Effect of microbes on Patterns of Labeled White Blood Cells in Osteomyelitis

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How to cite this article: Saroj Kumar Sahu, Sudatta Ray. Effect of microbes on Patterns of Labeled White Blood Cells in Osteomyelitis. Indian Journal of Public Health Research and Development 2023;14(3).

Abstract

Aim: This study investigates the effect of microbiological characteristics of causative organisms on the scintigraphic patterns of labelledwhite blood cell (WBC) scans in cases of proven osteomyelitis.

Methods: Retrospective analysis of 25 patients referred with suspected osteomyelitis and had both bone and labelled WBC scans performed and complete records of the microbiological culture of the causative organism. The bone and labelled WBC scans were retrieved and reviewed by two nuclear medicine physicians. Any definite focal accumulation of labelled WBCs within the bone was considered positive for osteomyelitis. Diagnosis of osteomyelitis in the discharge summary was considered the reference standard and was based on a combination of the clinical scenario, imaging, and laboratory findings including microbiology. A correlation of the pattern of labelled WBC and the type of microorganisms was done.

Results: A total of 16 patients were included in this study, seven females and nine males. Of these, seven patients had Gram-positive whereas nine patients had Gram-negative organisms. The majority (85.7%) of Gram-positive organisms showed increased accumulation of labelled WBCs, whereas only one-third (33.3%) of patients with Gram-negative organisms had such findings.

Conclusion: The pattern observed in this study shows that the falsenegative results of labelledWBC scans were mainly noted in patients with Gram-negative as opposed to Gram-positive infections. This confirms the experimental animal study findings that the secretion of anti-chemotactic factors by Gram-negative organisms, seems to be inhibiting the migration of labelled WBCs to the site of infection. The inhabitation is decreasing the accumulation of labelled WBCs and consequently resulting in a falsenegative finding. The study adds to evidence that microbiological characteristics of the causative organisms are another explanation for the falsenegative WBC in proven osteomyelitis.

Keywords: Bone scan, Gram-negative bacteria, Gram-positive bacteria, labelled white blood cells, osteomyelitis.

Introduction

The diagnosis of osteomyelitis can be challenging and depends on clinical findings, laboratory tests, and imaging studies supported by tissue culture. Gram-positive bacteria are the primary causative agents of osteomyelitis and *Staphylococcus aureus* is by far the most common in all age groups.[1] Osteomyelitis can

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also be caused by Gram-negative bacteria, especially in patients with certain predispositions such as sickle cell disease, diabetes, and trauma.[2] Multiple morphologic and functional imaging modalities have been used with different sensitivities and specificities. Conventional radiographs are usually used first due to their wide availability and low cost. However, plain radiographs usually have limited value due to their low sensitivity for acute infections as the anatomical changes are usually seen in the late stages of the disease.[3] Computed tomography (CT) provides good anatomical information. However, its ability to differentiate bone from softtissue infection is limited. In addition, its value is diminished in postsurgical cases with a metallic implant.[3] On the other hand, magnetic resonance imaging (MRI) provides high-resolution images with a good ability to differentiate soft tissue from a bone infection.[4] It has high sensitivity in detecting osteomyelitis, but its specificity is limited by many causes of false positive results such as postsurgical changes, bony infarction, and Charcot's joint.[5] Multiple nuclear medicine procedures are used in diagnosing osteomyelitis. Multi-phase bone scintigraphy using technetium99 mdiphosphonates (Tc99 m) provides an early diagnosis with comparable sensitivity to MRI. However, it has low specificity as multiple potential causes can result in false positive results.[6] The specificity of a bone scan can be improved when it is combined with a labelled white blood cell (WBC) scan. The labelled WBC study is the most accurate in diagnosing acute osteomyelitis. The combined approach using bone and labelledWBC scans provide comparable sensitivity and superior specificity in comparison to MRI.[7] Although the combined approach has generally acceptable accuracy (ranging from 79% to 100%), many factors should be considered before choosing this approach. The duration (acute or chronic) and the location (axial or appendicular) of the infection may affect the accuracy of this technique.[8] Furthermore, the microbiological characteristics of the causative organisms may also influence the accuracy of the procedure.

Multiple studies reported low sensitivity of labelled WBC scans for the detection of vertebral osteomyelitis. Palestro *et al*. reported a low sensitivity (39%) of In-111 WBC in 28 patients with proven vertebral osteomyelitis.[9]Another study on 22 patients with spine infections reported asensitivity of only 17%.[10] Hovi also reported three cases of histologically proven osteomyelitis detected by MRI but none by Tc-99 m-hexamethyl-propylene-amine oxime (99mTc-HMPAO)-labelled WBC studies.[11]

The exact reason for this observation has been uncertain, and there are several possible explanations. The diagnosis of vertebral osteomyelitis is often delayed, and most infections become chronic which lowers the sensitivity of labelled WBC scans.[12] Another possible explanation is that infection results in very high pressure in the vertebra and therefore, labelled WBCs could not migrate to the focus of infection during the available time for imaging. Palestro and Love proposed that marrow uptake of labelled WBCs in the normal spine may be greater than in the infected bone, masking the abnormality or causing a relative photogenic region.[13]Finally, Fernandez-Ulloa *et al*. suggested that Gram-negative organisms could be behindthis finding since they secrete anti-chemotactic factors.[14]A preliminary animal study with rabbits confirmed that Gram-negative infections in vertebral and femoral osteomyelitis are associated with low to no accumulation of labelled WBCs.[12] This study aims to investigate the microbiological characteristics of labelledWBC scans in cases of nonvertebral osteomyelitis since the high-pressure theory is not a factor in such sites.

Materials and Methods

Retrospective review of cases referred to the Department of Nuclear Medicine for suspected osteomyelitis from September 2009 to September 2013. Only 25 patients, who had both bone and labelledWBC scans performed were diabetics, and only two out of the seven patients with Gram-positive infections are diabetics (Table 1). Seven patients had Gram-positive bacterial infection, whereas nine had Gram-negative infection (Table 2). Out of the seven cases with Gram-positive infection, six (85.7%)had increased uptake of labelled WBCs [Table 3]. The majority of the positive labelledWBC scan cases of Gram-positive bacterial infection were due to *eus* (83.3%). Out of the nine Gram-negative bacterial-infected cases (Table 4), only three had increased uptake (33.3%), whereas the other six had nouptake (66.7%) on their labelledWBC scans. Two of the three patients with increased labelled WBCs uptake were infected with *Enterobacter aerogenes* while the third patient was infected with multiple Gram-negative organisms (Table 3). Figure 1 illustrates an example of Gram-negative bacterial infection at the foot with nouptake of labelled WBCs and complete records of the microbiological culture of the causative organism, were included in the study. The data were collected and attained from the nuclear medicine Department picture archiving and communication system, patients' hospital files and Laboratory Information System. Data from these selected 25 patients with complete records were analyzed.The three-phase bone scan was performed following the intravenous administration of 740–1,110 MBq (20–30 mCi) of Tc-99m-methylene diphosphonate. The dynamic blood flow images were acquired immediately after the radiotracer administration over the region of interest. The blood pool images were obtained after 5 min. Delayed whole-body images, as well as spot images, were obtained after 3 h. Single-photon emission CT (SPECT) and SPECT/CTimages were also obtained on selected cases when an accurate anatomical correlation was needed.

Labeled-WBC scan was performed the following day. The patient WBCs were labelled with 25 mCi of 99mTc-HMPAO and re-injected intravenously. Whole-body and spot images of the area of interest were acquired at 2 and 4 h after the radiotracer administration. Furthermore, SPECT andSPECT/ CT images were acquired on selected patients for correlation with the bone scan.The bone and labelledWBC scans were retrieved and reviewed by two nuclear medicine physicians. The images were interpreted based on a visual assessment of the uptake pattern of labelled WBC in correlation to the bone scan. Any definite focal accumulation of labelled WBCs within the bone was considered positive for osteomyelitis. All patients selected in the analysis had also microbiological and inflammatory markers performed. The final diagnosis of osteomyelitis in the discharge summary was considered the reference standard and was based on a combination of the clinical scenario, imaging, and laboratory findings including microbiology.

Results

Among the 25 patients studied, seven patients were excluded from further analysis since osteomyelitis was not their final diagnosis. In addition, two patients with proven osteomyelitis were excluded from the final analysis as they had a combination of mixed Gram-positive and Gram-negative bacteria in the culture. The remaining 16 patients including seven females and nine males with ages ranging between 20 and 75 years (mean 51.2) were included in the final analysis. None of the patients was given antibiotics for >48 h before scans. In the investigatedcases, the osteomyelitis was located at the tibia in fivecases, three cases at the metatarsal, two at each of thetalus, femur, and calcareous, as well as one case at each ofthe radius and posterior talocalcaneal joint. Eight out of the16 patients included were diabetics. The majority of the patients withGram-negative infections (six out of nine, 66.7%) were diabetics, and only two out of the seven patients with Gram-positive infections are diabetics (Table 1). Seven patients had Gram-positive bacterial infection, whereas nine had Gram-negative infection (Table 2). Out of the seven cases with Gram-positive infection, six (85.7%) had increased uptake of labelled WBCs (Table 3). The majority of the positive labelledWBC scan cases of Gram-positive bacterial infection were due to *eus* (83.3%).

Table 1: Demographics Data of patients

No. of Patient	Scan	Gram stain	Site of infection	Diabetic
1	+	+	Talus	-
2	-	-	Metatarsal	+
3	+	+	Femur	-
4	-	-	Tibia	+
5	+	-	Tibia	+
6	+	+	Tibia	-
7	-	-	Talus	-
8	-	-	Tibia	-
9	-	-	Metatarsal	-
10	+	+	Calcaneus	+
11	+	+	Calcaneus	+
12	-	-	Metatarsal	+

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13	+	-	Posterior talocalcaneal Joint	+
14	-	+	Radius	-
15	+	+	Femur	-
16	+	-	Tibia	+

Table 2: Type of micro-organisms and labelled white blood cells scan findings

Organism	Number ofcases	Positivescan (%)	Negativescan (%)
Gram-positive	8	6(85.7)	2(14.3)
Gram-negative	7	3(33.3)	4(66.7)

Table 3: Results of labelled white blood cell scan in patients with Gram-positive bacterial infections

Patient number	Scan results	Organism
1	+	Staphylococcus aureus
2	+	Enterococcus faecalis
3	+	Staphylococcus aureus
4	+	Enterococcus faecalis
5	+	Staphylococcus aureus
6	+	Enterococcus faecalis
7	+	Staphylococcus aureus

Table 4: Results of labelled white blood cell scan in patients with Gram-negative bacterial infections

Patient number	Scan results	Organism
8	-	Morganella morgagnii *Bacteriods spp.*
9	-	*Klebsiella pneumoniae*
10	-	Enterobacter sakazakii
11	-	Enterobacter aerogenes Morganella morgagnii
12	+	Escherichia coli Pseudomonas spp. Bacteriods fragalis
13	+	Enterobacter aerogenes
14	-	Enterobacter aerogenes
15	+	Enterobacter aerogenes
16	-	Citrobacter koseri

Out of the nine Gram-negative bacterial-infected cases (Table 4), only three had increased uptake (33.3%), whereas the other six had nouptake (66.7%) on their labelledWBC scans. Two of the three patients with increased labelled WBCs uptake were infected with *Enterobacter aerogenes* while the third patient was infected with multiple Gram-negative organisms (Table 3). Figure 1 illustrates an example of Gram-negative bacterial infection at the foot with nouptake of labelled WBCs.

Discussion

Gram-positive organisms are known to be the main causeof osteomyelitis; however, Gram-negative organismssuchas *Pseudomonasaeruginosa* and *Klebsiella pneumoniae*can also cause osteomyelitis, especially in the diabeticfoot, hospital-acquired infections, and intravenous drugabusers.[15,16] In this study, seven of the 16 patients withproven osteomyelitis were infected with Gram-positiveorganisms while nine patients had Gram-negativebacterial infections. The majority of patients (85.7%)with Gram-positive infections had positive labelledWBCscans. As well, the majority of patients (66.7%) withGram-negative infections had negative labelledWBC scans.The majority of patients in this study were infectedwith Gram-negative organisms which were likely due tothe higher presence of diabetes (66.7%) in this group.However, the main goal of this study was to investigate thepattern of labelledWBC uptake in Gram-negative-causedosteomyelitis. The pattern observed in our small groupof patients with nonvertebral osteomyelitis is consistentwith the findings advocated by Fernandez-Ulloa *et al*. intheir five cases of vertebral osteomyelitis. They notedthat the abnormally decreased uptake of labelled WBCs inpatients with proven vertebral osteomyelitis was mainlyobserved in the presence of Gram-negative organisms.[14]Kim *et al*. in 1987[17] and Palestro *et al*. in 1991[9] havealso reported false-negative labelledWBC scans in patientswith proven osteomyelitis mainly in cases associated with Gram-negative infections. It has been proposed that false-negative labelled WBCscans of vertebral osteomyelitis are likely due to increasedcompartmental pressure which may prevent the accumulationof labelled WBCs.[14] However, observing the same trend ofdecreased accumulation of labelled WBCs in nonvertebralosteomyelitis in animal and human studies indicates.

Conclusion

The pattern observed in this study shows that the falsenegative results of labelledWBC scans were mainly noted in patients with Gram-negative as opposed to Gram-positive infections. This confirms the experimental animal study findings that the secretion of anti-chemotactic factors by Gram-negative organisms, seems to be inhibiting the migration of labelled WBCs to the site of infection. The inhabitation is decreasing the accumulation of labelled WBCs and consequently resulting in a falsenegative finding. The study adds to evidence that microbiological characteristics of the causative organisms are another explanation for the falsenegative WBC in proven osteomyelitis.

Ethical statement: This study was approved institutional ethical committee

Conflict of Interests: The authors declare that they have no conflicts of interest

Source of Funding: Self-financed

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