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The diagnosis of osteomyelitis of the foot in diabetes: microbiological examination vs. magnetic resonance imaging and labelled leucocyte scanning

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Abstract

Aims Foot infections and their sequelae are among the most common and severe complications of diabetes mellitus. As diabetic patients with foot infections develop osteomyelitis and may progress to amputation, early diagnosis of osteomyelitis is critical.

Methods We compared the diagnostic values of labelled leucocyte scanning with $Tc^{99}m$, magnetic resonance imaging (MRI) and microbiological examination of bone tissue specimens with histopathology, the definitive diagnostic procedure. Thirty-one diabetic patients with foot lesions were enrolled in the study and histopathological examination was performed in all. Patients had clinically suspected foot lesions of ≥ grade 3 according to the classification of Wagner.

Results Bone specimens were obtained for histopathological examination. Microbiology had a sensitivity of 92% and specificity of 60%. Labelled leucocyte scanning had a sensitivity of 91%, specificity of 67%, and MRI a sensitivity of 78%, specificity of 60%.

Conclusions Microbiological examination may be as useful as and less costly than other diagnostic procedures and is the only method which can guide the choice of antibiotic therapy.

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Keywords diabetic foot, diagnosis, osteomyelitis

Abbreviations CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MDP, methylene diphonate; MRI, magnetic resonance imaging; MRSA, methicillin-resistant *Staphylococcus aureus*; WBC, white blood cell

Introduction

Foot infections are one of the most frequent and severe complications of diabetes. Diabetic patients with foot infections may develop osteomyelitis and progress to amputation. Many studies have demonstrated that foot infections are the most common cause of non-traumatic amputation of the lower extremity in diabetic patients [1–4]. The presence of osteomyelitis alters the approach to therapy and, moreover, the rate of amputation is increased [2]. Diabetes is the most common cause of non-infectious osteopathy and the foot is the most frequently affected site. Neuropathy, vascular disease and defects in host immunity predispose to foot infections and make the diagnosis and treatment of osteomyelitis more difficult [1,4].

In the diagnosis of osteomyelitis, the first approach is clinical examination. Not all diabetic patients with foot infection are febrile and one cannot differentiate whether the signs of inflammation are due to cellulitus or osteomyelitis. The size and the depth of the skin ulcer as well as elevation of erythrocyte sedimentation rate (ESR) have been shown to be predictive of

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the presence of osteomyelitis [5,6]. However, a high ESR is not specific for osteomyelitis.

The other diagnostic procedures are imaging studies. Bony abnormalities related to osteomyelitis are generally not evident on plain films until 10–20 days after infection [7]. These X-ray changes may take longer to develop. Thus, in the diagnosis of acute infection, most investigators recommend a 4-phase radionuclide bone scan coupled with labelled leucocyte scan and magnetic resonance imaging (MRI) [7–10]. Bone biopsy is the gold standard for the diagnosis of osteomyelitis, specimens being processed by both histopathological and microbiological procedures [1,5,9].

This study was performed to determine the role of labelled leucocyte scanning, MRI and microbiological procedures in the diagnosis of osteomyelitis of the foot in diabetic patients.

Methods

Thirty-one diabetic patients with foot lesions were enrolled in the study. Patients had clinically suspected foot lesions with ≥ grade 3 according to the classification of Wagner [11]. Informed consent was obtained from all patients. The size (≥ 2 cm^2) and depth (≥ 2 cm) of the ulcer, C-reactive protein (CRP) levels, ESR and white blood cell (WBC) count were measured. MRI (in 28 patients) and/or 4-phase radionuclide bone scanning and labelled leucocyte scanning (in 26 patients) were performed as the first step of investigation. Invasive diagnostic procedures were the second step. Histopathological examination was performed regardless of the presence of osteomyelitis according to MRI and scintigraphy results. Bone specimens to determine histopathological characteristics were obtained from all patients. Bone tissue was obtained by surgical procedures under aseptic conditions during either debridement or amputation.

Microbiological processing

Microbiological processing was performed in all patients. Bone specimens for anaerobic cultures were cultured in Schaedler agar and than placed in an anaerobic chamber. Bone specimens for aerobic culture were processed in the laboratory using 5% sheep blood agar, MacConkey's agar and Sabouraud agar. All aerobic and anaerobic plates were incubated for 24–48 h at 35°C. The identification of anaerobic bacteria was performed using An-ident Discs Code DD6® (Oxoid Ltd, Basingstoke, UK). Aerobic isolates were stained using the Gram stain method. Gram-negative organisms were identified according to the following properties: dextrose, sucrose and lactose fermentation, citrate usage, motility, urease and indole production, ornithine decarboxylase activity and oxidase reaction. Gram-positive organisms were identified according to the following properties: catalase and haemolysis reaction, coagulase production, optochin, bacitracin and trimethoprim-sulphamethoxazole susceptibility, growth in media including bile esculine and growth in media including 6.5% saline solution. The antibiotic susceptibility of species was determined using a disk-diffusion test as described in NCCLS M2-A7 and M100-S11 [12,13]. Microbiological diagnosis of osteomyelitis was based on the presence of bacteria in bone-tissue culture.

Imaging studies

Magnetic resonance imaging

MRI was performed on a Siemens Vision 1.5T (Siemens, Erlangen, Germany) using a knee coil. The foot was immobilized with a blanket. Precontrast imaging included conventional spinecho (SE TR/TE: 547/13; FOW: 180; matrix 256 × 256; slice thickness 4 mm) and T1 Fat sat (TR/TE: 893/13; FOW: 180; matrix 256 × 256, slice thickness 4 mm) sequences in sagittal and axial orientation. Turbo inversion recovery magnitude (TIRM TR/TE: 5349/71; FOW: 200; matrix 256 × 256, slice thickness 4 mm) in sagittal orientation was also obtained. After the injection of 0.1 mmol/kg gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA), T1 SE and F1 Fat sat sequences in sagittal and axial orientation were repeated. We used high signal intensity on TIRM, low signal intensity on T1 sequence and contrast enhancement as the definition of osteomyelitis.

Radionuclide studies

All images were obtained using a Siemens Orbiter gamma camera connected to a Pegasys computer (ADAC, Milpitas, CA, USA) equipped with a collimator. Four-phase bone scintigraphy was performed using 740 MBq (20 mCi) Tc^{99}m methylene diphonate (MDP). Immediately after injection, dynamic images of the feet (1 s/frame) were obtained for 1 min. The blood-pool phase at 60 s/frame followed this for 5 s. After 4 h, 500 000-count static images of the feet (plantar, lateral and medial views) were obtained for 3P-MDP. An additional plantar image for 50 000 counts was obtained 24 h after injection (4P-MDP).

Twenty-four hours later, Tc^{99}m WBC scans were obtained. The labelling procedure of the leucocytes was as follows: 6 ml acid dextrose solution and 9 ml 6% hydroxyl-ethyl-starch were drawn into a 60-ml sterile plastic syringe. Forty-five millilitres of the patient's blood was withdrawn into the syringe and mixed gently. The erythrocytes were allowed to settle for 30–60 min. Leucocyte-platelet-rich plasma was obtained by drawing blood into a sterile vacuum tube and centrifuging for 10 min at 150 *g* for leucocyte separation. Leucocytes were labelled with 400–500 MBq (10.8–13.5 mCi) Tc^{99}m Tc-hexamethylpropyleneamine (HMPAO) (Ceretec; Amersham, Little Chalfont, UK), incubated at room temperature for 10 min and centrifuged at 150 *g* for 5 min. The labelled cells were re-suspended in saline and re-injected intravenously.

Combined 4P-MDP and Tc^{99}m WBC scans were considered positive for osteomyelitis when there was an abnormal accumulation of leucocytes in a zone concordant with the area of uptake on bone scintigraphy. These scans were considered negative for osteomyelitis in the presence of abnormal accumulation of leucocytes in a zone not concordant with the area of uptake on bone scintigraphy (soft-tissue infection) or when no leucocyte accumulation was observed (no infection or aseptic inflammation).

Histopathology

Histopathological diagnosis of osteomyelitis was based on the presence of osteonecrosis and infiltration with leucocytes or chronic inflammatory cells such as lymphocytes or plasma cells.

Statistical analysis

The sensitivity and specificity rates of microbiological examination were determined in 31 patients, of MRI in 28 patients and of scintigraphy in 26 patients. Positive imaging or culture results were classified as true positive (TP) or false positive (FP) for osteomyelitis if biopsy results were positive or negative, respectively, whereas negative imaging or culture results were classified as true negative (TN) or false negative (FN) if biopsy results were negative or positive, respectively. Sensitivity was calculated as (no.TP)/[(no.TP) + (no.FN)], specificity as (no.TN)/[(no.TN) + (no.FP)], positive predictive value (PPV) as (no.TP)/[(no.TP) + (no.FP)], negative predictive value (NPV) as (no.TN)/[(no.TN) + (no.FN)].

Results

The characteristics of the 23 male (74%) and eight female (26%) patients were as follows: age (mean ± SD) 62 ± 8.8 years (range 40–77 years); duration of diabetes 16.8 ± 8.9 years (range 1–35 years); duration of foot infection 3.6 ± 3.1 months (range 0.5–12 months); ESR 87 ± 25 mm/h (range 37–120 mm/h); CRP 7.17 ± 5.66 mg/dl (range 1–25.3 mg/dl); serum creatinine 121 ± 91.9 μmol/l (range 62–115 μmol/l); WBC count 11 022 ± 5131/mm^3 (range 5020–31 880/mm^3).

According to the classification of Wagner, 11 patients (36%) had Grade 3, 15 patients (48%) had Grade 4 and five patients (16%) had Grade 5 foot lesions. One of the patients died due to septic shock during the follow-up period.

Pseudomonas aeruginosa (33%) was the most common organism isolated from bone tissue cultures and methicillin-resistant *Staphylococcus aureus* (MRSA) (24%) and *Acinetobacter* spp. (12%) were the other major pathogens. Anaerobic cultures yielded only *Peptostreptococcus* spp. (3%). Forty-seven microorganisms were isolated from bone cultures and 1.06 pathogens per case of osteomyelitis were identified (Table 1).

Bone scintigraphy, MRI and bone tissue biopsy were completed in 24 patients. Table 2 shows the comparison of bone scintigaphy and MRI results in patients with and without osteomyelitis confirmed by histopathology. Microbiology had a sensitivity of 92%, specificity of 60%, PPV of 92% and NPV of 60%. Labelled leucocyte scanning had a sensitivity of 91%, specificity of 67%, PPV of 95%, NPV of 50% and MRI had a sensitivity of 78%, specificity of 60%, PPV of 90% and NPV of 37.5%.

Table 1 Pathogens isolated from bone tissue culture

Pathogen	Bone tissue
Pseudomonas aeruginosa	11
Staphylococcus aureus	9
Methicillin resistance	8
Methicillin sensitive	1
Acinetobacter spp.	4
Enterococcus spp.	1
Coagulase-negative staphylococci	3
Methicillin resistance	2
Methicillin sensitive	1
Streptococcus spp.	2
Escherichia coli	1
Peptosterptococcus spp.	1
Serratia marsences	1
Total	33

Table 2 Comparison of labelled leucocyte scan, magnetic resonance imaging and microbiological examination results in patients with osteomyelitis confirmed or not confirmed by histopathology

	Histopathology	
	Osteomyelitis positive	Osteomyelitis negative
Labelled leucocyte scan		
Osteomyelitis positive	21	1
Osteomyelitis negative	2	2
Total (n = 26)	23	3
Magnetic resonance imaging		
Osteomyelitis positive	18	2
Osteomyelitis negative	5	3
Total (n = 28)	23	5
Microbiological examination		
Positive	24	2
Negative	2	3
Total (n = 31)	26	5

Discussion

Osteomyelitis is a limb- or life-threatening complication in patients with diabetes and can be prevented with an integrated, multidisciplinary approach [7,9]. A Wagner classification of ≥ grade 3 suggests osteomyelitis but provides a limited description of foot ulcers in diabetes. Use of the Armstrong or PEDIS classifications for detailed assessment of diabetic foot ulcers is recommended [9]. In this study, we used the Wagner classification as an inclusion criterion only. Diagnosing osteomyelitis in a diabetic patient with a foot infection is difficult [10]. There is no established consensus on the diagnosis of foot osteomyelitis in diabetes. Major problems include differentiating soft-tissue infection from bone infection and infectious from non-infectious bone disorders [1]. Non-infectious disorders have been given many names, including Charcot's joint and neuroosteoarthropathy, but are most simply referred to as osteopathy [8]. Once patients have a foot infection, it is difficult to distinguish chronic osteopathy, superficial soft-tissue infection and osteomyelitis either by clinical examination or diagnostic tests [10]. Bone biopsy is the gold standard for the diagnosis of osteomyelitis [1]. Several imaging techniques have been widely used in diagnosis. Plain films can demonstrate late abnormalities related to osteomyelitis [1,14]. ^{111}Indium-labelled leucocyte scanning is considered to be the most accurate radionuclide study [15,16]. MRI is more sensitive

than other imaging studies for determining the extent of soft tissue and bone involvement, as well as for surgical planning [1,14,15]. However, imaging tests are unable to identify pathogenic organism(s) or guide antibiotic therapy, which can be done only by microbiological examination. In most cases it is safe to wait for the results of microbiological analysis instead of prescribing empirical antibiotic therapy.

In our study, the definitive diagnosis was based on histopathological examination of bone specimens in all patients. Our findings have shown that the sensitivities and specificities of scintigraphy (sensitivity 91%, specificity 67%) and MRI (sensitivity 78%, specificity 60%) and the sensitivity and specificity of microbiological processing (92% and 60%, respectively) were similar.

In a meta-analysis by Eckman *et al.* [10], the sensitivity and specificity rates of labelled leucocyte scanning were determined as 86 ± 5.9% and 45 ± 8.9%, respectively, similar to our study. The reported sensitivity and specificity of MRI of 99% and 71%, respectively, are different from our results. However, in this meta-analysis, the specificity rates of MRI differed by approximately 20% in the studies included. This variation is supported by other studies in the literature. In a review by Lipsky [1], the sensitivity and specificity rates of MRI ranged between 29 and 100% and 71 and 100%, respectively. This difference may be due to case mix of the studies. On the other hand, the main technical limitation of MRI is the relatively poor resolution for the cortex, which may cause some false-negative results in cases of isolated cortical infection. The typical marrow signal changes of osteomyelitis detected by MRI can be detected in any process that results in marrow replacement or infiltration, including osteoarthropathy. These technical difficulties with MRI will adversely influence sensitivity and specificity rates. Eckman *et al.* [10] also performed a cost-effectiveness analysis and concluded that non-invasive testing adds significant expense for patients in whom osteomyelitis is suspected and may result in little improvement in health outcomes. The investigators suggested that in non-toxic patients, tissue culture guided antibiotic therapy following surgical debridement may be a better approach. Because of the expense of scintigraphy and MRI, cost-effective diagnostic procedures such as 'probing to bone' or high-resolution ultrasound are being examined in clinical studies [17,18]. Nevertheless, these methods also cannot provide any information about infectious aetiology or antibiotic regimen.

In the early stages of foot osteomyelitis in diabetes, the causative bacteria are *Staphylococcus* spp. and/or *Streptococcus* spp. [19,20]. *Pseudomonas aeruginosa* is a rare pathogen in these patients. In our study, *P. aeruginosa* (33%) was the most common organism isolated from bone tissue cultures and MRSA (24%) and *Acinetobacter* spp. (12%) were the other major pathogens. The possibility of contamination must be considered, but in our study bone tissue was obtained by surgical procedures under aseptic conditions during either debridement or amputation. Thus, contamination was very unlikely. The predominance of *P.aeruginosa* is probably due to prolonged hospitalization and the long duration of foot infection in our diabetic patients. As these patients underwent frequent surgical debridement, isolation of hospital-acquired microorganisms such as *P. aeruginosa*, MRSA and *Acinetobacter* spp. was an expected result. All these microorganisms are responsible for severe infections and in patients with advanced disease the frequency of these organisms is high [21]. The relatively low rate of isolation of anaerobic bacteria (3%) was noteworthy. However, in some series up to 34% of bone cultures have yielded anaerobic bacteria [19,20,22,23]. This difference is probably due to the high frequency of surgical debridement in our patients, which avoids the growth of anaerobic bacteria. In contrast, 15% of patients had polymicrobial infections (1.06 pathogens per case of osteomyelitis) in our study. In superficial diabetic foot infections, a high average number of organisms is isolated per case (two to five pathogens per case). However, in diabetic foot osteomyelitis, the average number of organisms isolated per case is low (two pathogens per case) [1,8,9,20–22].

Diabetic foot infections tend to increase as the age of the population increases. Morbidity and mortality in diabetic foot infections are still high despite costly diagnostic tests and new therapeutic approaches.

In general, microbiological examination is a useful tool for diagnosing osteomyelitis in diabetic foot ulcers. However, using microbiological examination alone, one cannot differentiate between soft tissue infection and osteomyelitis. As open biopsy is an invasive technique, selection of patients to undergo this surgical procedure is critical. Clinical presentation and ESR can guide the selection of these patients. If osteomyelitis is suspected on clinical criteria, bone tissue should be obtained by open biopsy and microbiological examination performed. In our study, microbiological examination of bone tissue specimen was effective and less costly than MRI and scintigraphy in the diagnosis of osteomyelitis, but it is not the definitive diagnostic procedure. Histopathological examination of bone tissue is the gold standard for the diagnosis of osteomyelitis. The advantage of microbiological examination is that it is the only method which can guide the choice of antibiotic therapy.

Competing interests

None declared.

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