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Comparison of the histopathological characteristics of osteomyelitis, medication-related osteonecrosis of the jaw, and osteoradionecrosis

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Abstract. The purpose of this study was to compare the histopathological parameters of chronic/suppurative osteomyelitis, medication-related osteonecrosis of the jaw (MRONJ), and osteoradionecrosis (ORN), and to examine the hypothesis that distinct histological features can be related to a specific disease, allowing for diagnosis based on microscopic evaluation alone. One hundred and ten samples were reviewed by two examiners in a blinded fashion, and a semi-quantitative histomorphometric analysis was performed. The parameters evaluated included the presence or absence of necrotic bone, inflammation, reactive bone formation, bacteria, and osteoclasts. No statistically significant differences were found between groups for any parameter. Necrotic bone was common to all three diagnoses. Inflammation and reactive bone formation were present in all three diagnoses. The presence of bacteria was a prominent feature in all cases. Osteoclasts were scarce in MRONJ and osteomyelitis, and non-existent in ORN. The results of this study failed to identify distinctive microscopic characteristics in any of the three entities that could be used to differentiate between them. Therefore, it is impossible to reach a specific final diagnosis based on microscopic findings alone. The role of microscopic analysis is to serve as an aid to diagnosis that must be complemented by the patient's history and imaging.

Key words: osteomyelitis; MRONJ; osteoradionecrosis; histopathology; necrotic bone.

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Medication-related osteonecrosis of the jaw (MRONJ) is a well-known complication of treatment with bisphosphonates, denosumab, and other drugs, such as anti-angiogenic agents and novel anti-cancer drugs. It has been described in patients treated with these medications for various malignant diseases, but also in patients treated for osteopenia and osteoporosis. MRONJ is a chronic and difficult to treat condition that emerged as a small-scale epidemic in the early years of the 21st century.

In 2014, the American Association of Oral and Maxillofacial Surgeons (AAOMS) published a position paper that highlighted the definition and staging of this disease and addressed the management of patients with MRONJ[1]. The diagnosis of MRONJ is currently based on clinical parameters alone. Histological analysis and radiographic features are not considered a pre-requisite for diagnosis, and most patients do not undergo a biopsy from the exposed necrotic bone.

A blinded study by Marx and Tursun compared the histopathology of three diseases – chronic/suppurative osteomyelitis, MRONJ, and osteoradionecrosis (ORN) – all of which have the presence of necrotic bone in common[2]. The authors concluded that each of these three entities showed a distinctive histopathological pattern, indicating different pathophysiology and treatment options[2]. These conclusions are inconsistent with those of previous studies[3], and have been challenged by some, due to the lack of expertise in pathology of both investigators and the lack of statistical validation[4].

The purpose this study was to compare in a blinded fashion the histopathological parameters of chronic/suppurative osteomyelitis, MRONJ, and ORN and to examine the hypothesis that distinct histological features can be related to each disease, allowing for diagnosis based on microscopic evaluation alone.

Materials and methods

Based on the archives of the Institute of Pathology, Tel Aviv Medical Center, the study included all biopsies from the jaws of patients with a diagnosis of osteomyelitis, MRONJ, or ORN, submitted between January 2011 and July 2016. Only cases with detailed clinical and radiological data available were included.

The histological slides were presented separately to an oral pathologist (IK) and a head and neck pathologist (LT) in a blinded manner, with no patient identification information or medical records. All archival slides were stained with haematoxylin and eosin. In all cases in which bacteria were evident on haematoxylin and eosin staining, periodic acid–Schiff (PAS) and Gram stains were added.

The set of parameters evaluated in the histomorphometric analysis included the presence or absence of necrotic bone, inflammation, reactive bone formation, bacteria (based on PAS and Gram stains), and osteoclasts.

In addition, the percentage bone circumference surrounded by inflammatory cells was evaluated in a semi-quantitative manner on a five-tier scale from 0 to 4, as follows: 0 = absence of inflammatory cells; 1 = up to 10% of bone circumference; 2 = 10–25%; 3 = 25–50%; 4 = >50% of bone circumference. The inflammatory infiltrate was examined under ×10 magnification. Each slide was searched for the area with the highest density of inflammation and the score was given accordingly.

The level of agreement between examiners was assessed with Cohen's kappa test for all criteria examined, and with the intraclass correlation coefficient (ICC) for the percentage of bone circumference surrounded by inflammation. Correlation of each criterion with the final diagnosis was assessed using Pearson's χ^2 test.

The study was approved by the institutional review board.

Results

One hundred and nineteen samples were reviewed by the pathologists. Nine samples were excluded from the study: three slides because they did not contain necrotic bone and six slides because they contained only small fragments of bone that were not suitable for analysis. The remaining 110 cases were included in the analysis.

Eight patients had more than one biopsy; however, only one was chosen to be included in the analysis for each patient. Of the cases included, MRONJ was the original diagnosis in 76 (69%), osteomyelitis in 28 (25.5%), and ORN in six (5.5%). The demographic characteristics of the patient sample are presented in Table 1.

The 76 MRONJ patients had been administered the following drugs: zoledronic acid (n = 41), alendronate (n = 12), denosumab (n = 9), pamidronate (n = 4), risedronate (n = 4), risedronate and denosumab (n = 2), alendronate and denosumab (n = 2), denosumab and bevacizumab (n = 1), and pemetrexed (n = 1).

Table 2 depicts the presence of reactive bone, bacteria, *Actinomyces*, and osteoclasts for each diagnosis and the number of slides with no inflammation. Figure 1 (parts a–e) demonstrates typical examples for each of the grading scores (1–4), as mentioned in the Materials and methods section. In the study group, some degree of inflammation (score of 1–4) was present in 25–26 of the osteomyelitis slides (89.3–92.9%), with some variation between examiners. Seventy-one MRONJ slides (93.4%) and three ORN slides (50%) had an inflammation score of 1–4. Reactive bone was present in 16.6–36.8% of all slides. Bacterial presence was a prominent feature in all cases, irrespective of the original diagnosis, with colonies consistent with *Actinomyces* present in the majority of cases (validated with PAS and Gram stains). Osteoclasts were scarce in MRONJ and osteomyelitis, and non-existent in ORN.

No histological differences were found between the slides of patients treated with bisphosphonates and other medications in the MRONJ group. Candida pseudohyphae were observed in four cases (two osteomyelitis and two MRONJ). One MRONJ case contained a focus of metastatic renal cell carcinoma.

Kappa values were used to determine the measure of agreement between examiners,

Table 1. Demographic characteristics.

	Osteomyelitis	MRONJ	ORN
Sex			
Male	16	29	4
Female	12	47	2
Age range (mean), years	22–83 (57)	45–86 (69)	46–82 (68)

MRONJ, medication-related osteonecrosis of the jaw; ORN, osteoradionecrosis.

Table 2. Presence of reactive bone, bacteria, *Actinomyces*, and osteoclasts for each diagnosis: averages for the examiners.

	Osteomyelitis	MRONJ	ORN
No inflammation	8.9%	6.6%	50.0%
Reactive bone	30.0%	36.8%	25.0%
Bacteria	84.0%	93.4%	83.0%
Actinomyces	73.0%	90.8%	66.6%
Osteoclasts	12.5%	21.7%	0.0%

MRONJ, medication-related osteonecrosis of the jaw; ORN, osteoradionecrosis.

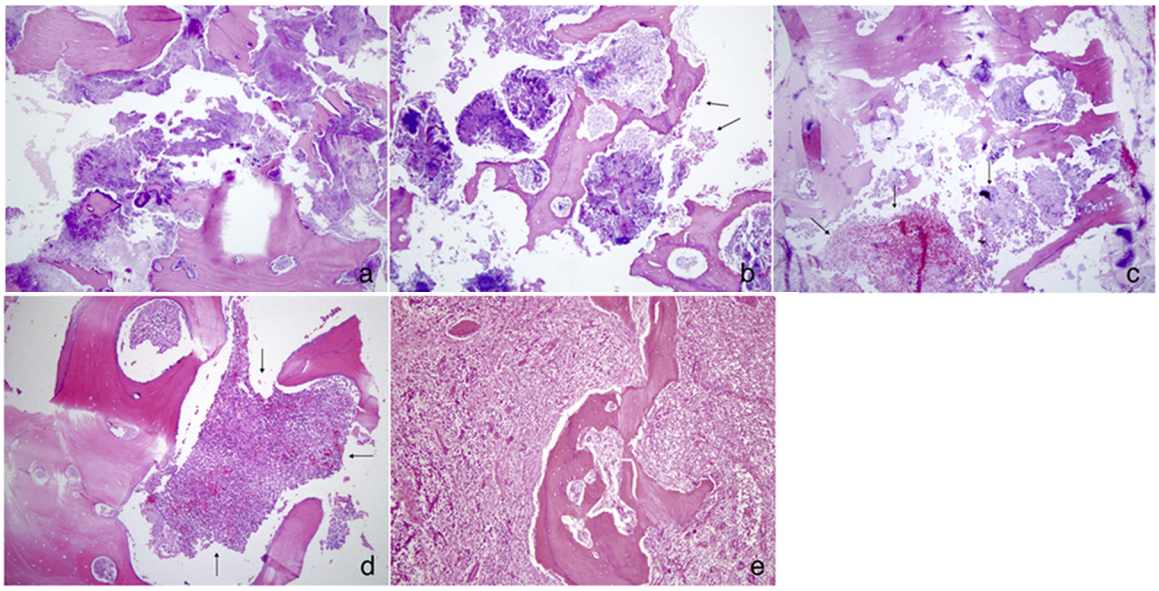

Fig. 1. Inflammation scores 0–4 based on the percentage of bone with adjacent inflammation: (a) score 0, (b) score 1, (c) score 2, (d) score 3, (e) score 4. Arrows point to inflammatory foci.

Table 3. Agreement between the examiners: Cohen's kappa values.

	κ	*P*-value
Inflammation	0.310	<0.0001
Reactive bone	0.920	<0.0001
Bacteria	0.842	<0.0001
Actinomyces	0.964	<0.0001
Osteoclasts	0.695	<0.0001

as presented in Table 3. Kappa values vary between $\kappa = 1$ indicating perfect agreement and $\kappa < 1$ indicating less than complete agreement. In the present analysis, all κ values were positive and high, with *P*-values of less than 0.0001 for all parameters, except inflammation around the bone circumference. The κ value for inflammation around the bone circumference was 0.31. Although statistically significant, since the value was relatively low, another statistical test was used for this category, the ICC. The ICC value was 0.849, with a *P*-value of less than 0.0001.

Correlation of each criterion with the diagnosis of osteomyelitis, MRONJ, and ORN was assessed using Pearson's χ^2 test. For analysis purposes, inflammation scores were re-grouped into three categories: 0 + 1, 2, and 3 + 4. All tests were statistically non-significant, with no correlation between any of the parameters examined and any of the three diagnostic groups, except *Actinomyces* and MRONJ. For the correlation of *Actinomyces* and MRONJ, results were significant for examiner 1 ($P = 0.024$) and close to significance for examiner 2 ($P = 0.052$), as presented in Table 4.

Table 4. Correlation of *Actinomyces* and MRONJ according to the examiner: Pearson's χ^2 test *P*-values.

	Examiner 1	Examiner 2
Inflammation	0.061	0.122
Reactive bone	0.982	0.359
Bacteria	0.088	0.585
Actinomyces	0.024	0.052
Osteoclasts	0.098	0.442

MRONJ, medication-related osteonecrosis of the jaw.

Discussion

Necrotic bone is a common finding in all three conditions investigated in the present study – MRONJ, ORN, and suppurative osteomyelitis – and it has an important role in the disease process. However, according to existing recommendations, a biopsy is not mandatory for the diagnosis of MRONJ or ORN[1,5–7]. The diagnosis is based on a combination of clinical features (history of relevant medications used or of radiotherapy or dental infection, pain, swelling, suppuration, and the presence of exposed bone), supported by certain radiographic findings (osteolysis, sclerosis or a combination, persisting alveolar sockets, sequestra, and involucrum formation)[8–13].

A review of the literature found only scarce evidence-based data regarding the microscopic features of these entities, with one study (describing 23 cases of osteomyelitis, 37 cases of MRONJ, and 45 cases of ORN) suggesting clear differences could be shown between these lesions[2]. In particular, the study reported the presence of inflammation in 100% of osteomyelitis cases and 0% in MRONJ and ORN, the presence of bacteria only inside marrow spaces in osteomyelitis versus bacteria only on the surface in MRONJ and ORN, and osteoclasts in 96% of osteomyelitis versus none in MRONJ and ORN. These conclusions are inconsistent with those of previous studies[3], and have been seriously challenged by some, due to the lack of expertise in pathology of both investigators and the lack of statistical validation[4].

The impression of the pathologists in the present study investigators' team was that inflammation was detectable in the majority of cases encountered in their daily work experience, as were colonies of bacteria, which is in sharp contrast with the findings of Marx and Tursun[2]. This discrepancy led to the planning of the present study. For a more accurate evaluation of the role of inflammation, a semi-quantitative evaluation was introduced in the present study, with the measurement of the percentage of bone surrounded by inflammation; this is in contrast to the study by Marx and Tursun, in which inflammation was reported as heavy, moderate, or slight.

The majority of cases retrieved during the time period included in the study were MRONJ ($n = 76$), almost three times the number of osteomyelitis cases ($n = 28$). This trend is consistent with that reported by Marx and Tursun[2]. The smallest group was ORN ($n = 6$), reflecting a decrease in the frequency of ORN following the introduction of improved radiotherapy methods (such as intensity-modulated radiation therapy) at Tel Aviv Sourasky Medical Center since 2010.

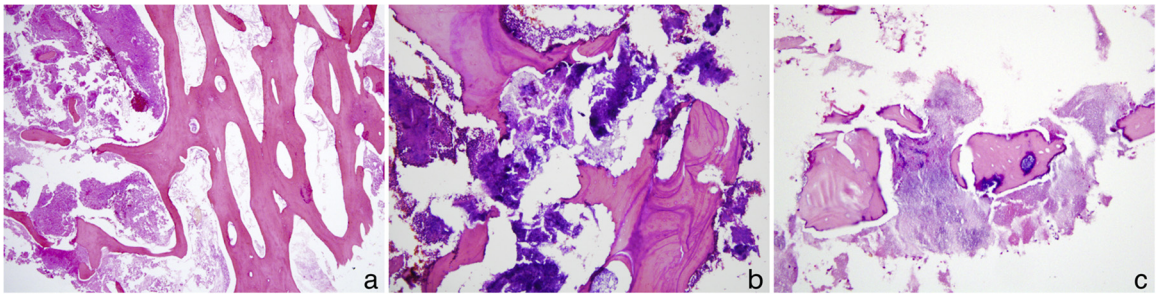

Fig. 2. Fragments of necrotic bone with large aggregates of microorganisms: (a) osteomyelitis, (b) MRONJ, (c) ORN; haematoxylin and eosin.

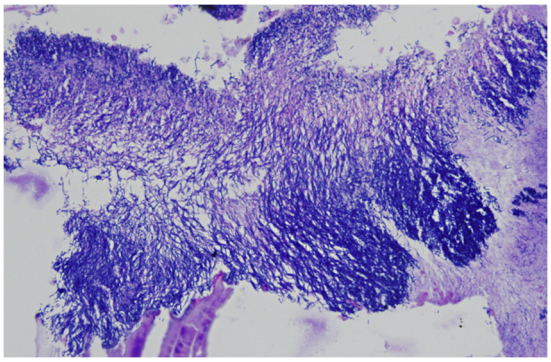

Fig. 3. Gram stain showing a variety of bacterial morphologies (cocci, rods, and filaments), mostly Gram-positive with some Gram-negative; magnification ×100.

The present study included a significantly larger group of MRONJ in comparison with the study by Marx and Tursun[2], and a lower number of ORN, with a significant discrepancy in results. Inflammation was observed in over 90% of osteomyelitis cases, but also in over 93% of MRONJ and 50% of ORN cases; thus as a parameter by itself, inflammation cannot be used to differentiate between the groups.

Regarding the assumption that there may be a difference in the amount of inflammation present between groups, with a focal versus diffuse pattern, the results of the study failed to prove any significant difference between groups for this parameter. Thus, the fraction of bone circumference involved with inflammation is not predictive of the diagnosis either.

Bacteria were found to exist in the majority of cases in all three groups (78–93%); however, it was not possible to determine whether the bacteria involved only marrow spaces or only surface areas (as was reported by Marx and Tursun). This was due to fragmentation of the specimens in most cases. Both surface and marrow harboured the bacterial colonies in many cases (Fig. 2a–c).

In the majority of cases, a plethora of mixed bacterial morphologies was present (cocci, rods, and filaments), both Gram-positive and Gram-negative (Fig. 3). Most slides exhibited bacteria with the typical morphology of *Actinomyces* (clusters of branching filaments), supported by PAS and Gram staining. Decalcification procedures did not affect the staining of microorganisms. The presence of *Actinomyces* colonies was the only parameter that did correlate with the diagnosis of MRONJ (for examiner 1), but not with the other two diagnostic groups, in which the threshold for significance was not reached. *Actinomyces* has been described as a consistent histological finding in many cases of MRONJ[14–17]. Some researchers even think that this species plays a role in the pathogenesis of MRONJ[18,19]. The present investigators' assumption correlates with that of Marx and Tursun[2] – the infectious process in MRONJ and ORN is secondary and not the primary cause. Bone exposed to the oral cavity may become heavily colonized by diverse species of microorganisms (bacteria, fungi, viruses) quite rapidly. *Actinomyces* is more evident in histological slides due to its striking morphological appearance, but the infected bone is actually paved with multiple species, a mixed microbiota, which is a key feature of any odontogenic infection.

There are no clear data on the role of infection (with specific species) in the pathophysiology of MRONJ, and it is certain that *Actinomyces* is only one genus in the vast diversity of bacterial species found in samples of MRONJ bone[20–23].

Osteoclasts were rare but found in both osteomyelitis and MRONJ (in 21.7% and 12.5%, respectively), as opposed to the reported 96% in osteomyelitis and 0% in MRONJ[2]. No osteoclasts were recorded in ORN, a finding that is consistent with the report by Marx and Tursun[2]. The present investigators found that even in cases in which osteoclasts were identified, it was necessary to search through many fields to find any, and their presence was focal and only in small numbers (Fig. 4a–c). Thus again, this parameter was found not to be useful as a diagnostic feature in this context.

Reactive bone formation was also recorded in all study groups, in between 1/4 and 1/3 cases, frequently adjacent to necrotic bone (Fig. 5a–c). This is a lower frequency of new bone formation than reported by Marx and Tursun (74–81%), but follows the same general tendency in all three groups in a comparable proportion.

The presence or absence of new bone may be related to the type of surgical procedure from which the biopsy was submitted: in larger resections there is probably an increased chance of finding viable bone at the periphery than in local sequestrectomy or marginal resections. The presence of reactive bone indicates the body's gradual and slow response to the dead bone beneath.

In conclusion, this study included a larger number of cases than have been investigated before, was performed by a combined team including an oral and maxillofacial surgeon and two specialists in pathology and oral pathology, used histomorphometry for analysis, and submitted results for statistical evaluation. The results of the analysis showed, with a high degree of confidence, that there are no distinctive microscopic characteristics in

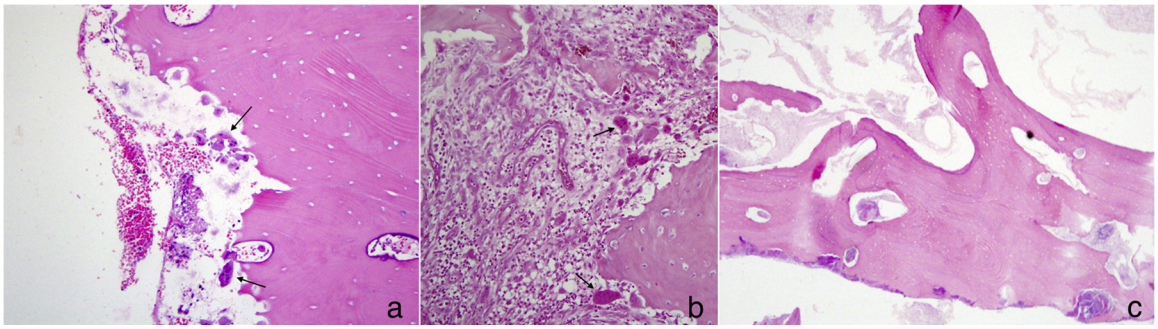

Fig. 4. (a) Osteomyelitis, showing multiple multinucleated cells (osteoclasts) and resorption lacunae (haematoxylin and eosin, original ×200). (b) MRONJ, showing loose connective tissue with chronic inflammation between fragments of reactive bone, with clusters of multinucleated giant cells (osteoclasts) (haematoxylin and eosin, original ×200). (c) ORN, showing a large fragment of necrotic bone without any multinucleated cells (haematoxylin and eosin, original ×40).

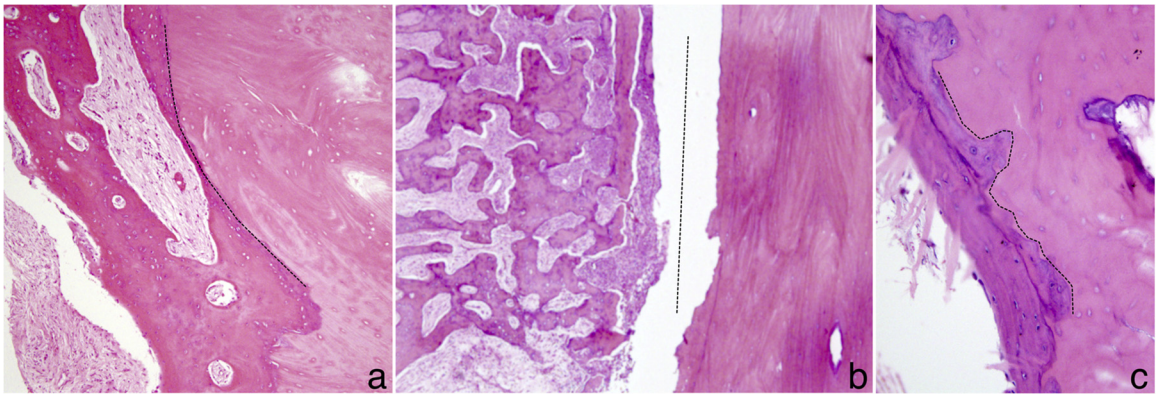

Fig. 5. (a) Osteomyelitis: a clear border (c) ORN is evident between the pale-stained necrotic bone and the new reactive bone formation, which is more intensely stained. (b) MRONJ: a large fragment of non-vital bone (left) bordered by new bone formation and loose vital connective tissue. (c) ORN: a clear margin (arrow) between the pale-stained necrotic bone and the new reactive bone formation, which is more intensely stained.

any of the three types of osteomyelitis investigated that could be used to differentiate between them. Therefore, it is impossible to reach a specific final diagnosis based on microscopic findings alone. The essential role of microscopic analysis in these cases is as an aid to diagnosis that should be complemented by the patient's history and imaging.

Funding

None.

Competing interests

None.

Ethical approval

The study was approved by the institutional review board (0310-16-TLV).

Patient consent

Not required.

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