Reliability and Safety of Bedside Blind Bone Biopsy Performed by a Diabetologist for the Diagnosis and Treatment of Diabetic Foot Osteomyelitis

Diabetes Care 2021;44:2480–2486 | https://doi.org/10.2337/dc20-3170

Florine Féron,[1] Gauthier Péan de Ponfilly,[2] Louis Potier,[3,4] Diane-Cécile Gauthier,[1] Laurence Salle,[1] Marie Laloi-Michelin,[1] Anne-Lise Munier,[5] Hervé Jacquier,[2] Tiphaine Vidal-Trécan,[1] Jean-Baptiste Julla,[1,4] Aurélie Carlier,[3] Yawa Abouleka,[3] Nicolas Venteclef,[4] Nathalie Grall,[6] Frédéric Mercier,[7] Jean-Pierre Riveline,[1,4] Éric Senneville,[8] Jean-François Gautier,[1,4] Ronan Roussel,[3,4] and Jean-Philippe Kevorkian[1]

OBJECTIVE

Bone biopsy (BB) performed by a surgeon or an interventional radiologist is recommended for suspicion of osteomyelitis underlying diabetic foot ulcer (DFU). To facilitate its practice, we developed a procedure allowing bedside blind bone biopsy (B4) by a diabetologist.

RESEARCH DESIGN AND METHODS

We conducted a three-step observational study consisting of a feasibility and safety phase (phase 1) to assess the success and side effects of B4, a validity phase (phase 2) to compare DFU outcomes between positive (B4+) and negative (B4−) bone cultures, and a performance phase (phase 3) to compare B4 with the conventional surgical or radiological procedure basic bone biopsy (B3). Primary end points were the presence of bone tissue (phase 1) and complete DFU healing with exclusive medical treatment at 12 months (phases 2 and 3).

RESULTS

In phase 1, 37 consecutive patients with clinical and/or radiological suspicion of DFU osteomyelitis underwent B4. Bone tissue was collected in all patients with few side effects. In phase 2, a B4+ bone culture was found in 40 of 79 (50.6%) participants. Among B4+ patients, complete wound healing after treatment was 57.5%. No statistical difference was observed with patients with B4− bone culture not treated with antibiotics (71.8%, $P = 0.18$). In phase 3, the proportion of patients with positive BB was lower in B4 (40 of 79, 50.6%) than in B3 (34 of 44, 77.3%, $P < 0.01$). However, complete healing was similar (64.6% vs. 54.6%, $P = 0.28$). No difference in rate of culture contamination was observed.

CONCLUSIONS

B4 is a simple, safe, and efficient procedure for the diagnosis of DFU osteomyelitis with a similar proportion of healing to conventional BB.

During their lifetime, 15–25% of patients with diabetes will develop a diabetic foot ulcer (DFU) related to neuropathy and/or peripheral arterial disease (1). At least one-half of all DFUs are clinically infected when the patient presents to clinicians (2,3). Osteomyelitis occurs in 40–80% of infected ulcers, which is a severe

[1]*Department of Diabetes and Endocrinology, Lariboisière Hospital, Assistance Publique-Hôpitaux de Paris, Université de Paris, Paris, France*
[2]*Department of Microbiology, Lariboisière Hospital, Assistance Publique-Hôpitaux de Paris, Université de Paris, Paris, France*
[3]*Department of Diabetes, Endocrinology, and Nutrition, Bichat Hospital, Assistance Publique-Hôpitaux de Paris, Université de Paris, Paris, France*
[4]*Cordeliers Research Centre, INSERM, Immunity and Metabolism in Diabetes Laboratory, Sorbonne University, Université de Paris, Paris, France*
[5]*Department of Infectious Disease, Lariboisière Hospital, Assistance Publique-Hôpitaux de Paris, Université de Paris, Paris, France*
[6]*Department of Microbiology, Bichat Hospital, Assistance Publique-Hôpitaux de Paris, Université de Paris, Paris, France*
[7]*Department of Surgery, Parc Monceau International Clinic, Paris, France*
[8]*Department of Infectious Disease, Gustave Dron Hospital, Tourcoing, France*

Corresponding author: Florine Féron, florine.feron@aphp.fr

Received 30 December 2020 and accepted 2 August 2021

F.F., G.P.d.P., and L.P. contributed equally to this study.

complication of DFU (4). Diabetic foot osteomyelitis (DFO) leads to minor or major limb amputations in almost 20% of patients (4). It has been shown that medical treatment of DFO may prevent amputations with early diagnosis of osteomyelitis and appropriate use of antibiotics (5,6). Empirical antimicrobial treatment is not recommended as it is for other chronic infections (7). On the other hand, as previously shown, both swabs and needle aspirations cannot be used as surrogate tools for bone infection identification (8,9). Moreover, bone biopsy (BB) performed through the wound (per-wound BB) might be a potential alternative but gives results that reach only 48.4% concordance with those obtain from percutaneous BB performed in healthy skin afar from the wound (10). Per-wound BB is associated with a higher risk of contamination of the specimen. In agreement with generally accepted basic rules for medical treatment of any other bone and joint infection, antimicrobial treatment should be based on bone culture results. As suggested in previous studies, percutaneous BB is a safe procedure that can be performed either by a surgeon or by an interventional radiologist (6–9). Although BB is not universally carried out because of the lack of availability, it remains the most accurate method to identify microbiological pathogens involved in DFO (9) and is recommended as the microbiologic key diagnosis reference by the International Working Group of Diabetic Foot (IWGDF) (7). Some DFO infections need an isolated or associated surgical treatment. Nevertheless, BB is a diagnostic procedure that is not always available, making it underused in most diabetic foot centers. Indeed, a study reported that only 20% of clinicians use BB in cases of suspected DFO (11) and consequently use an empirical or delayed tailored antibiotic strategy in most. In 2015, to overcome this difficulty, our clinical unit set up a bedside blind bone biopsy (B4) performed by the diabetologist (medical specialist) in our diabetes unit in cases of suspected DFO. Here, we report the feasibility and reliability of B4 with respect to the quality of bone samples, its side effects, and the healing rate in patients who underwent this procedure.

RESEARCH DESIGN AND METHODS

Study Design

The study consisted of three phases (Fig. 1).

Phase 1 (Feasibility and Safety Phase)

This first single-center, prospective, observational evaluation was conducted from December 2015 to September 2017 to assess the feasibility and safety of B4 by a diabetologist (Department of Diabetes and Endocrinology, Lariboisière Hospital) in cases of suspected DFO. Diabetologists were trained by colleagues from the Department of Radiology.

Phase 2 (Validity Phase)

In phase 2, we increased the number of subjects by extending the inclusion period (from December 2015 to September 2018), and we prospectively compared the wound healing rate between patients with B4 positive bone culture (B4+) treated by antibiotics and those with B4 negative bone culture (B4−) not treated by antibiotics during a 12-month follow-up.

Phase 3 (Performance Phase)

The performance of B4 carried out in Lariboisière Hospital from December 2015 to September 2018 was retrospectively compared with the conventional surgical or radiological procedure, namely basic bone biopsy (B3), performed in another diabetes unit (Department of Diabetes, Bichat Hospital) from September 2013 to September 2018 (Figs. 1 and 2). The therapeutic strategy was similar in both groups (B3 and B4) and driven by the results of bone culture. Each patient's therapeutic strategy followed international guidelines (7,12,13). At least a 12-month follow-up after the date of the BB was mandatory to include the patients in this evaluation phase. When bone culture was positive (B3+ or B4+), a tailored antimicrobial treatment was introduced for at least 6 weeks according to current recommendations (7,14). When bone culture was negative or contaminated (B3− or B4−), no antimicrobial treatment was prescribed.

Study Patients

Eligibility requirements at screening consisted of consecutive inpatients with diabetes aged >18 years admitted for foot ulcer and suspected osteomyelitis on clinical and/or X-ray examination based on the presence of at least two of the following criteria: chronic evolution of the ulcer (over a 4-week period), ulcer area >2 cm^2, positive probe-to-bone test, or abnormal findings consistent with bone involvement on plain X-ray. In case of prior antimicrobial treatment (acute skin and soft tissue infection or any other situation), BB was performed at least 2 weeks after the end of the antibiotic treatment (15,16). Decision for BB was made during a multidisciplinary conciliation meeting.

At inclusion, age, sex, duration of diabetes, HbA_{1c} level, diabetes-related complications, and serum C-reactive protein values were collected. DFU was classified using IWGDF criteria at the time of indication of BB (10,14). Severe peripheral arterial disease was defined as a stenosis of at least 70% on a proximal artery or presence of only one functional distal artery of the leg. Very severe peripheral arterial disease was defined as both severe proximal and distal arteriopathy. Neuropathy was assessed by the presence of paresthesia or cramps or loss of

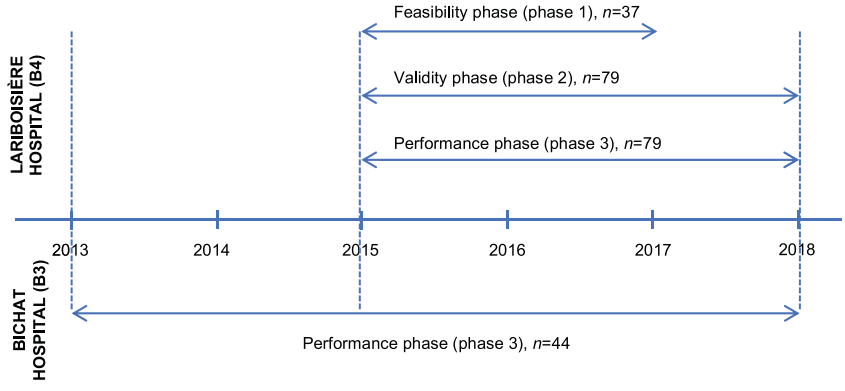

Figure 1—Design of the study.

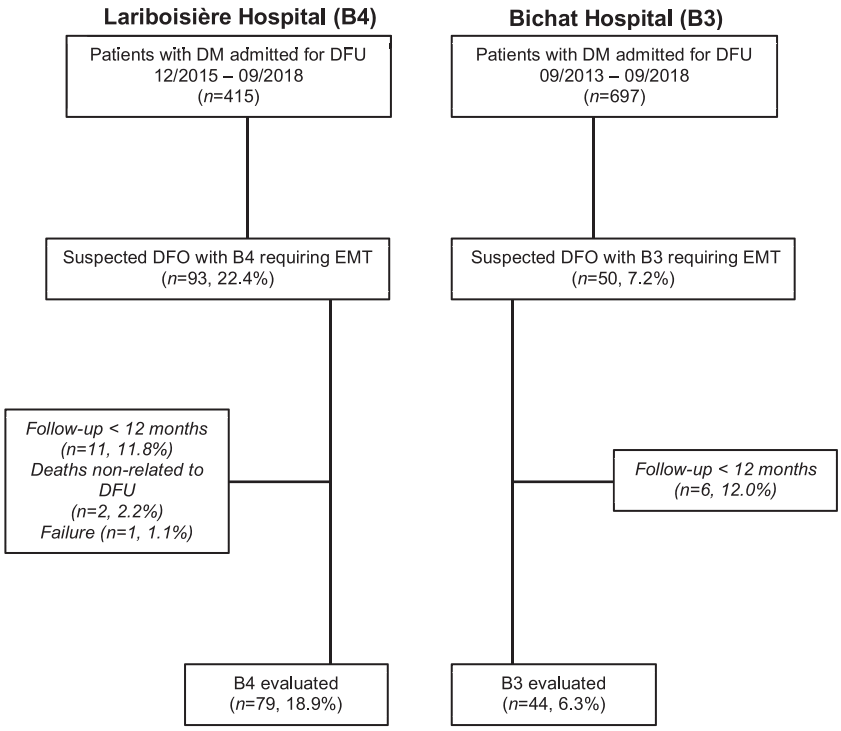

Figure 2—Phase 2/3 diagram of the study. DM, diabetes mellitus; EMT, exclusive medical treatment.

protective sensation with the 10g Semmes-Weinstein monofilament test.

In the B4 validity phase 2 and performance phase 3, only patients eligible for an exclusive medical treatment (including offloading; wound care, particularly mechanical debridement; dressing; and antimicrobial treatment in cases of positive microbiological culture) and in whom follow-up was available for at least 12 months after BB were included. Exclusion criteria were requirement for surgical treatment at inclusion (debridement or amputation), death unrelated to DFU, antimicrobial treatment prescribed within 2 weeks prior to BB, and/or incomplete follow-up. All subjects gave free and written informed consent to the use of their records for research purposes according to French legislation.

BB Procedures

B4

The whole procedure was performed in the diabetes unit in the patient's room under aseptic conditions. Pain relievers (step 1 ± 3) and anxiolytics were given orally within the hour preceding the procedure. X-ray with two or three pasted metallic markers, if necessary, within the last 15 days was required to fix the needle route of the biopsy for directly accessing the suspected osteomyelitic area. Superficial anesthesia was performed first subcutaneously and then on the periosteum (lidocaine 10 mg/mL; Aguettant, Lyon, France). Inhaled anesthesia with a mixture of equal parts of nitrous oxide and oxygen was started concomitantly (7–10 L/min). Following a short incision with a scalpel, the trocar inside a canula (Madison Bone Biopsy Mini Kit KDP 13/6; Merit Medical, South Jordan, UT) was inserted through healthy skin at a minimum distance of 2 cm from the ulcer edge if possible, close to the bone, preferentially on the dorsal foot side. When the trocar was firmly inserted into the bone, it was pulled out through the canula. The biopsy needle was then slipped into the canula and twisted into the bone clockwise. The biopsy needle was then pulled out, and the bone tissue was pushed out with the ejector pin into a sterile surgical drape and divided into two parts. The canula was extracted, and the procedure was repeated two times at two other sites (17,18). All three samples obtained were divided in two pieces: one for the microbiological department (in a dry environment within 3 h) and one for the histology laboratory (with formol). The size of each sample sent for microbiological and histological examination was close to 2–3 mm.

B3

B3 was performed in patients admitted for DFU at Bichat Hospital either by an interventional radiologist from Bichat Hospital or by an external surgeon working in another hospital. As for B4, B3 was done through healthy skin. Surgical B3 was performed in an operating theater under locoregional anesthesia. Biopsy was done with a trocar (Intraosseous Infusion Needle 16/3; Cook Medical, Bloomington, IN), if possible, at 2 cm from the edge of the ulcer. Samples were sent to the microbiological department of Bichat Hospital in a dry environment. When B3 was carried out by a radiologist, the needle route was CT or X-ray guided in the radiological suite. The biopsy was done with the trocar (Madison Bone Biopsy Mini Kit KDP 13/6). Histopathological examination was not performed for the B3 bone samples. Size of the samples obtained was from 2 mm to 1 cm.

Microbiological and Histological Procedures

Gram staining was performed on all bone biopsy samples (B4 and B3). Histological analysis was performed on dedicated specimens by evaluation of polymorphonuclear leukocyte count in high-power fields of frozen tissue sections (B4).

For samples from Lariboisière Hospital, tissues were placed in sterile Nalgene vials containing 10 mL of sterile water and 5 mL of sterile glass beads (1.5-mm diameter) and crushed by a Retsch MM301 Mixer Mill for 3.5 min at 30 Hz, as previously described (19,20). Crushed tissues and fluids were cultured at 35°C on blood agar plates (aerobically and anaerobically), on PolyViteX chocolate agar plates (under 5% CO_2), and in Rosenow broth for a 7-day incubation. After 1 week, Rosenow broth was systematically replated on a new set of agar plates and incubated in the same conditions for 7 more days.

For samples from Bichat Hospital, tissues were processed as follows. After grinding (ULTRA-TURRAX Tube Drive; IKA), each sample was plated onto standard agars for culture of aerobes and anaerobes and into Schaedler broth for 15 days.

A contamination was defined as the presence of one bacterium belonging to

the skin flora (e.g., coagulase-negative staphylococci, *Corynebacterium* species, or *Cutibacterium acnes*) in one biopsy over all samples per patient and/or associated with the decision of a multidisciplinary team not to take into account this bacterium in the antimicrobial treatment.

Outcomes

Phase 1 (Feasibility and Safety Phase)

The primary end point was defined by the presence of bone tissue at histological examination. Secondary end points were adverse events occurring within the following 72 h either locally (provoked ulcer/inflammation/necrosis, bleeding) or generally (fever, pain, positive systematic blood culture every hour in the following 3 h or in case of fever). Pain was assessed by a heteroevaluation scale during B4 (Algoplus scale [21]) and by pain reliever consumption in the following 24 h. Major bleeding was defined as life-threatening and/or a two-point reduction in hemoglobin rate (in g/dL), the need for a transfusion, or compression failure. Minor bleeding was defined as requiring no more than a 3-min compression to stop the bleed.

Phase 2 (Validity Phase)

The primary end point was the rate of patients with complete wound healing with exclusive medical treatment and no evidence of recurrence at 12 months with a 6-week tailored antimicrobial treatment in cases of proven osteomyelitis (B4+) or no antimicrobial treatment in cases of negative cultures (B4−). The choice of antibiotic therapy was left to the clinicians based on microbiology results. Complete wound healing was defined as complete skin epithelialization during the follow-up period with no DFU relapse. Failures were defined as lack of healing, requirement of surgical treatment (debridement, amputation), DFU relapse, and death related to DFU.

Phase 3 (Performance Phase)

In this comparative procedure evaluation, the primary end point was the same as in phase 2. The secondary end points were the comparison of microbiological results and the contamination rates per sample and per patient between the B3 and B4 groups.

Statistics

Comparisons between groups were performed using Fisher exact test for categorical variables and the Mann-Whitney test for continuous variables. Analyses were performed using R 3.1.3 statistical software, and $P < 0.05$ was considered statistically significant. Results are given as means and SDs unless otherwise stated.

RESULTS

Phase 1 (Feasibility and Safety Phase)

From December 2015 to September 2017, 37 of 291 (12.7%) consecutive inpatients with DFU were considered as displaying osteomyelitis and were included in the study. Baseline patient characteristics are presented in Table 1. DFU localizations were metatarsal 65%, proximal phalanges 16%, distal phalanges 11%, calcaneus 8%, and tarsus 0%. Eighty-seven samples were collected, with a mean of 2.4 samples per patient. Mean duration of the B4 procedure was 60 ± 8 min from first surgical drape settlement to biopsy needle extraction and drape withdrawal. The diabetologist needed to be assisted by two operating aides in most cases. Regarding the primary outcome, histological examination confirmed bone tissue in all samples (100% positivity rate). No local complication (bleeding, provoked ulcer, inflammation, and necrosis) was recorded. Only 3 (7%) patients complained of immediate pain, and 18 (50%) complained within 24 h (maximum step 2 pain relief). Fever was present in 21% of patients. Bacteremia was observed in three (8.1%) patients: *Staphylococcus aureus* was found for one patient (B4: *Citrobacter koseri, Serratia marcescens, Enterococcus faecalis*), *S. aureus* for the second patient (B4: *S. aureus, Finegoldia magna*), and *Fusobacterium nucleatum* for the third patient (B4: *Pseudomonas aeruginosa, Proteus mirabilis*). Thus, in one patient, *S. aureus* was the same in blood and bone cultures. For the two others, the germs found in blood culture were different from those found in bone culture but were taken into account to tailor antibiotic therapy.

Phase 2 (Validity Phase)

Ninety-three patients were included in the phase 2 evaluation. Fourteen (15%) were excluded: 11 were lost to follow-up, 2 died of causes unrelated to DFU, and 1 experienced procedure failure (no bone tissue sample) (Fig. 2). Seventy-nine (84.9%) patients were finally evaluated. Histological analyses confirmed the presence of bone tissue for each sample. B4+ cultures were found in 50.6% participants (40 of 79). Patients' characteristics were similar in B4+ and B4− except for age and C-reactive protein level, which were both significantly higher in B4+ versus B4− (Table 1). Sixty-seven percent (52 of 79) of patients received antibiotics during the previous 3 months. Regarding the primary outcome, the healing rate with exclusive medical treatment at 12 months was similar between groups, occurring in 23 (57.5%) and 28 (71.8%) of B4+ and B4− patients, respectively ($P = 0.18$) (Table 1). B4− and healing rate were similar in patients who had previous antibiotics and those with no antibiotics ($P = 0.15$ and $P = 1$, respectively). No statistical difference was observed in B4− and healing rate according to the severity of arteriopathy ($P = 0.5$ and $P = 0.25$, respectively). In the B4+ group, 17 (42.5%) patients had no complete wound healing with exclusive medical treatment. Among them, eight underwent surgical treatment (with complete healing for four), one had DFU relapse (but finally healed during the follow-up period), five died as a result of DFU, and three needed a second biopsy (no healing progression during the 12-month follow-up). The causes of death of the five patients were as follows: severe sepsis in the context of advanced cancer need for palliative care in one, geriatric cachexia with limitations of care (advanced age, severe diabetic complications) in two, sudden death at home with suspected iatrogenic origin in one, and sepsis-induced cardiogenic shock in one with coronary heart disease and severe kidney failure. In the B4− group, 11 patients did not heal with exclusive medical treatment as follows: 4 had surgical treatment (with complete healing for 3), 4 had DFU relapse (3 finally healed), and 3 needed a second biopsy (no healing after the 12-month follow-up).

Phase 3 (Performance Phase)

Population Screening

A total of 1,112 consecutive patients with DFU were admitted to Bichat Hospital or Lariboisière Hospital (Fig. 2). Among

Table 1—Patient characteristics

Characteristic	Phase 1	Phase 2		Phase 3			
	B4 (n = 37)	B4+ (n = 40)	B4− (n = 39)	B4 (n = 79)	B3 (n = 44)	P*	P**
Male/female sex, n	28/9	33/7	26/13	59/20	32/12	0.10	0.81
Male sex, %	75.7	82.5	66.7	74.7	72.7		
Age (years)	71 ± 14	75 ± 13	68 ± 12	71 ± 13	68 ± 11	0.003	0.78
Type 2 diabetes	35 (94.6)	31 (77.5)	36 (92.3)	67 (84.8)	42 (95.5)	0.07	0.07
Duration of diabetes (years)	19 ± 10	20 ± 11	21 ± 9	20 ± 10	22 ± 10	0.90	0.73
HbA_{1c} (%)						0.34	0.52
%	7.6 ± 1.8	7.7 ± 1.7	7.9 ± 2.4	8 ± 2.1	7.7 ± 2.1		
mmol/mol	60 ± 11.1	61 ± 10.5	63 ± 14.9	64 ± 13	61 ± 13		
Insulin	32 (86.5)	29 (72.5)	31 (79.5)	60 (75.9)	29 (65.9)	0.47	0.23
History of DFU	18 (48.7)	24 (60.0)	18 (46.2)	42 (53.2)	23 (52.3)	0.22	0.92
History of amputation	12 (32.4)	16 (40.0)	11 (28.2)	27 (34.2)	18 (40.9)	0.27	0.46
eGFR (mL/min/1.73 m^2)	66 ± 31	66 ± 29	70 ± 29	70 ± 29	55 ± 30	0.20	0.03
Proteinuria (g/L)	0.16 ± 0.70	0.1 ± 0.5	0.19 ± 0.9	0.13 ± 0.7	0.21 ± 0.7	0.13	0.10
Retinopathy	26 (70.3)	22 (55.0)	27 (69.2)	49 (62.0)	34 (77.3)	0.19	0.08
Neuropathy	30 (81.1)	36 (90.0)	33 (84.6)	69 (87.3)	35 (79.5)	0.47	0.25
Arteriopathy							
Severe	11 (29.7)	10 (25.0)	12 (30.8)	22 (27.8)	10 (22.7)	0.57	0.53
Very severe	7 (18.9)	11 (27.5)	5 (12.8)	16 (20.3)	6 (13.6)	0.10	0.36
Coronaropathy	8 (21.6)	7 (17.5)	10 (25.6)	17 (21.5)	10 (22.7)	0.38	0.88
Albuminemia (g/L)	33 ± 5.3	31 ± 5.9	31 ± 5.3	31 ± 5.6	33 ± 4.8	0.34	0.23
IWGDF grade 4	14 (37.8)	8 (20.0)	7 (17.9)	15 (19.0)	4 (9.1)	0.82	0.15
CRP (mg/L)	27 ± 73	33 ± 67	5 ± 29	16 ± 55	13 ± 43	0.001	0.64
Total healing rate§		29 (72.5)	33 (84.6)	62 (78.5)	37 (84.1)	0.19	0.55
Healing EMT		23 (57.5)	28 (71.8)	51 (64.6)	24 (54.6)	0.18	0.28
Healing EMT delay/B4 (days)		124 ± 94	103 ± 111	105 ± 103	105 ± 131	0.60	0.77

Data are n (%) or mean ± SD unless otherwise indicated. CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; EMT, exclusive medical treatment. *Phase 2 B4+ vs. B4−. **Phase 3 B4 vs. B3. §Including surgical intervention, recurrences, and second biopsy.

them, 143 (12.8%) had a suspected DFO requiring BB: 79 B4 and 44 B3 patients were evaluated. Histological analyses confirmed bone tissue in each B4 sample. Among the 44 B3 patients, BB was surgically performed in 32 (72.7%) and radiologically in 12 (27.3%). Baseline characteristics of patients were similar between the two groups except for estimated glomerular filtration rate, which was significantly lower in the B3 group (Table 1).

Microbiology Bacterial Strains

Microbiological results were discussed in multidisciplinary conciliation meetings. The number of patients diagnosed with osteomyelitis based on positive biopsies was significantly higher in the B3 group (77.3% vs. 50.6%), whereas the number of bone samples per patient was significantly higher in the B4 group (1.3 vs. 2.8) (Table 2). Overall, 162 different pathogens were obtained (90 and 72 in the B4 and B3 groups, respectively). The main pathogen was *S. aureus* in both groups (21.1% vs. 20.8% in the B4 and B3 groups, respectively, P = 0.97), with methicillin resistance observed in three patients in the B4 group and two in the B3 group. All bacterial stains are reported in Table 2. The proportion of polymicrobial osteomyelitis was similar between the two groups as well as the mean number of pathogens for polymicrobial osteomyelitis. Therapeutic strategy was often started as the first pathogen was identified in BB (∼3 days).

Outcomes

The rate of patients with complete wound healing with exclusive medical treatment and no relapse at 12 months was similar between the B4 and B3 groups (Table 1). No statistical difference was found between B3− and B3+ healing rate (30% and 61%, respectively, P = 0.14). In addition, the healing rate was not statistically different between the B4+ and B3+ groups (58% vs. 61%, respectively, P = 0.81). The contamination rate was similar in both groups (30 of 220 samples [13.6%] vs. 6 of 59 samples [10.2%] in B4 and B3, respectively, P = 0.59). Time duration between BB and complete wound healing was similar in both groups (Table 1).

CONCLUSIONS

In this observational study assessing the reliability, safety, and accuracy of a bedside BB for diagnosis of DFO, we showed that this easily accessible procedure was safe and allowed microbiological identification in most cases with a similar performance

Table 2—Microbiological data of bones cultures for phase 3 patients

	B4 (n = 79)	B3 (n = 44)	P
Samples by patients (mean)	2.8 ± 0.8	1.3 ± 0.7	<0.01
Osteomyelitis with pathogen found	40 (50.6)	34 (77.3)	<0.01
Isolates by pathogen	90 (100.0)	72 (100.0)	1
Gram-positive findings	49 (54.4)	43 (59.7)	0.50
Staphylococci	24 (26.7)	23 (31.9)	0.46
S. aureus	19 (21.1)	15 (20.8)	0.97
MRSA (% among species)	3 (15.8)	2 (13.3)	
Coagulase-negative staphylococci	5 (5.6)	8 (11.1)	0.20
Enterococci	8 (8.9)	7 (9.7)	0.86
Streptococci	13 (14.4)	10 (13.9)	0.92
Streptococcus pyogenes	1 (1.1)	0 (0)	0.37
Streptococcus agalactiae	3 (3.3)	2 (2.8)	0.84
Streptococcus dysgalactiae	3 (3.3)	3 (4.2)	0.78
Other streptococci	6 (6.7)	5 (6.9)	0.94
Other gram-positive cocci	0 (0)	1 (1.4)	0.26
Corynebacteria	4 (4.4)	2 (2.8)	0.58
Gram-negative findings	30 (33.3)	27 (37.5)	0.58
Escherichia coli	3 (3.3)	3 (4.2)	0.78
Klebsiella spp	0 (0)	4 (5.6)	0.02
Proteus spp	6 (6.7)	2 (2.8)	0.26
Enterobacter spp	3 (3.3)	4 (5.6)	0.49
Other *Enterobacteriaecae*	10 (11.1)	3 (4.2)	0.11
Pseudomonas aeruginosa	8 (8.9)	7 (9.7)	0.86
Other	0 (0)	4 (5.6)	0.02
Anaerobes	11 (12.2)	2 (2.8)	0.03
Bacteroides spp	3 (3.3)	2 (2.8)	0.84
Other	8 (8.9)	0 (0)	<0.01
Polymicrobial osteomyelitis	27 (67.5)	21 (61.8)	0.61
Pathogens per episode	2.3 ± 1.3	2.1 ± 1.2	0.62

Data are n (%) or mean ± SD. EMT, exclusive medical treatment; MRSA, methicillin-resistant *S. aureus*; spp, species.

in terms of healing compared with the surgical or radiological standard BB procedure. The diagnosis of osteomyelitis in cases of DFU is a difficult challenge of major therapeutic interest. Although BB is well recognized as the gold standard for the diagnosis, it is often impractical because the procedure requires some time, experience, and expense. Thus, there is a clear need in clinical practice for a safe, reliable, and simple method to determine the causative pathogen as recommended in DFO. Here, we investigated whether B4 performed by a diabetologist would be a helpful alternative.

Our findings show that B4 is a feasible, safe, informative, and highly successful procedure. A few patients experienced pain, but in no case did we observe secondary infection, necrosis, or hemorrhage.

To evaluate its validity, we assessed the DFU-associated outcomes according to the presence (B4+) versus lack (B4−) of proven osteomyelitis from microbiological culture. In most cases, DFU had a favorable outcome whatever the result of B4. Indeed, the healing rate in B4− (no antibiotics) was similar to that of B4+ (tailored antibiotics). Thus, this procedure allows a rapid tailored antibiotic treatment and avoidance of the unnecessary use of antibiotics in cases of negative bone culture. We found that serum C-reactive protein level was higher in cases of positive bone culture, which has not yet been described (4,15). Whether serum C-reactive protein level may be used as a predictor of osteomyelitis remains to be confirmed.

Ducloux et al. (22) retrospectively evaluated percutaneous BB also performed by a diabetologist at bedside. They found in 50 patients with positive bone culture a 66% healing rate within 20 ± 11.9 weeks during a mean follow-up of 9.9 ± 10.2 months after healing. Coupling bedside BB with hybrid ^{67}Ga single-photon emission CT/CT scanning to optimize the needle route, Aslangul et al. (23) reported a 50% positive bone culture among 15 of 24 patients (62.5%) who experienced a complete healing with no relapse during 1-year follow-up. Thus, the results of our B4 procedure are in line with previous studies assessing a similar procedure.

Our standard BB procedure (B3 used in phase 3) performed either by an orthopedic surgeon or by a radiologist allowed the diagnosis of osteomyelitis in more than three-quarters of subjects, which is quite similar to what has been previously published (8–10,14,24–28). Of note, contaminated cultures were low probably because biopsies were performed through intact skin, while some studies evaluated biopsies performed through the wound (10,28,29). *S. aureus* was the most prevalent pathogen, accounting for 30% of osteomyelitis, which is consistent with what has been previously published (8–10,14,24–27) and reported in a recent meta-analysis (30).

To our knowledge, our study is the first to compare bedside with standard BB procedures in a large cohort of patients. Although the proportion of negative bone cultures was higher with the bedside procedure, a complete healing rate with no antibiotics was observed in more patients (although nonsignificantly) compared with the standard procedure. However, we cannot exclude technical failures owing to the blind method of the procedure or the quality of samples. In addition, no patients received antibiotics during the 2 weeks prior to the procedure. The contamination rate was similar in both procedures, while the number of samples per patient was twofold higher in the bedside procedure. Pathogens causing osteomyelitis were similar in both procedures except for anaerobes, which were more prevalent in the bedside procedure. This may be explained by a longer delivery time and, consequently, a longer oxygen exposure time for samples from the standard procedure.

Our study has some limitations. First, it was not a randomized study. Second, B3 and B4 were performed in two different diabetes wards with potential differences in care, which led to obvious limitations in comparing BB types (center effect bias). However, the two units are part of the same hospital group and are unified in a diabetes federation, sharing similar therapeutic protocols for

diabetic foot care. Note that the outcomes were based on a complete wound healing and no bone X-ray normalization. Thus, no clear recommendation regarding the use of B4 instead of B3 can be drawn from our results since it was not the purpose of our study. However, our study clearly demonstrates the safety of such a procedure with a very low rate of adverse effects.

In conclusion, our results suggest that B4 could be a simple-to-perform, safe, and valid diagnostic tool with a similar healing rate with respect to more sophisticated and expensive procedures. Further studies, especially randomized clinical studies, are warranted to recommend and integrate it in the daily clinical practice of diabetes centers to improve decision making on appropriate therapeutics for osteomyelitis and, consequently, to reduce disabilities linked to amputation.

Acknowledgments. The authors would like to thank Prof. Jean-Denis Laredo (Department of Radiology, Lariboisière Hospital) for support and teaching the technique of bedside BB.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. F.F., G.P.d.P., L.P., and J.-P.K. wrote the manuscript and researched data. D.-C.G. and H.J. researched data and contributed to the discussion. L.S.-T., M.L.-M., A.-L.M., T.V.-T., J.-B.J., A.C., Y.A., N.G., F.M., and R.R. researched data. N.V. reviewed/edited the manuscript. J.-P.R. and E.S. contributed to the discussion and reviewed/edited the manuscript. J.-F.G. wrote the manuscript. F.F. and J.-P.K. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. JAMA 2005;293:217–228
2. Lavery LA, Armstrong DG, Wunderlich RP, Mohler MJ, Wendel CS, Lipsky BA. Risk factors for foot infections in individuals with diabetes. Diabetes Care 2006;29:1288–1293
3. Prompers L, Huijberts M, Apelqvist J, et al. High prevalence of ischaemia, infection and serious comorbidity in patients with diabetic foot disease in Europe. Baseline results from the Eurodiale study. Diabetologia 2007;50:18–25
4. Lavery LA, Peters EJG, Armstrong DG, Wendel CS, Murdoch DP, Lipsky BA. Risk factors for developing osteomyelitis in patients with diabetic foot wounds. Diabetes Res Clin Pract 2009;83:347–352
5. Lázaro-Martínez JL, Aragón-Sánchez J, García-Morales E. Antibiotics versus conservative surgery for treating diabetic foot osteomyelitis: a randomized comparative trial. Diabetes Care 2014;37:789–795
6. Zeun P, Gooday C, Nunney I, Dhatariya K. Predictors of outcomes in diabetic foot osteomyelitis treated initially with conservative (nonsurgical) medical management: a retrospective study. Int J Low Extrem Wounds 2016;15:19–25
7. Lipsky BA, Senneville É, Abbas ZG, et al.; International Working Group on the Diabetic Foot (IWGDF). Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). Diabetes Metab Res Rev 2020;36(Suppl. 1):e3280
8. Senneville E, Melliez H, Beltrand E, et al. Culture of percutaneous bone biopsy specimens for diagnosis of diabetic foot osteomyelitis: concordance with ulcer swab cultures. Clin Infect Dis 2006;42:57–62
9. Elamurugan TP, Jagdish S, Kate V, Chandra Parija S. Role of bone biopsy specimen culture in the management of diabetic foot osteomyelitis. Int J Surg 2011;9:214–216
10. Couturier A, Chabaud A, Desbiez F, et al. Comparison of microbiological results obtained from per-wound bone biopsies versus transcutaneous bone biopsies in diabetic foot osteomyelitis: a prospective cohort study. Eur J Clin Microbiol Infect Dis 2019;38:1287–1291
11. Richard J-L, Lavigne J-P, Got I, et al. Management of patients hospitalized for diabetic foot infection: results of the French OPIDIA study. Diabetes Metab 2011;37:208–215
12. Lipsky BA, Berendt AR, Cornia PB, et al.; Infectious Diseases Society of America. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis 2012;54:e132–e173
13. Société de Pathologie Infectieuse de Langue Française. Management of diabetic foot infections. Med Mal Infect 2007;37:26–50 [in French]
14. Tone A, Nguyen S, Devemy F, et al. Six-week versus twelve-week antibiotic therapy for nonsurgically treated diabetic foot osteomyelitis: a multicenter open-label controlled randomized study. Diabetes Care 2015;38:302–307
15. Witsø E, Persen L, Løseth K, Bergh K. Adsorption and release of antibiotics from morselized cancellous bone. In vitro studies of 8 antibiotics. Acta Orthop Scand 1999;70:298–304
16. Berthol N, Robineau O, Boucher A, et al. Two-step sequential approach for concomitant skin and soft tissue infection and osteomyelitis complicating the diabetic foot. Diabetes Care 2017;40:e170–e171
17. Peel TN, Sedarski JA, Dylla BL, et al. Laboratory workflow analysis of culture of periprosthetic tissues in blood culture bottles. J Clin Microbiol 2017;55:2817–2826
18. Bémer P, Léger J, Tandé D, et al.; Centre de Référence des Infections Ostéo-articulaires du Grand Ouest (CRIOGO) Study Team. How many samples and how many culture media to diagnose a prosthetic joint infection: a clinical and microbiological prospective multicenter study. J Clin Microbiol 2016;54:385–391
19. Roux A-L, Sivadon-Tardy V, Bauer T, et al. Diagnosis of prosthetic joint infection by beadmill processing of a periprosthetic specimen. Clin Microbiol Infect 2011;17:447–450
20. Jonasson E, Matuschek E, Kahlmeter G. The EUCAST rapid disc diffusion method for antimicrobial susceptibility testing directly from positive blood culture bottles. J Antimicrob Chemother 2020;75:968–978
21. Rat P, Jouve E, Pickering G, et al. Validation of an acute pain-behavior scale for older persons with inability to communicate verbally: Algoplus. Eur J Pain 2011;15:198.e1–198.e10
22. Ducloux R, Tazi O, Abou-Rjeili M, et al. Percutaneous bone biopsy to identify pathogens in diabetic foot chronic osteitis: useful and harmless. Wounds 2016;28:182–193
23. Aslangul E, M'bemba J, Caillat-Vigneron N, et al. Diagnosing diabetic foot osteomyelitis in patients without signs of soft tissue infection by coupling hybrid ^{67}Ga SPECT/CT with bedside percutaneous bone puncture. Diabetes Care 2013;36:2203–2210
24. Senneville E, Gaworowska D, Topolinski H, et al. Outcome of patients with diabetes with negative percutaneous bone biopsy performed for suspicion of osteomyelitis of the foot. Diabet Med 2012;29:56–61
25. Senneville E, Yazdanpanah Y, Cazaubiel M, et al. Rifampicin-ofloxacin oral regimen for the treatment of mild to moderate diabetic foot osteomyelitis. J Antimicrob Chemother 2001;48:927–930
26. Senneville E, Lombart A, Beltrand E, et al. Outcome of diabetic foot osteomyelitis treated nonsurgically: a retrospective cohort study. Diabetes Care 2008;31:637–642
27. Senneville E, Morant H, Descamps D, et al. Needle puncture and transcutaneous bone biopsy cultures are inconsistent in patients with diabetes and suspected osteomyelitis of the foot. Clin Infect Dis 2009;48:888–893
28. Letertre-Gibert P, Desbiez F, Vidal M, et al. Blood cultures after bone biopsy in diabetic foot osteomyelitis. Diagn Microbiol Infect Dis 2017;89:78–79
29. Lesens O, Desbiez F, Vidal M, et al. Culture of per-wound bone specimens: a simplified approach for the medical management of diabetic foot osteomyelitis. Clin Microbiol Infect 2011;17:285–291
30. Schechter MC, Ali MK, Risk BB, et al. Percutaneous bone biopsy for diabetic foot osteomyelitis: a systematic review and meta-analysis. Open Forum Infect Dis 2020;7:ofaa393