Culture of per-wound bone specimens: a simplified approach for the medical management of diabetic foot osteomyelitis

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Abstract

Surgical percutaneous bone biopsy specimen after a 14-day antibiotic-free period represents the gold standard of care for diabetic foot osteomyelitis but may be difficult to implement in many institutions. We evaluate a simplified strategy based on the results of per-wound bone specimen culture. For that purpose, we retrospectively reviewed the charts of 80 consecutive patients with diabetic osteomyelitis and bone sample obtained via the wound after a careful debridement. The outcome was defined as favourable if there was a complete healing of the wound with no sign of infection and stable or improved bone X-ray 6 months after antibiotic therapy completion. Culture of bone specimens was positive in 96% of patients, although half of the patients did receive a course of antimicrobials within 14 days of the bone specimen being obtained. A total of 129 bacterial isolates were obtained from bone cultures with a mean of 1.6 ± 1 isolates per patient (*Staphylococcus aureus*: 33%; coagulase-negative staphylococci: 14%; streptococci: 9%; enterococci: 12%; corynebacteria: 4%; Gram-negative bacilli: 20%; anaerobes: 4%). Forty-six percent of cultures were monomicrobial. The mean duration of follow-up from diagnosis was 17 ± 1 months. Six months after discontinuation of antibiotics, six patients (7.5%) had died, nine were considered as therapeutic failures and 65 were considered as cured. Fifty-four of these 65 patients had follow-up data available at 1 year and remained in remission. In conclusion, a simplified procedure based on the culture of bone sample obtained via the ulcer after a careful debridement of the wound is effective in the medical management of diabetic foot osteomyelitis.

Keywords: Bone biopsy, chronic ulcer, diabetic foot, exposed bone, osteomyelitis
Original Submission: 24 November 2009; **Revised Submission:** 29 January 2010; **Accepted:** 30 January 2010
Editor: F. Allerberger
Article published online: 11 February 2010
Clin Microbiol Infect 2011; **17:** 285–291
10.1111/j.1469-0691.2010.03194.x

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Introduction

Diabetic foot osteomyelitis is usually a consequence of a neglected chronic ulcer, which may produce silent soft tissue infection with contiguous bone involvement [1]. Exploration after ulcer debridement may reveal an exposed bone or joint, which is highly suggestive of osteomyelitis [2]. The clinical suspicion is usually confirmed by radiological investigations (repeated X-rays, magnetic rsonance imaging or computed tomography scans) [3,4]. The optimal management of diabetic osteomyelitis is still a matter of debate and there is no universally accepted strategy [5,6]. One radical option for severe diabetic foot osteomyelitis is surgical amputation, including total ray, transmetatarsal and limb amputation [1]. Lower limb amputations are the most dramatic complications associated with diabetes and are 12-fold more frequent in the diabetic population than in the nondiabetic one [7]. The consequences of amputation are multiple and include psychosocial issues, nonhealing surgical wounds, postoperative infection and postural instability with recurrent ulceration and further amputation [8]. Accordingly, efforts have been made to limit amputation to patients with diabetic foot osteomyelitis [9]. One option is to perform a conservative surgery without local or high-level amputation, followed by prolonged antibiotic therapy [10,11]. Some physicians advocate conservative nonsurgical management of diabetic osteomyelitis [8]. In these cases, it is extremely important to obtain a high-quality specimen for culture and to administrate a long course (several months) of adequate antibiotic therapy. Several sampling techniques are used in

clinical practice. Simple swabbing of the ulcer is often performed but should be proscribed because of inaccurate results [5]. Kessler *et al.* [12] advocated the use of needle aspiration through the normal skin surrounding the foot ulcer. However, this method was recently shown to be inconsistent with transcutaneous bone biopsy culture [13]. Deep specimens obtained after wound debridement are usually recommended but surgical percutaneous bone biopsy is likely to represent the gold standard method for reliable identification of the causal bacteria [14]. However, because bone biopsy is an expensive and invasive technique that requires an experienced surgeon to carry out the procedure, it is difficult to implement in most health care centres [15]. Furthermore, although Senneville *et al.* [14] did not report any side effects with this technique, percutaneous bone biopsy may compromise wound healing in patients with arteriopathy and severe neuropathy.

We evaluated a simplified procedure for the medical management of severe diabetic foot osteomyelitis with exposed bone, based on the results of bone sample culture obtained via the ulcer after a careful debridement of the wound.

Materials and Methods

Setting

Patients were retrospectively recruited at the Diabetic Foot Infection Day Care Unit of the Hôpital Saint Jacques in Clermont-Ferrand, France, a 742-bed university tertiary care hospital. Only patients with confirmed or suspected diabetic foot infection attend the clinic, so the prevalence of osteomyelitis was high. Patient data were recorded on dedicated charts and included medical history, a picture of the wound with a detailed description of its exploration, the method used to obtain the microbiological sample, and details of prescription medications and outpatient wound care.

Patients

Data were abstracted from patients who had been referred to the Diabetic Foot Infection Day Care Unit from January 2005 to January 2009. After chart review, patients were included in the study if they fulfilled the criteria: (i) clinical signs suggestive of infection (discharge, swelling, pain, inflammation, chronic nonhealing wound); (ii) positive probe-to-bone test (i.e. this 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 attendance at the unit; (vi) no surgery involving the bone within the first week after diagnosis (i.e. subsequent surgery involving the bone was a criteria defining treatment failure; see below).

Bone samples

After a careful debridement, the wound was cleaned with polyvidone iodine and then washed with sterile saline solution. When bedside bone testing revealed fragments of infected bone or sequestra, these were removed until healthy bone was identified. Bone samples were sent to the microbiology laboratory within 2 h in a sterile tube with a few drops of sterile saline solution. When bone testing was positive but the bone was not perceived as contaminated, a small sample was harvested and sent to microbiology laboratory. All samples were taken by the same operator, who wore sterile gloves and a gown. A disposable needle holder was used to harvest the fragment of infected bone.

Microbiology analysis

Aerobic and anaerobic cultures were performed for each sample for 6 days. Bacterial isolates were identified at the species level with the VITEK2 or API Systems (Biomérieux, La Balme, France). Antibiotic susceptibility testng was performed using the VITEK2 or API Systems or the disk diffusion method of the Comité de l'Antibiogramme de la Société Française de Microbiologie (Comité de l'Antibiogramme de la Société Française de Microbiologie, 2009; Communiqué 2009). Resistant microorganisms were defined as: for Gram-negative bacilli, resistance or intermediate susceptibility to ceftazidime; for staphylococci, resistance to methicillin; and, for enterococci, resistance to vancomycin.

Management

Patients are usually referred to our unit by the general practitioner or a nurse, when they are asked to stop any antibiotic therapy until attending the unit. Bone samples were taken at the unit (see below) after clinical assessment. When osteomyelitis was associated with severe deep soft tissue infections or systemic signs of infection, the patient was hospitalized and an intravenous empirical antibiotic therapy (a combination of high-dose amoxicillin–clavulanic acid and levofloxacin when there was no risk of multiresistant bacteria and piperacillin-tazobactam plus teicoplanin when a multiresistant bacteria had been previously found in the wound) was started when awaiting bone samples. Other patients received oral or intravenous home therapy. Antibiotic therapy was adjusted according to the culture results to enable use of the antimicrobial with the narrowest effective spectrum. Oral antibiotic therapy consisted of a combination of drugs with high oral absorption and good penetration of bone, mainly rifampicin, fusidic acid, levofloxacin, clindamycin and trimethoprim/sulphamethoxazole.

Amoxicillin–clavulanic acid was used when cultures showed susceptible mixed bacteria. Outpatient intravenous antibiotic therapy consisted mainly of teicoplanin, ceftriaxone or a continuous infusion of ceftazidime or piperacillin–tazobactam, and was used in cases of resistant bacteria or poor patient adherence to oral therapy.

Data recording

The following data were recorded after chart review: demographic data; prior history of hospitalization, amputation or osteomyelitis; location and description of the wound; joint and bone infected on X-ray with subsequent development; antibiotic therapy before bone sampling; need for surgery for abscess drainage. The outcome was defined as favourable if there was a complete healing of the wound with no signs of infection and stable or improved bone X-ray, 6 months after the completion of antibiotic therapy. Outcome was also assessed 1 year after antibiotic completion for patients whose treatment finished before September 2008. For patients who did not attend our clinic during the follow-up period required by the study, the general practitioner and patient were contacted by phone to confirm favourable outcomes. Other outcomes were defined as failure and were classified as amputation (for any cause), relapse (new episode of infection at the same or a contiguous site during follow-up, whatever the microbial cause), persistent infection (no response to the antibiotic therapy active against the organisms found in bone culture) and stability (if the wound was not healed at 6 months but fulfilled other criteria for favourable outcome).

Statistical analysis

For univariate analysis, chi-square or Fisher's exact test were used for comparisons between categorical variables. For multivariate analysis, a logistic stepwise backward regression was used. Categorical variables with $p < 0.1$ on univariate analysis were used as candidate variables for multivariate analysis. Statistical analysis was performed using SPSS software, version 10.1 (SPSS, Inc., Chicago, IL, USA).

Results

Patients

From January 2005 to January 2009, 80 patients met the inclusion criteria. Most patients were diagnosed in 2007 and 2008 (35% and 36%, respectively).The mean age was 68 ± 1 (range 39–89) years and 84% were men. Other patients characteristics are shown Table 1. Of the 49 patients with peripheral arteritis, 26 had angioplasty and 11 underwent vascular surgery; there was no possibility of revascularization for the others. Of the patients, 37.5% had a past history of amputation as a result of vascular (16%), infectious (9%) or mixed (12.5%) causes. Amputation involved the first toe for 11%, other toes for 22.5%, the fore-foot for 2.5% and the limb for 2.5%. Of the patients, 47.5% had a history of hospitalization within 3 months before the bone sample was obtained.

TABLE 1. Characteristics of the 80 patients with diabetic osteomyelitis and exposed bone

Patient characteristics	Frequency
Diabetic neuropathy	99%
Arteritis	60%
Nondialysed chronic renal failure	26%
Dialysis	5%
Past history of osteomyelitis	50%
Past history of amputation	37.5%
Osteoarthritis location	
Hallux	
Distal phalanx	6% (5)
Interphalangeal joint	5% (4)
Metatarsophalangeal joint	14% (11)
Other toes (second to fifth)	
Distal phalanx	7.5% (6)
Interphalangeal joints	19% (15)
Metatarsophalangeal joints	35% (28)
Calcaneum	9% (7)
Other	5% (4)
Course of antibiotic therapy >24 h preceding the bone specimen	
Within 3 days	45%
Within 14 days	53%
Within 3 months	72%

In 49% of cases, the infection involved a metatarsophalangeal (MTP) joint (Table 1). Seven (9%) had a calcaneum infection and ten (8%) had osteomyelitis after amputation because of arteritis. Twenty-one percent had visible, exposed bone or joint on physical examination. Fifteen patients had bone specimens sent for histology, which showed compatibility with an infectious process. Only one patient (the only one who did not have any neuropathy) required a painkiller (morphine sulphate, 5 mg) before the bone specimen was drawn.

Microbiological findings

There were 129 isolates obtained from bone cultures from the 80 patients, with a mean of 1.6 ± 1 species per patient (*Staphylococcus aureus*: 33%; central nervous system: 14%; streptococci: 9%; enterococci: 12%; corynebacteriae: 4%; Gram-negative facultative aerobic rods: 12%; *Pseudomonas aeruginosa*: 8%; and anaerobes: 4%). Culture of bone specimen was positive in 96% of patients (Table 2), although half ($n = 42$) of the patients received a course of antimicrobials within 14 days of bone sampling (Table 1).

Of these 42 patients, 30 had severe deep infection of the foot and were initially treated by their general practitioners with a combination of antibiotics (quinolone and rifampicin in six patients, quinolone and another antibiotic in 15, and a

TABLE 2. Bacteria found in 80 bone biopsies from the 80 patients included in the present study

Variables	Present study	Senneville *et al.* [14]	Aragon-Sanchez *et al.* [11]
Number of samples	80	76	176
Number of isolates	129	125	204
Mean number of isolates per sample	1.6 ± 1	1.54	–
Number of culture negative samples (%)	2 (2.5%)	2[a]	20 (11%)
Number (%) of isolates, by pathogen			
Gram-positive			
Staphylococci	61 (47%)	65 (52%)	117 (57%)
Staphylococcus aureus	43 (33%)	33 (26%)	95 (47%)
MRSA	24 (19%)	12 (10%)	35 (17%)
Central nervous system	18 (14%)	32 (26%)	22 (11%)
Streptococci	12 (9%)	15 (12%)	7 (3%)
Enterococci	15 (12%)	10 (8%)	2 (1%)
Corynebacteriae	5 (4%)	3 (2%)	–
Gram-negative bacilli	26 (20%)	23 (18%)	59 (29%)
Pseudomonas aeruginosa	10 (8%)	3 (2%)	18 (9%)
Anaerobes	5 (4%)	6 (5%)	–

[a]Not included in the present study.

combination of other antibiotics in nine). The 12 remaining patients received a single antibiotic. Forty-six percent of cultures were monomicrobial. Two bacterial species were found in 37.5% of bone cultures, three in 11%, and five in 1%. Two patients (2.5%) had negative cultures: both received antimicrobial treatment within 3 days before the bone sampling and were successfully treated with a 6-week course of oral therapy with no relapse after 18 months of follow-up.

Methicillin-resistant *S. aureus* (MRSA) was the most frequently isolated organism, especially in samples with only one pathogen (Table 2). There was a significant association between a monomicrobial culture and MRSA ($p < 0.001$). Other bacteria and prior antibimicrobial therapy were not associated with monomicrobial culture. Fidty-five percent of patients had at least one multiresistant microorganism. Variables associated with resistance in the univariate analysis are detailed in Table 3. In multivariate analyses, two variables were independently predictive for resistance: prior antibiotic therapy within 14 days (OR 5; 95% CI 2–13; p 0.005) and arteritis (OR 5; 95% CI 2–16; p 0.002).

TABLE 3. Risk factors associated with resistant bacteria in bone culture in univariate analysis

Variables	At least one resistant bacteria isolated (n = 44)	No resistance (n = 36)	p
Age >70 years	50%	36%	NS
Prior antibiotic therapy			
Within 3 months	82%	61%	0.04
Within 14 days	64%	36%	<0.02
Hospitalization within 3 months	61%	31%	<0.01
Arteritis	72	44	0.01
Chronic renal failure	32%	31%	NS
History of osteoarthritis	50	50	NS
History of amputation	48%	25%	0.04

NS, not significant.

Outcome

The mean duration of follow-up from time of specimen collection was 17 ± 1 months. No patient was no lost to follow-up. Thirty percent of patients were hospitalized at the time of diagnosis for a mean duration of 18 ± 3 days (range 5–54 days). The osteomyelitis was associated with abscess in 14% of patients, of whom two required a surgical drainage and nine were drained at the Diabetic Infection Day Care Unit. Antibiotic therapy is shown in Fig. 1. Of the 80 patients, 39% received a course of intravenous antibiotic therapy for a mean duration of 36 ± 3 days. Of these, 29% received intravenous antimicrobials as initial therapy and 10% were started on oral and then switched to intravenous therapy (six because of gastrointestinal irritation and two

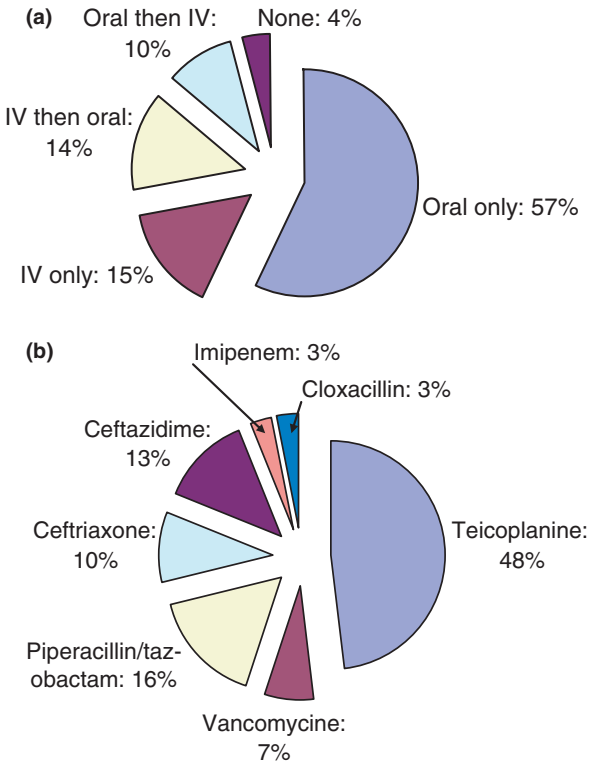

FIG. 1. (a) Route of antibiotic therapy prescribed for the treatment of the 80 patients with diabetic osteomyelitis; 'oral then IV' means that patients were started on oral and then switched to intravenous therapy (six because of gastrointestinal irritation as a result of oral antibiotics and two because of unsatisfactory outcome after oral therapy during follow-up); 'IV then oral' means that patients were switched to oral therapy after a course of 7–14 days of intravenous therapy. (b) Distribution of the intravenous antimicrobials prescribed in the 30 patients having received a course of intravenous therapy.

because of unsatisfactory response). Intravenous treatments were administrated at the hospital in four patients, at the hospital then at home in five, and at home only in 21. The mean duration of intravenous home therapy was 38 ± 3 days. Of the patients, 57.5% were treated exclusively with a combination of oral antimicrobials. Three patients did not receive any antibiotic therapy because of the culture of multiresistant bacteria and allergy or intolerance to multiple antimicrobials. They were instead treated with removal of sequestra or infected bone at the bedside. All these three patients were considered as cured 6 months after antibiotic completion. After 1 year of follow-up, all were still relapse-free but two experienced a new episode of osteomyelitis at another site.

Of the remaining 77 patients, 34% were treated for 6 weeks, 36% for 9 weeks, and 30% for 12 weeks or more.

At 6 months after antibiotics discontinuation, 7.5% had died, including five patients who did not complete their course of antimicrobials and died within 3 months after the diagnosis of osteomyelitis. Of these five patients, four were considered as being in remission (healed wound and stable X-ray) during follow-up and osteomyelitis did not contribute to death; one died from cirrhosis before the end of antibiotic therapy with an MRSA bacteraemia originating from the bone infection; and one died 5 months after completion of antibiotics and was considered as cured. Of the 74 patients surviving 6 months after completion of antimicrobial therapy, nine were considered as failures: two had amputations for vascular reasons within 3 months of antibiotic completion; two relapsed after being considered as cured at the 3-month review; and five remained stable (including four with calcaneum infection and severe arteritis and one with osteomyelitis involving several sectioned bones after amputation because of arteritis). Sixty-five of 74 patients were considered as cured 6 months after antibiotic completion; however, seven experienced a distinct episode of osteomyelitis, with different locations and causal bacteria from the initial infection. Follow-up data 1 year after antibiotic completion were available for 54 of the 65 patients considered as cured at 6 months: they had neither relapse, nor an amputation. However, six of the patients had a distinct new episode of osteomyelitis at a different location.

Discussion

We evaluated a simplified strategy to manage diabetic osteomyelitis, based on culture results of bone samples obtained via the ulcer after a careful debridement of the wound. The bone sample was taken even if antibiotics prescribed by the general practioner before attendance to our clinic had not been discontinued. Patients received antimicrobials that were effective against the organisms found in the bone culture. Cultures were positive in 97.5% of patients and 88% were considered as cured 6 months after the end of antibiotic therapy.

Of the 54 patients with follow-up data available 1 year after completion of antibiotics and considered as cured at 6 months, there was no relapse of the osteomyelitis. This pragmatic and simplified approach has several advantages: (i) the infected area can be visualized and thus cannot be missed when the specimen is drawn; (ii) the necrotic bone specimen is assumed to have a high bacterial load that may increase the likelihood of positive culture, even with recent antibiotic therapy; (iii) the medical treatment can be quickly implemented after the bone specimen was drawn; and (iv) the method does not create a new wound, which is especially important in patients with severe arteritis.

Our cohort comprised 80 consecutive patients, with most of them having a severe osteomyelitis. Arteritis was associated with difficult to treat osteomyelitis such as calcaneum infections, which are known to have a poor prognosis [16]. Similar to other studies, we found a high frequency of multiresistant bacteria, especially MRSA [11,17,18]. Patients with resistant bacteria had similar risk factors to those reported in other studies, including previous exposure to various antibiotics and frequent hospitalization as a result of complicated diabetes [11,17,18]. This makes the medical treatment of these patients more challenging, in addition to the severity of the disease in our population.

The present study had several limitations. First, the study is observational and retrospective. Second, this simplified approach can be used only in patients with a positive probe-to-bone test and some diabetic patients may have osteomyelitis of the foot with no wound or with a negative probe-to-bone test. However, because the osteomyelitis is usually the result of a neglected chronic ulcer, the rate of a positive probe-to-bone test is high in patients with diabetic osteomyelitis; for example, 95% of the 185 patients with proven diabetic foot osteomyelitis in the series published by Aragon-Sanchez *et al.* [11]. Third, we cannot exclude contamination of the sample by the skin flora, which may partly explain the high rate of multiresistant bacteria.

Performing a biopsy via an infected open wound is not recommended because of the risk of contamination by colonizing flora. Surgical transcutaneous bone biopsy obtained via a normal skin area after incision has been described by Senneville *et al.* [14] and is now the gold standard for microbiological diagnosis [5]. Although the risk of contamination does exist with our approach, we consider the risk to be

small for several reasons. First, careful debridement and cleaning of the wound reduces surface contamination. Second, our microbiological results were quite similar to those found by Senneville *et al.* [14] and Aragon-Sanchez *et al.* [11] who performed surgical bone biopsy and surgical resections, respectively (Table 3). Third, all antibiotic therapy was based on our bone sample culture results and the outcomes were good, taking into account the severity of the osteomyelitis, the high frequency of resistant bacteria and the presence of peripheral arterial disease. At 6 months after antibiotic discontinuation, 65 patients recovered, six had died before assessment and nine were considered as stable or failures.

However, a new episode of infection at a different site and with pathogens distinct from the initial episode occurred in seven patients during the 6-month follow-up period (and six of 54 patients at 1 year). These new distinct episodes of osteomyelitis may be explained by the fact that most of our 80 patients live in a rural, mountainous area, far from the tertiary health care centre [19]. Furthermore, 37.5% of our patients had a past medical history of amputation that may increase the risk of re-ulceration and therefore re-infection at a distinct site.

Most of our patients received an empirical antibiotic therapy administrated by the general practitioners to treat suspected cutaneous infection, usually without any microbiological investigation. Senneville *et al.* [14] recommend that patients should not receive any antibiotic therapy for 2–4 weeks before biopsy aiming to avoid false negative results. Indeed, some anti-microbials such as fluoroquinolones or rifampicin may have a prolonged release in bone that may also affect culture results. This recommendation implies a delay in starting definitive treatment. However, all our patients had severe osteomyelitis with perception of a necrotic bone or sequestra on wound exploration. Many of them had a past history of amputation (37.5%) or osteomyelitis (50%) and a majority (60%) had arteritis, which represents the main factor leading to amputation that is associated with infection. Thus, for the vast majority of our diabetic population, we osteomyelitis treatment should not be delayed. Moreover, the bacterial load is likely to be high in sequestra or necrotic bone, whereas the antibiotic penetration in such a bone is weak, especially in arteritic patients. This may increase the efficiency of bone culture despite previous antibiotic therapy. This hypothesis is confirmed in the study by Aragon-Sanchez *et al.* [11] in which 71% of patients received antibiotics before the surgical procedure; of 176 bone specimen with histopathological confirmation of bone infection, 20 (11%) were negative for culture, although there was no statistical association between prior antibiotic therapy and negative results in culture.

In conclusion, although surgical transcutaneous bone biopsy obtained via an incision in a normal skin area followed by adequate antibiotic therapy should be the standard of care for treating diabetic osteomyelitis, medical treatment based on the results of cultures of bone sampled via the ulcer after a careful debridement of the wound is an attractive altrenative and is associated with satisfactory outcomes.

Transparency Declaration

There is no conflict of interest for any of the authors.

References

1. Hartemann-Heurtier A, Senneville E. Diabetic foot osteomyelitis. *Diabetes Metab* 2008; 34: 87–95.
2. Lavery LA, Armstrong DG, Peters EJ, Lipsky BA. Probe-to-bone test for diagnosing diabetic foot osteomyelitis: reliable or relic? *Diabetes Care* 2007; 30: 270–274.
3. Dinh MT, Abad CL, Safdar N. Diagnostic accuracy of the physical examination and imaging tests for osteomyelitis underlying diabetic foot ulcers: meta-analysis. *Clin Infect Dis* 2008; 47: 519–527.
4. Butalia S, Palda VA, Sargeant RJ, Detsky AS, Mourad O. Does this patient with diabetes have osteomyelitis of the lower extremity? *JAMA* 2008; 299: 806–813.
5. Berendt AR, Peters EJ, Bakker K *et al.* Diabetic foot osteomyelitis: a progress report on diagnosis and a systematic review of treatment. *Diabetes Metab Res Rev* 2008; 24: S145–S161.
6. Jeffcoate WJ, Lipsky BA. Controversies in diagnosing and managing osteomyelitis of the foot in diabetes. *Clin Infect Dis* 2004; 39: S115–S122.
7. Fosse S, Hartemann-Heurtier A, Jacqueminet S, Ha Van G, Grimaldi A, Fagot-Campagna A. Incidence and characteristics of lower limb amputations in people with diabetes. *Diabet Med* 2009; 26: 391–396.
8. 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.
9. Frykberg RG, Wittmayer B, Zgonis T. Surgical management of diabetic foot infections and osteomyelitis. *Clin Podiatr Med Surg* 2007; 24: 469–482.
10. Freeman GJ, Mackie KM, Sare J, Walsh AK, Pherwani AD. A novel approach to the management of the diabetic foot: metatarsal excision in the treatment of osteomyelitis. *Eur J Vasc Endovasc Surg* 2007; 33: 217–219.
11. Aragon-Sanchez FJ, Cabrera-Galvan JJ, Quintana-Marrero Y *et al.* Outcomes of surgical treatment of diabetic foot osteomyelitis: a series of 185 patients with histopathological confirmation of bone involvement. *Diabetologia* 2008; 51: 1962–1970.
12. Kessler L, Piemont Y, Ortega F *et al.* Comparison of microbiological results of needle puncture vs. superficial swab in infected diabetic foot ulcer with osteomyelitis. *Diabet Med* 2006; 23: 99–102.
13. 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.
14. 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.

15. van Baal JG. Surgical treatment of the infected diabetic foot. *Clin Infect Dis* 2004; 39: S123–S128.
16. Bollinger M, Thordarson DB. Partial calcanectomy: an alternative to below knee amputation. *Foot Ankle Int* 2002; 23: 927–932.
17. Hartemann-Heurtier A, Robert J, Jacqueminet S *et al.* Diabetic foot ulcer and multidrug-resistant organisms: risk factors and impact. *Diabet Med* 2004; 21: 710–715.
18. Kandemir O, Akbay E, Sahin E, Milcan A, Gen R. Risk factors for infection of the diabetic foot with multi-antibiotic resistant microorganisms. *J Infect* 2007; 54: 439–445.
19. Couret G, Desbiez F, Thieblot P *et al.* Emergence of monomicrobial methicillin-resistant *Staphylococcus aureus* infections in diabetic foot osteomyelitis (retrospective study of 48 cases). *Presse Med* 2007; 36: 851–858.