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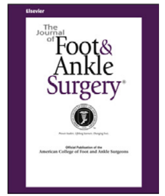

Are We Misdiagnosing Diabetic Foot Osteomyelitis? Is the Gold Standard Gold?

Lawrence A. Lavery, DPM, MPH, FACFAS[1], P. Andrew Crisologo, DPM, AACFAS[2], Javier La Fontaine, DPM, MS, FACFAS[1], Kavitha Bhavan, MD[3], Orhan K. Oz, MD, PhD[4], Kathryn E. Davis, PhD[5]

[1] Professor, Department of Plastic Surgery, University of Texas Southwestern Medical Center, Dallas, TX
[2] Fellow, Department of Plastic Surgery, University of Texas Southwestern Medical Center, Dallas, TX
[3] Associate Professor, Department of Internal Medicine, Infectious Diseases, University of Texas Southwestern Medical Center, Dallas, TX
[4] Professor, Department of Radiology, University of Texas Southwestern Medical Center, Dallas, TX
[5] Assistant Professor, Department of Plastic Surgery, University of Texas Southwestern Medical Center, Dallas, TX

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ABSTRACT

To compare the incidence of osteomyelitis based on different operational definitions using the gold standard of bone biopsy, we prospectively enrolled 35 consecutive patients who met the criteria of ≥21 years of age and a moderate or severe infection based on the Infectious Diseases Society of America classification. Bone samples were obtained from all patients by percutaneous bone biopsy or intraoperative culture if the patient required surgery. Bone samples were analyzed for conventional culture, histology, and 16S ribosomal RNA genetic sequencing. We evaluated 5 definitions for osteomyelitis: 1) traditional culture, 2) histology, 3) genetic sequencing, 4) traditional culture and histology, and 5) genetic sequencing and histology. There was variability in the incidence of osteomyelitis based on the diagnostic criteria. Traditional cultures identified more cases of osteomyelitis than histology (68.6% versus 45.7%, $p = .06$, odds ratio [OR] 2.59, 95% confidence interval [CI] 0.98 to 6.87), but the difference was not significant. In every case that histology reported osteomyelitis, bone culture was positive using traditional culture or genetic sequencing. The 16S ribosomal RNA testing identified significantly more cases of osteomyelitis compared with histology (82.9% versus 45.7%, $p = .002$, OR 5.74, 95% CI 1.91 to 17.28) and compared with traditional cultures but not significantly (82.9% versus 68.6%, $p = .17$, OR 2.22, 95% CI 0.71 to 6.87). When both histology and traditional culture (68.6%) or histology and genetic sequencing cultures (82.9%) were used to define osteomyelitis, the incidence of osteomyelitis did not change. There is variability in the incidence of osteomyelitis based on how the gold standard of bone biopsy is defined in diabetic foot infections.

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Diabetic foot osteomyelitis is a serious complication with increased risk of amputation, prolonged exposure to antibiotic therapy, and extended hospitalization (1). Misdiagnosis of osteomyelitis could expose patients to unnecessary antibiotics, surgery, and amputation. The gold standard to diagnose osteomyelitis is microbiologic and/or pathologic evaluation of bone (2–4). However, it is unclear whether culture, histology, or both should be used, or whether modern technology would improve the diagnosis of osteomyelitis (5). One more current technique is genetic sequencing 16S ribosomal RNA (rRNA) identification of bacteria. Genetic sequencing has been gaining popularity, especially with the growing concern that the involved pathogenic bacteria may not all be identified, because it does not rely on successful growth of the bacteria. Genetic sequencing for purpose of bacterial identification is not without its drawbacks: it does not make the distinction of living versus dead bacteria, high levels of genetic similarity with 16S rRNA do not necessarily correlate with DNA similarity, it does not traditionally provide susceptibilities, and it deals with multiple (public and private) nucleotide databases (6). This study's aim is to compare the incidence of osteomyelitis based on different operational definitions using bone culture with traditional culture techniques, cultures with genetic sequencing, and histology.

Patients and Methods

We prospectively enrolled 35 patients from July to October 2015 who met the criteria of ≥21 years of age and a moderate to severe infection based on the Infectious Diseases

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Address correspondence to: Lawrence A. Lavery, DPM, MPH, Department of Plastic Surgery, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, 75390 TX.

E-mail address: larry.lavery@utsouthwestern.edu (L.A. Lavery).

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Society of America classification with a suspicion of having diabetic foot osteomyelitis (4). The initial criteria were clinical presentation including a positive probe-to-bone test or a deep infection near bone or joint, radiographic changes, or magnetic resonance imaging (MRI) findings consistent with osteomyelitis. Exclusion criteria included patients with other acute infectious diseases, previously diagnosed osteomyelitis of the foot, organ or hematologic malignancy, or end-stage renal disease requiring dialysis or patients who were on immunosuppressive therapies. This study was approved by the University of Texas Southwestern Medical Center institutional review board (8843) under study number STU 022014-007.

Each patient received standard-of-care medical and surgical treatments as indicated for the infection. At baseline, demographics, medical and surgical history, and neurologic, vascular, and wound examination were documented. The vascular examination included ankle−brachial indices (Koven Technology Inc., St. Louis, MO), skin perfusion pressure measurements, and pulse volume recordings using the Sensilase Pad-IQ system (Väsamed, Eden Prairie, MN) (7,8). The neurologic examination included evaluation with a 10-g Semmes Weinstein monofilament and vibration threshold perception tests (8). Most of the patients received empiric antibiotic coverage with vancomycin and piperacillin/tazobactam on admission while in the emergency department. This was later tapered to pathogen-directed therapy after conventional cultures and sensitivities were obtained.

Bone samples were obtained from all patients by either a percutaneous bone biopsy (n = 10) or intraoperative surgical cultures (n = 25) and sent to the hospital's microbiology lab for conventional culture and to the pathology department for histology examination. For the surgical samples, bone was obtained after incision and drainage were performed and after the surgical site was irrigated with normal saline, using meticulous sample handling to avoid any cross-contamination. Samples were also sent for bacterial 16S rRNA genetic sequencing (Pathogenius Laboratory, Lubbock, TX). The standard approach in our community is to define osteomyelitis if there is either positive bone culture from traditional microbiologic examination or positive histology. We evaluated 5 methods of diagnosing osteomyelitis: 1) traditional culture, 2) histology, 3) genetic sequencing, 4) traditional culture and histology, and 5) genetic sequencing and histology. We compared the demographic and objective data collected among the operational definitions using the χ^2 test with an alpha of 0.05 for categorical variables and analysis of variance for continuous variables to observe trends. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using Microsoft Excel (Microsoft, Redmond, WA).

Results

We identified variabilities in the incidence of osteomyelitis based on the operational definitions that were used for the reference standard (Table). No significant differences were identified in the demographic data among the 3 operational definitions included in the Table. Among the objective data, significant trends were found with dorsal foot skin profusion pressure and erythrocyte sedimentation rate among the groups (Table). In our practice, we define osteomyelitis as having either a positive culture or positive bone histology. Using this approach, the incidence of osteomyelitis was 68.6%. Traditional cultures identified more cases of osteomyelitis than histology alone, although this difference was not observed to be statistically significant (68.6% versus 45.7%, p = .06, OR 2.59, 95% CI 0.98 to 6.87). In every case that histology reported osteomyelitis, the bone culture was positive using traditional culture methods and genetic sequencing. Thus, simply relying on histology did not identify any cases that were missed by traditional cultures.

When genetic sequencing was used to diagnose osteomyelitis, the same phenomenon was observed. The 16S rRNA testing identified more cases of osteomyelitis than histology (82.9% versus 45.7%, p = .002, OR 5.74, 95% CI 1.91 to 17.28), and all the positive histology cases also had positive cultures. When genetic sequencing was used to define osteomyelitis, there was a higher incidence of osteomyelitis, but the difference was not statistically significant compared with traditional cultures (82.9% versus 68.6%, p = .17, OR 2.22, 95% CI 0.71 to 6.87). When both histology and traditional culture (68.6%), or histology and genetic sequencing (82.9%) were used to define osteomyelitis, the incidence of osteomyelitis did not change compared to cultures alone.

Table
Demographics and admission values as stratified by operational definitions of osteomyelitis

Factor	Traditional Histology	Traditional Culture	Genetic Sequencing
Osteomyelitis diagnosis, % (n)	45.7 (16)	68.6 (24)	82.9 (29)
Male sex, % (n)	67.8 (11)	70.8 (17)	75.9 (22)
Median age, y	44.5 (13)	45.5 (16)	46.0 (17)
Body mass index >30 kg/m^2, % (n)	37.5 (6)	50.0 (12)	37.9 (11)
Type 2 diabetes mellitus, % (n)	81.3 (13)	87.5 (21)	86.2 (25)
Glycated hemoglobin >10%, % (n)	31.3 (5)	41.7 (10)	48.3 (14)
Any history of tobacco use, % (n)	56.3 (9)	58.3 (14)	58.6 (17)
History of diabetic foot ulceration, % (n)	81.3 (13)	70.8 (17)	65.5 (19)
Vibrotactile perception threshold >25 Hz, % (n)	75.0 (12)	79.2 (19)	79.3 (23)
Ankle−brachial index <0.9, % (n)	18.8 (3)	20.8 (5)	24.1 (7)
Median ankle−brachial index	1.1 (0.2)	1.08 (0.2)	1.07 (0.2)
SPP great toe, mm Hg	57.0 (58)	69.0 (60)	74.0 (54)
SPP plantar medial forefoot, mm Hg	75.5 (23)	76.5 (28)	77.0 (23)
SPP plantar lateral forefoot, mm Hg	90.5 (53)	88.5 (42)	86.0 (47)
SPP dorsal foot, mm Hg*	94.5 (62)	80.0 (74)	87.0 (62)
White blood cell count on admission, × 10^9/L	8.4 (5.9)	6.7 (5.7)	7.3 (6.5)
Erythrocyte sedimentation rate on admission, mm/h *	97.5 (74)	67.5 (70)	70.0 (69)
C-reactive protein on admission, mg/dL	7.5 (12.8)	7.0 (12.3)	7.7 (12.6)

Values expressed as median (interquartile range as the difference between the third and first quartiles) unless otherwise noted.
* Significant trend based on alpha ≤0.05.

Discussion

No test to identify a disease state is perfect; however, some reference standard is required to define the presence of a disease process. Bone biopsy is the accepted reference standard for diagnosis of diabetic foot osteomyelitis (3,9), but the operational definition of what constitutes a positive bone biopsy has not reached consensus and warrants further discussion. It is a process with well-recognized limitations, but we continue to expect the ideal theoretical reference standard. The results of this study suggest that there is considerable variability in the incidence of osteomyelitis based on which operational definition of the gold standard was used. Genetic sequencing is a more sensitive method to identify bacterial pathogens (10,11) than traditional culture techniques. The highest incidence of osteomyelitis was based on genetic sequencing with bacterial 16S rRNA (82.9%). Traditional bacterial cultures alone identified an incidence of osteomyelitis of 68.6%. The lowest incidence of osteomyelitis was reported when histology was the sole criterion (45.7%). Histology did not identify any new cases that were missed by traditional cultures or genetic sequencing.

These tests have limitations. Genetic sequencing identifies all the bacterial genetic material in the wound, from both living and dead pathogens, but the test does not provide antibiotic sensitivity data, whereas traditional culture methods may not be able to effectively grow certain pathogens, such as anaerobes, in the laboratory. Traditional bone cultures theoretically could be affected by systemic antibiotic treatment before cultures are obtained, and there is a concern that this could reduce culture yield. The common perception is to hold antibiotics before bone biopsy; however, this practice does not have convincing evidence (12−23). Pathogen-directed therapy has been reported to have a higher rate of success, so regardless, cultures are needed to plan therapy. Perhaps one of the reasons for the high rate of treatment failures for osteomyelitis is that pathogen-directed therapy is not used (24).

We previously reported that genetic sequencing identified significantly more pathogens, especially anaerobic pathogens, in patients with osteomyelitis (10). Likewise, in a report that compared traditional cultures and genetic sequencing in diabetic foot ulcers, the number and diversity of pathogens was significantly higher when 16S rRNA genetic sequencing was used (11). Both bone culture techniques could be contaminated if the specimen were obtained through abscess or infected soft tissue. Another source of potential contamination is if contaminated instruments are used to obtain a clean margin sample or if the back table does not maintain proper attention to sterile technique and

specimen handling. In patients with percutaneous bone biopsy, it is important to set up a sterile field with adequate preparation of the site and obtain the bone specimen 2 cm away from any open wound to avoid cross-contamination (25).

Histologic examination has a relatively subjective criterion for diagnosing osteomyelitis. There are several reports that discuss poor interobserver reliability of histologic examination for osteomyelitis (26,27) and other disease processes (28–30). Surprisingly, in this study, histology was positive for osteomyelitis every time the bone culture was positive. Other studies report contradictory findings. For instance, Weiner et al (27) reported disagreement in 34% of cases (15 of 44) based on microbiologic and histologic diagnosis. In contrast, a study by Cecilia-Matilla et al (31) endorsed an excellent interrater reliability rating when well-defined criteria were used for acute osteomyelitis, chronic osteomyelitis, and acute or chronic osteomyelitis, with kappa indices of 0.97, 0.95, and 0.92, respectively. In 7 of these cases, histology was positive and bone cultures were negative, and in 8 cases, cultures were positive and histology was negative.

Practice guidelines for diabetic foot infections recommend using bone culture and/or histology to diagnose osteomyelitis. The Infectious Disease Society of America suggests that osteomyelitis is optimally defined by histology and culture (4), and the International Working Group on the Diabetic Foot (3) states that “definitive diagnosis usually requires positive results on microbiological (and, optimally, histological) examination.” However, bone biopsy is the exception rather than the rule in osteomyelitis publications. There is variability in the use of bone culture and bone histology in the published work on osteomyelitis. Many studies used a combination of probe to bone, radiographs, MRI, bone scans, and even clinical judgment as criteria (32–37) to define cases of osteomyelitis, without bone biopsy to verify the diagnosis or identify the pathogen. Radiographic changes and probe-to-bone testing would likely identify chronic osteomyelitis with severe bone destruction, but it would probably miss subtle cases of acute osteomyelitis before radiographic changes are seen and when probe-to-bone testing is negative. In contrast, imaging techniques are more sensitive and likely to identify early bone changes. However, there can be high rates of false-positive results when MRI (20.6%) and single-photon emission computed tomography (SPECT) (26.9%) are used to identify osteomyelitis (38). Thus, the risk of misdiagnosing and overtreating a soft tissue infection as a bone infection are high when SPECT and MRI are used, and acute osteomyelitis may be missed more frequently when radiographs and probing the ulcer are used to define osteomyelitis.

Even in prospective studies, the gold standard is not always used. We identified 11 prospective studies of osteomyelitis; 6 of the studies used bone biopsy to define the disease. Tone et al (39) and Shults et al (40) used positive culture from bone biopsy to define osteomyelitis in a randomized controlled trial that evaluated different durations of therapy to treat osteomyelitis and in a study that compared radiographs, bone scans, and wound cultures. Enderle et al (41) and Wang et al (42) used bone histology to define osteomyelitis in studies that evaluated ultrasound and MRI to diagnose osteomyelitis. Newman et al (43) used either histology or bone culture in a study to evaluate leukocyte scans to diagnose osteomyelitis. Cecilia-Matilla et al (31) used microbiology as well as histology for diagnosis. The other studies used a combination of tests to define osteomyelitis. Lazaro-Martinez et al (32) and Vouillarmet et al (36) used a combination of the probe-to-bone test and radiographs without verification of bone culture results in prospective studies of osteomyelitis outcomes. Grayson et al (34), Croll et al (44), and Johnson et al (6) used a combination of bone culture, histology, clinical follow-up, or radiographs to define osteomyelitis in studies to evaluate probing to bone, MRI, and bone scans, respectively.

Limitations to this study are not to be overlooked. This study cannot identify a superior operational definition, because it is underpowered and a pilot study. For example, the lack of statistical significance between traditional cultures and genetic sequencing is probably owing to a type B error. The aim is not to be misconstrued as to define accuracy of the operational definitions but rather to report on relative sensitivities of these for diagnosis of osteomyelitis. Although the intricacies of traditional culture are outside the purview of this study, traditional culture has its own limits, because it relies on the ability to grow the organism and then identify it based on metabolic and phenotypic characteristics of the bacteria. Traditional culture methods are also known to be difficult for growing anaerobic organisms. Although genetic sequencing methods appear to be more efficient at identifying difficult-to-culture organisms (because this method does not rely on growing the organism), they have their own detriments, such as the inability to identify whether the organism is alive or dead and the general lack of susceptibilities. Histopathologic diagnosis of osteomyelitis, although not limited by the ability to grow an organism, is limited by suboptimal interrater agreement as discussed previously.

In conclusion, every test to diagnose a disease process is flawed. The results of this study use the relatively new technology of genetic sequencing to add to the discussion. This study demonstrates the variability in the diagnosis of osteomyelitis, even when different criteria using bone biopsy are implemented. For example, studies report widely varying pathogen recovery, with 50% to 90% of patients with vertebral osteomyelitis (45–47); in the diabetic foot, that number has reached as high as 95% (17). Flaws in the interpretation of the reference standard to diagnose diabetic foot osteomyelitis are important to identify. As identified in this pilot study, depending on which operational definition was used within the accepted reference standard of bone biopsy, the diagnosis of diabetic foot osteomyelitis changed up to 37%. This study also identifies processes that we need to improve. Given its limitations, genetic sequencing is not a viable reference standard alone, and it may be more prudent to use traditional culture to have the benefit of sensitivities. But in contrast, traditional cultures may miss important pathogens that genetic sequencing could identify. Future investigation should be made to address the current shortcomings of genetic sequencing, such as determining whether the pathogen is alive and alternate methods to determine susceptibilities. Furthermore, standardization of the histopathologic evaluation of bone for signs of osteomyelitis may address the apparent discrepancy between histologic and traditional culture diagnosis of diabetic foot osteomyelitis.

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