Culture of Bone Biopsy Specimens Overestimates Rate of Residual Osteomyelitis After Toe or Forefoot Amputation

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Investigation performed at the University Hospital Basel, Basel, Switzerland

Background: Guidelines recommend both histological analysis and culture for definite diagnosis of osteomyelitis. It is not clear if histological and culture criteria can be used interchangeably in the clinical scenario of toe amputation. We therefore prospectively compared the results of intraoperative culture and those of histological examination in this setting.

Methods: Consecutive patients requiring toe or forefoot amputation at the University Hospital Basel during a 2-year period were included in the study. Biopsy specimens from the residual bone were cultured according to microbiological standards. Histological analysis was performed using standardized criteria for osteomyelitis. Clinical outcomes were assessed retrospectively via chart review.

Results: Of 51 patients included in the study, 33 (65%) had a positive culture of residual bone and 14 (27%) showed histological signs of osteomyelitis. A negative histological result but a positive culture was found for 21 (41%) of the patients, suggesting that culture has a high false-positive rate if histological analysis is used as the reference to rule out osteomyelitis. The recommended criteria of both positive histological findings and positive culture were fulfilled by 12 (24%) of the 51 patients.

Conclusions: Positive cultures of residual bone after forefoot or toe amputation overestimate the true rate of osteomyelitis as defined by histological analysis, presumably because of contamination from soft tissue at the time of surgery. Additional studies are needed to evaluate the indications for, and the duration of, antibiotic treatment according to these findings.

Clinical Relevance: Our results cast doubt on the strategy of relying solely on culture of bone biopsy specimens when deciding whether antibiotic treatment for osteomyelitis is necessary after toe or forefoot amputation.

Osteomyelitis is common in the setting of toe or forefoot amputation due to diabetes mellitus or vascular disease and leads to high morbidity and costs[1,2]. During amputation, the surgeon determines the level of resection according to macroscopic criteria[3]. However, it is often not clear whether the residual bone is still affected by osteomyelitis, which would require prolonged antibiotic treatment[4]. International guidelines recommend processing bone specimens for both histological analysis and culture[5], but the diagnosis of osteomyelitis is often based on only one of the two[6,7]. As a result of the decalcification process, it may take longer for the results of histological analysis to become available. Furthermore, there are no standardized criteria for the histological diagnosis of osteomyelitis in this setting. Culture of percutaneously acquired bone biopsy specimens has been shown to be reliable for diagnosing and guiding treatment of osteomyelitis in diabetic foot ulcers[8]. However, the retrieval of a bone biopsy specimen during a toe amputation differs from a percutaneous bone biopsy because there is an inevitable risk of contamination of the bone specimen from adjoining infected soft tissue

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in the operative field, no matter how carefully rules of aseptic surgery are followed. The relevance of the findings of cultures of residual bone after toe or forefoot amputation has never been addressed in detail, to our knowledge. The aim of the present study was to prospectively compare cultures and histological analyses of biopsy specimens obtained intraoperatively from the bone remaining after toe or forefoot amputation.

Materials and Methods

Patients

During a period of 2 years, we prospectively enrolled consecutive patients requiring a toe or forefoot amputation because of gangrene and/or infection at the Department of Vascular Surgery at the University Hospital Basel, Switzerland. Patients requiring emergency amputation because of severe soft-tissue infection or trauma were excluded. The decision to perform the amputation as well as the timing were at the discretion of the treating surgeon. Radiographic examinations were not required for inclusion into the study since the indication for amputation was based on clinical grounds in all cases. The local ethical committee approved the study protocol, and all patients gave written informed consent. All patients underwent assessment for peripheral arterial disease with ankle brachial index measurements as well as segmental volume plethysmography. If necessary and possible, peripheral arterial disease was treated with endovascular techniques or bypass surgery before or after amputation. Age; sex; presence of diabetes mellitus; stage of peripheral arterial disease; previous revascularization procedures; C-reactive protein (CRP) level; presence of necrosis and/or cellulitis; PEDIS (perfusion, extent, depth, infection, and sensation) classification; number of amputated toes; amputation level; wound closure; and antibiotic treatment before, during, and after amputation were documented. Patient charts from regular outpatient visits or from rehospitalizations were analyzed retrospectively to assess outcomes. Treatment was considered to have failed if another, more proximal amputation had to be performed.

Surgical Technique of Amputation

Surgery was performed by 1 of 3 senior consultants. The skin of the entire foot was disinfected using an alcohol-based iodine solution (Braunoderm; B Braun) for 3 minutes, and sterile drapes were applied. The surgeon determined the extent of amputation on the basis of the macroscopic aspect, taking into account the extent of ischemia and infection. If necrosis had not reached the interdigital fold, exarticulation of the metatarsophalangeal joint was performed followed by removal of the cartilage from the metatarsal head. If necrosis or infection was more extensive, transmetatarsal amputation of individual digits or the entire forefoot was performed using an oscillating saw.

Bone Biopsy

The protocol for retrieving biopsy specimens from the residual, presumably uninfected bone ("proximal biopsy") is described in Figure 1. Toe amputation was performed with use of all possible precautions to avoid contamination of the residual bone, including wrapping the toe dedicated for amputation in sterile gauze and removing it before retrieving biopsy specimens from the residual bone. The technique of proximal bone biopsy differed according to whether the patient underwent exarticulation of the metatarsophalangeal joint or transmetatarsal amputation. In the case of exarticulation, 2 bone cylinders (1 for culture and 1 for histological analysis) were retrieved from the metatarsal head using an 8G Jamshidi biopsy needle (BD). In the case of transmetatarsal amputation, a 3 to 5-mm slice of corticocancellous metatarsal bone was removed using an oscillating saw. The slice was then cut in half, with 1 half used for culture and the other used for histological analysis. All biopsy specimens were transferred directly into sterile closable transport tubes and sent in parallel for microbiological culture and histological analysis immediately after surgery.

Histological Analysis of Bone Biopsy Specimens

Histological analysis was performed using EDTA (ethylenediaminetetraacetic acid) decalcification (in combination with ultrasound at 30°C) of formalin-fixed probes followed by paraffin embedding and staining with hematoxylin and eosin. The time until decalcification was noted. The size of each biopsy specimen was measured.

Our histological criteria for diagnosing osteomyelitis were adapted from the approaches described by Mirra et al.[9] and Spangehl et al.[10], in their work on periprosthetic joint infections, and in general pathology textbooks[11] and were standardized to enhance reproducibility. Our criteria were based on the hypothesis that osteomyelitis should be absent from the bone

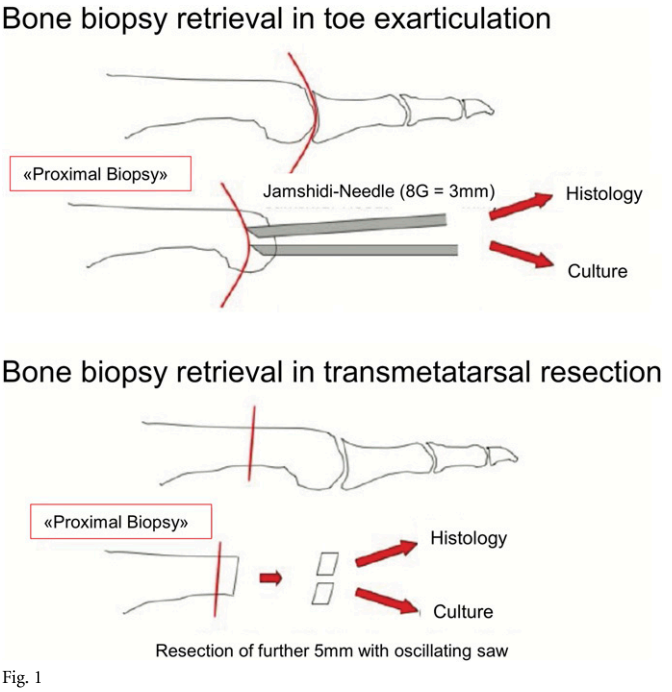

Fig. 1

Technique for retrieving bone biopsy specimens from residual bone ("proximal biopsy").

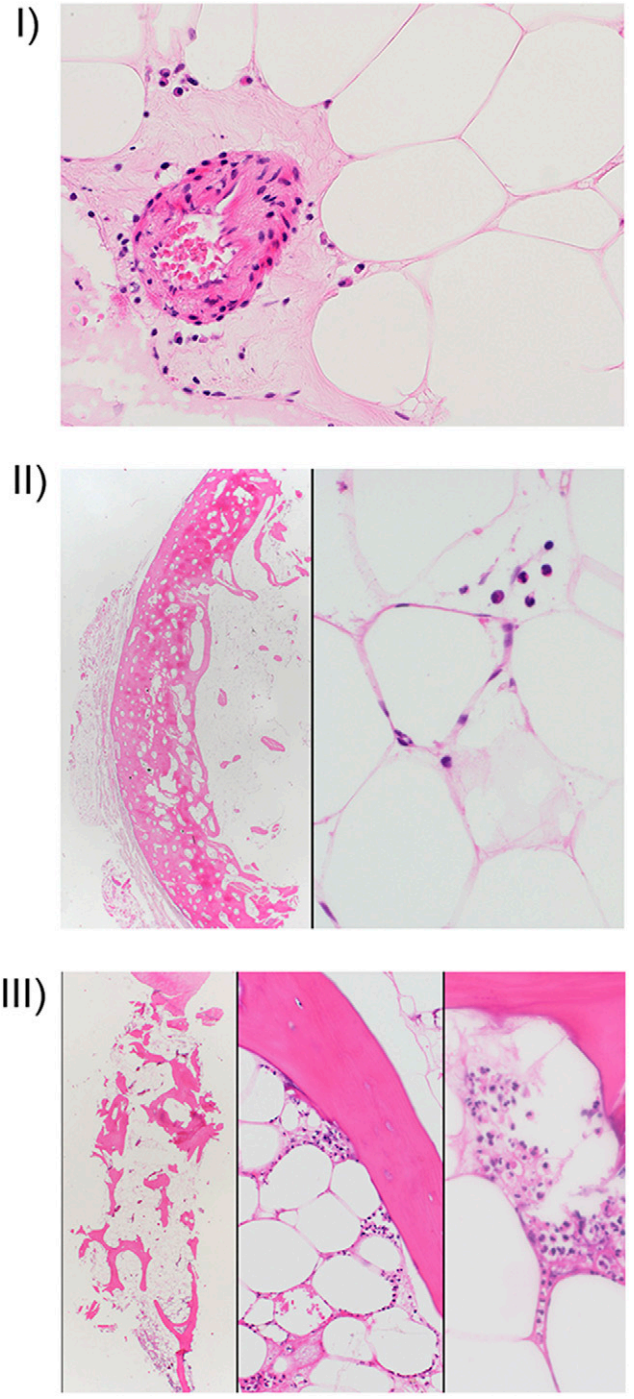

Fig. 2

Representative histological images of bone specimens taken from diabetic patients (hematoxylin and eosin). I = non-infectious changes in bone marrow of bone with perivascular fibrosis and plasmacytic infiltration in the absence of neutrophilic granulocytes (400×). II = possible osteomyelitis with <5 neutrophilic granulocytes (12.5× and 600×). III = definite osteomyelitis with ≥5 neutrophilic granulocytes, showing fatty marrow necrosis with loss of nuclear staining (image in the center) and microabscess-like infiltration accompanied by edema (image on the right) (20×, 200×, and 400×).

obtained with the proximal biopsy. Because published findings on histological alterations in the bone marrow of patients with diabetes in the absence of osteomyelitis demonstrated perivascular fibrosis and lymphoplasmacytic infiltrations[12], we decided not to use fibrosis, marrow necrosis, or perivascular lymphoplasmacytic infiltration alone, without the presence of neutrophilic granulocytes, as an indicator of residual osteomyelitis. The entire biopsy specimen was processed for histological study and analyzed. If any high-power field contained at least 1 neutrophilic granulocyte in combination with marrow necrosis, fibrosis, lymphoplasmacytic infiltration, edema, or reactive bone formation, the diagnosis of possible osteomyelitis was made. If any high-power field contained ≥5 neutrophilic granulocytes in combination with the findings listed above, a definitive diagnosis of osteomyelitis was made. The diagnosis of osteomyelitis was ruled out if the analyzed fields did not contain any neutrophilic granulocytes. Representative images are shown in Figure 2. The examining pathologist (G.J.) was blinded to clinical information.

Culture of Bone Biopsy Specimens

Firm bone samples were placed directly into thioglycolate broth, whereas soft portions were crushed and plated onto agar media. Plates containing Columbia blood agar with 5% sheep blood agar were incubated in 5% CO_2, Brucella agar plates were incubated in an anaerobic workstation, and thioglycolate broth tubes were incubated in ambient air at 36°C to 37°C. All cultures were checked daily for growth, and the thioglycolate broth tubes were incubated for up to 7 days. Aerobic and anaerobic subcultures were performed in cases of suspected growth. Standard microbiological techniques were applied to identify recovered microorganisms. All results were supervised by a senior microbiologist blinded to the results of the histological analysis.

To avoid misinterpreting them as infection, common low-virulence skin colonizers such as coagulase-negative staphylococci, Corynebacterium species, or Propionibacterium species were classified as irrelevant and cultures that showed growth of only these organisms were counted as negative.

Antibiotic Treatment After Amputation

After amputation, the surgeon together with the infectious disease specialist decided whether the patient required antibiotic treatment for residual osteomyelitis, taking into account the clinical aspect of the wound during and after amputation and the results of bone biopsy cultures. Histological results were not available for this initial decision and also were often disregarded during follow-up. Postoperative antibiotic therapy for osteomyelitis consisted of intravenous treatment for 2 weeks followed by oral treatment, usually for an additional 10 weeks.

Statistical Analysis

Statistical analysis was done with a Fisher exact test, with a p value of <0.05 considered to be significant, using the SPSS version-022.0.0.0 software package (IBM).

TABLE I Patient Characteristics

Characteristic	
Total no. of patients*	51 (100)
Age† *(yr)*	77 (73-84)
Male*	36 (71)
Diabetes mellitus*	36 (71)
Peripheral arterial disease*[21]	46 (90)
Stage I	13 (25)
Stage II	4 (8)
Stage III	2 (4)
Stage IV	27 (53)
CRP >50 mg/L at time of operation*	22 (43)
Cellulitis*	26 (51)
Cumulative PEDIS classification*	
<7	19 (37)
≥7	32 (63)
Revascularization procedure*†	32 (63)
Type of amputation*	
Exarticulation of toe	39 (76)
Transmetatarsal	12 (24)
Postop. antibiotic treatment for osteomyelitis	17 (33)
Duration† *(days)*	
Total	84 (84-84)
Initial intravenous (IV) therapy	14 (14-14)
Oral therapy	70 (70-70)
Agent*	
Amoxicillin/clavulanate (IV)	13 (25)
Flucloxacillin (IV)	1 (2)
Ertapenem (IV)	1 (2)
Clindamycin (oral)	7 (14)
Quinolone (levofloxacin, ciprofloxacin) (oral)	4 (8)
Quinolone in combination (rifampicin, clindamycin, or amoxicillin) (oral)	6 (12)

*The values are given as the number with the percentage in parentheses. †The values are given as the median with the IQR in parentheses. ‡Endovascular technique or bypass surgery.

Results

The characteristics of the 51 patients enrolled in the study are summarized in Table I, with Table E-1 (see Appendix) showing data for each patient. Culture of the proximal bone biopsy specimen was positive for 33 (65%) of the 51 patients. However, only 14 (27%) of the 51 specimens demonstrated histological evidence of osteomyelitis, with 5 of the 14 showing <5 neutrophilic granulocytes (i.e., only "possible" osteomyelitis according to our criteria). A negative histological finding but positive culture was found for 21 (41%) of the 51 patients, whereas 12 of the 14 patients with a positive histological finding had a positive culture and 16 of the 18 with negative culture also had a negative histological result (Table II).

Decalcification of the bone biopsy specimens required a median of 6 days (interquartile range [IQR] = 5.0 to 8.0 days) overall, with a median of 6 days (IQR = 5.0 to 7.0 days) for the specimens obtained with needle biopsy and 8 days (IQR = 6.25 to 11.25 days) for those retrieved with a transmetatarsal biopsy. The bone biopsy specimens had a median volume of 68 mm^3 (IQR = 44 to 153 mm^3).

Histological analysis revealed a positive result in 10 (26%) of the 39 patients who underwent exarticulation compared with 4 (33%) of the 12 who had transmetatarsal amputation ($p = 0.71$). Ten (30%) of the 33 specimens with positive culture findings were found to have polymicrobial growth (see Appendix Table E-2). *Staphylococcus aureus* was the most commonly identified pathogen, and it was found twice as often in specimens with histologically proven osteomyelitis (6 of 14, 43%) than in those with negative histological findings (8 of 37, 22%), although the difference was not statistically significant ($p = 0.16$). However, 8 (57%) of the 14 specimens that showed growth of *Staphylococcus aureus* on culture had no histological signs of osteomyelitis. No methicillin-resistant *Staphylococcus aureus* was detected. *Enterobacteriaceae* were more often found in histologically negative (9 of 37, 24%) than in histologically positive (1 of 14, 7%) biopsy specimens, although again there was no significant difference ($p = 0.25$). *Pseudomonas aeruginosa* was grown on culture of 4 (8%) of the 51 biopsy specimens. Coagulase-negative staphylococci were the only microorganism found in the cultures of 3 (6%) of the 51 biopsy specimens. However, coagulase-negative staphylococci (other than *Staphylococcus lugdunensis*) were considered irrelevant due to their low virulence, and these cultures were not considered positive. Initiation of antibiotic therapy before the amputation did not influence the rate of positive cultures, with 20 (74%) of the 27 patients with preoperative antibiotic therapy having a positive culture compared with 13 (54%) of the 24 who did not undergo preoperative antibiotic therapy ($p = 0.16$).

Seventeen (33%) of the 51 patients received postoperative antibiotic therapy for osteomyelitis. The median duration of treatment was 84 days (IQR = 84 to 84 days). Details regarding the antibiotic therapy are shown in Table I. One patient died of heart failure before wound closure and was excluded from the outcome analysis. The wounds had completely healed at the time of follow-up in 33 (66%) of the 50

TABLE II Relationship Between Histological and Culture Findings in Proximal Bone Biopsy Specimens*

	Positive Histologically	Negative Histologically	Total
Positive culture	12	21	33
Negative culture	2	16	18
Total	14	37	51

*$P = 0.09$.

TABLE III Clinical Failure of Wound-Healing After Amputation in Patients with and without Postoperative Antibiotic Therapy for Osteomyelitis

	Failure			
Proximal Bone Biopsy	Antibiotic Therapy	No Antibiotic Therapy	Odds Ratio (95% Confidence Interval)	P Value
Positive culture	3/17 (18%)	8/18 (44%)	3.73 (0.78-17.68)	0.14
Negative culture	0/0 (0%)	6/15 (40%)	Not applic.	Not applic.
Positive histologically	0/4 (0%)	5/10 (50%)	Not applic.	0.22
Negative histologically	3/13 (23%)	9/23 (39%)	2.14 (0.46-9.97)	0.46
Total	3/17 (18%)	14/33 (42%)	3.43 (0.82-14.30)	0.11

patients, who were followed for a median of 90 days (IQR = 39 to 123 days). Seventeen (34%) of the 50 patients required an additional, more proximal amputation. There was a trend toward a better outcome for patients who received osteomyelitis therapy (Table III). This effect was most pronounced in patients with a positive result on histological analysis, with failure occurring in 0 of the 4 with antibiotic treatment versus 5 of 10 without such treatment ($p = 0.22$). A preoperative CRP level of >50 mg/L was significantly associated with treatment failure, which was seen in 11 (52%) of 21 patients with a CRP level of >50 mg/L versus 6 (21%) of 29 with a level of <50 mg/L ($p = 0.033$). There was no significant difference in the rates of treatment failure between patients with toe exarticulation and those with transmetatarsal resection (11 [28%] of 39 versus 6 [55%] of 11; $p = 0.15$).

Discussion

We believe that this is the first study comparing the results of microbial culture and histological analysis of intraoperative biopsy specimens of residual bone after toe or forefoot amputation. We found that the cultures were frequently positive in the absence of any histological signs of osteomyelitis. With the histological analysis considered as the reference, those cultures must be considered false-positive.

According to guidelines of the Infectious Diseases Society of America[4] and an international expert consensus[5], the results of both histological analysis and culture have to be positive for a definite diagnosis of osteomyelitis[5]. Nevertheless, one or the other criterion has been used for the diagnosis in the literature and the two appear to have been used interchangeably[6,13]. In some studies on osteomyelitis of the foot in diabetic patients, percutaneous biopsy was performed through intact skin from the dorsum of the foot[8]. In our view, the results of these studies cannot be extrapolated to the setting of a bone biopsy performed during toe or forefoot amputation. A risk of bacterial contamination of the bone at the resection margin from surrounding infected soft tissue or ulcerated skin remains, no matter how carefully rules of aseptic surgery are followed. We thus interpret the high rate of positive cultures in association with negative histological findings in biopsy specimens as being due to intraoperative bacterial contamination of the specimens. While this assertion remains hypothetical, other possible explanations seem far less plausible. Major immunosuppression could result in nonreactivity of infected bone. However, only 2 patients were undergoing immunosuppressive therapy. One of them showed definitive osteomyelitis. with ≥5 neutrophilic granulocytes per high-power field in the histological analysis of the proximal biopsy specimen, and the other demonstrated negative results on both the histological analysis and culture. Necrotic sequestered bone without any connection to the blood supply would be another explanation; however, the biopsy specimens were taken from macroscopically vital and bleeding bone.

The few available studies on osteomyelitis after toe amputation differ from our investigation in several important ways (Table IV). Kowalski et al.[14] and Weiner et al.[15] used the resected "distal" bone for analysis whereas we biopsied the remaining "proximal" (i.e., clinically uninfected) bone to determine the subsequent treatment. The resected joint may have served as an anatomical barrier against the spread of the infection, particularly in cases of exarticulation. Analyzing the resected bone is likely to overestimate the rate of "true" residual

TABLE IV Differences Between Present Study and Comparable Studies in the Literature

	Study Design	No. of Biopsies	Location of Biopsies	Histological Analysis Performed	Culture Performed	Positive Histologically *(no. [%])*	Positive Culture *(no. [%])*
Present study	Prospective	51	Residual bone	Yes	Yes	14 (27)	33 (65)
Kowalski et al.[14]	Retrospective	111	Resected bone margin	Yes	Yes (usually)	39 (35)	84 (76)
Weiner et al.[15]	Prospective	44	Resected bone margin	Yes	Yes	32 (73)	31 (70)
Atway et al.[16]	Retrospective	27	Resected bone margin	No	Yes	—	11 (41)

osteomyelitis. It is of note that the 73% rate of histologically proven osteomyelitis found by Weiner et al.[15] in amputated toes is virtually identical to our 67% rate of histologically confirmed osteomyelitis in biopsy specimens taken from the resected bone (data not shown). Furthermore, Kowalski et al.[14] found positive cultures to be twice as frequent as positive histological findings in their study of specimens from the resected bone (Table IV); however, they did not discuss this observation. Atway et al.[16] analyzed biopsy specimens from the resected bone margin after amputation but performed only cultures; they did not carry out histological analysis.

Compared with other studies[15,17], we found a very low rate of false-negative cultures. This may be explained by the standardized technique of biopsy retrieval, short times for transport to the laboratory, and our extensive microbiological experience in handling bone cultures.

There is no consensus on the precise histological criteria for diagnosing osteomyelitis in patients with diabetes mellitus or vascular disease requiring toe or forefoot amputation, which may lead to significant interobserver variability[18]. Previous studies of uninfected bone from diabetics showed non-inflammatory changes such as perivascular fibrosis and plasmacytic, but not neutrophilic, infiltrates[12]. Available criteria are generally based on the presence of neutrophilic granulocytes and have mainly been used for the diagnosis of hematogenous osteomyelitis[11] or were adapted from criteria used in the diagnosis of periprosthetic joint infections[9,19]. For this study, only the complete absence of neutrophilic granulocytes in the entire biopsy specimen was considered as a negative histological finding. We used this very low threshold in order to rule out even minor osteomyelitic residues and to eliminate any controversy when labeling bacterial results as "false-positive." The rate of negative histological studies and thus of false-positive cultures would have been even higher if we had excluded cases with <5 neutrophilic granulocytes per high-power field (regarded as possible osteomyelitis in our analysis) and included only cases with ≥5 neutrophilic granulocytes, such as has been done for the diagnosis of periprosthetic joint infection[9,10]. However, our criteria were chosen for the specific purpose of this study, and these criteria—including the low threshold for defining granulocytic infiltration—have to be validated before being applied in clinical practice. Additionally, the histological analyses in our study were performed by a single blinded pathologist, and we did not analyze intraobserver variability.

The strength of our study lies in the prospective design, the rigorous protocol for retrieving bone samples from clearly defined sites, the systematic histological and microbiological analyses, and the complete follow-up. Nevertheless, our study has limitations. The study population was heterogeneous with regard to peripheral arterial disease, diabetes, gangrene, and cellulitis. Furthermore, there was no rigorous standardized preoperative assessment of whether osteomyelitis was already present. However, our treatment strategy meets prevalent accepted standards of care. Moreover, the study population was too small to rigorously investigate the impact of patient and treatment characteristics on clinical outcome. Finally, our study was not designed to investigate the correct indication for, or value of, antibiotic therapy for osteomyelitis after toe amputation. It may well be reasonable to use a short period of antibiotic therapy for patients with culture-positive but histologically negative results since the documented intraoperative contamination of the bone may lead to propagation of the infection. Prospective randomized studies comparing treatment regimens are required to address this issue and determine whether osteomyelitis therapy should be restricted to patients with histologically proven residual osteomyelitis.

In conclusion, cultures of residual bone after toe or forefoot amputation are frequently positive without any histological evidence of osteomyelitis. We hypothesize that such false-positive results are due to contamination from adjacent infected soft tissue. The rate of residual osteomyelitis in patients who have undergone toe amputation appears to be much lower than postulated by studies that relied on culture of bone biopsy specimens alone[16,20]. We recommend that both histological analysis and culture of bone biopsied from the resection margin be used to diagnose residual osteomyelitis after toe amputation. Histological analysis is useful to rule out osteomyelitis, whereas culture will permit guided antibiotic therapy. Additional studies are needed to evaluate the indications for, and duration of, antibiotic treatment according to these findings.

Appendix

eA Tables showing individual patient characteristics and details on surgery, bone biopsy results, and wound-healing as well as histological and microbiological characteristics are available with the online version of this article as a data supplement at jbjs.org (http://links.lww.com/JBJS/E814). ■

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