Pathogens Isolated From Deep Soft Tissue and Bone in Patients With Diabetic Foot Infections

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Background: We sought to determine the similarity of pathogens isolated from soft tissue and bone in patients with diabetic foot infections. It is widely believed that soft-tissue cultures are adequate in the determination of causative bacteria in patients with diabetic foot osteomyelitis. The culture results of specimens taken concurrently from soft-tissue and bone infections show that the former does not predict the latter with sufficient reliability. We sought to determine the similarity of pathogens isolated from soft tissue and bone in patients with diabetic foot infections.

Methods: Forty-five patients with diabetic foot infections were enrolled in the study. Patients had to have clinically suspected foot lesions of grade 3 or higher on the Wagner classification system. In patients with clinically suspected osteomyelitis, magnetic resonance imaging, scintigraphy, or histopathologic examination were performed. Bone and deep soft tissue specimens were obtained from all patients by open surgical procedures under aseptic conditions during debridement or amputation. The specimens were compared only with the other specimens taken from the same patients.

Results: The results of bone and soft-tissue cultures were identical in 49% (n = 22) of cases. In 11% (n = 5) of cases there were no common pathogens. In 29% (n = 13) of cases there were more pathogens in the soft-tissue specimens; these microorganisms included microbes isolated from bone cultures. In four patients (9%) with culture-positive soft-tissue specimens, bone culture specimens remained sterile. In one patient (2%) with culture-positive bone specimen, soft-tissue specimen remained sterile.

Conclusion: Culture specimens should be obtained from both the bone and the overlying deep soft tissue in patients with suspected osteomyelitis whose clinical conditions are suitable. The decision to administer antibiotic therapy should depend on these results. (J Am Podiatr Med Assoc 98(4): 290-295, 2008)

Foot infections are one of the most frequent and severe complications of diabetes and are the most common proximate cause of nontraumatic amputation of the lower extremity in diabetic patients.[1-4] Patients with diabetic foot infections may develop osteomyelitis, which alters the approach to therapy and increases the risk of amputation.[2] Diagnosing osteomyelitis in a diabetic patient with a foot infection can be difficult.[5] Even when the diagnosis of osteomyelitis is proven or highly likely on the basis of noninvasive techniques (magnetic resonance imaging (MRI) or scintigraphy), the causative bacteria should be de-

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termined. Only microbiological examination of a bone specimen can identify pathogenic organisms and guide antibiotic therapy.

Microbiological examination of bone tissue specimens is as sensitive and specific as imaging tests in the diagnosis of osteomyelitis.[6] Microbiological examination is especially important with osteomyelitis because it usually requires prolonged antibiotic therapy. Prognosis of osteomyelitis depends heavily on proper identification and treatment of the bone-infecting organism. Bone biopsy for histopathologic examination is the standard for diagnosing osteomyelitis. If bone is unavailable, therapy is often based on the results of cultures of overlying soft tissues, because osteomyelitis generally develops by contiguous spread of soft-tissue infection to underlying bone.[1, 7] This approach would be acceptable if the etiologic agents in soft-tissue and bone infections were similar. If not, incorrect therapy may lead to adverse drug effects or antibiotic resistance. This aspect of management of the infection is a matter of concern given the present worldwide spread of bacterial resistance.[8] Studies comparing the microbiological results of soft-tissue cultures with those of reliable underlying bone biopsy specimens for patients with diabetes are lacking. This study compared the culture results of specimens taken from soft-tissue and bone infections in diabetic persons with a foot infection.

Materials and Methods

A total of 45 consecutive hospitalized diabetic patients with foot lesions were enrolled in the study for a 2-year period. Patients had to have clinically suspected foot lesions with a grade of 3 or higher according to the Wagner classification system.[9] Informed consent was obtained from all patients. As a part of the clinical evaluation, the size (≥ 2 cm^2) and the depth (≥ 2 cm) of any skin ulceration, C-reactive protein levels, erythrocyte sedimentation rate, and white blood cell counts were noted for each patient. In patients with clinically suspected osteomyelitis, one or more tests were performed. As the first step of investigation, MRI was performed on 28 patients and 4-phase radionuclide bone scanning and labeled leukocyte scanning were performed in 26 patients.

Invasive diagnostic procedures were the second step of investigation. Bone specimens to determine histopathologic characteristics were obtained from 31 patients. Infection in bone and soft tissue was confirmed by histopathology, scintigraphy, or MRI. Microbiological examination of both bone and deep soft tissue (DST) specimens obtained by either open surgical debridement or amputation under aseptic conditions was performed in all patients. All surgical procedures were performed with local or general anesthesia. Both bone and soft-tissue specimens from each patient had to be taken during the same open surgical procedure. Concordance was defined as the finding of exactly the same bacterial species with identical susceptibility pattern in both specimens. None of the patients had received either local or systemic antibiotic therapy for at least 72 hours before the surgical procedure.

Microbiological Processing

Specimens of bone and DST were cultured for anaerobes in Schaedler agar placed in an anaerobic chamber, and for aerobes in 5% sheep blood agar, MacConkey's agar, and Sabouraud's agar. All aerobic and anaerobic plates were incubated for 24 to 48 hours at 35°C. The identification of anaerobic bacteria was performed with An-ident Discs Code DD6 (Oxoid Ltd, Basingstoke, England). Aerobic isolates were stained using Gram's stain. Gram's-negative organisms were identified according to the following properties: dextrose, sucrose, and lactose fermentation; citrate usage; motility; urease and indole production; ornithine decarboxylase activity; and oxidase reaction. Gram's-positive organisms were identified according to the following properties: catalase and hemolysis reaction, coagulase production, optochin, bacitracin and trimethoprim-sulfamethoxazole susceptibility, growth in media that included bile esculine, and growth in media that included 6.5% saline solution. Antibiotic susceptibility of species was determined with a disk-diffusion test as described elsewhere.[10, 11]

Imaging Studies

Magnetic resonance imaging. Twenty-eight patients had MRI with Siemens Vision 1.5 T (Siemens, Erlangen, Germany). We used the presence of high–signal intensity on turbo inversion recovery magnitude, low–signal intensity on T1 sequence, and contrast enhancement to define osteomyelitis.

Radionuclide studies. Multiphase bone scintigraphy coupled with technetium-99m white blood cell scan was performed in 26 patients. All of the images were obtained with a Siemens Orbiter gamma camera (Erlangen, Germany) connected to a Pegasys computer (ADAC, Milpitas, California) equipped with a collimator. Four-phase bone scintigraphy was performed with 740 MBq (20 mCi) technetium-99m methylene diphonate. Twenty-four hours after the bone scintigraphy, technetium-99m white blood cell scans were obtained.

Combined 4P-methylene diphonate and techne-

tium-99m white blood cell scans were considered positive for osteomyelitis when there was an abnormal accumulation of leukocytes in a zone concordant with the area of uptake on bone scintigraphy. These scans were considered negative for osteomyelitis in the presence of abnormal accumulation of leukocytes in a zone not concordant with the area of uptake on bone scintigraphy (soft-tissue infection) or when no leukocyte accumulation was observed (no infection or aseptic inflammation).

Histopathology

Histopathologic examination was performed in 31 patients. Histopathologic diagnosis of osteomyelitis was based on the presence of osteonecrosis and infiltration with leukocytes or chronic inflammatory cells, such as lymphocytes or plasma cells.

Statistical Analyses

Continuous variables were compared with the Student *t* test. The χ^2 test was used for categorical data. Differences between groups were considered to be significant for variables yielding a *P* value less than 0.05.

Results

Among the 45 patients enrolled in the study, 33 (73%) were male and 12 (27%) were female. Table 1 shows demographic and clinical characteristics of the enrolled patients. Fifteen patients (33.3%) had grade 3, 20 patients (44.5%) had grade 4, and 10 patients (22.2%) had grade 5 foot lesions on the Wagner classification system.

All subjects had infection both in bone and soft tissue confirmed through histopathology, scintigraphy, or MRI. We failed to isolate any pathogens in four patients' bone cultures and one patient's DST culture. Cultures of bone yielded 47 microorganisms. The mean number of isolates per bone specimen was 1.04 (range, 1–2). Of the 47 isolates cultured from bone specimens, 23 (49%) were aerobic Gram's stain–positive bacteria, 23 (49%) were aerobic Gram's-negative bacilli, and 1 (2%) was strict anaerobe.

Culture of soft-tissue specimens identified 69 microorganisms. The mean number of isolates per soft-tissue specimen was 1.53 (range, 1–4). Of the 69 isolates cultured from DST specimens, 27 (39%) were aerobic Gram's-positive bacteria, 39 (57%) were aerobic Gram's-negative bacilli, two (3%) were strict anaerobes, and one (1%) was of the genus *Candida*. Overall, pathogens were equally represented in cultures of bone and DST specimens. The percentage of bone cultures yielding *Staphylococcus aureus* (27.6%) is higher than the DST (20.3%). However, the difference was not statistically significant ($P > .05$). A total of 27 *S aureus* isolates were isolated from bone and DST. Of these, 21 were methicillin resistant. The distribution of the pathogens identified in cultures of bone and DST specimens is shown in Table 2.

In six (13%) patients' bone cultures and in 21 (47%) patients' DST cultures, more than one causative microorganism was isolated. The mean number of microorganisms isolated in bone cultures was not significantly lower compared with that isolated in DST cultures: 1.04 versus 1.53 ($P > .05$). Culture results of DST versus bone specimen in the same patient are shown in Table 3. Of note is that culture results for the two specimens were identical in slightly less than half the cases. In almost 10% of cases, the bone was sterile but the DST grew at least one organism. In 11% of cases there were no organisms in common between the two specimens.

Discussion

Bone biopsy is the standard for determining causative bacteria of osteomyelitis.[1, 7, 12-14] Some believe that bone biopsy is unsafe or unnecessary and that culturing the soft tissue near the bone infection is adequate.[15, 16] However, some studies have shown that

Table 1. Demographic and Clinical Characteristics of Enrolled Patients

Variable	Normal Range	Mean ± SD	Range
Age (years)	NA	62.7 ± 9.1	40–83
Duration of diabetes mellitus (years)	NA	17.3 ± 9	1–40
Duration of diabetic foot infection (mo)	NA	3.3 ± 2.8	0.5–12
Erythrocyte sedimentation rate (mm/h)	0–20	89.6 ± 24.6	37–120
C-reactive protein concentration (mg/dL)	0–0.6	7.8 ± 6.2	1–25.3
Creatinine level (mg/dL)	0.6–1.3	1.4 ± 1.3	0.6–7.4
White blood count (cells/mm^3)	4,000–9,000	11,148.4 ± 5,141.2	5,020–31,880

Abbreviation: NA, not applicable.

Table 2. Number of Microorganisms Isolated From Bone and Soft-Tissue Cultures

Microorganism	Soft-Tissue (n = 69)	Bone (n = 47)
Pseudomonas aeruginosa	23	14
Staphylococcus aureus		
Methicillin resistant	11	10
Methicillin sensitive	3	3
Acinetobacter spp	8	5
Enterococcus spp	4	2
Coagulase-negative staphylococci		
Methicillin resistant	3	3
Methicillin sensitive	2	2
Streptococcus spp	4	3
Nonhemolytic	1	2
Alpha hemolytic	1	0
Beta hemolytic	2	1
Proteus mirabilis	1	1
Proteus vulgaris	1	1
Escherichia coli	2	1
Enterobacter spp	2	0
Peptostreptococci	3	1
Serratia marsences	1	1
Candida spp	1	0

Note: More than one organism could be found in one culture.

soft-tissue cultures from close to the bone infection are not adequate.[17, 18] The culture results of specimens taken concurrently from soft-tissue and bone infections show that the former does not predict the latter with sufficient reliability.[1] Because wound cultures may be contaminated by colonizing flora, a percutaneous bone biopsy sample obtained without traversal of an open wound is the ideal method of guidance for antibiotic therapy.[19]

Zuluaga et al[18] found a concordance rate of 28%, which was lower than our result of 49%. They excluded chronic osteomyelitis secondary to diabetic foot infections. The greater variability composition of the colonizing flora of diabetic foot wounds, compared with the composition of fistula of chronic osteomyelitis, may explain the discrepancy.[8]

Senneville et al[8] found a concordance rate of 17.4% in patients with diabetic foot osteomyelitis. Their specimen collection techniques—percutaneous bone biopsy and swab samples from the bottom of the ulcer—were different from those in our study. We obtained DST and bone specimens through open surgical debridement or amputation under aseptic conditions. Lavery et al[20] found that only 13% of cases showed an exact match between the bone and soft-tissue culture results. In 36% of their cases, more pathogens were present in the soft-tissue specimens; in 22% of their cases, more pathogens were present in the bone specimens. In 29% of our cases, more than one pathogen was present in DST specimens, and bone cultures yielded at least one of these pathogens. In 9% of our cases with culture-positive DST specimens, bone culture specimens remained sterile; in one patient (2%) with culture-positive bone specimen, the DST specimen remained sterile (Table 3). Approaching the same problem with different methodologies may lead to different concordance rates. There is still no clear understanding on selecting the best specimen for microbiological diagnosis.

In our study, one patient's bone specimen culture was positive whereas the soft-tissue culture remained sterile. On the other hand, different microorganisms were isolated from the bone and the soft tissue of five patients. The disconcordance rate was 13%. Lavery et al[20] found a rate of 6%.

Treatment on the basis of organisms found in soft tissue, but not bone, is likely to lead to unnecessary broad-spectrum therapy. If the patient does not respond well to antibiotic therapy covering only soft-tissue isolates, a different pathogen responsible for osteomyelitis should be considered. Both bone and soft-tissue specimens should be obtained in diabetic foot osteomyelitis, and antibiotic therapy should proceed on the basis of these results. Isolation of different bacteria species in soft-tissue samples and corresponding bone cultures points out that the causative bacteria of osteomyelitis may infect bone and soft tissue differently. However, this seems unlikely. Diabetic foot osteomyelitis is a chronic infection. In the acute phase of the disease, microbiology of soft tissue may be similar to that of the bone. On the other hand, the dynamics of bacterial populations in soft tissues and bone differ greatly over time.[17]

Table 3. Culture Results of Soft-Tissue Specimen Compared With Bone Specimen From the Same Patient

Result	Specimen (No. [%]; n = 45)
Identical	22 (49)
More organisms in soft tissue	
When organisms found in bone	13 (29)
When bone was sterile[a]	4 (9)
Soft tissue sterile, organisms found in bone	1 (2)
No common organisms	5 (11)

[a]Positive histopathology in two patients; positive magnetic resonance imaging or scintigraphy in two patients lacking histopathologic data.

In patients with acute diabetic foot osteomyelitis, repeated surgical procedures may alter the microbiological status of the tissues. Soft tissues exposed to the environment are easily and rapidly colonized by noninvading flora. This explains the lack of microbiological concordance between soft-tissue and bone specimens in diabetic foot osteomyelitis.

In diabetic foot osteomyelitis, the most isolated causative bacteria are usually Gram's-positive cocci.[12, 13, 20-22] *Pseudomonas aeruginosa* is a rare bone pathogen in these patients. In our study, *P aeruginosa* was the most common organism isolated from bone tissue cultures, and methicillin-resistant *S aureus* and *Acinetobacter* spp were the other major pathogens (Table 2). The high incidence of drug-resistant organisms is probably because of the long hospitalization period, the long duration of diabetic foot infection, and the administration of various antibiotic therapies in our patient group. Early use of broad-spectrum antibiotics may have encouraged the involvement of drug-resistant species.[23, 24]

Hartemann-Heurtier et al[25] reported that previous hospitalization is a significant risk factor for drug-resistant organisms in diabetic foot infections. Because our patients underwent frequent surgical debridements in the hospital, the isolation of hospital-acquired microorganisms such as *P aeruginosa*, methicillin-resistant *S aureus*, and *Acinetobacter* spp was expected. Surgery necessitates admission to the hospital, which increases the chances of secondary infection with drug-resistant organisms.[24] These microorganisms are responsible for severe infections. In patients with advanced disease, the incidence of these organisms is high.[26] Nevertheless, few data are available on risk factors for drug-resistant organisms in diabetic foot osteomyelitis. In our study, the relatively low rate of isolating anaerobic bacteria (2%) was notable. However, in some studies, up to 34% of bone cultures have yielded anaerobic bacteria.[20, 21, 27] This difference is probably because of the high frequency of surgical debridement and previous use of antibiotics, which help avoid infection with anaerobic bacteria, in our patients. On the other hand, 13% of our patients had polymicrobial infections (an average of 1.04 pathogens per case of osteomyelitis). As polymicrobial infections are frequent in diabetic foot osteomyelitis, this was an expected result.[1, 7, 20, 26, 28]

Conclusion

The major advantages of our study were performing bone cultures in all patients and histopathology of bone on most patients, taking bone and soft-tissue specimens at the same time, having available MRI or nuclear medicine scans for many patients, and excluding ongoing antibiotic treatment at the time of the cultures. In conclusion, culture specimens should be obtained from both the bone and the overlying DST in patients with suspected osteomyelitis whose clinical conditions are suitable. The decision to administer antibiotic therapy should depend on these results.

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