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ARTICLE

Application of white blood cell SPECT/CT to predict remission after a 6 or 12 week course of antibiotic treatment for diabetic foot osteomyelitis

Julien Vouillarmet[1] · Myriam Moret[2] · Isabelle Morelec[3] · Paul Michon[1] · Julien Dubreuil[3]

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Abstract
Aims/hypothesis Diabetic foot osteomyelitis is a major risk factor for amputation. Medical treatment allows remission in 53–82% of cases. However, the optimal duration of antibiotic therapy remains controversial as a validated marker of osteomyelitis remission is lacking. The aim of this cohort study was to assess prospectively the remission rate of diabetic foot osteomyelitis medically treated using white blood cell (WBC)-single-photon emission computed tomography (SPECT)/computed tomography (CT) as a predictive marker of remission.
Methods Individuals with diabetic foot osteomyelitis that was non-surgically treated between April 2014 and December 2015 were included. All participants were treated with antibiotics alone. WBC-SPECT/CT was performed at 6 weeks and antibiotic treatment discontinued if the clinical signs of soft-tissue infection had resolved and there was no abnormal uptake of labelled WBCs. Treatment was otherwise continued for a total of 12 weeks and then discontinued. For these individuals, another WBC-SPECT/CT was performed at 12 weeks. Remission was defined as the absence of recurrence of osteomyelitis at the same location at 1 year.
Results Forty-five individuals were included; overall remission rate was 84% at 1 year. A 6 week course of antibiotics was used in 23 participants, 22 of whom were in remission at 1 year (96%); a 12 week course was used for 22 participants, 16 of whom were in remission at 1 year (73%). Sensitivity of WBC-SPECT/CT at 12 weeks was 100%, specificity 56%, positive predictive value 46% and negative predictive value 100%.
Conclusions/interpretation The study suggests that WBC-SPECT/CT could predict remission at the end of antibiotic treatment.
Trial registration: ClinicalTrials.gov NCT02927678

Keywords Diabetic foot · Nuclear imaging · Osteomyelitis · Single photon emission computed tomography/computed tomography · Treatment

Abbreviations

CRP	C-reactive protein
CT	Computed tomography
DFO	Diabetic foot osteomyelitis
ESR	Erythrocyte sedimentation rate
HMPAO	Hexamethylpropyleneamine oxime
IDSA	Infectious Diseases Society of America
IWGDF	International Working Group on the Diabetic Foot
SPECT/CT	Single photon emission computed tomography
$TcPO_2$	Transcutaneous oxygen pressure
WBC	White blood cell

Introduction

Diabetic foot osteomyelitis (DFO) is a frequent and important concern for people with diabetes, in whom the prevalence is about 20% for those presenting with a mild diabetic foot

✉ Julien Vouillarmet
julien.vouillarmet@chu-lyon.fr

[1] Hospices Civils de Lyon, Service d'Endocrinologie, Diabète et Obésité, Centre Hospitalier Lyon-Sud, 69495 Pierre Bénite, France

[2] Hospices Civils de Lyon, Service d'Endocrinologie et Diabète, Groupement Hospitalier Est, Bron, France

[3] Hospices Civils de Lyon, Service de Médecine Nucléaire, Centre Hospitalier Lyon-Sud, Pierre Bénite, France

infection and up to 60% for those hospitalised with severe infection [1]. Despite controversies in the management of DFO, the purely medical approach based on antibiotic therapy alone is efficient [2, 3]. Indeed, the remission rate of DFO in those treated medically is reported to range from 53% to 82% [4–14].

Several factors are probably involved in the success of the medical treatment, including the choice of the appropriate antibiotics active against the bacterial strain located in the bone, the vascular status of the individual and the associated clinical details, such as absence of chronic kidney disease. Two other factors may also contribute to the variability in efficacy between studies. The first is the difficulty in predicting DFO remission; there is currently no clinical, biological or radiological factor predictive of remission to help physicians decide when to discontinue treatment. Some factors have been proposed for this (such as a decrease in previously elevated inflammatory markers, resolution of any underlying soft-tissue infection, healing of any wound, and radiographic changes that suggest healing), but without any strong supporting evidence in the literature [1]. The recent International Working Group on the Diabetic Foot (IWGDF) guidelines consider that the best way to define treatment success is the absence of infection recurrence at the initial site for 12 months after cessation of the treatment [15]. Some studies did not use a specific duration for evaluation (mainly because of a retrospective design) [4–6, 8, 11] or used other variables, such as survival with limb intact [10] or ulcer healing [7, 11, 13].

The second point is the controversy over the optimal duration of antibiotic treatment. When we planned the present study, most published work had treatment durations longer than 12 weeks for some or all of the individuals [4, 5, 7–12]. Based on these studies, the 2012 Infectious Diseases Society of America (IDSA), recommended more than 3 months' antibiotic treatment [1]. Tone et al in 2015 published the only randomised controlled study in this area, which compared 6 weeks with 12 weeks of treatment in 40 participants [14]. The remission rate was 60% in those treated for 6 weeks and 70% in those treated for 12 weeks. The difference was not statistically significant and the key message of the study was to recommend 6 weeks of antibiotic therapy. This was retained in the last IWGDF guidelines in 2016 [15]. Studies to investigate the optimal duration of antibiotic treatment in DFO and to determine when DFO has been arrested after treatment were requested by IDSA experts to improve management [1]. Long-term use of antibiotics is associated with side effects such as acute kidney injury and also bacterial resistance, and thus a test or imaging method to assess the need for antibiotics would help clinicians.

Our retrospective study of individuals with a wide range of antibiotic treatment durations showed that a negative white blood cell (WBC)-single photon emission computed tomography (SPECT)/computed tomography (CT) could be an effective predictive marker of osteomyelitis remission at the end of antibiotic treatment [12]. This study examines the potential of WBC-SPECT/CT to predict remission after a 6 or 12 week course of antibiotic treatment for DFO.

Methods

Study design We prospectively assessed DFO remission at 1 year after completion of the antibiotic treatment in participants with non-surgically treated DFO. The duration of antibiotic treatment was predefined according to clinical findings (soft-tissue inflammation) and the results of WBC-SPECT/CT performed after 6 weeks of antibiotic treatment. If there was no sign of soft-tissue infection, and the examination was interpreted as negative, antibiotics were discontinued. If there was persistent soft-tissue infection and/or positive WBC-SPECT/CT (defined by an abnormal uptake of labelled WBCs), antibiotics were continued for a further 6 week cycle and then discontinued for all participants. Another WBC-SPECT/CT was performed at 12 weeks. Participants without either clinical signs of inflammation or a positive SPECT/CT (at 6 or 12 weeks) were followed until ulcer healing and/or 1 year after antibiotic discontinuation. Participants with a positive WBC-SPECT/CT at 12 weeks were followed monthly with clinical and/or radiological assessment until relapse (recurrent bone infection at the initial site) or for at least 1 year. In the case of relapse, a medical and/or surgical treatment was instituted.

Population Consecutive individuals with DFO seen in an outpatient setting or during hospitalisation were prospectively included in two diabetic foot centres in Lyon, France, between April 2014 and December 2015. Those with an indication for bone resection, or minor or major amputation, were not included, nor were those with a contraindication to WBC-SPECT/CT or who refused the WBC-SPECT/CT. Individuals with peripheral arteriopathy or renal insufficiency were not excluded in the absence of an indication for bone resection or amputation. As in recent randomised controlled studies on DFO [13, 14], at least 40 participants were included, with a minimum of 20 participants treated for 6 weeks and 20 participants treated for 12 weeks, with an expected rate of DFO remission above 70%.

The investigations were carried out in accordance with the principles of the Declaration of Helsinki. The study was registered with the institutional office of the national data protection agency (Commission Nationale de l'Informatique et des Libertés; ID: 15-010) and registered on ClinicalTrials.gov (NCT02927678). In accordance with legislation in place in France at the time of the study, ethics approval was not required, and oral informed consent was collected for all participants.

Outcome assessment DFO was defined in accordance with IWGDF guidelines by the association of soft-tissue infection and/or positive probe-to-bone test, and signs suggestive of osteomyelitis on plain radiographs (cortical disruption, periosteal elevation, a sequestrum or involucrum, or gross bone destruction) [15]. Systematic bone biopsy was not performed. The primary outcome was overall remission of DFO, defined by the absence of recurrent infection at the initial site and the absence of need for surgical bone resection or amputation at least 12 months after completion of antibiotic treatment [15]. Relapse was defined as a recurrent bone infection at the initial site. The secondary outcomes included remission at least 12 months after completion of antibiotic treatment in those treated for 6 weeks, and the diagnostic qualities of WBC-SPECT/CT at the end of antibiotic treatment to determine remission (sensitivity, specificity, positive and negative predictive values for those treated for 12 weeks, and negative predictive value for those treated for 6 weeks). None of the participants with a negative SPECT/CT during the study had another examination at follow-up to observe a potential positive test result.

Medical treatment Individuals were treated according to guidelines [1, 15]: first, with an empirical antibiotic therapy based on infection severity, and second with treatment according to the results of microbiological culture. Antibiotic administration was intravenous, oral or intravenous followed by oral. The type of antibiotic was left to clinical judgement, taking into account allergies and comorbidities (notably renal function). A combination of at least two antibiotics was recommended. Deep wound samples were collected after debridement and cleansing of the ulcer with sterile NaCl solution. Bone biopsy was not routinely performed. All physicians adopted the same approach to wound care, including daily dressing and off-loading of the ulcer. The duration of antibiotic treatment was predefined, as described above.

Variables Information on diabetes history and chronic complications was collected, as well as characteristics of the diabetic foot ulcer. Healing was defined by complete epithelialisation of the wound. Vascular assessment was performed at baseline and included pressure index measurement and transcutaneous partial oxygen pressure. Peripheral arteriopathy was defined by an ankle pressure index < 0.9 and/or a toe systolic pressure < 70 mmHg and/or a transcutaneous oxygen pressure ($TcPO_2$) measurement < 30 mmHg. Blood samples were collected at baseline and after 6 and/or 12 weeks of antibiotic treatment to measure C-reactive protein (CRP), neutrophil count and creatininaemia. Nephropathy was defined by the presence of at least one of the following signs: eGFR < 60 ml min^{-1} 1.73 m^{-2}, positive microalbuminuria or positive proteinuria. All participants had radiographs centred on the site of infection at the initial visit, at the end of antibiotic treatment and at 1 year.

WBC-SPECT/CT imaging Blood samples (42 ml) were collected on citric acid dextrose to label WBCs with 99mTc-hexamethylpropyleneamine oxime (HMPAO). Cell-rich plasma was obtained after sedimentation for 30–90 min at 37°C in the presence of 6.5 ml heafusine. Granulocytes were labelled with 650 MBq of freshly prepared 99mTc-HMPAO (Ceretec; Amersham, Mississauga, ON, Canada); incubation was for 15 min at room temperature (20°C). The labelled cells were washed and suspended in cell-poor plasma, then re-injected intravenously with a delay of no more than 3 h after collection of the initial blood sample.

Scintigraphic images were acquired 2 h and 20 h after injection of the 99mTc-HMPAO-labelled cells with a gamma camera (Philips Skylight; Philips, Amsterdam, the Netherlands). An incidence focus on the feet (a 256 × 256 matrix, pre-time = 10 min at 2 h and 15 min at 20 h) was used (plantar with zoom and lateral views). A hybrid SPECT/CT system (Siemens Symbia T2; Siemens, Munich, Germany) was used for imaging at 20 h. Interpretation of WBC-SPECT/CT was based on uptake of labelled WBCs, on recruitment between 2 h and 24 h acquisitions, and with the proximity with bone on SPECT/CT. All examinations were interpreted prospectively as positive or negative by a nuclear physician informed of the initial site of the DFO and with over 20 years' experience of WBC-SPECT/CT.

Statistical analysis Continuous variables were described using mean and SD; categorical variables were described using group size and percentage. Continuous variables were compared using a two-sample *t* test and categorical variables with Fisher's exact test. A p value < 0.05 was fixed as the threshold of statistical significance. Analyses were performed using Prism software version 5.0c (GraphPad Software, La Jolla, CA, USA).

Results

Population Of the 98 individuals identified as eligible, 45 were included in the study. Of the 53 who did not join, 30 had an episode of DFO with an indication for bone resection, minor or major amputation (11 with necrosis and/or severe arteriopathy, 11 with important tissue loss, four with important foot deformities, two with poor general health, one uncontrolled sepsis and one severe adverse reaction) and a further 23 had a contraindication for scintigraphy (two dementia, one non-adherent, one difficult venous access) or refused to undergo this procedure (n = 19). One patient presented with a second DFO episode at another site after a relapse and only the

first episode was included. All participants were followed for 1 year after completion of the antibiotic treatment.

Descriptive data The main characteristics of the participants are summarised in Table 1. Of the 45 participants, 82% ($n = 37$) required hospitalisation, the mean ± SD duration of which was 15 ± 9 days. All but one individual had a wound at baseline. Osteomyelitis was located on the toes in 19 participants (42%), the metatarsal bone in 25 participants (56%), and on the rear foot in one participant (2%). A total of 14 participants had received antibiotic treatment before inclusion: 4/23 (17%) in the group treated for 6 weeks and 10/22 (45%) in the group treated for 12 weeks ($p = 0.21$). All but one were treated orally.

The pathogens identified in deep wound samples are presented in Table 2. Methicillin-resistant *S. aureus* was not observed. Blood culture was positive for five participants (11%). An intravenous antibiotic regimen was used as first-line treatment in 38% ($n = 17$) of the participants, the mean ± SD duration of which was 14 ± 12 days, followed by a course of oral antimicrobial therapy. Regarding mode of treatment, 13% ($n = 6$) were treated exclusively with intravenous broad-spectrum β-lactams and/or glycopeptides and 49% ($n = 22$) exclusively with an oral antibiotic regimen. Antibiotics were used in combination; the most frequent antibiotic used was ofloxacin (82%, $n = 37$), followed by amoxicillin/clavulanic acid (49%, $n = 22$), trimethoprim–sulfamethoxazole (15%, $n = 7$) and pristinamycin (13%, $n = 6$); other antibiotics were used in five or fewer episodes each.

Table 1 Baseline characteristics of the population ($n = 45$)

Characteristic	n (%)/mean ± SD
Age (years)	64 ± 11
Male sex	37 (80)
Diabetes duration (years)	18 ± 11
Type 2 diabetes	40 (87)
HbA_{1c} (%)	8.3 ± 1.9
HbA_{1c} (mmol/mol)	67 ± 9
Neuropathy	42 (91)
Retinopathy	28 (62)
Nephropathy[a]	29 (64)
eGFR < 60 ml min^{-1} 1.73 m^{-2}	13 (29)
Peripheral arteriopathy[b]	12 (27)
Cardiovascular disease	15 (33)
Diabetes treatment	
OAD or incretin	20 (43)
OAD or incretin-insulin	10 (22)
Insulin	16 (35)
History of osteomyelitis	18 (39)
History of amputation	16 (35)

[a] Defined by the presence of at least one of: eGFR < 60 ml min^{-1} 1.73 m^{-2}, positive microalbuminuria or positive proteinuria

[b] Defined by an ankle pressure index < 0.9 and/or a toe systolic pressure < 70 mmHg and/or $TcPO_2$ < 30 mmHg

OAD, oral glucose-lowering drug

The overlying soft-tissue wound was healed at the end of antibiotic treatment (either 6 or 12 weeks) in 49% ($n = 22$) of participants; a reduction in wound area > 50% or healing was observed in 75%. The mean ± SD time to healing at the end of antibiotic treatment was 14.1 ± 9 weeks; it was 11.4 ± 6.8 for individuals treated for 6 weeks, and 17.1 ± 10.2 for those treated for 12 weeks ($p < 0.01$). Non-healing of the wound at the end of follow-up was observed in five participants, including one participant in remission.

A 6 week course of antibiotics was used for 23 participants (51%), and a 12 week course for 22 participants (49%) according to the results of WBC-SPECT/CT and clinical findings (Fig. 1). One patient had persistent signs of soft-tissue inflammation at 6 weeks, with abnormal WBC-SPECT/CT, and therefore antibiotic treatment was extended to 12 weeks. Wound, infectious and vascular variables at baseline, 6 and 12 weeks according to the duration of antibiotic treatment are presented in Table 3. There were no statistically significant differences between the two groups at baseline for wound duration before DFO, wound size, presence of cellulitis, fever, positive probe-to-bone test, CRP level or the presence of peripheral arteriopathy. At 6 weeks, there were no differences between the two groups for wound size, reduction of wound area > 50% or healing, neutrophils or CRP level. Participants treated for 12 weeks had a significantly higher level of neutrophils ($p = 0.04$) and intravenous antibiotic treatment was more frequently used ($p < 0.01$) at baseline but also a significantly higher rate of persistent positive probe-to-bone test at 6 weeks ($p = 0.04$) compared with those treated for 6 weeks.

Outcome data At 1 year after completion of antibiotic treatment, remission of DFO was observed in 84% ($n = 38$) of the participants (Fig. 1). A relapse occurred in seven individuals after a median (range) of 5 months (1–10 months); at the end of treatment, 6/7 had an abnormal WBC-SPECT/CT (Fig. 2), 6/7 had a persistent wound, 2/7 a positive probe-to-bone test, the mean ± SD level of neutrophils was 3.44 ± 0.44 × 10^9/l, and that of CRP was 57 ± 29 nmol/l. Relapses were managed by minor amputation in five participants and by second-line antibiotic treatment in two individuals. Remission of DFO was 96% ($n = 22$) in participants treated for 6 weeks and 73% ($n = 16$) in those treated for 12 weeks. The presence of peripheral arteriopathy and renal insufficiency did not impact on remission of DFO; the remission rates were 91.7% and 100%, respectively.

For those treated for 6 weeks, the negative predictive value of WBC-SPECT/CT at the end of treatment was 96%. For those treated for 12 weeks, the sensitivity of WBC-SPECT/CT at the end of treatment was 100%, specificity was 56%, positive predictive value was 46% and the negative predictive value was 100%.

Table 2 Distribution of pathogens identified from deep wound samples

Pathogen	Incidence		
	Total (n = 103)	6 weeks (56 cases)	12 weeks (47 cases)
Gram-positive cocci	54 (52)	27 (48)	27 (57)
Methicillin-sensitive *S. aureus*	24 (23)	14 (25)	10 (21)
Coagulase-negative staphylococci	5 (5)	1 (2)	4 (9)
Streptococcus spp.	17 (16)	7 (12)	10 (21)
Enterococcus faecalis	5 (5)	4 (7)	1 (2)
Enterobacter cloacae	3 (3)	1 (2)	2 (4)
Gram-negative bacilli	34 (33)	23 (41)	11 (23)
Proteus spp.	10 (10)	7 (12)	3 (6)
Morganella morganii	6 (6)	3 (5)	3 (6)
Pseudomonas aeruginosa	5 (5)	4 (7)	1 (2)
Klebsiella spp.	6 (6)	4 (7)	2 (4)
Other	7 (7)	5 (9)	2 (4)
Obligate anaerobes	10 (10)	4 (7)	6 (13)
Gram-positive bacilli	4 (4)	1 (2)	3 (6)
Gram-negative cocci	1 (1)	1 (2)	0 (0)

Data are n (%)

Discussion

The study suggests that WBC-SPECT/CT predicts remission after a 6 or 12 week course of antibiotic treatment, and found that the overall remission rate was higher than most of the rates observed in the literature [4–14]. As is generally observed in studies dealing with DFO, the overwhelming majority of the population included in the present study had neuropathy and there was a high rate of chronic complications associated with long-term diabetes. However, contrary to recent studies [13, 14], the population included individuals with peripheral arteriopathy and one-quarter had renal

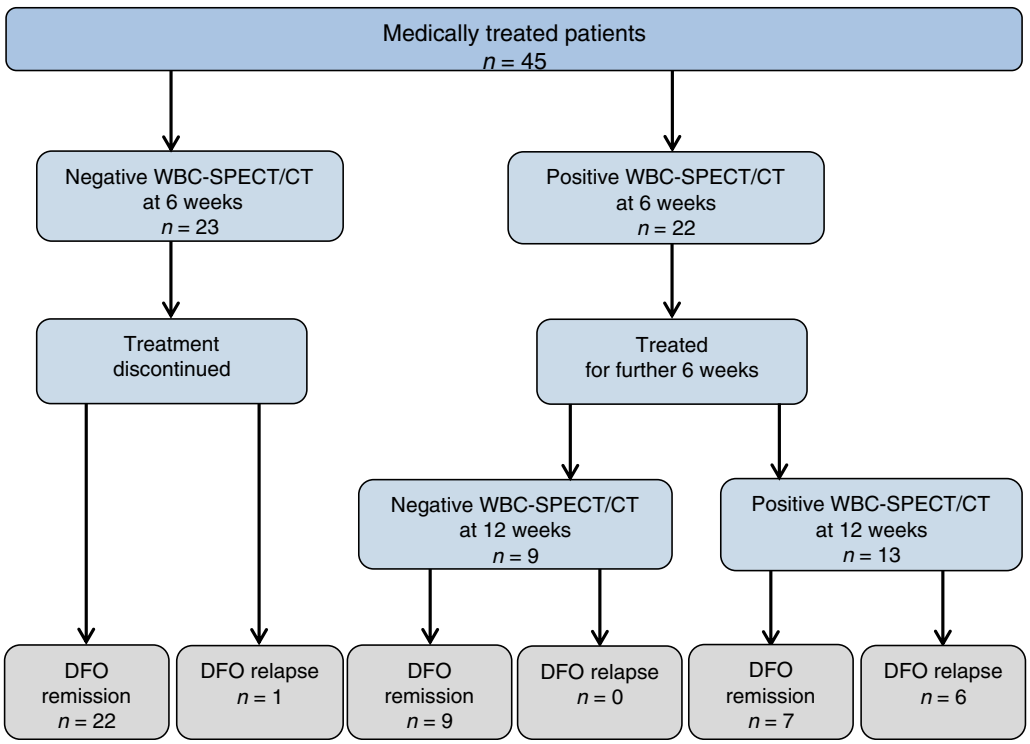

Fig. 1 Flow of participants through the study. Relapse was classed as infection at the initial site. Remission was the absence of relapse after 1 year of follow-up following antibiotic discontinuation

Table 3 Wound, infectious and vascular variables according to the duration of antibiotic treatment

Variable	6 weeks' treatment (n = 23)		12 weeks' treatment (n = 22)		
	Baseline	6 weeks	Baseline	6 weeks	12 weeks
Wound duration (weeks)[a]	19.7 ± 36.9	NA	15.7 ± 25.7	NA	NA
Wound size (mm^2)[a]	124 ± 117	77 ± 120	226 ± 240	122 ± 127	110 ± 165
Wound healing[a]	0	15 (65)	1 (5)	2 (9)	7 (32)
Healing or reduction wound area > 50%[a]	NA	19 (83)	NA	14 (64)	15 (68)
Probe-to-bone test	17 (74)	0*	13 (59)	5 (23)*	3 (14)
Cellulitis > 2 cm	18 (78)	0	17 (77)	1 (5)	1 (5)
Fever (%)	2 (9)	0	6 (27)	0	0
Neutrophils ($X10^9$/l)	5.54 ± 2.01*	3.67 ± 1.33	7.03 ± 2.88*	3.61 ± 1.57	3.79 ± 1.42
CRP (nmol/l)	724 ± 686	67 ± 143	867 ± 771	57 ± 57	48 ± 29
Peripheral arteriopathy[b]	8 (35)	NC	4 (18)	NC	NC
Previous antibiotic therapy	4 (17)	NA	10 (45)	NA	NA
Initial parenteral antibiotic therapy	7 (30)*	NA	18 (82)*	NA	NA

Data are n (%) or mean ± SD

[a] one participant treated for 12 weeks did not have a wound at baseline

[b] Defined by an ankle pressure index < 0.9 and/or a toe systolic pressure < 70 mmHg and/or $TcPO_2$ < 30 mmHg

* $p < 0.05$ between episodes in those treated for 6 weeks and those treated for 12 weeks

NA, not applicable, NC, not collected

insufficiency; although both are linked with poor prognosis, their prevalence does not seem to have negatively affected the rate of DFO remission at 12 months after completion of antibiotic treatment.

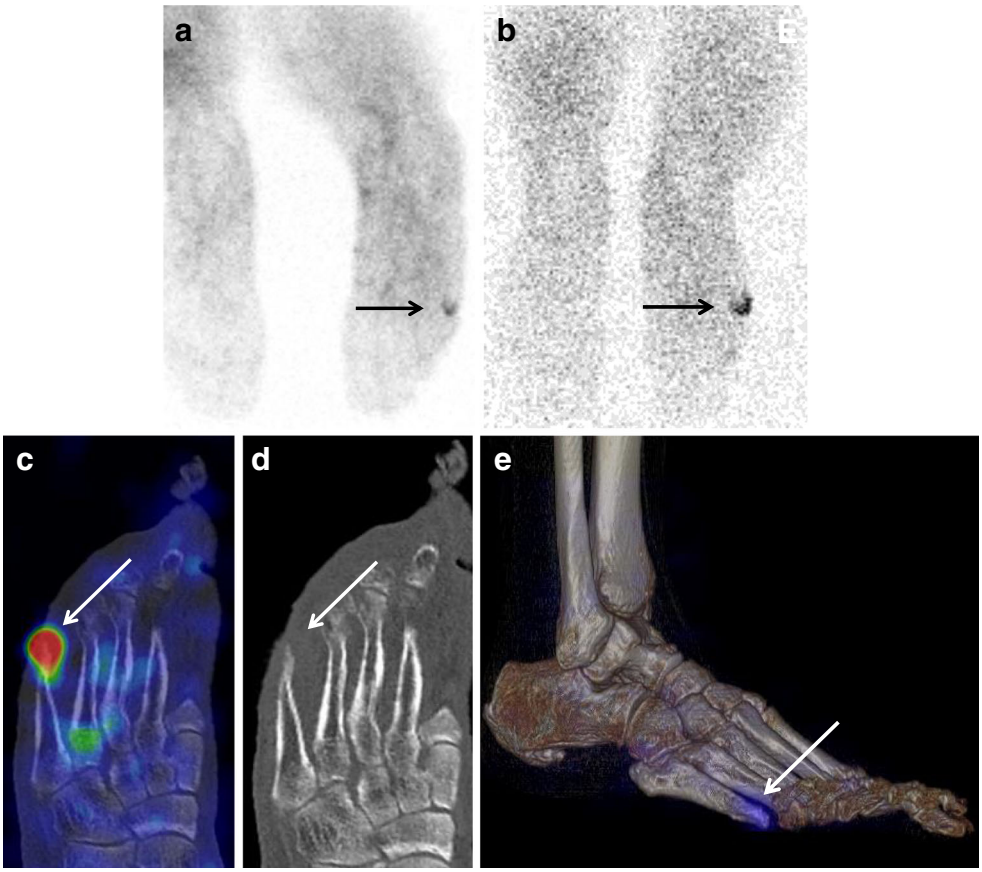

Fig. 2 WBC-SPECT/CT after 12 weeks of treatment. There is a significant accumulation of leucocytes at the right fifth metatarsal bone 2 h after injection (arrow) (**a**), with recruitment at 20 h (arrow) (**b**) extending to the cortex of the bone and causing cortical erosion on SPECT-CT (arrows) (**c**–**e**). This individual relapsed at 4 months after the end of treatment

There are few reported data dealing with the duration of antibiotic treatment in DFO. The paucity of data contrasts with the importance of this question, as the duration of antibiotic treatment is associated with the frequency of side effects and the overall cost of care [14]. At the time we planned the study, and on the basis of data available in the literature, guidelines recommended at least a 12 week course of antibiotic treatment in the absence of surgery [1]. We hypothesised that a shorter 6 week course may be sufficient for some individuals and thus used this period of treatment in the study design. Interestingly, the study by Tone et al [14] was subsequently published and the IWGDF guidelines were modified to recommend a 6 week course of antibiotic treatment [15]. However, as mentioned by Tone et al in the discussion of their study, the small population size could have induced a high risk of beta error, leading them to conclude that a difference was absent when, in fact, it existed [14]. Furthermore, the authors did not find any factor predictive for remission, and thus it cannot be excluded with certainty that some individuals may benefit from treatment for longer than 6 weeks.

The IWGDF also underline that the question of the duration of antibiotic therapy to treat DFO remains controversial [15]. The difficulty in choosing the optimal duration of antibiotic treatment relates to the absence of a validated tool to predict DFO remission. Guidelines suggest different markers that could be helpful to predict the efficiency of medical treatment in DFO: radiographical changes, healing of any wound, decrease in previously elevated inflammatory markers and resolution of any underlying soft-tissue infection [1]. However, evidence to support these recommendations is poor. For instance, the reproducibility [16] and diagnostic characteristics [12] of radiological examinations to predict remission are too poor for application in routine clinical care. More generally, healing of any wound is clearly an important factor to prevent any new foot infection, but it is of note that, although all but one individual with relapse in our study had a non-healed wound at the end of treatment, not all of those who had a non-healed wound had relapse. Thus, wound healing may not be used exclusively to assess the remission of DFO, as it has been in some studies [7, 11, 13]. Factors other than infection affect the healing process, such as the size of the ulcer at baseline, compliance with off-loading, tissue perfusion, nutritional status and so on [17]. Some studies suggest a predictive role for erythrocyte sedimentation rate (ESR) [18–20] and CRP trajectories when monitoring DFO treatment [19, 20]. Yet none of these studies assessed the remission rate of DFO according to the level of these markers of inflammation at the end of treatment. In this study, CRP level at baseline and during follow-up was not associated with DFO remission. As ESR varies according to many factors (e.g. age, sex, anaemia, obesity), this variable was not routinely measured in the participating centres and so was not collected in this study. It cannot be formally excluded that ESR, which normalises more slowly than other inflammatory markers, could be more valuable [18–20].

The last point is the remission of any underlying soft-tissue infection. This factor was included in our decision to discontinue antibiotic treatment at 6 weeks. Interestingly, only one participant had a persistent soft-tissue inflammation at 6 weeks, and this person also had an abnormal WBC-SPECT/CT at 6 weeks. This suggests that persistence of soft-tissue inflammation alone may have a good positive predictive value but a low sensitivity.

Animal and human studies have shown that nuclear imaging could be of interest to predict osteomyelitis remission [21–24]. However, these studies used isotopes with a high radiation burden and low spatial resolution. Lipsky later suggested that a leucocyte scan might be useful in demonstrating that infection has been arrested [25]. We published in 2014 a paper suggesting that WBC-SPECT/CT could be used to predict DFO remission at the end of medical treatment with an optimal negative predictive value (100%) and that it could therefore be a very useful tool to guide the duration of antibiotic treatment in DFO [12]. The interest in WBC-SPECT/CT to assess DFO remission was also confirmed by Lazaga et al, who found a negative predictive value of 83% [26]. This was further confirmed for those treated for 6 weeks in the present study. The limited value of this examination is, however, related to the high rate of false positives, with positive predictive values ranging from 50% [26] to 70% [12]; a high false-positive rate could lead to overtreatment. For instance, in the current study some individuals who were treated for 12 weeks may have had the same outcomes if they had been treated for 6 weeks.

WBC-SPECT/CT seems to better select individuals who need only a 6 week course of treatment: all but one participant treated for 6 weeks in our study was in remission at least 12 months after completion of antibiotic treatment, which is far from the 60% observed by Tone et al [14], although comparing studies is difficult. Conversely, this technique could also help to select individuals who need a longer course of antibiotic treatment, and it is of note that such participants were probably more severely ill than those treated for 6 weeks (as evidenced by a higher neutrophil count and a more frequent use of intravenous antibiotic treatment at baseline). However, WBC-SPECT/CT is costly, not widely available and also time consuming, and so cannot be recommended for routine care. It could be proposed that WBC-SPECT/CT be performed at 6 weeks of treatment in a subset of individuals (for example, those with a persistent wound, probe-to-bone test, soft-tissue inflammation and/or elevated inflammatory marker), but this approach needs to be confirmed in studies. It is also of note that, in addition to WBC-SPECT/CT, other recent nuclear imaging techniques appear to be promising for the diagnosis and management of DFO, though none is currently validated to predict DFO remission [27–31].

The study has some limitations that are important to discuss. First, bone biopsy was not performed to confirm diagnosis of DFO. However, the most recent IWGDF guidelines recommend a bone biopsy only when the diagnosis is in doubt or determining the antibiotic susceptibility is crucial [15]. It is important to note that, even for research purposes, bone biopsy is not commonly performed [4–6, 10–13]; only one retrospective study in the literature has assessed its capacity to improve DFO remission [9]. Moreover, bone biopsy, through sampling errors, may lead to false-negative microbiological culture results. Indeed, even in trained teams, the rate of negative bone culture despite suspicion of DFO is reported to be around 20% [14]. More generally, in the absence of bone specimen, we cannot be sure to target specifically the bacterial specimen located in the bone based only on deep wound samples [32, 33]. It remains possible that participants in our study who had a relapse received an antibiotic regimen not adapted to the bacterial species in the bone, which is a well-known factor for failure [9]. Conversely, some of the participants were treated exclusively by broad-spectrum β-lactams and/or glycopeptides and it is possible that a bone biopsy could have helped to narrow the spectrum used. It can be noticed that the use of rifampicin was rare (three participants, data not shown), following recommendations to limit the development of additional bacterial resistance [9] in the absence of bone biopsy. It is important to note that, despite the absence of WBC-SPECT/CT at baseline, differential diagnoses such as active Charcot disease, recent trauma or non-septic arthritis were unlikely. Charcot disease usually presents with moderate widespread uptake without any WBC recruitment at 20 h [34]. Recent trauma could involve recruitment of WBC owing to debridement by neutrophils and macrophages, but the fusion images with thin CT slices often allow diagnosis [35, 36]. It is also possible to differentiate non-septic arthritis from septic forms as the latter present WBC recruitment at 20 h [37]. Furthermore, the physician who interpreted the data (IM) has 20 years' experience in this field.

To conclude, the study suggests that WBC-SPECT/CT could predict remission at the end of the antibiotic treatment, but these results need to be confirmed in other centres and with larger numbers of people.

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Data availability The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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Contribution statement JV conceptualised and designed the study, contributed to data analysis and interpretation, and wrote the manuscript. MM, PM, IM and JD contributed to the acquisition, analysis and interpretation of the data. All authors were involved in revising the manuscript critically for important intellectual content and for final approval of the version to be published. JV is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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