The Journal of Foot & Ankle Surgery 59 (2020) 75–85

Contents lists available at ScienceDirect

The Journal of Foot & Ankle Surgery

journal homepage: www.jfas.org

Diagnosing Osteomyelitis: A Histology Guide for Pathologists

Amelia B. Sybenga, DO[1], Daniel C. Jupiter, PhD[2], V.O. Speights, DO[3], Arundhati Rao, MD, PhD[3]

[1] *Clinical Fellow, Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN*
[2] *Associate Professor, Department of Preventive Medicine and Community Health, Department of Orthopaedic Surgery and Rehabilitation, The University of Texas Medical Branch, Galveston, TX*
[3] *Professor, Department of Pathology and Laboratory Medicine, Scott & White Medical Center, Baylor Scott and White Health, Texas A&M Health Science Center, Temple, TX*

ARTICLE INFO

Level of Clinical Evidence: 2

Keywords:
diagnosis
diagnostics
histopathology
infection
involucrum
neutrophil
osteomyelitis
periosteal reaction

ABSTRACT

Histopathologic examination of bone specimens coupled with bone culture is considered the gold standard for the diagnosis of osteomyelitis (OM). Despite this, studies have demonstrated interpathologist agreement in the diagnosis of OM as low as 30%, largely stemming from a lack of specific definitions and diagnostic criteria. Review of the literature has provided insight into the lifecycle of OM, illustrating the histologic progression of OM phases from acute to chronic, and provides support for defining subcategories of OM. Using an algorithmic histopathologic tool consisting of 15 criteria, each with an associated score, we defined 5 categories of OM: (1) acute OM, (2) acute and chronic OM, (3) chronic OM, (4) chronic active OM, and (5) chronic inactive OM. We reviewed 462 microscopic slides from 263 patients with suspected OM, and for each slide, we determined an algorithm-derived diagnosis, which was then used to calculate a total histopathologic load score (Jupiter score). Algorithm-derived diagnoses recapitulated original clinical diagnoses and diagnosed cases as OM that had not been originally diagnoses. These novel cases were more likely to have subsequent clinical complications. Finally, pathologic load scores were assessed for association with the category of OM.

© 2019 by the American College of Foot and Ankle Surgeons. All rights reserved.

Histologic examination of bone specimens coupled with bone culture is considered the gold standard for the diagnosis of osteomyelitis (OM), and requests from clinicians for bone examination by a pathologist in cases of suspected OM are not uncommon. The disease itself is far from rare; up to 25% of patients with diabetes will develop lower extremity ulcers during their lifetime (1). Depending on the severity of infection, between 10% and 72% of diabetic foot ulcers are accompanied by OM (2–5). Under controlled circumstances, bone culture is a highly reliable diagnostic modality; however, in practice, preprocedural antibiotic administration, soft tissue contamination, sampling errors, and culture failure limit the use of bone cultures (6–10), necessitating that histologic diagnosis stands alone (11). However, unlike other pathologic diagnoses, strict histologic criteria for OM are not well defined, resulting in significant interpathologist variation in diagnosis.

Few studies have attempted to examine the effectiveness of histopathologic examinations as an independent diagnostic modality. Although a study by Lipsky et al (12) suggested 95% sensitivity and 99% specificity for histologic bone examination, a small study by Meyr et al (13) demonstrated that concordance among pathologists in the diagnosis of OM using only histopathologic criteria is as low as 30%. Despite these issues, studies exploring other diagnostic modalities, such as probe-to-bone test, positron emission tomography, and magnetic resonance imaging (MRI), have compared results against this "gold standard" (14–17).

These concerns are of great clinical importance, because delay in the appropriate management of OM significantly increases morbidity and mortality. For example, 40% to 47% of patients with residual OM in an amputation margin had nonhealing wounds or further proximal amputation within 2 years of the initial amputation (18). Amputation is associated with high mortality; for example, a study demonstrated that there is an associated 61% 5-year mortality risk after below- or above-knee amputation in patients with diabetes (19). Coupled with poor patient outcomes are the significant healthcare costs associated with treatment failure, particularly in the case of further proximal amputation (20).

Through examination of the salient histopathologic features of OM in the literature, we illustrate a lifecycle of OM as it progresses from early infection to end-stage disease and organize those features into a criteria-based guide to assist pathologists in the diagnosis of OM in the distal extremity.

Materials and Methods

Literature Review

To guide informed decisions about which pathologic criteria to include in a diagnostic tool for OM, we searched PubMed for articles concerned with the diagnosis and treatment

Financial Disclosure: None reported.
Conflict of Interest: None reported.

Address correspondence to: Amelia B. Sybenga, DO, Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, 2200 Children's Way, Nashville, TN 37232.
E-mail address: amelia.b.sybenga@vumc.org (A.B. Sybenga).

1067-2516/$ - see front matter © 2019 by the American College of Foot and Ankle Surgeons. All rights reserved.
https://doi.org/10.1053/j.jfas.2019.06.007

Table 1
Descriptions of histologic features of osteomyelitis in the distal lower extremity

	Diagnostic Features/Major Criteria (Score 3)	Category	Description	Differential Diagnosis
1	Neutrophils	AOM/ACOM/CAOM	1 actively destroying bone, or aggregate of 10 or more in 1 hpf	Instrumentation, hemorrhage, acute fracture
2	Microabscess	AOM/ACOM/CAOM	Aggregate of neutrophils and other inflammatory cells	Brown tumor, plasma cells, tumor, hematopoeisis
3	Fibrinoid necrosis	AOM/ACOM/CAOM	Fibrillary, eosinophilic, acellular	Fat necrosis, serous atrophy, early fibrosis
4	Bacteria	AOM/ACOM	Colonies, rods, cocci, filamentous; also yeast with or without hyphae	Contamination, necrosis, bone processing debris
5	Sequestrum	COM/ACOM/CAOM	Sharply angulated, acellular, surrounding inflammation	Bone dust, avascular necrosis
6	Involucrum	COM/ACOM/CAOM	Irregular foci of woven bone in marrow or soft tissues	Periosteal reaction
7	Periosteal reaction, rapid growth	CAOM/ACOM/COM	Woven bone extending from the cortical surface into the soft tissues	Involucrum, adjacent tumor
	Supportive Features/ Intermediate Criteria (Score 2)			
8	Fibroplasia	COM/ACOM/CAOM	Early to late fibrosis usually with plasmacytic inflammation	Tumor desmoplasia, repair/reactive changes, fibrinoid necrosis
	General Features/ Minor Criteria (Score 1)			
9	Necrotic bone	AOM/ACOM/CAOM	Avascular necrosis; pale, acellular; usually no tissue reaction	Bone dust, sequestrum
10	Erosion	AOM > COM	Irregular, “chewed” or scalloped boney surfaces	Cortical soft tissue attachments
11	Destruction	AOM > COM	Bone with “punched out” areas extending from the surface	Sharp-force trauma, postoperative changes (curetting)
12	Remodeling	COM > AOM	Angulated and rhomboid shaped, usually with increased osteocytes	Periosteal reaction
13	Plasma cells	COM > AOM	Eccentric nucleus with perinuclear hof	Microabscess
14	Granulation tissue	All types	Foci of increased vascularity, can be thick-walled with prominent endothelial cells, often with background fibrosis	Chronic ischemia, normal vessels, atherosclerotic or hypertensive changes, adjacent tumor
15	Periosteal reaction, slow growth	CIOM/COM	Thick, lamellated bone in undulating or onion skinning pattern, extending from cortex into soft tissue	Repair, sclerosis, ischemia

Abbreviations: ACOM, acute and chronic osteomyelitis; AOM, acute osteomyelitis; AOM > COM, more commonly seen in AOM; CAOM, chronic active osteomyelitis; CIOM, chronic inactive osteomyelitis; COM, chronic osteomyelitis; COM > AOM, more commonly seen in COM; hpf, high-power field.

of OM, as well as specific pathologic findings of interest. In addition to the PubMed search (Supplemental Table 1), we used several pathology textbooks, both general and bone pathology specific; articles and journals we had previously collected; and references gleaned from the systematic search. Although our literature search revealed a paucity of work on grading schemes and a lack of consensus regarding diagnostic criteria for OM, we were able to identify 15 generally agreed-on histologic features of OM (Table 1). The search also allowed specification of the association of these criteria with differential diagnoses and ample evidence of diagnostic categories of OM, including AOM, ACOM, and COM. Last, we found evidence to support 2 additional, novel, histologic categories of OM: CAOM and CIOM.

Development of the Diagnostic Algorithm

Guided by the literature, we classified the 15 histologic features above into acute, chronic, and general components—general components being those found in both acute and chronic inflammatory processes that are associated with a broader differential diagnosis. We also weighted the criteria according to their emphasis in the literature as major, intermediate, and minor criteria. Based on the weighted criteria, we specifically defined diagnostic categories of OM: if slides from a patient specimen revealed at least 1 major acute criterion, the patient was diagnosed as having AOM; if slides from a patient specimen revealed at least 1 major chronic criterion, the patient was diagnosed as having COM; if slides from a patient specimen revealed at least 1 major acute and at least 1 major chronic criterion and the acute features were as or more extensive than the chronic features, the patient was diagnosed as having ACOM; if slides from a patient specimen revealed at least 1 major acute and at least 1 major chronic criterion and the chronic features were more extensive than the acute features, the patient was diagnosed as having CAOM; and in the absence of any major criteria, a diagnosis of NOM, or reactive/repair was rendered. There was 1 caveat to this diagnostic process: if slides from a patient sample revealed thick periosteal reaction, remodeling, marrow fibrosis, and plasma cell infiltrate, without the presence of major criterion, the patient was diagnosed with CIOM.

Finally, to determine whether we could establish a rigorous cutoff, or set of cutoffs, for defining NOM, AOM, COM, ACOM, CAOM and CIOM, we arbitrarily assigned scores to each criterion: 3 points for each major criterion, 2 points for each intermediate criterion, and 1 point for each minor criterion. Each slide could then be assigned a histologic load score—the sum of the points for the criteria observed on the slide, which could then be associated with diagnosis.

Patients and Specimens

Using our electronic pathology database, we searched for all bone specimens submitted between January 1, 2012, and July 1, 2013. for suspected OM of the distal lower extremity, including all foot and ankle biopsies, amputations, and clearance margins, limited to patients 18 years of age and older at the time the specimen was received. Patient demographics and comorbidities were recorded, as was whether bone culture or radiographic studies were performed and the use of antibiotics before biopsy. Demographics included race, sex, and age, and comorbidities included diabetes, neuropathy, peripheral vascular disease, hyperlipidemia, anemia, and venous stasis. We chose bone specimens distal to the tibia, because this reduces the differential diagnoses compared with other areas of the body (21). We thus could focus largely on the bony changes in OM and changes associated with the diabetic foot. Bone specimens submitted for or with a diagnosis of extra digit, neoplasm, synovitis, hammer toe, Charcot reconstruction, or fracture were excluded, as were any specimens described as coming from above the distal tibia. Above- and below-knee amputations performed for foot or ankle OM and/or nonhealing ulceration were also excluded.

Archived glass slides, made from bone tissue fixed in 10% buffered formalin, decalcified with 23% hydrochloric acid decalcifier, paraffin embedded, and stained with hematoxylin-eosin (H&E), for these cases were reviewed, and the presence or absence of each diagnostic criterion was recorded. Adhering to these definitions, we then made a diagnosis for each slide. After the diagnosis was rendered, a histopathologic load score was also calculated for each specimen: this is the total of the scores from all assigned criteria and is referred to as the Jupiter score.

Finally, we compared the rates of failed treatments between those who were diagnosed with OM both originally and using criteria and those who were originally diagnosed with NOM but rediagnosed with OM using criteria (NOM-OM discordance). These failed treatments resulted in a nonhealing wound, and subsequent proximal amputation, within a year of the original diagnosis. Notably, only 1 sample was originally diagnosed as OM and criteria diagnosed as NOM; thus, we did not consider this type of discrepancy.

For the purposes of comparison with original diagnoses to evaluate patient outcomes, CAOM was treated as ACOM, CIOM was treated as COM, and reactive/repair and other were treated as NOM. When examining the agreement of diagnoses, we treated each slide as an individual specimen; however, when looking at treatment failure, many patients had >1 slide associated with their case. For those patients, we considered only the margin, regardless of the agreement or disagreement with the other slides associated with the case, assuming only the margin diagnosis would affect patient outcomes. For the

same reasons, if a patient had multiple margins, we considered the case had NOM-OM discordance if there was disagreement regarding any of those margins. This also ensured that each patient was counted only once in the analysis of clinical outcomes.

Statistical Analysis

Original and criteria-based diagnoses were compared for agreement by using simple percentages. Characteristics were compared between those with and without discordant diagnoses by using χ^2 tests. Scores were compared between diagnostic categories by using analysis of variance (ANOVA). All components of these studies were reviewed and approved by the Institutional Review Board of Scott & White Medical Center.

Results

Patient Demographics

Before the application of exclusion criteria, we found 427 cases (patients) of bone specimens from the foot and ankle. Of these, 164 cases were excluded: 26 cases involved accessory digit, neoplasm, synovitis, hammer toe, Charcot reconstruction, or fracture; 88 were cases submitted from outside facilities not connected with our electronic medical records (extended care and private practice facilities); 39 cases lacked sufficient clinical information for inclusion; and 11 cases had missing slides. After the application of exclusion criteria, 263 cases remained for analysis, comprising 462 H&E slides from amputations and biopsies.

Eight comorbidities occurred with high frequency: 230 (87.45%) patients had hypertension, 206 (78.3%) had diabetes mellitus, 154 (58.56%) had peripheral neuropathy, 114 (43.51%) had peripheral vascular disease, 94 (35.74%) had chronic kidney disease, 187 (71.1%) had hyperlipidemia, 112 (42.59%) had anemia at the time of biopsy or amputation, and 18 (6.9%) had venous stasis. Nearly 90% (n = 239) of patients had ≥3 of these diagnoses. Race and sex demographics were consistent with the statistical census information for central Texas (22). Eighty-three percent (n = 215) of patients were taking antibiotics before and at the time of bone biopsy, debridement, and/or amputation (Table 2).

Only 19.77% (n = 52) of patients had cultures performed at the time of biopsy (Table 2), but it is unclear if all of the cultures were bone cultures or if some were soft tissue cultures. There were several cases in which clinicians documented in their notes that bone culture had been ordered, but the actual test performed by the microbiology laboratory was listed as a body tissue culture rather than a bone culture. Some of those body tissue cultures were performed during wound debridement, before bone biopsy or amputation, whereas others that were listed as body tissue cultures had "bone" written in the specimen description. Our criteria-based diagnoses of the slides yielded 80 diagnoses of AOM, 95 diagnoses of COM, 94 diagnoses of ACOM, 48 diagnoses of CAOM, 37 diagnoses of CIOM, 37 diagnoses of reactive/repair, 67 diagnoses of NOM, and 4 diagnoses of other (ischemic bone with secondary bacterial colonization).

Table 2
Patient demographics (N = 263)

Age, y	Mean	60.74
	Range	22-94
	SD	14.88
Race, n (%)	White	166 (63.12)
	Hispanic	58 (22.05)
	Black	22 (8.37)
	Unknown	17 (6.46)
Sex, n (%)	Male	160 (60.84)
	Female	103 (39.16)
Smoker, n (%)	No	194 (74.33)
	Yes	67 (25.67)
Preprocedural antibiotics, n (%)	No	44 (16.99)
	Yes	215 (83.01)
Bone culture, n (%)	No	211 (80.23)
	Yes	52 (19.77)
Comorbidities, n (%)	DM	206 (78.33)
	HTN	230 (87.45)
	PVD	114 (43.51)
	HLD	187 (71.1)
	PN	154 (58.56)
	Anemia	112 (42.59)
	CKD	94 (35.74)
	VS	18 (6.9)
Comorbidity counts, n (%)	1+	261 (99.24)
	2+	252 (95.82)
	3+	239 (90.87)
Previous amputation, n (%)		109 (41.44)
Nonhealing wounds, n (%)		141 (53.61)
Further proximal amputation, n (%)		83 (31.68)

Abbreviations: CKD, chronic kidney disease; DM, diabetes mellitus; HLD, hyperlipidemia; HTN, hypertension; PN, peripheral neuropathy; PVD, peripheral vascular disease; VS, venous stasis.

Patient Outcomes

When comparing agreement between an original diagnosis of OM and the criteria-based diagnoses of OM, there were 116 patients with NOM-OM discordance, representing 44.6% of the patients. Of the patients with NOM-OM discordance, 65.5% experienced nonhealing wounds, compared with 43% among patients for whom there was diagnostic agreement ($p = .00031$). Similarly, among patients with NOM-OM discordance, 37.9% experienced further proximal amputation, compared with 25.7% among patients for whom there was diagnostic agreement ($p = .03419$).

When comparing agreement for the specific category of OM (AOM, ACOM, COM, and NOM), the discordance increased to 64%. Importantly, some of the original pathology reports associated with these patients contained descriptions of COM, including chronic inflammation and marrow fibrosis, even though the final diagnoses were NOM. Descriptive diagnoses rarely mentioned the presence of sequestrum (2 cases) and never mentioned involucrum or periosteal reaction. Table 3 details the original and algorithm-based diagnoses.

Not surprisingly, the risk of a nonhealing wound and further proximal amputation increased with increasing number of comorbidities, as well as with history of previous amputation. Interestingly, patients with anemia were more likely to have poorer outcomes and were particularly more likely to require further proximal amputation, with a relative risk of 1.86 ($p = .00077$).

Table 3
Agreement

Summary of Diagnoses				
OM and NOM	OM	NOM		
Original diagnoses	201	261		
Criteria-based diagnoses	354	108		
Subcategory	AOM	ACOM	COM	NOM
Original diagnoses	164	17	20	261
Criteria-based diagnoses	80	142	132	108
Agreement				
All Subtypes	Original Diagnosis			
Criteria diagnosis	AOM	ACOM	COM	NOM
AOM	54	3	0	23
ACOM	83	14	13	32
COM	20	0	7	105
NOM	7	0	0	101

Abbreviations: ACOM, acute and chronic osteomyelitis; AOM, acute osteomyelitis; COM, chronic osteomyelitis; NOM, no osteomyelitis; OM, osteomyelitis.
OM vs NOM: AOM, COM, and ACOM diagnoses combined as OM.
Subtypes: AOM, COM, and ACOM as separate diagnostic categories; categories of criteria-based diagnoses were merged as follows for evaluating agreement: CIOM and COM; ACOM and CAOM; reactive/repair, other and NOM merged for comparison.

Table 4
Scores

	Score		0 to 3		≤ 4		4 to 6		≥ 6		≥ 9		< 11		≥ 12	
Slides	n	Mean	n	%	n	%	n	%	n	%	n	%	n	%	n	%
ACOM	94	15.3	0	0.0	0	0.0	0	0.0	94	100.0	92	97.9	10	10.6	84	89.4
CAOM	48	13.6	0	0.0	0	0.0	0	0.0	48	100.0	43	89.6	16	33.3	32	66.7
AOM	80	9.9	0	0.0	2	2.5	14	17.5	74	92.5	51	63.8	56	70.0	24	30.0
COM	95	8.8	0	0.0	1	1.1	5	5.3	93	97.9	40	42.1	88	92.6	7	7.4
CIOM	37	5.2	1	2.7	8	21.6	34	91.9	14	37.8	0	0.0	37	100.0	0	0.0
REA	37	2.9	25	67.6	35	94.6	12	32.4	0	0.0	0	0.0	37	100.0	0	0.0
NOM	67	1.0	66	98.5	67	100.0	1	1.5	0	0.0	0	0.0	67	100.0	0	0.0
Other	4	4.3	1	25.0	1	25.0	3	75.0	1	25.0	0	0.0	4	100.0	0	0.0

Abbreviations: ACOM, acute and chronic osteomyelitis; AOM, acute osteomyelitis; CAOM, chronic active osteomyelitis; CIOM, chronic inactive osteomyelitis; COM, chronic osteomyelitis; NOM, no osteomyelitis; REA, reactive/repair.

Jupiter Score

The average scores in ACOM, CAOM, AOM, COM, CIOM, reactive/repair, NOM, and other specimens were 15.3 points, 13.6 points, 9.9 points, 8.8 points, 5.2 points, 2.9 points, 1.0 point, and 4.0 points, respectively (Table 4), significant at $p < .00001$ on ANOVA. All NOM slides scored ≤ 4 points, with 98.5% (n = 66) scoring ≤ 3 points. Similarly, 94.6% (36) of reactive/repair slides scored ≤ 4 points. The majority of AOM and COM slides had scores ranging between 6 and 15 points, 86.3% (n = 69) and 96.8% (n = 92) respectively, with 93.8% (n = 56) of the AOM slides and 92.5% (n = 86) of the COM slides scoring < 12 points. Only 3% (n = 6) of AOM slides and 2% (n = 2) of the COM slides scored < 6 points. The 6 slides of AOM scoring < 6 points—5 points (4 slides) and 4 points (2 slides)—were all early AOM, containing focal aggregates of neutrophils with minimal other changes. The only slide of COM scoring 4 points contained an obvious marrow sequestrum with surrounding plasmacytic inflammation and few other changes. The majority of ACOM and CAOM slides scored ≥ 9 points: 97.9% (n = 92) and 89.6% (n = 43), respectively. Even starker, 89.4% of ACOM slides scored ≥ 12 points. The majority of CIOM slides ranged between 4 and 6 points (91.9%), with 97.3% scoring between 4 and 7 points; however, with 21% of cases scoring ≤ 4 points, this diagnostic category shows overlap with reactive/repair, which showed 94.6% (n = 35) scoring ≤ 4 points, and highlights potential diagnostic difficulty differentiating reactive changes and CIOM.

Discussion

Diagnostic Categories

Our review of the literature revealed a paucity of clear histopathologic definitions and diagnostic criteria for OM. The literature describes various categories of OM and various histologic appearances, each caused by specific causative organisms, such as syphilis, tuberculosis, and fungi (23–25). We also found descriptions of the most common types of OM affecting the diabetic foot: suppurative (bacterial) OM and neuropathic OM (23,24). However, in contrast to many non-neoplastic diseases for which acute or active disease, chronic active disease, and chronic inactive disease are specifically defined by criteria-based histopathologic findings, we found no such accepted criteria for OM, although a few incomplete recent attempts to define COM have been made (26,27).

We encountered 5 distinct diagnostic categories of OM in the pathology, clinical, and radiology literature: AOM, ACOM, COM, CAOM, and CIOM (Fig. 1). Of the 5 categories, AOM was the best established in the literature, both in terms of making a diagnosis and in terms of treatment (4,23,24). ACOM, sometimes called subacute OM or acute and chronic OM, was actually described as 2 separate entities. The first, subacute OM, is a specific diagnosis, a distinct subtype of hematogeneous OM, classically associated with Brodie's abscess and not associated with the direct extension of a superficial wound to the bone as seen in diabetic foot ulceration (28). The second mention of ACOM in the literature is as a diagnostic category, the intermediary point in the pathologic progression of AOM to COM (27,29). We focused on the latter, viewing ACOM as a diagnostic category, rather than as a specific diagnosis. In histologic descriptions, ACOM contains elements of both COM and AOM and therefore seems to inhabit its own histopathologic category (23,30); however, there is an absence of literature to allow a determination as to whether ACOM should be treated clinically as AOM or COM, and we found no mention of the specific timeline of the progression from AOM to ACOM to COM, CAOM, or CIOM.

COM is described throughout the literature as the consequence of long-standing or untreated AOM and is a controversial diagnostic category, from diagnosis to treatment. The definition of "long-standing" is not well established, as evidenced by 1 author referencing 10 days of clinical symptoms of AOM, or relapse of previously treated AOM, as consistent with a diagnosis of COM (31), and in a later article stating that COM is a poorly defined evolution occurring over months to years (30).

We also found that COM is far more difficult to diagnose than AOM, both clinically and radiologically. Probe-to-bone is frequently used with some specificity, but poor sensitivity (16) and other clinical diagnostic methods, such as persistent nonhealing wound, are even more limited in their predictive abilities (32). Although MRI seems to be the most sensitive test for AOM (17), it does not readily differentiate Charcot arthropathy from COM, and there is some support of the use of positron emission tomography-computed tomography for COM diagnoses (14,15). Additionally, we found that some of the radiology literature has more specific definitions for features associated with COM (involucrum and sequestrum) and that these definitions are not unlike the histopathologic descriptions (29,33). Interestingly, we also found some radiology literature that uses MRI to differentiate between CAOM and CIOM based on the appearance of periosteal reaction with or without new involucrum or periosteal reaction, respectively (29,34).

We did find consensus that COM is frequently culture negative, and despite its reliability in controlled settings, culture can often yield false-positive results that should be interpreted with caution (31). In our study, only a minority of patients had cultures performed. Consistent with the literature, the reliability of those cultures is suspect because of the inconsistency of the type of culture ordered, because of the testing of "bone culture" before the patient has a bone biopsy, and because 87% of our patients were taking antibiotics before the culture. We also found little agreement on how COM should be treated. Some authors suggested increased duration and variation of antibiotic therapy for COM as well as antibiotic implants (35), whereas others claimed that surgical excision was the only effective treatment (36).

Finally, when looking at the pathology literature, we noted that, until very recently, no attempt had been made to develop a clear

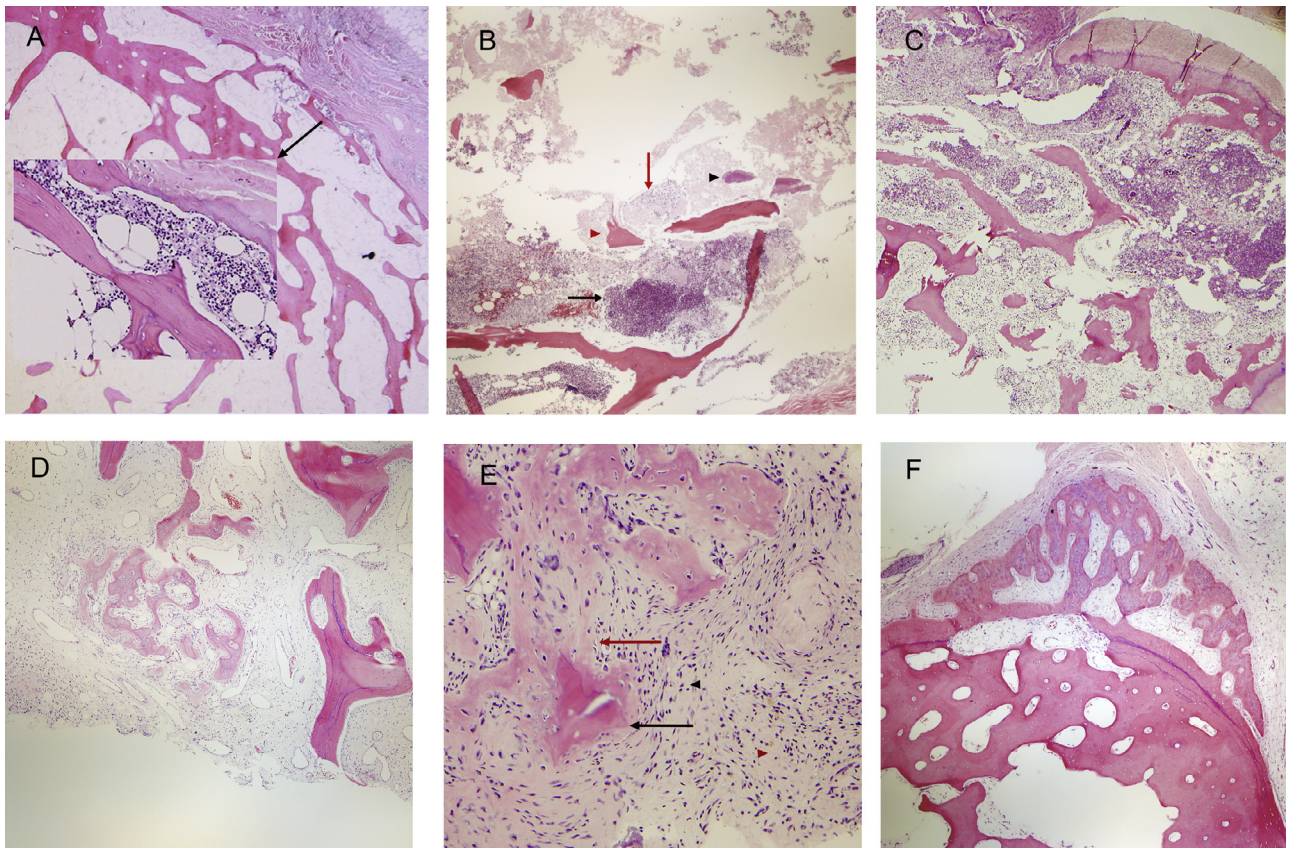

Fig. 1. Categories of osteomyelitis. (*A*) Acute osteomyelitis (early): small focus of neutrophils in the marrow space eroding the bone (magnification ×4 and ×20, hematoxylin-eosin [H&E] stain). (*B*) Acute osteomyelitis: abscess formation (black arrow), fibrinoid necrosis (red arrow), bacteria (black arrowhead), necrotic bone (red arrowhead) and bone erosion (asterisk) (magnification ×4, H&E stain). (*C*) Acute and chronic osteomyelitis: acute inflammation and abscess (right), draining fistula (top) with numerous sequestra and early fibroplasia with chronic inflammation (bottom left) (magnification ×4, H&E stain). (*D*) Chronic osteomyelitis: involucrum and bony remodeling in a background of marrow fibrosis with plasmacytic inflammation and granulation tissue (red arrowhead) (magnification ×4, H&E stain). (*E*) Chronic active osteomyelitis: Sequestrum (black arrow) and early involucrum (red arrow) in a background of dense marrow fibrosis with numerous plasma cells (black arrowhead) and a small aggregate of neutrophils (red arrowhead). (*F*) Chronic inactive osteomyelitis: undulating, thick periosteal reaction with dense marrow fibrosis, scattered plasma cells and bone remodeling.

definition for COM. In a study from 2013 by Cecilia-Matilla et al (27), the authors attempt to provide histopathologic definitions of COM by documenting the presence of the various features in "histopathologically proven" cases of OM. The authors describe OM as a progression from AOM to ACOM to COM, with which we agree. However, the vast majority of their cases had imaging, probe-to-bone, and cultures that were positive for OM, meaning that only clinically obvious cases of OM were used in their study, limiting the generalizability of their study. The authors determined, based on these observations, that COM should be defined simply as marrow fibrosis with chronic inflammation, predominantly plasma cells, without significant sequestra; this is in contrast to the majority of the literature. They support this argument by stating that 78% of their patients with the presence of pure-marrow fibrosis were culture positive. This last finding is interesting, given that the majority of reports have demonstrated that COM is typically culture negative, and positive results are viewed with skepticism (2,32). Although remodeling was considered in their description of COM, sequestrum, periosteal reaction, and involucrum were not mentioned (27).

In a 2016 study by Turi et al (26), the authors define AOM and COM, but not ACOM. Here, the authors demonstrate that 50% of COM cases contained portions of necrotic bone surrounded by fibrosis and chronic inflammation, consistent with the literature. In this study, broad conclusions about the importance of histopathologic exam are made; however, how they reached these conclusions, as well as what their histopathologic observations were, is unclear, as data-gathering techniques were not described, nor were any significant associations with outcomes or literature provided. Again, neither periosteal reaction nor involucrum was mentioned (26).

Finally, a brief study published in October 2014 developed a complex scoring system with a much smaller sample size than that in the current study that is dependent on access to clinical, radiologic, and culture information and for which minimal supporting data were provided for their conclusions (37).

The Criteria: Major Acute Criteria

Pathologists have historically relied on the presence of neutrophils actively involving bone, or larger microabscesses in the marrow space, to render a diagnosis of AOM; in few other disease processes is there such a reliance on a single histopathologic finding to make a diagnosis. Throughout the literature, histopathologic descriptions of AOM consistently included neutrophils and microabscesses but also fibrinoid necrosis and bacteria. Most references include the mention of sequestrum, when discussing ACOM and COM. Fibrinoid necrosis and bacteria, present in fragmented biopsy samples from the center of a necrotic OM lesion, where obvious neutrophils may not be found, can provide invaluable diagnostic information. The absence of neutrophils in this setting should not preclude a diagnosis of OM. We thus chose as our major criterion the presence of at least 1 neutrophil actively destroying bone (23,38). Alternatively, erring on the side of diagnostic caution, aggregates of at least 10 neutrophils in at least 1 high-power field in

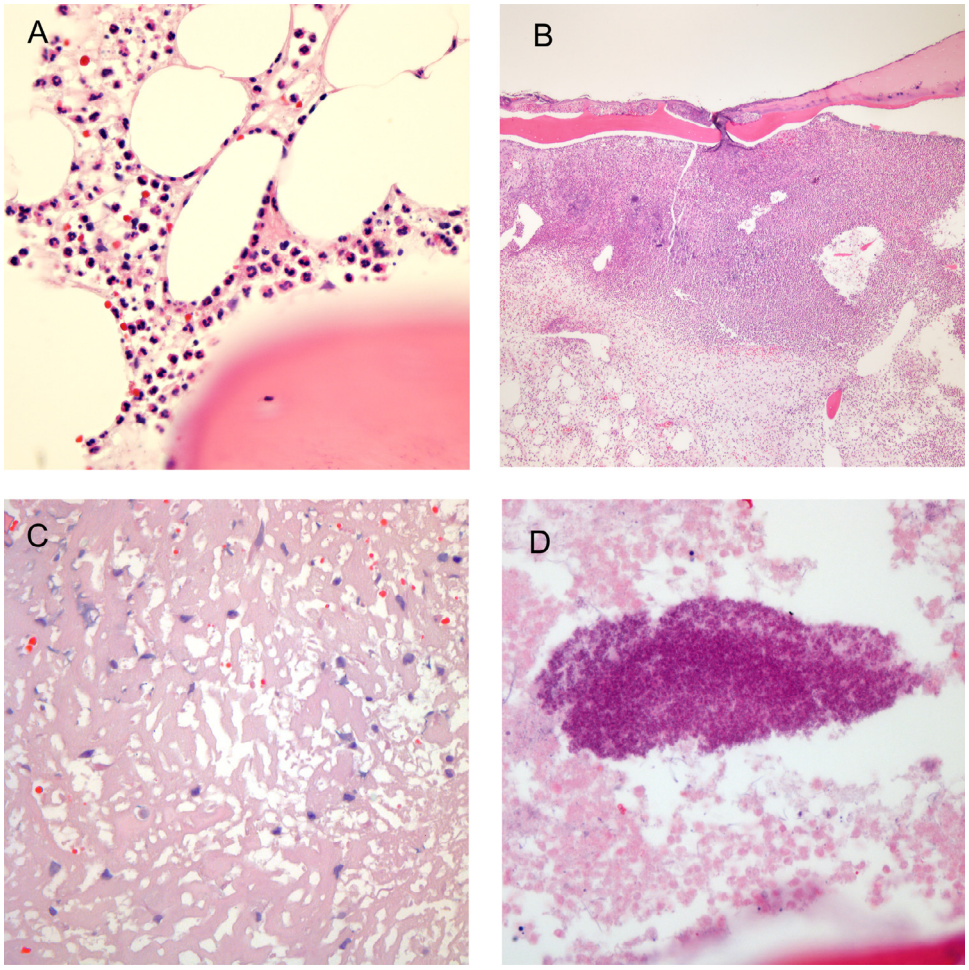

Fig. 2. Major acute criteria. (*A*) Neutrophils: clinging to edges of bone as seen here, or in aggregates of 10 or more per high powered field in the marrow space (magnification ×40, hematoxylin-eosin [H&E] stain). (*B*) Abscess: neutrophils admixed with other inflammatory cells forming a vaguely circumscribed aggregate in the marrow space (magnification ×2, H&E stain). (*C*) Fibrinoid necrosis: acellular, fibrillary, eosinophilic, necrotic debris (magnification ×10, H&E stain). (*D*) Bacteria: this particular case demonstrates a colony of cocci (magnification ×100 oil immersion, H&E stain).

the marrow space, independent of any hemorrhage or extravasated red blood cells, also satisfies this criterion (39,40). Hematopoiesis in this location and age was very rarely encountered. In addition, we included abscess, fibrinoid necrosis, and bacteria in our major acute criteria. The bacteria criterion can include fungi as well; however, we did not observe any cases of fungal OM in our patient samples (Fig. 2).

Major Chronic Criteria: Sequestrum

A sequestrum is defined in the pathology literature as a devascularized, necrotic, presumably infected bone fragment that breaks away from normal bony structures and is theorized to be responsible for maintaining chronic infection (30). Sequestra are seen in all types of OM but are considered one of the most diagnostic features of COM (23). A sequestrum is surrounded by fibrosis and inflammation (Fig. 3). A giant cell reaction can also be present. Absence of a chronic inflammatory response and fibrosis around fragments of bone, or fragments of bone embedded in a microabscess without fibrosis, is more consistent with bone infarct in AOM, which can be resorbed without inciting chronic disease; alternatively, the bone may represent artifactual bone dust. Importantly, if there is a concern that sequestra may represent bone dust, using the "necrotic bone" criterion instead (see "General Criteria") will eliminate this finding as a major criterion, and the examining pathologist must look for additional diagnostic criteria to make a case for a diagnosis of OM.

Of note, fragments of bone or cartilage embedded in the soft tissues, absent significant histopathologic changes in the primary bone, is most consistent with a destructive arthropathy, such as Charcot or rheumatoid arthritis (21). Review of clinical history and radiology is essential before deciding on a diagnosis of COM (21). Although we did not diagnose OM if sequestrum was present in the soft tissues in the absence of an obvious bony lesion, we do recommend mentioning it in the pathology report, because of the risk of persistent inflammatory response and nonhealing wound (30).

Involucrum and Rapid Periosteal Reaction

As previously mentioned, the histopathologic definitions of involucrum and periosteal reaction are somewhat controversial. One definition equates involucrum with a periosteal reaction, where periosteum has been lifted away from the surface of the bone secondary to suppuration and undergoes periosteal bone growth (34,41). The more common definition of involucrum is periosteal bone growth around a sequestrum (Fig. 3) (42). Each definition has a characteristic woven bone pattern. The former is present circumferentially around the cortical surface of the bone, whereas the latter is found within the marrow space or soft tissues. Periosteal reaction is periosteal bone growth in

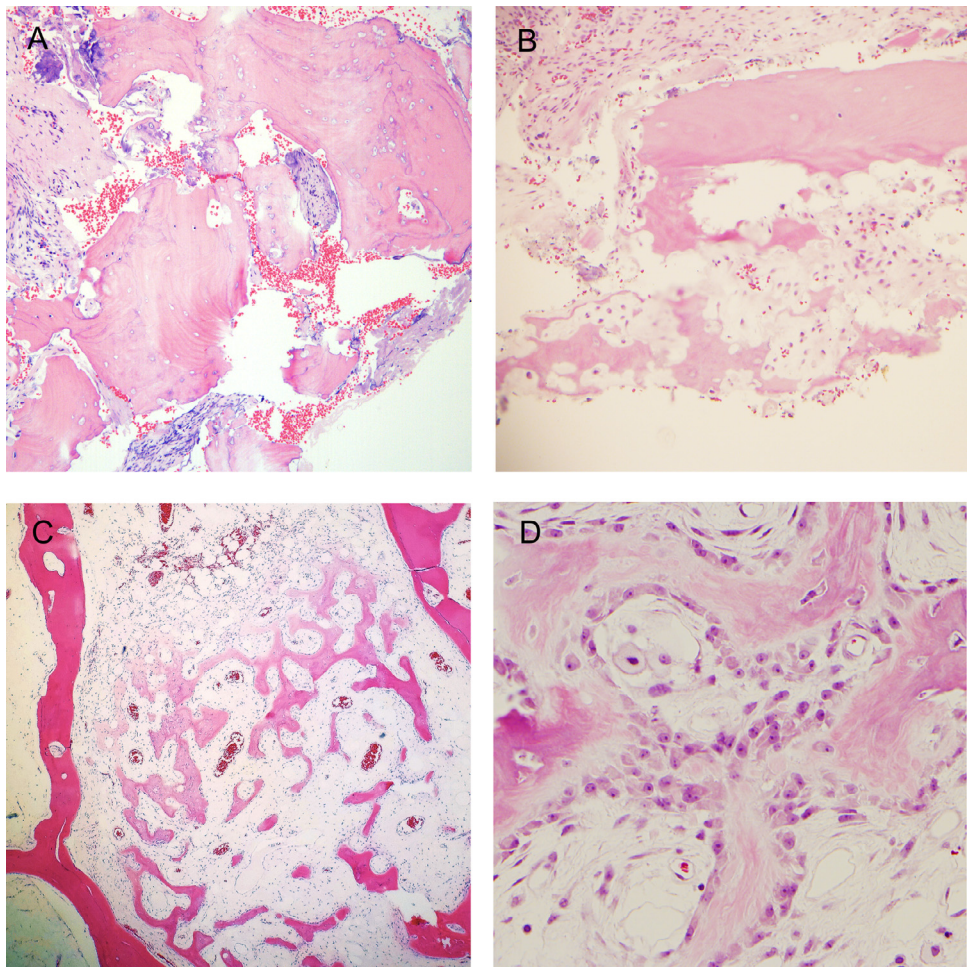

Fig. 3. Major chronic criteria. (*A*) Sequestrum: large fragment of necrotic bone with surrounding fibrosis and inflammation (magnification ×4, hematoxylin-eosin [H&E] stain). Note the empty lacunae. (*B*) Sequestrum: small fragments of necrotic bone with surrounding fibrosis and inflammation (magnification ×10, H&E stain). (*C*) Involucrum within the marrow space (magnification ×4, H&E stain). (*D*) Involucrum within the marrow space (magnification ×40, H&E stain). For rapid periosteal reaction, see Fig. 4.

response to injury and is nonspecific, with subtle variations in the appearance that can range from thin to thick, nearly solid, sclerotic, and onion-skinned, among others. In a 1981 report, Ragsdale et al (34) associated "single lamellar periosteal reactions" with active OM, whereas "undulating" and "lamellated" (onion-skinning) periosteal reactions were associated with slower processes, such as arthropathy, inactive or low-grade OM, and fracture (Fig. 4). Other patterns of periosteal reaction were associated with tumors. Most references encountered mention both involucrum and rapid periosteal reaction as being both a radiologic and a pathologic feature of ACOM and COM when present.

Intermediate Criteria

Fibrosis (marrow fibroplasia) is a common reactive change occurring in response to many insults. Fibroplasia, like most cellular changes, is a dynamic process, beginning with early fibroplasia, which is loose, amphophilic, and less organized, and progressing to dense, organized, eosinophilic fibrosis (Fig. 5). Fibroplasia is mentioned as an important component of ACOM and COM (23,43). Although it can be argued that aggregates of plasma cells in a background of fibroplasia represent active infection in end-stage COM and should be considered diagnostic of COM (27), we found insufficient support for this argument. Specifically, these cases are usually culture negative; it may be that this finding does not represent OM but rather its sequelae. This would explain why many cases of COM fail antibiotic treatment and ultimately require complete surgical excision (44). An alternative explanation is that this might represent a culture-negative infection. Polymerase chain reaction and other techniques for detecting bacterial DNA or ribosomal RNA have demonstrated this possibility (45,46). In the absence of other features, fibroplasia with plasma cells should place arthropathy and chronic fracture high on the differential (21).

General Criteria

Through our literature review, we discovered that OM is described and viewed as a dynamic, progressive process that involves background histopathologic changes that can support a diagnosis of OM. For example, the background changes present in AOM tend to involve bony erosion and destruction, as well as bone infarct necrosis, rather than remodeling. On the other hand, the background changes present in COM correspond more to bony remodeling, fibrosis, and granulation tissue than do those in AOM. This is reflected in the notations in Table 1, in the section of minor criteria. The general criteria include bone erosion, bone destruction, necrotic bone, remodeling, granulation tissue, plasmacytic inflammation, and thick periosteal reaction (Fig. 6). Each criterion was selected from histopathologic changes associated with both general tissue reactions and bone-specific reactive changes (21,23–25,47,48). The importance of the general criteria is to establish a pattern of background changes that should raise the suspicions of the pathologist and align with what is observed in major criteria. They

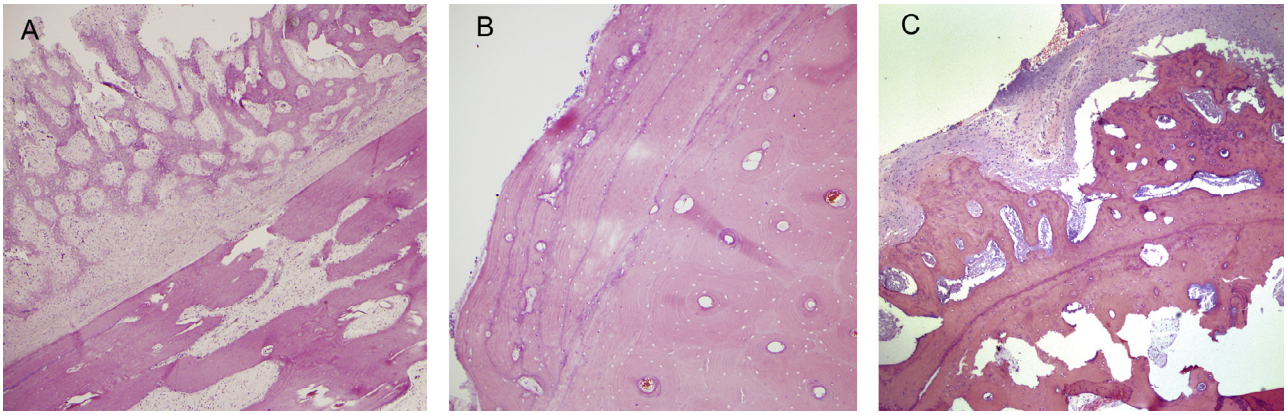

Fig. 4. Periosteal reactions. (*A*) Rapid periosteal reaction (single lamellar): thin projections of woven bone encase the cortex in a case of acute and chronic osteomyelitis (magnification ×10, hematoxylin-eosin [H&E] stain). (*B*) Lamellated ("onion skin") type periosteal reaction in a patient with a clinical history of Charcot arthropathy (magnification ×4, H&E stain). (*C*) Thick periosteal reaction, undulating pattern: irregular, thick, lobulated bone growth extending from the cortex into the soft tissues (magnification ×10, H&E stain).

should also serve to caution the pathologist to consider a broader differential in their absence. For instance, we diagnosed 4 slides containing bacteria (see "Major Criteria") as other rather than AOM. This was done because bacteria were present but clearly represented secondary colonization in large bone infarcts, evidenced by the absence of all other criteria in the samples. Aggregates of plasma cells in a background of fibrosis, for some pathologists, is diagnostic of COM (27), although we did not find enough support in the literature to warrant recommending these findings alone as being diagnostic. On the other hand, aggregates of plasma cells present in conjunction with marrow fibrosis, erosion, vascular proliferation, thick periosteal reaction, and bone remodeling—each of which is separately nonspecific—collectively point to OM with destructive arthropathy (Charcot or rheumatoid arthritis) and chronic fracture in the differential. Despite the nonspecific nature of thick periosteal reaction, we consider the specific pattern of reaction in the presence of background changes (fibroplasia and plasmacytic inflammation) associated with OM as being diagnostic of CIOM for this study (29,34).

Other Criteria Considerations

We identified other non-specific histopathologic findings for which we felt there was insufficient evidence to warrant inclusion among our criteria. Among these are osteopenia and narrowing of the bone shaft, giant cells, and bone sclerosis. One study suggested that giant cells were actually displaced, activated osteoclasts, or an osteoclast-like metaplasia (48). Similarly, we observed osteoclast activation in a number of cases. Again, thick periosteal reaction with fibrosis and soft-tissue giant-cell reaction in these cases makes Charcot arthropathy a likely differential diagnosis (21). Half of our patients were over 61 years of age, and many had chronic kidney disease, making the presence of osteopenia as a criterion less significant. We did not include gross findings in our study.

The Life Cycle of OM

In 2011, Myer et al (13) published a small study demonstrating a surprising degree of interobserver variability in the histologic diagnosis of OM, with agreement as low as 30%, despite previous studies purporting to show the reliability of histopathologic examination (12). Although this study was very small, only 2 pathologists were used and they had no access to clinical or radiologic information; we believe this lack of concordance would be substantiated by larger studies and is due, at least in part, to an observed overall lack of exposure to inflammatory bone pathology during pathology training. The myriad histologic changes that can be identified in wounds, repair, and infections are also limited in their descriptions in general pathology texts. Through examination of > 400 bone slides with simultaneous correlation with the available literature,

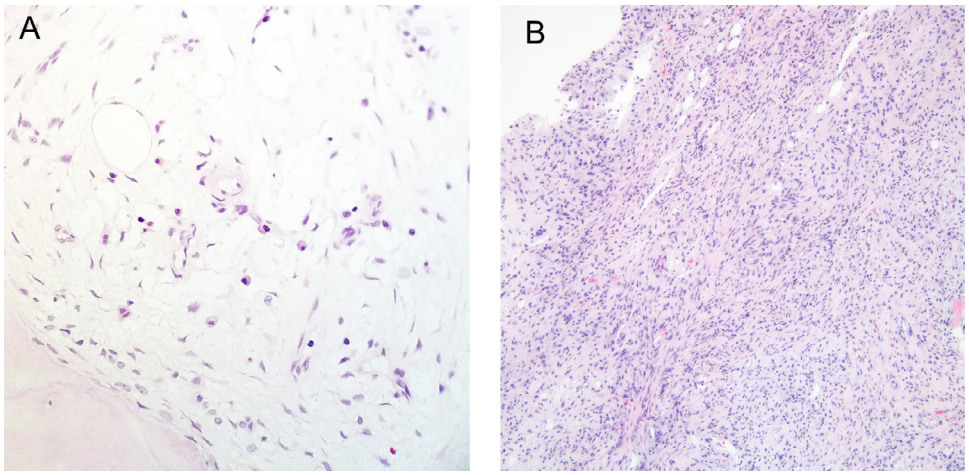

Fig. 5. Fibroplasia. (*A*) Early fibroplasia: loosely organized fibrous tissue with scattered plasma cells (magnification ×10, hematoxylin-eosin [H&E] stain). (*B*) Late fibroplasia: dense and organized fibrosis with scattered chronic inflammation (magnification ×4, H&E stain).

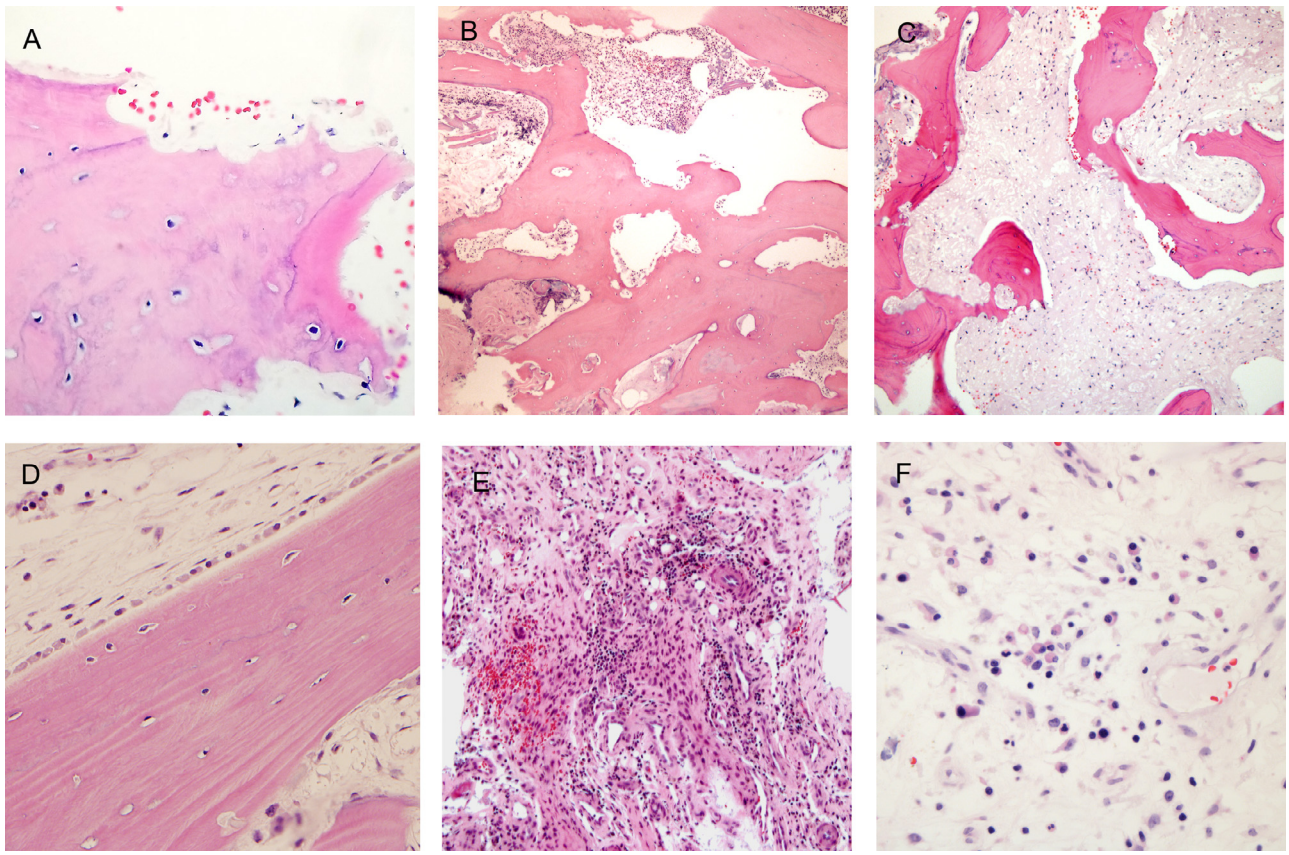

Fig. 6. General criteria. (*A*) Erosion: surface of bone shows scalloping (magnification ×10, H&E stain). (*B*) Destruction: bone demonstrates "punched out" appearance (magnification ×4, hematoxylin-eosin [H&E] stain). (*C*) Necrotic bone: bone with features of avascular necrosis in a background of fibrinoid necrosis (magnification ×4, H&E stain). Note the empty lacunae and lack of fibrosis. (*D*) Remodeling: note the prominent osteoblastic rimming (magnification ×4, H&E stain). (*E*) Granulation tissue: proliferation of small blood vessels in a background of dense marrow fibrosis and inflammation (magnification ×10, H&E stain). (*F*) Plasmacytic inflammation (magnification ×40, H&E stain). For thick periosteal reactions, see Fig. 4.

we were able to observe the spectrum of OM from early infection to end-stage fibrosis (Fig. 1). Infection of the bone follows a similar course and results in similar tissue reactions as other tissues of the body, with some differences that are unique to bone.

OM in the foot and ankle typically begins by direct extension of a skin infection or, less commonly, by direct traumatic inoculation. Like other tissues, the presence of foreign organisms and dying cells leads to release of chemical mediators, beginning a complex cascade of events facilitating recruitment of the immune response (24). Vasodilation followed by migration of neutrophils is the earliest indication of OM. Neutrophils begin to superficially erode bone (Fig. 2*A*, Fig. 6*A*) where bacteria have colonized (Fig. 2*D*), recruiting macrophages. Their combined oxidative burst causes tissue damage, including destruction of vessel walls, resulting in segmental bone infarcts and the appearance of necrotic bone fragments. Increased vascular permeability allows fibrin to pass into the marrow space, creating a fibrinous exudate. This is early AOM (Fig. 1*A*).

Progressive infection leads to further bone damage, which takes on a punched-out appearance (destruction) (Fig. 6*B*). Additional recruitment of immune factors and tissue damage leads to abscess formation (Fig. 2*B*), and suppurative inflammation composed of neutrophils, fibrin, and dead cellular material (fibrinoid necrosis) (Fig. 2*C*, Fig. 6*C*), particularly in cases of pyogenic bacteria, like *Staphylococcus aureus* (the most common causative organism in OM) (Fig. 2*D*) (24). This is fulminant AOM (Fig. 1*B*) (23). The continued release of inflammatory mediators by neutrophils and macrophages results in recruitment of plasma cells (Fig. 6*F*) and activation of fibroblasts, which begin laying down collagen in the marrow space, leading to progressive marrow fibrosis (Fig. 5*A*, *B*) (24). Histologically, we begin seeing the features of early ACOM (Fig. 1*C*).

As the acute inflammatory onslaught continues, subperiosteal abscesses and further vascular destruction lead to fragmentation of necrotic bone into the soft tissues and marrow space (sequestrum) (Fig. 3*A*, *B*) and draining fistulae into the soft tissues (Fig. 2*B*). Simultaneous with the lifting of the periosteum, the living periosteum around fragments of sequestra undergoes rapid, reactive growth (involucrum and rapid periosteal reaction) (Fig. 3*C*, Fig. 4*A*). Inflammatory mediators and bone destruction activate osteoclasts, which begin the process of bone remodeling (Fig. 6*D*). Release of calcium from bones for immune modulation results in local osteopenia. Ischemia and fibroblasts signal vascular endothelial growth factor, and neovascularization accelerates (granulation tissue) (Fig. 6*E*) (24). At this point, the biopsy sample will show fulminant ACOM (Fig. 1*C*).

The acute inflammatory reaction eventually fades, and neutrophils are replaced by plasma cells (Fig. 6*F*) and osteoclastic giant cells, and the marrow is replaced by fibrosis of increasing density (Fig. 5*B*) (24). Histologically, this is CAOM (Fig. 1*D*), which eventually becomes COM after the complete resolution of neutrophils, abscesses, and fibrinoid necrosis (Fig. 1*D*–*E*). As the infectious organism is eliminated or enters osteoblasts, the initial acute periosteal reaction slows and thickens, resulting in various patterns of thick, circumferential lamellae (Fig. 4*B*, *C*), whereas progressive remodeling leads to irregular, dense bone thickening with abnormal cement lines (Fig. 3*A*, Fig. 6*A*) (34). Damaged cortical bone becomes sclerotic, and fracture calluses appear at the sites of fractures and fistulae. Soft tissue and marrow sequestra are resorbed, osteoclastic giant cells disappear, and plasma cells decrease to a mild

infiltrate. This is histologically and radiologically consistent with CIOM (Fig. 1*F*) (29).

Chronically damaged, scarred tissues and chronic disease states, such as diabetes, peripheral neuropathy, and peripheral vascular disease, increase the risk of reinfection or reactivation of low-grade, intracellular infections after antibiotic cessation. This would appear as COM, with patchy foci of neutrophils, fibrinoid necrosis, microabscesses, and rapid periosteal reaction. Histologically and radiologically, this is CAOM (34).

Jupiter Score

The score provides a guide for the pathologist to make more consistent diagnoses by providing specific criteria to reduce the use of descriptive diagnoses and by considering the various pieces of a pathologic assessment in their totality, independent of access to culture or radiology results. Importantly, only those cases falling between a score of 4 and 6 points should result in descriptive diagnoses, and then, assuming no mass lesion is identified by radiology, a specific list of differential diagnoses is preferable (reactive/repair, CIOM, treated low-grade OM, neuropathic arthropathy, and chronic fracture). Finally, clinicians can easily understand the scoring system: initial results indicate that a score of ≥ 6 means that there is histopathologic evidence of OM, whereas a score of ≤ 4 means the sample is likely not OM. A score of 5, although harder to understand, should be given a nonspecific/descriptive diagnosis with a list of differential diagnoses, such as those described in earlier sections of the discussion. Although there is room for improvement and addition of further criteria to improve discrimination between the various types of OM, this study provides evidence of the utility of our score as a diagnostic tool that could significantly improve communication between pathologists and clinicians through clarity and simplification.

Study Limitations

Our study does have several limitations. First, as with any literature search, we may have missed references of interest. Second, we have data from only 1 center, and diagnostic patterns may be different at different institutions. However, we did not control for variability in diagnoses between pathologists at our institution. Strengths of the study include large sample size and allowing examination of suspected OM rather than being restricted to confirmed cases. Future work guided by this initial report will include replicating the study using other institutions' data. Further, the ability of pathologists to reliably and repeatably apply the algorithm needs to be assessed.

In conclusion, we have developed a histopathologic scoring system that demonstrates the potential to improve accuracy and consistency in the diagnosis of OM, improve interpathologist agreement, and assist pathologists in diagnostically challenging cases. We do, however, recommend a multidisciplinary approach to the diagnosis of OM that incorporates microbiology studies, radiology, and clinical findings. In that regard, our scoring system may be exceedingly useful in conjunction with other scoring systems that take into account a multidisciplinary approach. Anemia may play a more significant role in the progression of OM and have a negative impact on patient outcomes. Additional research into the role of anemia in these processes is needed. Finally, we have defined specific histopathologic criteria for diagnosis of 5 categories of OM: AOM, ACOM, COM, CAOM, and CIOM. Using pure fibroplasia with plasmacytic inflammation is insufficient to render a diagnosis of COM, which we have defined as the presence of sequestrum and/or involucrum with marrow fibroplasia, bone remodeling, and plasmacytic inflammation. In the absence of involucrum or sequestrum, a diagnosis of CIOM can be rendered but listed in a specific differential that should include neuropathic arthropathy and repair. Consensus among experts is required to determine the use of CAOM and CIOM as pathologic diagnostic categories.

Acknowledgments

We would like to thank Mitchell Moosavi, MD, for his extensive work reviewing patient charts during the development of our tool, with assistance from Lindsey A. Stockton, MD.

V.O. Speights, DO, our coauthor, dear friend, and colleague, passed away during the course of this study. When most would have spent their last weeks with family, Dr Speights dedicated himself to completing this study, understanding the impact it could have on the future of patient care. A past president of the Texas Society of Pathologists, an active member of numerous committees in Texas and for the College of American Pathologists, and recipient of numerous awards, Dr Speights was one of the strongest advocates for physician trainees, the pathology profession, and patient access to care. His impact in the medical profession over the years is only surpassed by his graciousness and compassion, and although he is profoundly missed, his legacy is evident in the lives of all of those he touched.

Supplementary Materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1053/j.jfas.2019.06.007.

References

1. Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. JAMA 2005;293:217–228.
2. Berendt AR, Peters EJ, Bakker K, Embil JM, Eneroth M, Hinchliffe RJ, Jeffcoate WJ, Lipsky BA, Senneville E, Teh J, Valk GD. Diabetic foot osteomyelitis: a progress report on diagnosis and a systematic review of treatment. Diabetes Metab Res Rev 2008;24 (suppl 1):S145–S161.
3. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, Armstrong DG, Deery HG, Embil JM, Joseph WS, Karchmer AW, Pinzur MS, Senneville E, Infectious Diseases Society of A. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis 2012;54:e132–e173.
4. Lipsky BA, Peters EJ, Senneville E, Berendt AR, Embil JM, Lavery LA, Urbancic-Rovan V, Jeffcoate WJ. Expert opinion on the management of infections in the diabetic foot. Diabetes Metab Res Rev 2012;28(suppl 1):163–178.
5. Malhotra R, Chan CS, Nather A. Osteomyelitis in the diabetic foot. Diabet Foot Ankle 2014;5. https://doi.org/10.3402/dfa.v3405.24445.
6. Jeffcoate WJ, Lipsky BA. Controversies in diagnosing and managing osteomyelitis of the foot in diabetes. Clin Infect Dis 2004;39(suppl 2):S115–S122.
7. Senneville E, Melliez H, Beltrand E, Legout L, Valette M, Cazaubiel M, Cordonnier M, Caillaux M, Yazdanpanah Y, Mouton Y. Culture of percutaneous bone biopsy specimens for diagnosis of diabetic foot osteomyelitis: concordance with ulcer swab cultures. Clin Infect Dis 2006;42:57–62.
8. Elamurugan TP, Jagdish S, Kate V, Chandra Parija S. Role of bone biopsy specimen culture in the management of diabetic foot osteomyelitis. Int J Surg 2011;9:214–216.
9. Akinyoola AL, Adegbehingbe OO, Aboderin AO. Therapeutic decision in chronic osteomyelitis: sinus track culture versus intraoperative bone culture. Arch Orthop Trauma Surg 2009;129:449–453.
10. Senneville E, Morant H, Descamps D, Dekeyser S, Beltrand E, Singer B, Caillaux M, Boulogne A, Legout L, Lemaire X, Lemaire C, Yazdanpanah Y. Needle puncture and transcutaneous bone biopsy cultures are inconsistent in patients with diabetes and suspected osteomyelitis of the foot. Clin Infect Dis 2009;48:888–893.
11. Weiner RD, Viselli SJ, Fulkert KA, Accetta P. Histology versus microbiology for accuracy in identification of osteomyelitis in the diabetic foot. J Foot Ankle Surg 2011;50: 197–200.
12. Lipsky BA. Osteomyelitis of the foot in diabetic patients. Clin Infect Dis 1997;25: 1318–1326.
13. Meyr AJ, Singh S, Zhang X, Khilko N, Mukherjee A, Sheridan MJ, Khurana JS. Statistical reliability of bone biopsy for the diagnosis of diabetic foot osteomyelitis. J Foot Ankle Surg 2011;50:663–667.
14. Pineda C, Vargas A, Rodriguez AV. Imaging of osteomyelitis: current concepts. Infect Dis Clin North Am 2006;20:789–825.
15. Guhlmann A, Brecht-Krauss D, Suger G, Glatting G, Kotzerke J, Kinzl L, Reske SN. Chronic osteomyelitis: detection with FDG PET and correlation with histopathologic findings. Radiology 1998;206:749–754.
16. Lavery LA, Armstrong DG, Peters EJ, Lipsky BA. Probe-to-bone test for diagnosing diabetic foot osteomyelitis: reliable or relic? Diabetes Care 2007;30:270–274.
17. Fujii M, Terashi H, Tahara S. Efficacy of magnetic resonance imaging in diagnosing osteomyelitis in diabetic foot ulcers. J Am Podiatr Med Assoc 2014;104:24–29.
18. Kowalski TJ, Matsuda M, Sorenson MD, Gundrum JD, Agger WA. The effect of residual osteomyelitis at the resection margin in patients with surgically treated diabetic foot infection. J Foot Ankle Surg 2011;50:171–175.

19. Tentolouris N, Al-Sabbagh S, Walker MG, Boulton AJ, Jude EB. Mortality in diabetic and nondiabetic patients after amputations performed from 1990 to 1995: a 5-year follow-up study. Diabetes Care 2004;27:1598–1604.
20. Ragnarson Tennvall G, Apelqvist J. Health-economic consequences of diabetic foot lesions. Clin Infect Dis 2004;39(suppl 2):S132–S139.
21. Wold L, Unni KK, Slim F, Sundaram M. *Atlas of Orthopedic Pathology*. 3rd ed, Saunders, Philadelphia, PA; 2008.
22. Bureau USC. Population estimates, July 1, 2018, (V2018). 2016. https://www.census.gov/quickfacts/fact/table/tx/PST045218. Accessed February 13, 2019.
23. Klein MJ, Bonar SF, Freemont T, Tuyethoa NV, Lopez-Ben R, Siegel HJ, Siegel GP. *Non-neoplastic Diseases of Bones and Joints: Atlas of Nontumor Pathology*. American Registry of Pathology, Annapolis Junction, MD; 2012.
24. Kumar V, Abbas AK, Robbins SL, Cotran RS. *Robbins and Cotran Pathologic Basis of Disease*. 7th ed, Elsevier Saunders, Philadelphia, PA; 2005.
25. Rosai J, Ackerman LV. *Rosai and Ackerman's Surgical Pathology*. 10th ed, Mosby, Edinburgh/New York; 2011.
26. Turi GK, Donovan V, DiGregorio J, Criscitelli TM, Kashan B, Barrientos S, Balingcongan JR, Gorenstein S, Brem H. Major Histopathologic Diagnoses of Chronic Wounds. Adv Skin Wound Care 2016;29:376–382.
27. Cecilia-Matilla A, Lazaro-Martinez JL, Aragon-Sanchez J, Garcia-Morales E, Garcia-Alvarez Y, Beneit-Montesinos JV. Histopathologic characteristics of bone infection complicating foot ulcers in diabetic patients. J Am Podiatr Med Assoc 2013;103:24–31.
28. Hayes CS, Heinrich SD, Craver R, MacEwen GD. Subacute osteomyelitis. Orthopedics 1990;13:363–366.
29. Hankin D, Bowling FL, Metcalfe SA, Whitehouse RA, Boulton AJ. Critically evaluating the role of diagnostic imaging in osteomyelitis. Foot Ankle Spec 2011;4:100–105.
30. Lew DP, Waldvogel FA. Osteomyelitis. Lancet 2004;364:369–379.
31. Lew DP, Waldvogel FA. Osteomyelitis. N Engl J Med 1997;336:999–1007.
32. Lipsky BA. Bone of contention: diagnosing diabetic foot osteomyelitis. Clin Infect Dis 2008;47:528–530.
33. Jennin F, Bousson V, Parlier C, Jomaah N, Khanine V, Laredo JD. Bony sequestrum: a radiologic review. Skeletal Radiol 2011;40:963–975.
34. Ragsdale BD, Madewell JE, Sweet DE. Radiologic and pathologic analysis of solitary bone lesions. Part II: periosteal reactions. Radiol Clin North Am 1981;19:749–783.
35. McNally MA, Ferguson JY, Lau AC, Diefenbeck M, Scarborough M, Ramsden AJ, Atkins BL. Single-stage treatment of chronic osteomyelitis with a new absorbable, gentamicin-loaded, calcium sulphate/hydroxyapatite biocomposite: a prospective series of 100 cases. J Bone Joint Surg Br 2016;98:1289–1296.
36. Kanoun ML, Khorbi A, Khmiri C, Tebourbi A, Hadded N, Boughzala W, Ben Maitig M, Chebil M, Hachem A, Essadem H. [Diagnosis and treatment of Brodie's abcess in adults: about twenty cases]. Tunis Med 2007;85:857–861.
37. Tiemann A, Hofmann GO, Krukemeyer MG, Krenn V, Langwald S. Histopathological Osteomyelitis Evaluation Score (HOES) - an innovative approach to histopathological diagnostics and scoring of osteomyelitis. GMS Interdiscip Plast Reconstr Surg DGPW 2014;3:Doc08.
38. Nielsen GP, Rosenberg A. *Diagnostic Pathology*. Bone,1st ed, Amirsys, Salt Lake City, UT; 2013.
39. Tsaras G, Maduka-Ezeh A, Inwards CY, Mabry T, Erwin PJ, Murad MH, Montori VM, West CP, Osmon DR, Berbari EF. Utility of intraoperative frozen section histopathology in the diagnosis of periprosthetic joint infection: a systematic review and meta-analysis. J Bone Joint Surg Am 2012;94:1700–1711.
40. Bauer TW, Parvizi J, Kobayashi N, Krebs V. Diagnosis of periprosthetic infection. J Bone Joint Surg Am 2006;88:869–882.
41. Radiology L. Acute osteomyelitis. Available at: http://learningradiology.com/notes/bonenotes/osteomyeltispage.htm. Accessed February 13, 2018.
42. Krasnokutsky MV. The button sequestrum sign. Radiology 2005;236:1026–1027.
43. Roderick MR, Ramanan AV. Chronic recurrent multifocal osteomyelitis. Adv Exp Med Biol 2013;764:99–107.
44. Henke PK, Blackburn SA, Wainess RW, Cowan J, Terando A, Proctor M, Wakefield TW, Upchurch GR Jr., Stanley JC, Greenfield LJ. Osteomyelitis of the foot and toe in adults is a surgical disease: conservative management worsens lower extremity salvage. Ann Surg 2005;241:885–892. discussion 892–884.
45. Trouillet-Assant S, Lelievre L, Martins-Simoes P, Gonzaga L, Tasse J, Valour F, Rasigade JP, Vandenesch F, Muniz Guedes RL, Ribeiro de Vasconcelos AT, Caillon J, Lustig S, Ferry T, Jacqueline C, Loss de Morais G, Laurent F. Adaptive processes of Staphylococcus aureus isolates during the progression from acute to chronic bone and joint infections in patients. Cell Microbiol 2016;18:1405–1414.
46. Kobayashi H, Oethinger M, Tuohy MJ, Hall GS, Bauer TW. Distinction between intact and antibiotic-inactivated bacteria by real-time PCR after treatment with propidium monoazide. J Orthop Res 2010;28:1245–1251.
47. Mills SE. *Histology for Pathologists*. 4th ed, Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, PA; 2012.
48. Kataoka M, Torisu T, Tsumura H, Hirayama T, Fujikawa Y. Role of multinuclear cells in granulation tissue in osteomyelitis: immunohistochemistry in 66 patients. Acta Orthop Scand 2000;71:414–418.