SUMMARY - Background: The aim of this prospective study was to assess the role of 99mTc-HMPAO leucocyte scintigraphy combined with a 99mTc-MDP bone scintigraphy in the diagnosis of the diabetic foot infection (HMPAO-Leu/MDP).

Methods: 75 diabetic patients with suspected osteomyelitis were included. The HMPAO-Leu/MDP scan was considered to be consistent with osteomyelitis when the HMPAO-Leu uptake was concordant in all the incidences with an MDP bone uptake. A HMPAO-Leu uptake without concordant bone MDP activity was considered as a soft-tissue infection. The results of the HMPAO-Leu/MDP scan were compared to the following diagnostic criteria: bone infection was confirmed by radiological follow-up or bone biopsy; the absence of bone infection was confirmed by clinical (healing of the ulcer without antibiotherapy) and radiological follow up.

Results: According to these criteria, among the 83 ulcers, bone infection was observed in 41 (49.4%): the HMPAO-Leu/MDP scan was positive in 38 cases, including 14 ulcers with normal or doubtful radiographs at inclusion. In the group of 42 ulcers without proven bone infection, the HMPAO-Leu/MDP scan was negative in 41 cases, including 17 lesions with a soft-tissue infection.

Conclusion: With a sensitivity of 92.6%, a specificity of 97.6%, the HMPAO-Leu/MDP scan is a reliable tool for the diagnosis of osteomyelitis in the diabetic foot. Neuroarthropathy did not affect the performances of the HMPAO-Leu/MDP scan. Owing to a high spatial resolution this test is very helpful to differentiate bone infection from soft-tissue infection especially in case of neuroarthropathy.

Key-words: diabetic foot, bone infection, nuclear medicine.
The diagnosis of osteomyelitis remains a difficult challenge in the diabetic foot. Routine radiographic techniques have a low sensitivity since they do not reveal changes until the bone has been significantly eroded; furthermore a concomitant neuroarthropathy may hinder the radiological interpretation. Three-phase bone scan with 99mTc-MDP (methylene diphosphonate) has a 100% sensitivity but a very low specificity due to the high incidence of neuroarthropathy or traumatic fractures [1]. White blood cells labelled with various radionuclides, especially 111In-dium oxine [2, 3] have been shown to be useful for the diagnosis of bone infection when combined with a 99mTc-MDP bone scan. In addition, recent studies have reported that 99mTc-HMPAO (hexamethylpropylene amine oxime) may contribute significantly to the diagnosis of bone and joint infections [4, 5]. Therefore we conducted a prospective study to evaluate the efficacy of the 99mTc-HMPAO-Leucocyte scintigraphy combined with a 99mTc-MDP bone scintigraphy (HMPAO-Leu/MDP scan) for the diagnosis of osteomyelitis in the diabetic foot.

**PATIENTS AND METHODS**

**Patients**

75 diabetic patients with suspected osteomyelitis from a foot ulcer (46 males, 29 females; median age: 61.3 years, range: 40-86; median duration of diabetes: 12 years, range 5-35; HbA1c: 8.7% range 6.9-12) were included from November 1993 to March 2001. 35 were treated with insulin and 40 with oral antidiabetic agents. Other significant medical history in this population included peripheral vascular or coronary diseases (n = 45), peripheral neuropathy (n = 53), previous foot ulcers (n = 48), neuroarthropathy with Charcot joint (n = 5). Inclusion criteria were: suspected bone or joint infection from a single or multiple foot ulcers and no history of vascular or foot surgery during the previous three months. Patients with acute limb-threatening infection (i.e., extensive necrotizing fasciitis) or systemic infection were not included since these clinical presentations are medical emergencies and require immediate antibiotic therapy. According to these criteria 101 foot ulcers were initially included in the study.

**Imaging protocol**

Standardized dorsi-plantar and lateral weight-bearing radiographs were taken on the day of inclusion, then repeated each week during at least three months when they were negative or doubtful for the diagnosis of osteomyelitis. Three-phase bone scintigraphy was performed 24 hours after plain films, using 600 MBq 99mTc-MDP. Immediately after injection, dynamic images of the feet were obtained for one minute. Five minutes after the injection, a blood pool image was obtained. After three hours, delayed images of the feet (anterior, plantar with zoom and lateral views) were acquired with a preset time of five minutes.

Leucocytes labelling with 99mTc-HMPAO: blood samples (42 ml) were collected on citric acid dextrose A. Cell-rich plasma was obtained after sedimentation for 30-60 minutes at 37°C in the presence of 2.5 ml Hespan. After sedimentation at 200 g for 10 minutes, granulocytes were isolated on Ficoll gradient. The granulocytes were labelled with 300 MBq of freshly prepared 99mTc-HMPAO (Ceretec, Amersham®); incubation lasted for 15 minutes at room temperature. The labelled cells were washed and resuspended in cell-poor plasma, then injected intravenously with a delay of less than 2.5 hours after the initial blood sampling. The mean injection dose was 200 MBq. In our population the labelling efficiency was 59% ± 12%. In vivo viability was assessed by pulmonary transit and hepatosplenic margination. Scintigraphic images were acquired four to five hours after injection with a gamma camera (rectangular Sopha DSX) used for bone scintigraphy. Special care was taken to place the patients in the same position as for bone scintigraphy. The ulcer dressing was carefully changed between the leucocytes injection and the first images acquisition.

The same static incidences centered on the feet were performed, together with an image centered on the thorax to assess cell viability. 99mTc-HMPAO-Leu and 99mTc-MDP scans were performed within a 2-day interval.

**Data analysis**

Each imaging study was independently evaluated by one experienced radiologist and one nuclear medicine physician who knew the site of interest but did not have any additional information. The HMPAO-Leu/MDP scan was considered to be positive for osteomyelitis when there was an accumulation of leucocytes concordant in all the incidences with an abnormal uptake on bone scintigraphy (Fig. 1). The HMPAO-Leu/MDP scan was considered to be negative for bone infection when there was an accumulation of leucocytes closed to the foot ulcer without concordant abnormal uptake on bone scintigraphy (soft-tissue infection; Fig. 2 and 3) or when no leucocytes accumulation was observed (absence of infection).

The final diagnostic procedure was performed using the following criteria:

- osteomyelitis was diagnosed by radiological examination at inclusion or during follow-up; in 28 ulcers a needle bone biopsy for bacteriological and histological studies was performed only if accurate cultures could be obtained through uninvolved tissue.
and when the radiograph at inclusion was negative or doubtful contrasting with a positive bone scintigraphy. Histopathologic criteria for osteomyelitis include necrotic bone with inflammatory exsudate adjacent to an extensive resorption;

- the absence of bone infection was confirmed by radiological follow-up during at least three months and healing of the ulcer without antibiotic treatment. A negative bone biopsy was not considered as a criteria to exclude the diagnosis of osteomyelitis. Among the 101 initial foot ulcers, 18 were excluded for the following reasons: antibiotic treatment indicated because of a second ulcer with evidence of bone infection (n = 8) or serious progressive cellulitis (n = 6), amputation of the ulcerated site during follow-up (n = 1), death (n = 1), absence of radiological follow-up (n = 2). The clinical characteristics of the 83 ulcers included in the final analysis are given in Table I.

### RESULTS

Among the 83 foot ulcers, 41 (49.4%) were associated with osteomyelitis diagnosed by radiology (n = 26) or bone biopsy (n = 15). The HMPAO-Leu/MDP scintigraphy was positive in 38 cases (Fig. 1) (three false-negatives) including 14 lesions with normal or doubtful radiography at inclusion but showing images of bone infection during follow-up. For the 42 ulcers without proven bone infection, the HMPAO-Leu/MDP scan was negative for 41 (one false-positive), including 17 ulcers with soft-tissue infection (leucocytes accumulation without concordant abnormal uptake on the bone scintigraphy; Fig. 2 and 3). With a specificity of 97.6%, sensitivity of 92.6% and accuracy of 95.1% the HMPAO-Leu/MDP scan has the best overall diagnostic performances as compared to plain radiography at inclusion or MDP scintigraphy alone (Table II). The MDP bone scintigraphy alone has a very high sensitivity (100%) but a low specificity (28%) especially in cases of previous foot ulcers or neuroarthropathy in the same site.

### DISCUSSION

This study confirms the results of our preliminary study [6] and demonstrates that the $^{99m}$Tc-HMPAO-Leu scintigraphy combined with a $^{99m}$Tc-MDP bone

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**Fig. 1. Ulcer of the right first toe. Focal MDP uptake on bone scintigraphy (bottom) concordant with HMPAO accumulation on leucocyte scintigraphy (top): Osteomyelitis of the right first toe.**

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scintigraphy is effective in the diagnosis of osteomyelitis of the diabetic foot.

Previous studies have reported that leucocyte imaging may prove useful for the detection of bone infection in the diabetic foot [3, 7, 8]. However, the major limitation of leucocyte imaging is the poor spatial resolution when performed without bone scintigraphy since this procedure cannot reliably separate a bone from a soft-tissue infection [7-9]. In our series, among the 17 ulcers with a focally increased leucocyte accumulation and a normal bone scan, the HMPAO-Leu scintigraphy alone was of poor value for distinguishing between soft-tissue infection and osteomyelitis. In these cases, the negative bone scan allowed to exclude osteomyelitis and this result was confirmed by the clinical and radiological follow-up. Moreover, the bone scintigraphy alone, even with the use of three-phase imaging, cannot be used to differentiate bone infection from neuroarthropathy, traumatic fractures or hyperemia that result from autonomic neuropathy. Each of these conditions yields positive images which remain positive for months. In that way, a negative bone scan virtually rules out bone infection in the diabetic foot but a positive one is inconclusive. Labelled leucocyte scan along with bone scintigraphy have been reported to have a sensitivity ranging from 86% to 100% and a specificity from 69 to 94% [2, 7]. These discrepancies are related in part to the inclusion criteria, the final diagnostic criteria used to classify the lesions with or without osteomyelitis and the method used for leucocytes labelling.

Leucocytes labelled with various radionuclides have been used for the detection of foot osteomyelitis. However, $^{111}$Indium oxine has become widespread and several studies have reported a high sensitivity and specificity when combined with a three-phase bone scan [2, 10]. Scintigraphy with leucocytes labelled with $^{99m}$Tc-HMPAO has the advantages of a lower radiation burden, a continuous availability, an ideal gamma-ray energy and a better spatial resolution than $^{111}$In-Leu scans. Several authors have recently reported that $^{99m}$Tc-HMPAO-Leu scan combined with
a 99mTc-MDP bone scan is helpful in the detection of peripheral bone and joint infection [5] even in chronic osteomyelitis [4]. A very few studies have focused on the accuracy of the 99mTc-HMPAO-Leu scintigraphy for the detection of pedal osteomyelitis in diabetic patients [11, 12]. However the aim of these studies was to compare the procedure with a three-phase bone scan and not to evaluate the performance of the combined scans as performed in our study. The performances of the combined HMPAO-Leu/MDP scan (sensitivity: 92.6%, specificity: 97.6%, accuracy: 95.1%) reported in the present study seems to be better than those with 111Indium-Leu scintigraphy combined with a bone scintigraphy.

In our study, the only false-positive scan resulted from a methodological error regarding leucocytes accumulation in a calcaneus ulcer dressing which had not been changed between the leucocytes injection and the acquisition of the first images four hours later.

The three false-negative scans occurred in ulcers involving the extremity of the big toe. The 99mTc-MDP bone scintigraphy was positive for these three patients, the 99mTc-HMPAO-Leu scintigraphy negative in two patients and positive in the third one but without exact concordance between the MDP and HMPAO uptakes. Histological and bacteriological examinations from a bone biopsy were negative for these three ulcers; the final diagnosis of bone infection was performed with the radiological follow-up showing osteomyelitis within a two to three-week interval from the initial radiographs. However, the radiographic findings suggestive of bone infection are not specific since osteolysis, irregular bone destruction and above all periosteal new bone formation can be seen despite

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**TABLE I. Clinical characteristics of the 83 ulcers.**

<table>
<thead>
<tr>
<th>Site of the ulcer</th>
<th>Count (%)</th>
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<tbody>
<tr>
<td>Forefoot</td>
<td>68 (82%)</td>
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<tr>
<td>Midfoot</td>
<td>3 (3.6%)</td>
</tr>
<tr>
<td>Hindfoot</td>
<td>12 (14.4%)</td>
</tr>
<tr>
<td>Ulcer size (cm²)</td>
<td>1.8 (range 0.6-5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of ulcer</th>
<th>Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuropathic</td>
<td>68 (82%)</td>
</tr>
<tr>
<td>Neuroischemic</td>
<td>13 (15.6%)</td>
</tr>
<tr>
<td>Non-neuropathic, non-ischemic</td>
<td>2 (2.4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade (Wagner classification)</th>
<th>Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>70 (84.4%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>10 (12%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>3 (3.6%)</td>
</tr>
<tr>
<td>Grade 4 and 5</td>
<td>0</td>
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</tbody>
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| Duration of the ulcer (weeks)  | 3.1 (range 1-9.2) |

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**TABLE II. Sensitivity, specificity and accuracy of plain radiography at inclusion, MDP scan alone and HMPAO-Leu/MDP scan (83 ulcers).**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial radiography</td>
<td>48 %</td>
<td>78.5 %</td>
<td>60.2 %</td>
</tr>
<tr>
<td>MDP Bone scan</td>
<td>100 %</td>
<td>28 %</td>
<td>62 %</td>
</tr>
<tr>
<td>HMPAO-Leu/MDP scan</td>
<td>92.6 %</td>
<td>97.6 %</td>
<td>95.1 %</td>
</tr>
</tbody>
</table>

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**Fig. 3. Ulcer of the fifth left metatarsal in a neuroarthropathic foot. Focal HMPAO uptake at the site of the ulcer (top) without concordant MDP uptake on bone scan (bottom): soft-tissue infection.**

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the absence of osteomyelitis, especially in neuropathic diabetic patients [1]. Nevertheless, the comparison of the successive radiographies was considered by the radiologist to be suggestive of bone infection. Despite the negative HMPAO-Leu/MDP scans these patients were considered to have bone infection and an antibiotic treatment was started.

The diagnosis of foot osteomyelitis is often difficult in patients with neuroarthropathy since many of the radiographic findings are similar (bone resorption, dislocation, osteolysis of the distal ends of the metatarsals) [14]. Moreover, both neuroarthropathy and osteomyelitis cause considerable bone remodeling, which results in increased $^{99m}$Tc-MDP uptake on bone scintigraphy. In these patients a combined $^{111}$In-Leu/$^{99m}$Tc-MDP scan has been reported to prove useful for the detection of osteomyelitis [2, 10]. However, in a retrospective study, Seabold [13] has reported several false-positive images at sites of rapidly progressing neuroarthropathy without proven bone infection. The mechanism of this $^{111}$In leucocytes uptake at noninfected acute fracture sites remains unclear. It could result from a late Indium marrow accumulation since it is mandatory with this radionuclide to acquire images 18 to 24 hours after the leucocytes injection. Moreover the poor spatial resolution of Indium is not sufficient to identify the bone infection despite of the metatarsal destruction induced by the neuroarthropathy.

In conclusion the HMPAO-Leu/MDP scan is a very sensitive and specific method to detect bone infection in the diabetic foot. After plain radiography, the $^{99m}$Tc-MDP bone scintigraphy is the first step. If negative, osteomyelitis is unlikely. If positive, a $^{99m}$Tc-HMPAO-Leu scintigraphy should be performed in order to exclude or to confirm the diagnosis of bone infection.

REFERENCES