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

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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 either by percutaneous bone biopsy, or by intraoperative culture if the patient required surgery. Bone samples were analyzed for conventional culture, histology, and 16S rRNA genetic sequencing. We evaluated five definitions for osteomyelitis: 1. traditional culture 2. histology 3. genetic sequencing 4. traditional culture and histology 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% vs. 45.7%, p=0.06, OR 2.59, 95% CI 0.98–6.87) but not significant. In every case that histology reported osteomyelitis, bone culture was positive using traditional culture or genetic sequencing. 16S rRNA testing identified significantly more cases of osteomyelitis compared to histology (82.9% vs. 45.7%, p=0.002, OR 5.74, 95% CI 1.91–17.28) and more compared to traditional cultures but not significant (82.9% vs. 68.6%, p=0.17, OR 2.22, 95% CI 0.71–6.87). 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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Keywords

diabetes; diagnosis; biopsy; foot ulcer; infection; osteomyelitis

Introduction

Diabetic foot osteomyelitis (DFO) is a serious complication with an 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 microbiological and/or pathological evaluation of bone (2–4). However, it is unclear if culture, histology, or both should be used, or if modern technology would improve the diagnosis of osteomyelitis (5). One such method of a more current technique is genetic sequencing 16S rRNA identification of bacteria. Genetic sequencing has been gaining popularity, especially with the growing concern that the involved pathogenic bacteria may not be all identified as it does not rely on successful growth of the bacteria. Genetic sequencing for purpose of bacterial identification is not without its drawbacks as it does not make the distinction of living versus dead bacteria, may have high levels of genetic similarity with 16S rRNA that doesn't correlate with DNA similarity, does not traditionally provide susceptibilities, and 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/or histology.

Patients and Methods

We prospectively enrolled 35 patients from July 2015 to October 2015 who met the criteria of ≥21 years of age and a moderate to severe infection based on the Infectious Diseases Society of America (IDSA) 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, with previously diagnosed osteomyelitis of the foot, on immunosuppressive therapies, organ or hematological malignancy, and end stage renal disease requiring dialysis. This study had institutional review board approval prior to enrolling patients.

Patients received standard of care medical and surgical treatments as indicated for their infection. At baseline, demographics, medical and surgical history, as well as neurological, vascular and wound examination were documented. The vascular exam included ankle brachial indices (ABI) (Koven Technology Inc., St. Louis, MO, USA), skin perfusion pressure measurements, and pulse volume recordings using the Sensilase Pad-IQ system (Väsamed, Eden Prairie, MN, USA) (7, 8). The neurological exam included evaluation with a 10g 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 was performed and after the surgical site was irrigated with normal saline and with meticulous sample handling to try to avoid any cross contamination. Samples were also sent for bacterial 16S rRNA genetic sequencing [Pathogenius Laboratory, (Lubbock, Texas)]. The standard approach in our community is to define osteomyelitis if there is either positive bone culture from traditional microbiological examination or positive histology. We evaluated five 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 demographical and objective data collected among the operational definitions using χ^2 test with an alpha of 0.05 for categorical variables and ANOVA for continuous variables to observe trends. Odds ratios and 95% confidence intervals were calculated using Microsoft Excel (Redmond, WA).

Results

We identified variabilities in the incidence of osteomyelitis based on the operational definitions that were used for the reference standard (Table 1). No significant differences were identified in the demographical data among the three operational definitions included in Table 1. Among the objective data, significant trends were found with dorsal foot skin perfusion pressures and erythrocyte sedimentation rate among the groups (Table 1). In our group, we define osteomyelitis as having either a positive culture or positive bone histology to define osteomyelitis. 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% vs. 45.7%, p=0.06, OR 2.59, 95% CI 0.98–6.87). In every case that histology reported osteomyelitis, the bone culture was positive using traditional culture methods and genetic sequencing. So 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. 16S rRNA testing identified more cases of osteomyelitis compared to histology (82.9% vs. 45.7%, p=0.002, OR 5.74, 95% CI 1.91–17.28), 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 it was not statistically significant compared to traditional cultures (82.9% vs. 68.6%, p=0.17, OR 2.22, 95% CI 0.71–6.87). 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.

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) compared to 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 criteria (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 while traditional culture methods may not be able to effectively grow certain pathogens in the laboratory, such as anaerobes. 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 to hold antibiotics prior to bone biopsy; however, 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 is 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 help 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 and colleagues reported disagreement in 34% of cases (15 of 44) based on microbiologic and histological diagnosis

(27). In contrast, a study by Cecilia-Matilla et al., endorsed an excellent inter-rater reliability rating when well-defined criteria were used for acute osteomyelitis, chronic osteomyelitis and acute on chronic osteomyelitis with kappa indices of 0.97, 0.95, and 0.92 respectively (31). 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 states “definitive diagnosis usually requires positive results on microbiological and optimally, histological examination (3).” 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 SPECT-CT (26.9%) are used to identify osteomyelitis (38). So, the risk of misdiagnosing and over-treating a soft tissue infection as a bone infection are high when SPECT-CT and MRI are used, and acute osteomyelitis may be missed more frequently when x-rays and probing the ulcer are used to define osteomyelitis.

Even in prospective studies the gold standard is not always used. We identified eleven prospective studies of osteomyelitis; six of the studies used bone biopsy to define the disease. Senneville and Shults used positive culture from bone biopsy to define osteomyelitis (39, 40) in an RCT that evaluated different durations of therapy to treat osteomyelitis and in a study that compared radiographs, bone scans and wound cultures. Enderle and Wang used bone histology to define osteomyelitis in studies that evaluated ultrasound and MRI to diagnose osteomyelitis (41, 42). Newman and colleagues used either histology or bone culture in a study to evaluate leukocyte scans to diagnose osteomyelitis (43). Cecilia-Matilla used microbiology as well as histology for diagnosis (31). The other studies used a combination of tests to define osteomyelitis. Lazaro-Martinex (32) and Vouillarmet (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 (34), Croll (44), and Johnson (6) used a combination of bone culture, histology, clinical follow-up, or x-ray 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 as this is underpowered and a pilot study. For example, the lack of statistical significance between traditional cultures and genetic sequencing is probably due to a type B error. The aim is not to be misconstrued as to define accuracy of the operational definitions, but to report on relative sensitivities of these for diagnosis of osteomyelitis.

While the intricacies of traditional culture are outside the purview of this study, traditional culture has its own limits as it relies on the ability to grow the organism and then identify it based off of metabolic and phenotypic characteristics of the bacteria. Traditional culture methods are also known to be difficult for growing anaerobic organisms. While genetic sequencing methods appear to be more efficient at identifying difficult to culture organisms, (as this method doesn't rely on growing the organism) it has its own detriments such as the inability to identify if the organism is alive or dead and the general lack of susceptibilities. Histopathologic diagnosis of osteomyelitis, while not limited by the ability to grow an organism, is limited by suboptimal inter-rater agreement as previously discussed in this study.

Every test to diagnose a disease process is flawed. The results of this study use 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 50–90% of patients with vertebral osteomyelitis (45–47) and 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%. It also identifies processes that we need to try to improve. Given the limitations in genetic sequencing techniques, it is not a viable reference standard alone and it may be more prudent to use traditional culture so as to have the benefit of sensitivities. But in contrast, traditional cultures may miss important pathogens that genetic sequencing could identify. Future investigation should be given to address the current shortcomings of genetic sequencing such as determining if 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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Table 1:

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)

* Significant trend based on alpha ≤0.05.