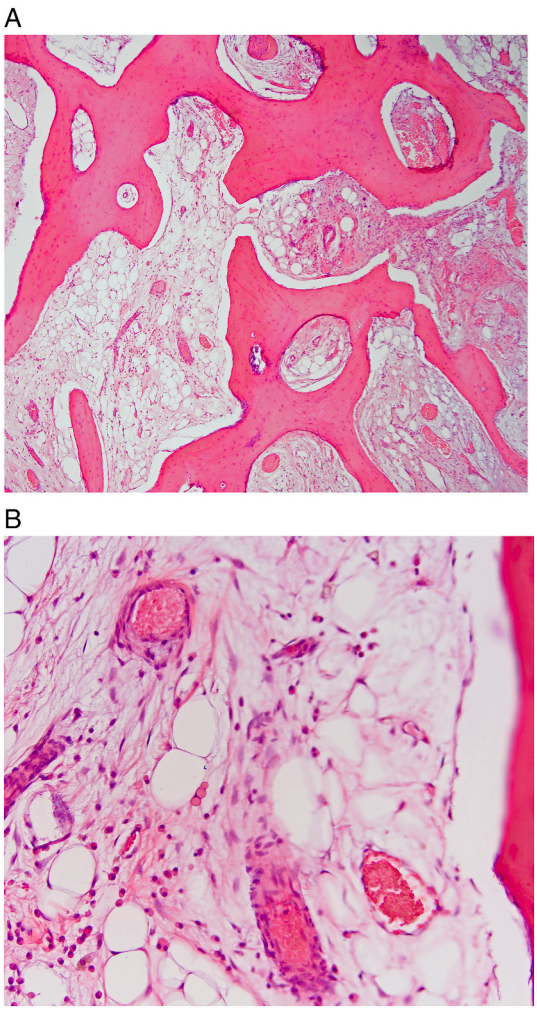


**Fig. 1.** Figure 1a and 1b: Osteomyelitis-like changes in degenerative joint disease include osteonecrosis (Figure 1a, H&E, 40x) and intramedullary inflammation (Figure 1b, H&E, 400x).

from different sites in the same patient were allowed. Chart review was conducted to determine a number of factors, including history, pertinent laboratory and radiographic studies, and demographic data. In each instance, the length of medullary bone present in the sample was recorded. Each biopsy had its histopathology assessed by a senior surgical pathologist (D.M.) for a number of features which included neutrophil infiltration, neutrophil aggregates, plasma cell infiltration, plasma cell aggregates, fibrosis, necrosis (sequestrum), and reparative change (including involucrum).

Neutrophil infiltration was defined as the presence of over 10 neutrophils in a 400× field in at least 1 field. A neutrophil aggregate was defined as a cluster of at least 5 touching neutrophils. Similarly, plasma cell infiltration was defined as over 10 plasma cells per 400× field in at least 1 field, and a plasma cell aggregate consisted of at least 5 touching plasma cells. Fibrosis was defined as the presence of eosinophilic fibrillary collagenous tissue, not resembling fibrin, sufficient in quantity to alter the architecture of the normally fatty marrow and filling at least one inter-trabecular space. Reparative changes were assessed qualitatively by noting the presence of involucrum, woven bone deposition, and/or osteoblastic rimming. All parameters were assessed at least 1 intertrabecular space away from the corticomedullary interface. For each parameter, a value of 1 was recorded if the histopathologic feature was present and 0 if it was not.

Some cases demonstrated a combination of findings, and these were categorized as 'fibro-granulocytic' (if demonstrating fibrosis with either

neutrophil infiltration or neutrophil aggregates), fibro-plasmacytic (if demonstrating fibrosis with either plasma cell infiltration or plasma cell aggregates), and mixed (having a value of 1 in both plasmacytic and neutrophilic categories). Lastly, it became apparent that certain cases with neutrophilic activity demonstrated foci in which neutrophils formed a 'micro-abscess' which we defined as an uninterrupted sheet of neutrophils filling at least one intertrabecular space. No analogous finding was discovered for plasmacytic inflammation.

Culture results were recorded. In our institution bone biopsies are routinely submitted for both culture and histopathology. In most instances, the biopsy is submitted directly to the microbiology section, from where, after material is garnered for culture, it is forwarded to histopathology. In some instances two biopsies are obtained, one sent directly to histology and another sent to microbiology; in the latter scenario, the tissue initially sent to microbiology is forwarded to histology to be added to the specimen for histopathologic examination.

In the microbiology laboratory, a small portion of bone, about pea-sized, is removed from the specimen and ground until homogenized in 5 mL of sterile saline using a sterile proprietary kit. For routine aerobic and anaerobic cultures, media are inoculated in the following order: chocolate agar aerobic, chocolate agar anaerobic, blood agar aerobic, blood agar anaerobic, phenylethyl alcohol (PEA) agar, Bacteroides bile esculin (BBE) agar with KV, methicillin-resistant *Staphylococcus aureus* (MRSA) agar, colistin nalidixic acid (CAN) agar, MacConkey, thioglycolate broth, and enriched thioglycolate broth. For fungal cultures, material was inoculated on Sabouraud dextrose (SAB-DEX), brain-heart infusion (BHI), Mycosel slant, and SAB-DEX slant. These are then incubated in appropriate conditions and the remaining tissue transported to histopathology.

Additional laboratory parameters - when available - were also gathered including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), white blood cell count (WBC), platelet count (Plt), and absolute neutrophil count. Radiographic findings, if available, were recorded. Lastly, chart review provided the duration of clinically presumed osteomyelitis.

## 3. Results

Of 174 cases identified in the initial search a total of 163 cases fulfilled criteria for inclusion, including 51 from a metatarsal bone, 36 from the sacrum, 25 from the calcaneus, 16 from the ischium, 11 from the femur, 6 vertebral, 6 coccygeal, 5 tibial, 4 fibular, 2 talar, and 1 navicular. Among the 71 long bones sampled, 41 biopsies were from the metaphysis, 17 from the epiphysis, and 13 from the diaphysis. 23 patients included in the study had biopsies from multiple sites, none more than 2 biopsies, and in all cases only a single core biopsy, though sometimes fragmented, was attempted. The patients averaged 54.2 years of age, 93 were males, and 70 were females. A majority of the patients, 131, carried a diagnosis of diabetes mellitus, while 96 had an underlying diagnosis of peripheral vascular disease, 41 were immobilized for a variety of reasons, and 14 had documented antecedent trauma preceding the onset of infection.

All bone biopsies were conducted after antibiotics had been withheld for at least 48 h. An 11-gauge Jamshidi needle was used in most cases, providing a biopsy with a diameter of approximately 2 mm. In some cases an 8-gauge needle was utilized. The specimens averaged 9.5 mm in length (range 2 to 24 mm). Cases in which cultures were positive averaged 10.1 mm in length, and culture-negative cases averaged 8.6 mm ($p < 0.01$).

Of the 163 biopsies included in this study, 104 were culture-positive for one or more organisms, and 59 were culture-negative (Table 1). Thirty-one were polymicrobial, and a variety of agents were represented (Table 2). Of the 104 culture-positive cases, 49 had plasmacytic activity and 63 had neutrophilic activity. Of these, 35 demonstrated plasma cell aggregates, 45 had plasma cell infiltration, 59 had neutrophil aggregates, and 58 had neutrophil infiltration (Table 2). There were 12 cases which had no inflammatory cell infiltration; however, all of those cases had medullary fibrosis (Fig. 2). Overall, of 104 culture-positive biopsies 99 had fibrosis. All culture-positive cases had at least one of the following: neutrophil activity, plasmacytic activity, or eosinophilic fibrosis. Bone reparative changes were evenly divided between culture negative and culture positive cases, and necrosis of lamellar bone was an unusual finding in both groups.

Medullary fibrosis was present in 8 of the culture-negative cases. Of these, 4 had no inflammatory cells and instead had bone reparative changes. Of the other four, 2 cases had neutrophilic activity and 2 had plasmacytic activity. Reparative changes were present in only 8 of the culture positive cases, while they were identified in 4 of the culture-negative ones.

Overall, the presence of any plasmacytic activity (either plasma cell aggregates or plasma cell infiltration) provided sensitivity of 48% (95% CI 37–57%), specificity 86% (95% CI 75–94%), and positive predictive value 86% (76–92%). Neutrophilic activity had sensitivity of 61% (50–70%), specificity of 88% (77–95%), and positive predictive value 90% (82–94%). Eosinophilic fibrosis gave a sensitivity of 95% (89–98%), specificity of 86% (75–94%), positive predictive value of 93% (87–96%) and negative predictive value of 91% (81–96%). The fibrogranulocytic pattern (Fig. 3) had a specificity of 96% (88–99%) and positive predictive value of 97% (89–99%). The fibroplasmacytic pattern (Fig. 4) had a specificity of 88% (77–95%) and positive predictive value of 87% (76–93%).

Lastly, three cases with neutrophilic activity demonstrated foci in which neutrophils formed a 'micro-abscess' which we defined as an uninterrupted sheet of neutrophils filling at least one intertrabecular space (Fig. 5). Each of these was culture positive for *Enterococcus avium*.

All cases included in the study had clinically presumed or suspected osteomyelitis of at least 28 days duration. There was no correlation between the nature of the inflammatory infiltrate (neutrophilic versus plasmacytic) and the duration of suspected osteomyelitis. In considering this question an interesting pattern emerged; neutrophils had a likelihood ratio of 1.86 for methicillin-resistant *Staphylococcus aureus* (MRSA) and 2.61 for all *Staphylococcus aureus* (SA). Neutrophil-rich inflammatory infiltrates tended to correlate with the presence of SA on cultures, and no cases with SA were completely negative for all features of OM.

Patients with positive cultures showed mean ESR of 90.9 mm/h (median 95, range 31 to 40, SD 28.1), mean CRP of 10.9 mg/L (median 8.4, range 1.6 to 38, SD 7.8), mean platelet count 341 (median 306, range 133 to 873, SD 156.6), and mean ANC 8.4 per $mm^3$ (median 8.2, range 3.2 to 22.1, SD 3.1) (Table 3). Patients with negative cultures had mean ESR 82.5 mm/h (median 93, range 25 to 140, SD 32.7), mean CRP of 9.7 mg/L (median 8.2, range 1 to 38, SD 7.4), mean platelet count 320 (median 288, range 55 to 873, SD 148), and mean ANC 7.9 per $mm^3$

**Table 1**
Bone biopsy histopathologic characteristics.

| | Number of cases | Length (mm of medullary tissue) | Plasma cell clusters | Plasma cell infiltrate | Neutrophil clusters | Neutrophil infiltrate | Eosinophilic fibrosis |
|---|---|---|---|---|---|---|---|
| Culture positive | 104 | 10.2 | 35 | 45 | 59 | 58 | 99 |
| Culture negative | 59 | 8.6 | 3 | 8 | 7 | 2 | 8 |

**Table 2**
Histologic features and microbial culture results.

| | Cases # (%)[a] | Plasmacytic activity # ł | Granulocytic activity # ł | Fibro-plasmacytic # ł | Fibro-granulocytic # ł | Mixed # ł | Eosinophilic fibrosis # ł | Eosinophilic fibrosis only # ł |
|---|---|---|---|---|---|---|---|---|
| *MRSA* | 31 (18.9) | 11 | 26 | 5 | 20 | 6 | 31 | 0 |
| *Enterococcus faecalis* | 16 (9.7) | 6 | 9 | 2 | 7 | 3 | 16 | 4 |
| *MSSA* | 13 (7.9) | 2 | 13 | 0 | 11 | 2 | 13 | 0 |
| *Pseudomonas aeruginosa* | 12 (7.3) | 6 | 8 | 4 | 6 | 2 | 12 | 0 |
| *Proteus mirabilis* | 10 (6.1) | 6 | 6 | 3 | 3 | 3 | 10 | 1 |
| *Corynebacterium* species | 7 (4.3) | 3 | 2 | 3 | 2 | 0 | 7 | 2 |
| *Escherichia coli* | 6 (3.6) | 5 | 2 | 4 | 1 | 1 | 6 | 0 |
| *Enterococcus avium* | 3 (1.8%) | 0 | 3 | 0 | 3 | 0 | 3 | 0 |
| *Klebsiella pneumoniae* | 3 (1.8%) | 3 | 2 | 1 | 0 | 2 | 3 | 0 |
| *Acinetobacter baumanii* | 3 (1.8%) | 2 | 3 | 2 | 3 | 3 | 2 | 0 |
| Group A beta-hemolytic streptococci | 2 (1.2%) | 2 | 0 | 2 | 0 | 0 | 2 | 0 |
| Group B beta-hemolytic streptococci | 2 (1.2%) | 0 | 2 | 0 | 2 | 0 | 2 | 0 |
| *Providencia stuartii* | 2 (1.2%) | 2 | 2 | 2 | 2 | 2 | 2 | 0 |
| *Staphylococcus epidermidis* | 2 (1.2%) | 0 | 2 | 0 | 2 | 0 | 2 | 0 |
| *Staphylococcus simulans* | 2 (1.2%) | 2 | 0 | 2 | 0 | 0 | 2 | 0 |
| *Staphylococcus anginosus* | 2 (1.2%) | 2 | 2 | 2 | 2 | 2 | 2 | 0 |
| *Eikenella corrodens* | 2 (1.2%) | 0 | 2 | 0 | 2 | 0 | 2 | 0 |
| *Bacteroides thetaiotaomicron* | 2 (1.2%) | 1 | 1 | 1 | 1 | 0 | 2 | 0 |
| Group C beta-hemolytic streptococci | 2 (1.2%) | 2 | 2 | 2 | 2 | 2 | 2 | 0 |
| *Streptococcus viridans* | 2 (1.2%) | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| *Candida species* | 1 (0.6%) | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| *Morganella morganii* | 1 (0.6%) | 1 | 0 | 1 | 0 | 0 | 1 | 0 |
| *Staphylococcus capitis* | 1 (0.6%) | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| Culture-negative | 59 (36%) | 8 | 7 | 2 | 0 | 0 | 8 | 4 |

Ł Of cases with this pathogen.
[a] Represents percentage of culture-positive cases. Number exceeds number of study cases due to polymicrobial cases.

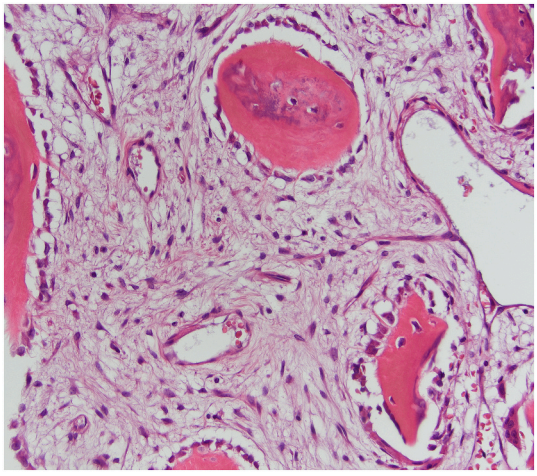

**Fig. 2.** Pauci-inflammatory osteomyelitis with only medullary fibrosis (H&E, 400x).

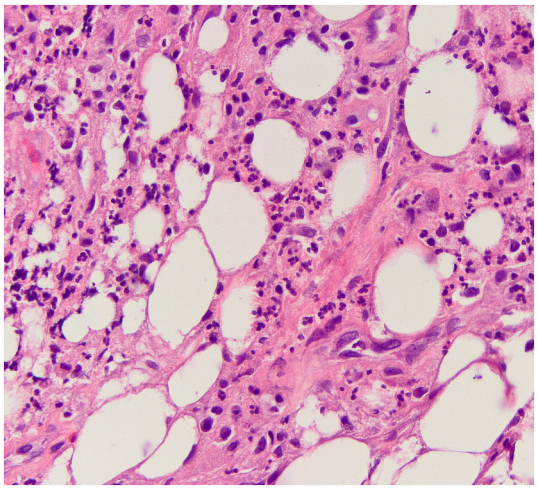

**Fig. 3.** Fibrogranulocytic pattern of injury in osteomyelitis (H&E, 400x)

(median 8.1, range 2 to 23, SD 3.9) (Table 3).

Radiographic studies were performed in 51 of the study patients, including plain films alone in 9, MRI in 41, and CT in 1. These were interpreted, according to the likelihood of osteomyelitis, as low, medium, or high likelihood of osteomyelitis. These categorical results did not correlate with histologic and culture findings.

## 4. Discussion

A preliminary study of histology as compared to microbial culture was performed. Results indicate that neither inflammation nor fibrosis is an exclusive or constant feature in culture-positive osteomyelitis. The nature of the inflammatory infiltrate, neutrophil-predominant versus plasma cell-predominant, did not affect the likelihood of bacterial isolates and did not coincide with the temporal course of the illness. Instead, the composition of the infiltrate correlated with specific isolates.

In a small number of cases we found medullary fibrosis without inflammation. These were associated with bone reparative changes in some cases, a feature that did not affect the likelihood of a culture isolate. While the evidence is insufficient to establish correlation, the observation comports with our clinical experience. We believe that reparative change may result in some of the radiographic and laboratory indicators of infection; alternatively, they may indicate the sampling of a portion of bone somewhat removed spatially from the nidus of infection. Further study is required for clarification.

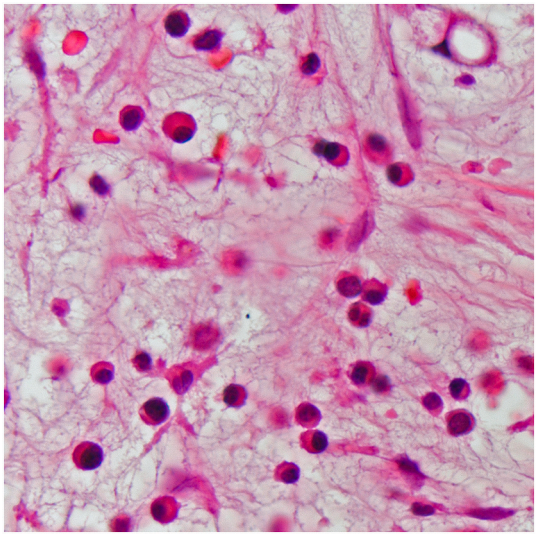

**Fig. 4.** Fibroplasmacytic pattern of injury in osteomyelitis (H&E, 600x).

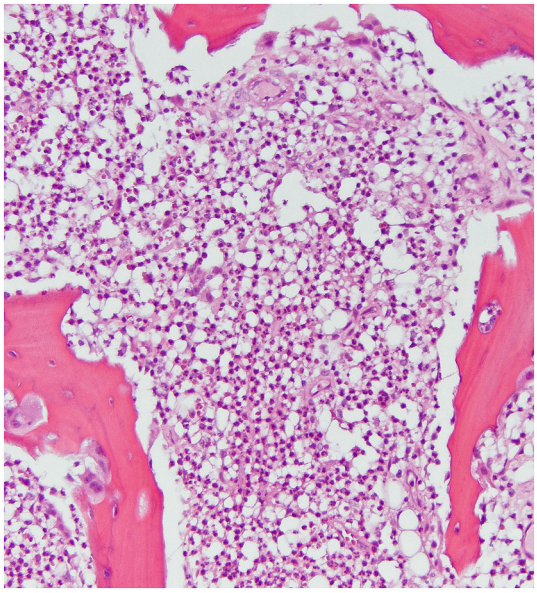

**Fig. 5.** Neutrophilic microabscess filling at least one intertrabecular space (H&E, 400x).

We found no significant value added by the distinction of inflammatory cell aggregates from inflammatory cell infiltration. We found no difference between plasma cell activity and neutrophil activity in correlation with the rate of culture positivity, and there were very few cases in which both cell types were present in abundance; instead, it seemed that most cases had either neutrophils or plasma cells. Furthermore, neutrophil-rich inflammation was correlated specifically with isolation of *Staphylococcus aureus*, while plasma cell predominance was found more frequently with other agents.

All of our cases had duration of suspected OM longer than 28 days. We believe this to be the result of time elapsed in medical management, securing radiographic tests, surgical consultation, and scheduling surgery, and no cases of 'acute' osteomyelitis were identified for inclusion in the study. Neutrophil-rich infiltrates were frequent in months-old cases of suspected osteomyelitis, as were plasma cell-rich infiltrates. This challenges our inclination to equate a neutrophil-rich infiltrate with 'acute osteomyelitis' and 'chronic osteomyelitis' with one rich in plasma cells. If this finding is supported by further study, then it may be that some factor other than time is responsible for influencing inflammatory cell constituents in these biopsies, that de novo plasma cell osteomyelitis may exist, and that the key factor determining the histologic manifestations may be the infectious agent itself, analogous perhaps to valvular endocarditis.

Laboratory parameters, while in aggregate behaving as expected (Table 3), were inconsistent in individual cases [3,32-34]. Radiographic parameters were likewise ambivalent. This likely reflects the overall clinical ambivalence that led to bone biopsy in this select group of cases, and no conclusions should be drawn about the performance characteristics of these tests.

The microbiologic findings were similar to those reported in prior studies [34-37]. Cultures were positive in 63.4% of our cases, and, considering all cases, 18.9% were polymicrobial and 44.5% were monomicrobial. The most common isolate was *Staphylococcus aureus* (26.8% of all cases), mostly MRSA, followed by *Enterococcus faecalis* (9.7% of cases). Gram negatives, all together, represented 20% of isolates. In the study by Sheehy et al. from the UK, 20% of cases were polymicrobial, 46% monomicrobial, 32% *Staphylococcus aureus*, and 5% *Enterococcus sp.*, with about 28% gram negatives [36]. Lavery et al., in a study conducted 20 years ago concerned solely with diabetic foot infections, found polymicrobial infections in 83% of cases, *Staphylococcus aureus* in 50% of cases, *Enterococcus* sp. in 22%, and gram negatives in 55% [37]. Weiner et al. obtained positive cultures in 70.4% of their cases of diabetic foot infections but did not report their specific isolates [35].

Our study design is hampered by the lack of a true "gold standard" and, in our attempt to find correlates, the susceptibility of culture to both false positive and false negative results [32,34,35]. Cultures may be falsely positive as a result of contamination from adjacent soft tissue. Cultures may be falsely negative owing to the zonal nature of the disease, in which pathogens may be localized to particular areas of the medullary space. Furthermore, essentially all patients were exposed to antibiotics, some for an extended time, prior to culture with variable duration of antibiotic clearing prior to biopsy [37-40].

## 5. Conclusion

Secondary osteomyelitis is a diagnostic challenge in which pathologists are increasingly called upon to assist. We report an early correlation between common histopathologic findings and culture isolates, which we believe deserves further study. The significance of pauci-inflammatory fibrosis was noted, as was the tendency of many cases of long standing to be neutrophil rich, including all cases in which *Staphylococcus aureus* was isolated. Additional testing of biopsy specimens,

**Table 3**
Comparison of standard laboratory values between culture-positive and culture-negative cases of osteomyelitis.

| | Erythrocyte sedimentation rate (mm/h) | C-reactive protein (mg/L) | White blood cell count ($\times 10^9$/L) | Platelet count ($\times 10^9$/L) | Absolute neutrophil count ($mm^3$) |
|---|---|---|---|---|---|
| Culture-positive average | 90.9 (SD 28.1) | 10.9 (SD 7.8) | 11.2 (SD 3.4) | 341 (SD 156.6) | 8.4 (SD 3.1) |
| Culture-negative average | 82.5 (SD 32.7) | 9.7 (SD 7.4) | 8.9 (SD 5.2) | 320 (SD148) | 7.9 (SD 3.9) |

SD: Standard deviation.

perhaps using PCR amplification and sequencing of bacterial 16S rRNA as a reference, may help to further elucidate these questions.

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## Declaration of competing interest

None.

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