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ORIGINAL ARTICLE

Comparison of microbiological results obtained from per-wound bone biopsies versus transcutaneous bone biopsies in diabetic foot osteomyelitis: a prospective cohort study

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Abstract

Transcutaneous bone biopsy (TCB) is the gold standard for taking microbiological specimens in diabetic foot osteomyelitis (DFO), but this technique is not widely used in diabetic foot care centers. We aimed to evaluate the reliability of per-wound bone biopsy (PWB) cultures by comparing them with concomitant TCB cultures obtained through healthy skin. This is a prospective monocentric study including patients seen in consultation for clinical and radiological diabetic foot osteomyelitis with positive probe-bone tests between April 2015 and May 2018. Two bone biopsies were performed on each consenting patient: TCB through a cutaneous incision in healthy skin, and PWB, after careful debridement of the wound. A total of 46 paired cultures were available from 43 eligible patients. Overall, 16 (42%) of the PWB and TCB pairs had identical culture results, but the TCB cultures were sterile in 8 (17%) cases. For 38 paired cultures with positive TCB, the correlation between PWB results and TCB results was 58.4%. PWB revealed all microorganisms found in the transcutaneous specimen in 26/38 samples (68.5%). In patients with DFO, the culture results of specimens taken by per-wound biopsies did not correlate well with those obtained by TCB. PWB should be reserved for cases where the transcutaneous biopsy is sterile or not feasible.

Keywords Diabetic foot · Osteomyelitis · Bone biopsy · Correlation · Diabetic foot osteomyelitis

Introduction

There is still no consensus on the most appropriate strategy to treat diabetic foot osteomyelitis (DFO) [1] and clinicians can consider using either primarily surgical or primarily medical strategies [2].

Pathogen identification and antibiotic susceptibility testing are thus important to provide the appropriate antibiotic therapy [3].

Several studies have shown that taking the bone biopsy specimens through healthy skin and not through the ulcer is currently the only reliable technique for determining the microbiology of DFO and is therefore the reference method [4, 5]. Despite these recommendations, the French prospective multicenter study, OPIDIA, showed that this technique is not well implemented in the care protocols of diabetic foot centers as it requires a multidisciplinary team [6]. Moreover, many developing countries do not have access to this technique due to limited means.

A retrospective single-center study of 80 patients with DFO demonstrated satisfactory clinical outcomes after antibiotic therapy adapted to the bacteriological findings of the bone biopsies taken via the wound after careful debridement. Follow-up in this study was for 12 months [7]. Of the 74 patients alive 6 months after the end of antibiotic therapy, 65 (88%) were considered cured. At 1 year, the data available for 54 of these 65 patients showed neither relapse nor amputation.

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The objective of this study was to correlate the microbiological results obtained from per-wound bone biopsies with the currently recommended bone biopsy through healthy skin for the diagnosis of diabetic foot osteomyelitis.

Methods

Setting

Patients were prospectively recruited at the Diabetic Foot Infection Day Care Unit of the University Hospital of Clermont-Ferrand, France, a 1916-bed university tertiary care hospital. Only patients with confirmed or suspected diabetic foot infection attend the clinic, so the prevalence of osteomyelitis is high. Patient data were collected from a French database, known as Happy@feet, which includes medical history, a picture of the wound with a detailed description of its exploration, the method used to obtain the microbiological sample, and the details on the patient's treatment including the antimicrobial agents prescribed [8].

Patients

Data were extracted for patients who attended the Diabetic Foot Infection Day Care Unit from April 2015 to May 2018. After a chart review, adult diabetic patients were included in the study if they met the following criteria: (i) clinical signs suggestive of infection (discharge, swelling, pain, inflammation, chronic non-healing wound); (ii) positive probe-to-bone test (the test is positive when the bone can be felt through a foot ulcer using a sterile blunt metal probe); (iii) initial or subsequent X-ray showing signs of osteomyelitis contiguous to the wound site; (iv) no clinical signs of active Charcot's disease; (v) bone sample obtained during a visit to the unit; (vi) have signed a consent for the collection of anonymized data and their analysis.

The non-inclusion criteria were an indication for immediate surgical management and unwillingness or inability to provide informed consent.

The study protocol was approved by the local ethics committee (no. 2014/CE85).

Bone samples

All samples were taken at the bedside by the same operator, who wore sterile gloves, gown, mask, and hat, in a large consultation room dedicated to bone biopsy.

After a careful debridement, the wound and the healthy skin were sterilized with polyvidone iodine and then washed with sterile saline solution.

Bone biopsies through healthy skin were performed by introducing a 13-gauge pediatric osteo-medullary biopsy trocar through a 3-mm incision made approximately 10 mm from the margins of the wound.

For per-wound biopsies, careful debridement of the necrotic and fibrinous tissues was first performed using a scalpel or curette before a bone sample was taken using metal forceps. When necessary, a bone debridement was performed in the same way. These procedures were sometimes performed under local anesthesia, though this depended on the severity of the patient's neuropathy.

Bone samples were sent to the microbiology laboratory in a sterile tube with a few drops of sterile saline solution within 2 h of sampling. All samples were transferred to, and processed by, the center's local clinical microbiology laboratory.

Microbiological assessment

The laboratory identified bacteria by MALDI MS technology using a VITEK MS system (Biomérieux, La Balme, France) and determined antibiotic susceptibility using the VITEK2 system or the disk diffusion method. Susceptibility results were interpreted according to the recommendations of the Antibiogram Committee of the French Microbiology Society.

Statistical analyses

Fisher's exact test was used to compare the microbiological results. The statistical significance level was set at $p < 0.05$. An estimate of the concordance between the results for per-wound bone biopsy cultures and transcutaneous bone biopsy cultures was provided by the percentage of paired cultures that resulted in the identification of the same pathogen in a given patient.

Results

Population

Between April 1, 2015, and May 31, 2018, we performed 46 paired cultures (per-wound bone biopsy and transcutaneous bone biopsy) collected from 43 patients with confirmed diabetic foot osteomyelitis. Patient characteristics are presented in Table 1. The clinical examinations during the consultations showed 19 (41.3%) cases of soft tissue infection. X-rays performed on 39 patients showed initial osteitis in 33 (84.6%). Six patients did not initially have radiological osteitis; however, it was visible on the X-ray performed 1 month later. Eighteen patients received antibiotic therapy in the 14 days prior to the biopsies; antibiotic therapy was not stopped before sampling for six of them.

Table 1 Demographic and clinical characteristics of enrolled patients

Variable	*N* (%) or ± SD
Mean age (± SD)	71 (± 11.6)
Male/female	37 / 9
Diabetes duration (years)	15.6 (± 9.1)
Mean (± SD) HbA1c (%)	7.25 (± 0.96)
Insulin therapy	32 (69.6)
Oral anti-diabetic treatment	16 (34.8)
Lower limb arteriopathy	29 (63)
Past history of amputation	11 (23.9)
Etiology of past amputation	
Infected	4 (8.7)
Ischemic	4 (8.7)
Both	3 (6.5)
Chronic renal failure	38 (82.6)
60 ml/mn ≤ clearance < 90 ml/mn	10 (21.7)
30 ml/mn ≤ clearance < 60 ml/mn	23 (50)
Clearance < 30 ml/mn	5 (10.9)
University of Texas grade/stage	
Grade 3 stage B	32 (69.6)
Grade 3 stage D	14 (30.4)
Mean (± SD) duration of ulcer evolution (weeks)	12 (23.5)
Ulcer localization	
Hallux	11 (23.9)
Other toes	22 (47.8)
Metatarsus	12 (26.1)
Calcaneum	1 (2.2)
Soft tissue infection	19 (41.3)
Dermo-hypodermitis	8 (17.4)
Sausage toe	11 (23.9)
Prior antibiotic therapy	29 (63)
Current	6 (13)
Within 14 days	12 (26.1)
Beyond 14 days	11 (23.9)
Mean (± SD) C-reactive protein (mg/L)	38.7 (74.9)

The University of Texas classification:
Stage: B = infected; D = infected and ischemic
Grade: 3 = wound penetrates to bone or joint

Microbiological findings

For the 46 paired cultures obtained from 43 patients, results were negative (i.e., sterile) for 1 (2%) of the per-wound biopsy cultures and 8 (17%) of the transcutaneous biopsy cultures ($p = 0.005$).

The distribution of the identified germs in the 46 paired bone biopsies is shown in Table 2. The different isolates were equally distributed in cultures from both types of bone biopsies. As shown in Table 3, the concordance ranged from 29.6 to 70.4%, with an overall concordance of 48.4%. The best results were observed for *S. aureus* strains, whereas the most discordant results were those with gram-negative bacilli and coagulase-negative staphylococci.

After exclusion of cases with sterile transcutaneous biopsy cultures, the mean concordance was 58.4% (36.4–79.2%). For the 38 paired bone biopsy cultures with positive transcutaneous biopsy cultures, 16 (42%) had identical results (Table 4). In 10 (26%) of these paired bone cultures, per-wound biopsy results included more isolates than the transcutaneous biopsy. Thus, in 26 (68%) of these pairs, per-wound biopsies revealed all microorganisms found in the transcutaneous specimen. In 5 (13%) cases, per-wound biopsy cultures did not have isolates found by transcutaneous biopsy cultures. Finally, in 7 (18.5%) cases, there were no common pathogens identified from transcutaneous biopsy and per-wound biopsy specimens.

From the 77 isolates found by transcutaneous bone biopsy cultures, 59 (77%) also occurred in paired per-wound bone biopsy cultures.

Discussion

IDSA guidelines suggest sending a sample for culture when bone is debrided to treat osteomyelitis but no study has compared the results of these cultures with those obtained by the gold standard method, the transcutaneous bone biopsy. Our results showed only a 48.4% concordance between per-wound bone biopsy and transcutaneous bone biopsy cultures in DFO. Furthermore, 18% of the patients had discordant results. With regard to the identification of *Staphylococcus aureus*, the concordance improved to 79.2%. If only the results of the per-wound biopsy cultures had been taken into account when prescribing antibiotics, 26 (68%) of our 38 patients with positive transcutaneous bone biopsy cultures would have received an effective antibiotic therapy, 10 of whom would have potentially had an antibiotic therapy with a too broad spectrum; 13% would have been undertreated; and 18.5% would have received an inappropriate antibiotic therapy.

The current gold standard for microbiological documentation is a bone biopsy through healthy skin, which does not require a surgeon and can be quickly done by a trained practitioner. It thus avoids a delay in therapeutic management and does not compromise the foot prognosis. However, the disadvantage is the risk of a false negative due to the absence of X-ray guidance [4]. Per-wound biopsy is easily achievable during ulcer debridement. This technique has shown several advantages: (i) the visual exposure of the infected bone eliminates the risk of sampling a healthy zone; (ii) the necrotic bone sample is assumed to have a higher bacterial load, which increases the probability of a positive culture, even with recent antibiotic therapy; (iii) antibiotic therapy can be started soon after sampling; (iv) this technique does not create a new skin lesion, in patients commonly suffering from arteritis.

Table 2 Distribution of microorganisms isolated from per-wound bone biopsy specimens and percutaneous bone biopsy specimens concomitantly obtained from 43 patients with diabetic foot osteomyelitis

Variable	Per-wound biopsy (PWB) specimens	Transcutaneous biopsy (TCB) specimens	PWB and TCB identical cultures	PWB specimens when TCB were sterile
No. of sample culture	46	46	16	8
Total no. of isolates	104	77	37	21
Mean no. of isolates per sample	2.4	1.7[a]	2.3	2.6
No. of sterile sample cultures (%)	1 (2)	8 (17)[a]	0	0
A single bacterial species per sample	11 (24)	14 (31)	5 (31)	1 (12.5)
Two bacterial species per sample	14 (31)	13 (28)	5 (31)	3 (37.5)
Three or more species per sample	20 (43)	11 (24)	6 (38)	4 (50)
Gram-positive cocci	66 (63.5)	49 (63.6)	27 (73)	12 (57)
Staphylococci	42 (40.4)	33 (42.9)	16 (43)	9 (43)
Staphylococcus aureus	27 (26)	19 (24.7)	10 (27)	3 (14)
MRSA	4 (3.8)	3 (3.9)	1 (3)	0
Coagulase-negative staphylococci	15 (14.4)	14 (18.2)	6 (16)	6 (29)
Enterococci	9 (8.7)	7 (9.1)	4 (11)	1 (5)
Streptococci	15 (14.4)	9 (11.7)	7 (19)	2 (10)
Corynebacteria	17 (16.3)	11 (14.3)	4 (11)	4 (19)
Gram-negative bacilli	19 (18.3)	16 (20.8)	5 (14)	5 (24)
Anaerobes	2 (1.9)	1 (1.3)	1 (3)	0

MRSA methicillin-resistant *S. aureus*

[a] $p < 0.01$

Transcutaneous bone biopsy cultures were sterile in 8 cases. These negative results could be explained by the biopsy-targeting technique used and the presence of antibiotics. Biopsies were not guided by X-ray and were not performed under ultrasound guidance. Although per-wound bone biopsies are easy to perform and therefore constitutes an attractive method for DFO microbiological identification, our results show that the technique is less reliable than transcutaneous bone biopsy, but can be useful the latter is not possible or is sterile.

Table 3 Correlation between per-wound bone biopsy and transcutaneous bone biopsy cultures

Isolates	No. of cultures					
	Total	Per-wound biopsy (PWB)	Transcutaneous biopsy (TCB)	Both	Correlation[a] (%)	Correlation[a] only in cases of positive TCB cultures (%)
Staphylococcus aureus	27	8	0	19	70.4	79.2
CNS	22	8	7	7	31.8	43.8
Enterococci	10	3	1	6	60	66.7
Streptococci	16	7	1	8	50	57.1
Corynebacteria	18	7	1	10	55.6	71.4
Gram-negative bacilli	27	11	8	8	29.6	36.4
Anaerobes	2	1	0	1	50	50
Total	122	45	18	59	48.4	58.4

[a] Percentage of transcutaneous bone biopsy and per-wound bone biopsy specimens obtained from a given patient that yielded the same pathogen

CNS coagulase-negative *Staphylococcus*

Table 4 Correlation between per-wound bone biopsy (PWB) and transcutaneous bone biopsy (TCB) cultures in the 38 samples with positive transcutaneous bone biopsy cultures

	No. of samples (%)
PWB contained all organisms found in TCB	26 (68)
Identical PWB and TCB cultures	16 (42)
PWB contained all organisms found in TCB plus additional organisms	10 (26)
PCB lacked organisms found in TCB	5 (13)
Completely different isolates in PWB and TCB cultures	7 (18.5)

Conclusion

Our study shows that, compared with transcutaneous bone biopsy specimens, per-wound bone biopsy cultures are not sufficiently accurate to identify the causative organisms in patients with an infected diabetic foot ulcer, except in the case of *Staphylococcus aureus*. This technique should be reserved for cases where the trs bone biopsy is sterile or not feasible.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study protocol was approved by the local ethical research committee (reference number 2014/CE85).

Informed consent Informed consent was obtained for the collection of anonymized data and their analysis.

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