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Utility of Culturing Marginal Bone in Patients Undergoing Lower Limb Amputation for Infection

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ABSTRACT

Guidelines suggest culturing clinically uninfected bone at the margin after surgical resection for osteomyelitis, but little published evidence supports this procedure. To investigate whether culturing marginal bone after completing resection of infected bone affected antibiotic use or further surgical intervention, we collected data on sequential patients undergoing amputation for a foot infection at our tertiary care hospital between January 2014 and May 2015. We recorded patient age, sex, presence of diabetes mellitus, level of amputation, whether marginal bone was sent for culture, microbiology of any marginal bone specimens, type and duration of antibiotic therapy, and any further surgical resection. Among 132 patients, the mean age was 71.9 years, 103 (78.0%) were male, and 79 (59.8%) had diabetes. Treating surgeons sent marginal bone in 58 (43.9%) of these patients, 50 (86.2%) of which were culture positive. Patients with a positive bone culture were significantly more likely to undergo further surgical intervention (20.0% vs 6.1%, $p = .047$). For patients with diabetes, compared with those without, surgeons did not send marginal bone for culture more often (46% vs 42%, $p = .72$), nor did they undertake further surgical interventions more frequently (13.4% vs 10.1%, $p = .89$). Our results suggest that the clinicians used the marginal bone culture findings to make clinical decisions but do not clarify if there is a benefit to performing this procedure. Although patients whose proximal bone specimens were culture positive were more likely to undergo a surgical intervention, the reasons for, and benefit of, this additional surgery were unclear.

An estimated 10,000 patients are hospitalized annually for diabetic foot ulceration in Australia (1). Of patients presenting with a diabetic foot ulcer, just over one half have clinical findings suggestive of infection (2). Among these, underlying osteomyelitis is present in 10% to 20% of patients with a mild infection, and in 50% to 60% of those who are hospitalized (2). As in most other countries, the prevalence of diabetes in Australia has dramatically increased over the past few decades (3), leading to a progressively greater clinical and financial burden for managing diabetes-related foot infections. Defining optimal management of diabetic foot infections is essential to minimize morbidity and mortality and improve quality of life for the affected patient (4), and to avoid inducing antibiotic resistance and reduce costs to the health system.

Osteomyelitis of the foot can generally be diagnosed by combinations of clinical findings, serum inflammatory markers, and imaging procedures (5), but the criterion standard remains the result of a culture of bone, preferably combined with those of histopathological examination (2). The need for and best method of obtaining bone samples for microbiological investigations at the time of lower amputation surgery is unclear (2,6). The surgeon typically decides the margin between uninfected and infected bone while in the operating room based on the bone's look and feel. Typical signs of infection are loss of bone firmness or an absent "paprika sign" (punctuate bleeding of bone edges on resection). Because even healthy-appearing bone (i.e., with no disruption, discoloration, or softness) may in fact be infected, a false-negative examination may be responsible for failure of initial treatment or a recurrence of infection. Often, the surgeon determines the appropriate level of amputation by assessment of preoperative imaging (usually plain radiography, magnetic resonance imaging, or both) combined with intraoperative assessment of bone appearance, texture, and strength. Surgeons as a result sometimes send a sample of the so-called marginal or proximal bone (i.e., the last bone left at the site of resection) for culture at the time of amputation. A positive culture presumably means that the infection has spread into what was thought to be a "clear" (uninfected)

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margin. This concept is akin to the principle of determining if there are "clear margins" after surgical resection for a malignancy. Clinicians must also be aware that bone cultures may be falsely positive because of specimen contamination or falsely negative because of recent antibiotic therapy or improper specimen transport or culture.

Recommendations from major guidelines on diabetic foot infection (2,6) and the experience of expert clinicians suggest that a patient with proximal margin bone infection should be further treated for osteomyelitis (i.e., with a prolonged course of antibiotic therapy, additional surgical resection, or both). Evidence supporting the idea that information gleaned from culture of these marginal bone samples is of any clinical benefit in determining the need for this further anti-infective treatment is limited to only 2 small pilot studies (7,8), however, which were conducted exclusively in patients with diabetes. We thus investigated our experience with marginal bone culture to help determine whether sending a bone sample from the presumed clear margin during amputation surgery for foot infections (in patients with and without diabetes) is therapeutically useful.

Patients and Methods

We retrospectively collected data on sequential patients who underwent any type of lower limb bone resection because of infection on the background of arterial insufficiency or diabetes between January 2014 and May 2015 at our tertiary-level teaching hospital in Sydney, Australia. There were no exclusion criteria. We assembled a database that included information on each patient's age, sex, presence of diabetes, and level of amputation. After completing the amputation, the operating surgeon made a decision as to whether or not to send a specimen for culture from the presumed "clear" (clinically uninfected) bone margin, based on his or her clinical concern for possible residual osteomyelitis.

When the surgeons collected an intraoperative marginal bone specimen, they followed a procedure standardized to our vascular surgery unit. After the amputation, the surgeon discarded the used instruments and then, using new, sterile gloves and instruments, nibbled samples of marginal bone to send for culture. The bone samples were sent from the operating room in sterile containers; they were initially processed in the microbiology laboratory in beef enrichment broth and subsequently inoculated onto a variety of agar plates. We reviewed the microbiology records to determine which, if any, microorganisms were isolated from these specimens, as well as their antibiotic sensitivities. We reviewed the hospital record to determine whether the treating clinicians prescribed any antibiotic therapy postoperatively and, if so, which agents. We also noted whether the patient underwent any further surgical intervention at the operative site. One of the several consultant vascular surgeons performed or supervised all of the amputation procedures because it is protocol at our institution for the vascular surgery service to manage patients with ischemic or diabetic foot infections.

All authors were involved in reviewing the data and composing the article. We conducted comparisons using independent sample Mann-Whitney *U* tests for continuous variables and Pearson's chi-square and binomial testing for categorical variables. We analyzed data using SPSS Statistics Standard Edition 20 software (IBM, New York, NY). We considered $p < .05$ to be statistically significant. The Royal Prince Alfred Hospital granted low- and negligible-risk ethics approval for this study.

Results

Between January 2014 and May 2015, 132 patients underwent lower limb amputation for infection at our medical center, either for confirmed osteomyelitis or severe soft tissue infection. The procedures included 9 transphalangeal amputations, 107 single-digit amputations (through the metatarsal), and 16 transmetatarsal (forefoot) amputations. Among the included patients, the mean age was 71.9 ± 12.4 years; 103 (78.0%) were male, and 79 (59.8%) had a history of diabetes mellitus. The operating surgeon elected to send marginal bone samples from 58 (43.9%) of the patients, all of which were cultured in our local microbiology laboratory by standard methods. The remaining 74 patients did not have any bone specimen sent. Based on our hospital's procedure, none of the specimens were sent for histopathological evaluation. All patients received preoperative antibiotic therapy with either piperacillin-tazobactam (for those with diabetes) or flucloxacillin (for those without diabetes) leading up to the operation; patients allergic to penicillin received the ciprofloxacin and clindamycin combination. The treating surgeon continued this antibiotic therapy until bone culture results were available. If the patient had a positive bone culture, the treating surgeon requested consultation on further management from the infectious diseases service. Antibiotic therapy was discontinued in all patients for whom the marginal bone culture was negative.

After the initial amputation surgery, 10 patients underwent a further surgical intervention at the same operative site. Of the patients who had a toe amputation, 4 underwent a subsequent major amputation (2 below the knee and 2 above the knee), 4 had an adjacent toe amputation, 1 had only wound debridement, and 1 had both wound debridement and amputation of 2 adjacent toes. Of patients undergoing a transmetatarsal amputation, 2 underwent subsequent below-knee amputation and 2 had a further debridement alone. Indications for further intervention were clinical infection in all patients. No patients had further interventions based on the marginal bone culture results.

Among the patients from whom the surgeon sent a bone specimen, culture results were positive in 50 (86.2%). Patients from whom marginal bone specimens were sent (regardless of the culture result) were significantly more likely to have further surgical intervention (5.4% vs 20.0%, $p = .02$). Patients from whom a bone specimen was culture positive were significantly more likely to undergo further surgical intervention than those whose bone samples were culture negative (20.0% vs 6.1%, $p = .047$). Patients with diabetes were not significantly more likely than patients without diabetes to require further surgical intervention (13.4% vs 10.1%, $p = .89$). There was also no significant difference in the frequency of surgical interventions between male and female patients (10.7% vs 13.7% respectively, $p = .813$), nor was age related to the likelihood of further surgical intervention ($p = .15$).

The most commonly isolated pathogens from bone specimens, as shown in Table 1, were gram-positive cocci (i.e., staphylococci [coagulase-positive and coagulase-negative] and enterococci). Among the gram-negative organisms, *Pseudomonas aeruginosa* and *Proteus* species were the most frequently isolated. In addition to the isolates shown in Table 1, there was a single isolate of each of the following: *Serratia marcescens, Morganella morganii, Acinetobacter* species, *Propionibacterium* species, *Raoultella ornithinolytica*, and group B *Streptococcus*.

Five patients who had a culture-positive bone specimen were not treated with additional (beyond the preoperative) antibiotic therapy. In 3, the bone isolate was a coagulase-negative *Staphylococcus*, which the treating physician, in consultation with the infectious disease team, believed to be a contaminant. None of these 5 required any further surgical intervention. The most commonly prescribed antibiotic agents, based on the results of culture-positive bone specimens, are shown in Table 2. Among 45 patients who received initial intravenous antibiotic therapy, 28 received subsequent oral therapy. Table 3 shows the most frequently prescribed agents for patients who had a "switch" from

Table 1
Bacteria isolated from marginal bone specimens

Isolated pathogens from marginal bone specimen	No. (%)
Coagulase-negative *Staphylococcus*	14 (28)
Staphylococcus aureus	
Methicillin sensitive	13 (26)
Methicillin resistant	4 (8)
Enterococcus species	
Vancomycin sensitive (*avium, cloacae, faecalis, raffinosus*)	11 (22)
Vancomycin resistant	2 (4)
Corynebacterium species	8 (16)
Pseudomonas aeruginosa	7 (14)
Proteus (*mirabilis, vulgaris*)	7 (14)
Citrobacter (*freundii, koseri*)	3 (6)
Stenotrophomonas maltophilia	2 (4)
Escherichia coli	2 (4)
Enterobacter cloacae	2 (4)

Values represent the number of times each microbe was grown, with the percentage of all isolates in parentheses.

Table 2
Initially selected antibiotic agent (and route of therapy) for the 45 patients who received further antibiotic therapy because of a positive specimen

Initial antibiotic therapy (route)	No. (%)
Piperacillin-tazobactam (IV)	27 (60.0)
Vancomycin (IV)	3 (6.7)
Cefepime (IV)	3 (6.7)
Amoxicillin-clavulanate (PO)	3 (6.7)
Trimethoprim-sulfamethoxazole (PO)	3 (6.7)
Cephazolin (IV)	2 (4.4)
Ceftriaxone (IV)	2 (4.4)
Ciprofloxacin (IV)	1 (2.2)
Flucloxacillin (IV)	1 (2.2)

Abbreviations: IV, intravenous; PO, oral.

intravenous to oral antibiotic therapy. The choice of antibiotic agents was adjusted according to culture and sensitivity results when necessary, in consultation from the infectious diseases service. The Fig. shows a flow diagram of the treatment approach to all included patients.

Discussion

Recent guidelines developed by the International Working Group on the Diabetic Foot state, "[a] specimen of [clinically infected] bone should be obtained at the time of surgery for analysis by culture and histopathology" (2). Similarly, the most recent diabetic foot infection guidelines of the Infectious Diseases Society of America recommend that "[w]hen bone is debrided to treat osteomyelitis, we suggest sending a sample for culture" (6). Furthermore, an expert consensus document on surgical management of diabetic foot osteomyelitis states that "[g]rossly abnormal or infected bone should be sent for microbiology" and "[a] sample of the proximal-most bone resected (i.e., a bone margin specimen) should be labeled separately and sent for microbiology" (9). None of these guidelines specifically addressed the question of interest in the current study (i.e., is there is any benefit in determining if, after surgical resection of apparently infected bone, the clinically uninfected bone is culture positive?) The answer to this question would help the clinician in deciding if the patient requires further medical or surgical treatment.

The results of 2 previous studies in patients with diabetes with a foot infection who underwent surgical resection found that the presence of residual osteomyelitis in the marginal bone was common (35% to 41%) and significantly associated with reulceration, further amputation, or death (7,8). In fact, patients with a positive culture from proximal bone margin were 2 (7) to 3 (8) times more likely to suffer a poor outcome. Presumably, if the marginal bone is shown to be uninfected, antibiotic therapy may safely be discontinued or significantly shortened (8). Of note, both of these studies reported on patients with diabetes only, and only 1 (7) discussed the methods used to obtain a proximal bone sample. Both were also small, retrospective studies from a single center.

Because of these reports suggesting high rates of clinically unsuspected osteomyelitis, the surgeons at our institution adopted the practice of frequently sampling marginal bone after performing a lower extremity amputation. Although the surgeons presumably considered the bone obtained by the method we described as being obtained "aseptically," there is some risk of contamination when a specimen is taken from an operative field. This possibility, and that microbiology laboratories will identify and report all isolates from operative tissue specimens, may account for the high rate (86.2%) of marginal bone specimens sent to microbiology in our study that had a positive culture. One other study of marginal cultures of bone after metatarsal resection in 19 patients found that cultures of the first 0.5 cm of bone were positive in 50% of cases, whereas those of the next 0.5 cm (i.e., total of 1.0 cm) were positive in only 9% (10). We do not have information from our surgeons on where they sampled bone.

We found that patients who had marginal bone specimens sent for culture, compared with those who did not have bone sent, were 4 times more likely to undergo further surgical intervention regardless of the microbiological findings. Because this a retrospective study, we can only speculate on potential explanations for this finding, but we believe that the surgeon is more likely to collect marginal bone specimens after bone resection if he or she is clinically concerned about the presence of residual osteomyelitis. That the surgeons likely made decisions based on the results of marginal bone cultures is suggested by the fact that patients whose marginal bone specimens were culture positive were 3 times more likely to undergo further surgical intervention than if bone cultures were negative. The reasons for this may include that (1) the proximal bone, although appearing clinically uninfected, was in fact infected and required further therapy, or (2) the surgeon was more likely to send a marginal bone specimen if he or she believed that further intervention might be needed. Furthermore, the surgeons were also probably aware that marginal bone samples are more likely to be contaminated when they are collected from more clinically severe or grossly infected wounds. Because there was no established protocol regarding the need to send a bone specimen in this retrospective study, it is possible that decisions of the surgeon affected the pretest probability of a positive marginal bone culture.

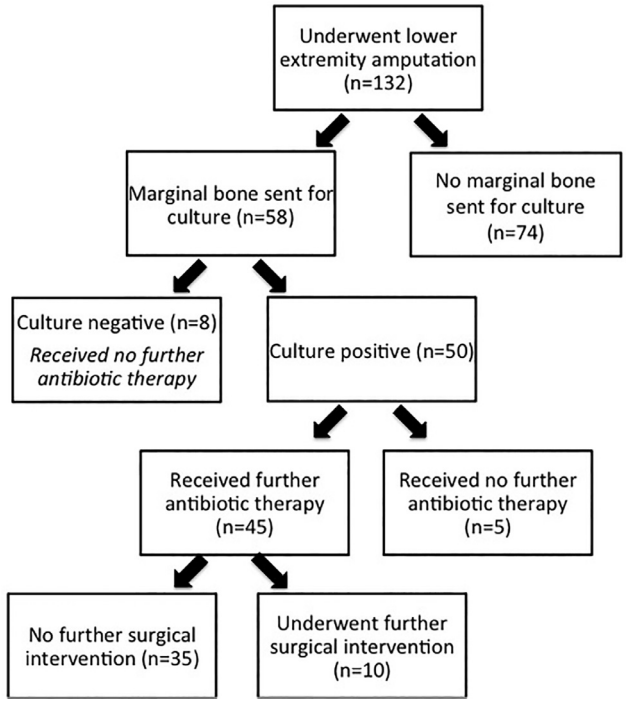

Fig. Culture results and treatments after lower extremity amputation for all included patients.

Table 3
Agents selected for the 28 patients who had oral "switch" therapy after initial intravenous treatment, with the percentage of total patients receiving therapy

Oral switch therapy	No. (%)
Ciprofloxacin	11 (39.3)
Amoxicillin-clavulanate	9 (32.1)
Flucloxacillin	3 (10.7)
Clindamycin	2 (7.1)
Cephalexin	2 (7.1)
Lincomycin	1 (3.6)

Of note, patients who were treated with additional antibiotic therapy after surgery, compared with those who were not, were still significantly more likely to undergo further surgical intervention at the affected site. This finding suggests that the treating clinicians used their clinical judgment in addition to the microbiological findings in their management decisions. In other words, patients with negative proximal bone cultures had their antibiotics discontinued with no subsequent infectious complications, suggesting that collecting a bone specimen was used (and perhaps useful) in decision making. In all cases, the treating surgeon discussed decisions about managing patients with a positive marginal bone culture with the infectious diseases team at our institution. Among the 5 patients with a culture-positive margin who were not treated with further antibiotic therapy, 3 cultures were believed to be contaminated (because of a coagulase-negative *Staphylococcus* growth), and the remaining 2 had their antibiotics discontinued because there had been contamination of the specimen intraoperatively and no clinical signs of infection postoperatively.

Selections of antibiotic regimens by the treating clinicians adhered to the current Australian therapeutic guidelines (11), suggesting that their antimicrobial choices were likely appropriate. Also of note is that the flora grown from the marginal bone specimens were similar to those reported from studies from other institutions (12) and generally covered by the types of agents most commonly selected.

Although collecting marginal bone specimens can provide useful information, it also has potential downsides. It requires extra time to collect the specimens during surgery, in addition to the special efforts to correctly transport them to the microbiology laboratory, where additional effort is required to process them. Furthermore, this procedure has financial costs, including those associated with processing the specimens; the cost for doing a culture and sensitivity is $48.15 AUD (1) at our institution. In addition, a positive culture result, whether true or false, will typically lead to further diagnostic testing and additional therapeutic interventions. This is especially relevant currently, when the debate regarding unnecessary use of diagnostic tests and medical (especially antibiotic) treatments has sparked initiatives such as “Choosing Wisely” (13). However, a negative marginal bone culture may (as in our study) result in the clinician electing a shorter course of antibiotic therapy that is appropriate for soft tissue, compared with bone, infection. This would be a major benefit in avoiding unnecessary cost, potential adverse effects, and the development of antibiotic resistance.

For a test to be clinically useful, it must first be shown to be sufficiently accurate and then to be helpful in changing management (14). Furthermore, performing the test should generally lead to improved patient outcomes (15), help reduce the likelihood of misdiagnosis, or increase quality of life (16). Our results suggest that culturing marginal bone specimens may provide a good indicator of the presence or absence of residual bone infection following amputation, as noted in the 2 previous studies of this issue. But it is also possible that some (if not most) of these positive cultures (which were predominantly coagulase-negative staphylococci) were contaminants, and that further antibiotic therapy was unnecessary and potentially harmful.

Our study had several limitations. First, we included a relatively small number of patients, although more than the 2 previously reported studies on this topic included (7,8). Second, this (like the previous studies) was a retrospective, single-center review. Third, we cannot know what information the treating surgeons used to make decisions about further antibiotic or surgical treatment. Fourth, our institution does not routinely send bone samples for histopathology, which can provide corroboratory evidence to help determine if the veracity of culture results. In addition, the degree of arterial insufficiency in each patient undergoing minor amputation was not recorded in this study, which is a likely confounder. However, all patients had lower limb arterial supply assessed by a vascular surgeon before surgery; patients assessed as requiring arterial intervention to support the healing of an amputation underwent this before the amputation. Further, few data were collected on the outcomes of patients for whom bone samples were not sent; this could potentially be investigated in further studies. Finally, we do not have follow-up information to report the clinical outcome of the patients included in our study. One strength of our study is that it included both diabetic and nondiabetic patients.

Given the limited patient numbers and retrospective nature of this and the other 2 published studies, a randomized controlled trial would clearly be the next step in determining the utility of collecting marginal bone specimens for culture during amputation procedures for infected lower limbs. Devising an ethical protocol might be difficult, but the study could compare outcomes of randomizing patients with a group for whom proximal bone specimens are sent and another group for whom they are not. Another possibility, which may be ethically dubious, would be to obtain bone specimens for culture from both groups but only provide the culture information to the treating clinicians in 1 group. Subgroups of the patients having bone samples sent could also be used to analyze the clinical outcomes based on microbiological versus histopathological findings. Further, it would be interesting to compare microbiological, clinical, and histologic features to help elucidate the efficacy of each diagnostic modality.

In conclusion, the results of our study suggest that culturing marginal bone after amputation of an infected lower extremity may be useful in that it affected clinical judgment, but did not clearly demonstrate whether this finding offered clinical utility. Although we found that patients from whom bone cultures were positive had a higher incidence of further surgical interventions, we do not know the reasons the treating clinicians made these decisions or if the surgery was necessary. We believe that this is an important issue in equipoise and would like to see further properly designed prospective studies.

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