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^{99m}Tc labeling white blood cells with a simple technique: Clinical application

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Several radionuclides and different methods have been employed as cellular labels to study inflammatory sites in man. Here we present the results obtained with white blood cells (WBC) labeled with ^{99m}Tc using a simple and low cost new technique (SnTec). WBC were incubated with 12 μg/m*l* of stannous chloride for 10 min at room temperature. Then ^{99m}Tc was added. After 10 min, the ^{99m}Tc-labeled WBC were washed and injected into the patient. Comparison studies with ^{99m}Tc-labeled WBC using the HMPAO technique were carried out in patients with suspected osteomyelitis. Since the results are similar with both methods, we suggest the use of SnTec to label WBC, in cases of inflammatory diseases.

Key words: white blood cells, ^{99m}Tc, SnTec, HMPAO, osteomyelitis

INTRODUCTION

SEVERAL AUTHORS have used radioactive compounds to detect inflammatory foci in cases of infection. Most of them use ^{67}Ga or ^{51}Cr, ^{111}In or more recently, ^{99m}Tc. ^{51}Cr was the first marker used for *in-vivo* detection but its high photon energy, the high rate of conversion and its long physical half life are obvious disadvantages.[1] ^{67}Ga localizes in bone to some extent because it is incorporated into calcium hidroxy appatite crystal as a calcium analogue, and in bone marrow because of its behavior as an iron analogue. Based on the bone incorporation mechanism, ^{67}Ga is subject to the same non-specificity as the ^{99m}Tc phosphonate bone scan.[2] ^{111}In-labeled leukocyte imaging is an important method to use in diagnosing infection and locating abscesses.[1,3] However, ^{111}In suffers the disadvantages of being expensive and inconvenient to supply; there is also concern about its dosimetry.[4,5] Because of these factors, there is interest in alternative methods, and we have developed one technique (SnTec) using ^{99m}Tc for labeling leukocytes.

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^{99m}Tc is widely used in Nuclear Medicine[6,7] and it has been shown that it is the most promising radionuclide for labeling white blood cells (WBC) when used with gamma camera imaging.

Since 1986, most work on ^{99m}Tc labeling of leukocytes has centered around the hexamethylpropyleneamine oxime (HMPAO),[8,9] which is trapped inside cells due to rapid conversion to a hydrophilic complex.[10] The high cost and the low labeling efficiency are the main disadvantages, as well as the decreased availability, inability to label mononuclear cells and the large amount of blood required.[8,9] Moreover, its labeling technique requires the use of Hespan that is forbidden in some countries.[10]

We have previously shown that the stannous chloride is a suitable and inexpensive method for reducing ^{99m}Tc and thus labeling WBC.[11–13] We now report *in vivo* comparison of SnTec and HMPAO methods in patients with suspected osteomyelitis.

MATERIALS AND METHODS

Leukocyte labeling procedure

Briefly,[14] 30 m*l* of patient whole blood was withdrawn in sterile heparinized tubes (Liquemine, Roche, Brazil). For mononuclear cells isolation, the Ficoll-Hypaque technique was used. The cells were washed by centrifugation (1000 rpm, 10 min) in saline solution (NaCl 0.9%) using

a clinical centrifuge. The pellet was resuspended in saline and transferred to a microcentrifuge tube for platelets removal (500 rpm, 1 min). This procedure was repeated 3 times until assured that the cell preparation was free of platelets. The final mononuclear cell concentration was 10^7 per m*l*. The cells were then incubated with 12 μg/m*l* of stannous chloride solution ($SnCl_2 \cdot 2H_2O$, Merck, Brazil) for 10 minutes at room temperature. Afterwards, ^{99m}Tc (370 MBq) was added and the incubation was continued for another 10 minutes. The preparation was washed once in saline. The pellet was resuspended in saline and injected into patients. Images were obtained immediately and at various times (30 min–3 hours), after injection of the labeled cells.[11] ^{99m}Tc-HMPAO scintigraphy was carried out in the same patient after one week.

^{99m}Tc-HMPAO was prepared by adding pertechnetate to a freeze-dried kit (Ceretec, Amersham Ltd.) according to the manufacturer's directions.

In vitro cell viability was demonstrated with the Trypan blue exclusion test.[15,16]

These procedures were performed in 25 patients, male and female, aged 18 to 60. Here we have selected 2 patients to be presented.

Histological studies were performed to aid the diagnosis confirmation.

RESULTS

Table 1 shows the final diagnosis for 25 studied patients.

Figure 1 shows a case report of a selected patient who had a painful infectious process in the left leg and difficulty in walking. X-ray studies only showed a pathological fracture of the left ankle with multiple lytic lesions (A and B). A bone biopsy was performed and was positive for osteomyelitis. The patient underwent scintigraphic examinations to determine the extention of the disease. A bone scintiscan (D) showed diffuse uptake throughout the left distal tibia and ankle, confirming X-ray findings. However, a left femural focal bone lesion was also demonstrated (C), prompting us to continue the investigation. Labeled WBC with SnTec (F and H) and ^{99m}Tc-HMPAO (E and G) scintigraphies were performed and an intensive diffuse abnormal activity involving all the left distal leg, but not the left femur, was found. A new biopsy was carried out with positive findings for osteomyelitis (I) and lymphoma (J).

Figure 2 shows a case report of a patient with sustained nontraumatic bone pain on the right knee. X-ray results suggested bone infarct (A). ^{99m}Tc-MDP scintigraphy revealed focal activity in the distal left femur (B) which might be compatible with the suspected diagnosis. Labeled WBC with SnTec (D) and ^{99m}Tc-HMPAO (C) was then performed and no abnormal activity was found, suggesting a non-infectious lesion. Histo-pathological findings (E) confirmed bone infarct.

Table 1 Final diagnosis of the evaluated patients

Patients (n)	Sex M	Sex F	Final Diagnosis (Histologically confirmed)
2	1	1	Normal
2	1	1	Bone infarct
19	11	8	Osteomyelitis
1	0	1	Lymphoma
1	0	1	Lymphoma/osteomyelitis

n = number of patients, M = male, F = female.

DISCUSSION

Radionuclide bone scan using ^{99m}Tc labeled phosphonate compounds depends upon bone blood flow and osteoblastic activity. Bone reacts to any traumatic or other insult by making new bone. Since the bone scan is very effective in detecting osteogenesis, it provides a sensitive tool for the detection of any insult to bone. The specificity of the test, however, is lower, since any insult to bone, including trauma, infection, vascular injury, or metabolic injury, may appear similarly on the bone scan.[2]

Thus, more specific methods for detecting inflammatory sites of infection should be developed.

Unlike from ^{67}Ga, ^{111}In and ^{99m}Tc WBC accumulate at the site of infection or inflammation because these cells migrate to those regions. Its accumulation does not depend on osteogenesis, but only on the migration of WBC.

An inexpensive and reliable means of labeling WBC with ^{99m}Tc is desirable, as this radionuclide is inexpensive, readily available and has favourable imaging and radiation dosimetry characteristics.

Several authors have reported different procedures for labeling leukocytes with ^{99m}Tc. However, they provided neither sufficiently high labeling yields nor satisfactory label stability. Furthermore, most of them use more steps and unnecessary manipulations.[17]

Inflammation is generally divided in two forms: acute and chronic. Acute inflammation is relatively short in duration, lasting from a few hours to 3 to 4 days. Chronic inflammation generally lasts much longer. The cell type present in inflammation varies according to the age of the inflammation. In most types of acute inflammation, infiltration of neutrophils within the first 6 to 24 hours predominates. However, in infection produced by certain organisms, neutrophils may predominate for 2 to 4 days. Neutrophils contain chemotactic factors for monocytes which begin to appear 24 to 48 hours later. In viral infections, monocytes may be the first cells to arrive.[18]

Chronic inflammation mostly consists of mononuclear cells, primarily macrophages, lymphocytes and plasma cells. In the lesions of osteomyelitis, neutrophils can persist for many months, but mononuclear cells may be predominant.[18]

^{99m}Tc-HMPAO is currently more expensive per patient

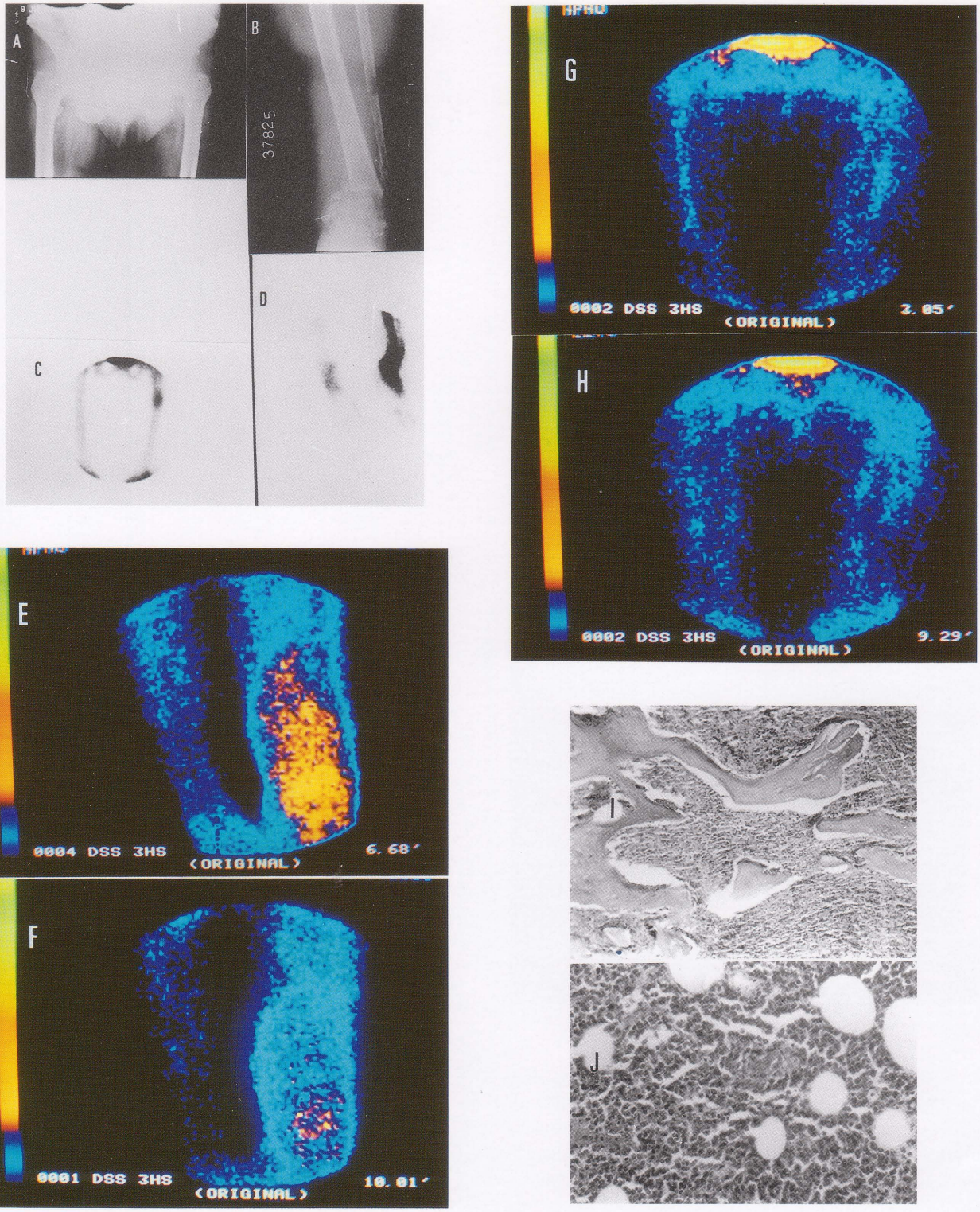
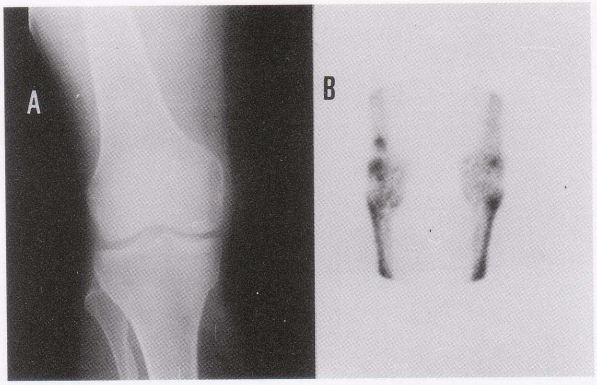
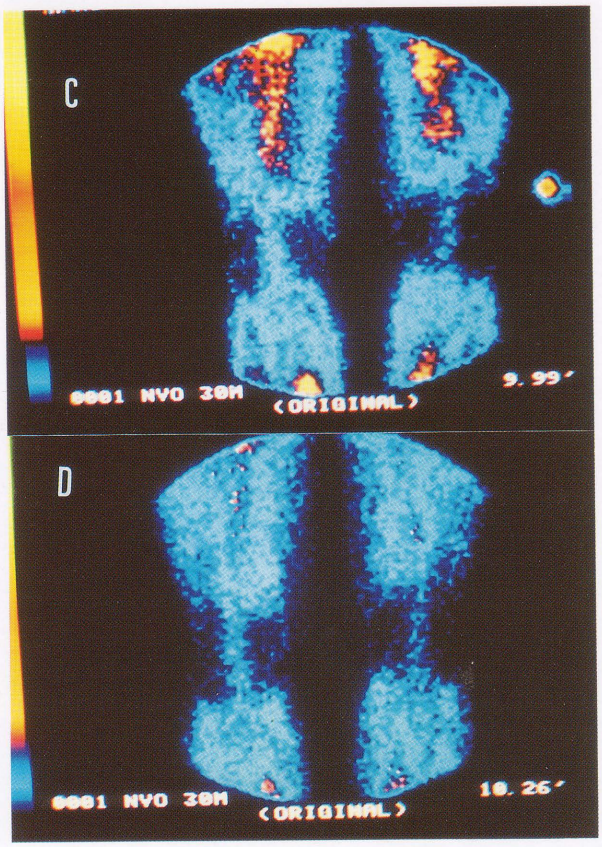


Fig. 1 A case report using ^{99m}Tc-WBC: comparison study.
(A) normal radiographs in left femur and (B) lytic lesions and pathological fracture in left ankle. (C) ^{99m}Tc-MDP shows abnormal uptake in femur and (D) and in distal left tibia and ankle. Scannings obtained with WBC labeled with HMPAO (E and G) and SnTec (LETC) (F and H). Images obtained with 3 hours after injection show an intensive abnormal uptake involving all the left distal leg. No abnormal femural uptake is found (G and H). (I) Histo-pathological study of left ankle confirms osteomyelitis and (J) Histo-pathological study of left femur confirms lymphoma.





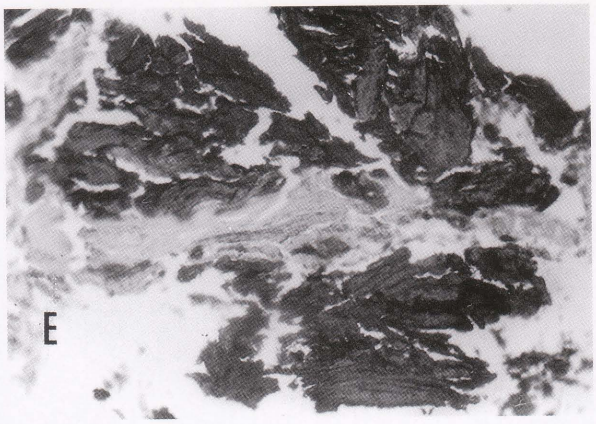


Fig. 2 A case report of a patient with bone infarct. (A) X-ray suggest a bone infarct involving the distal left femur. (B) ^{99m}Tc-MDP shows abnormal uptake at same area of X-ray findings. (C and D) No abnormal activity was found on the 30 minutes. (E) Histo-pathological study confirmed bone infarct.

dose than ^{111}In and is not easily obtainable in some countries, e.g Brazil; WBC labeling with HMPAO requires more blood and slightly more complex cell-separation techniques, including the use of Hespan, forbidden in some countries.[10] It is reported that ^{99m}Tc-HMPAO shows signs of *in vivo* instability and marker excretion of the free label into the renal and gastrointestinal tracts[19,20] which can reduce diagnostic specificity. False-positive cases could be accounted for by the uptake of labeled platelets which are present in the mixed WBC preparation.[21] In addition, it is not able to label the mononuclear fraction of WBC.[8,9]

Although ^{99m}Tc-HMPAO has disadvantages, it is an acceptable method for WBC labeling. Comparative studies with ^{99m}Tc-HMPAO and our technique (SnTec) show the same results.

In our study, the sensitivity of the technique developed by us (SnTec) and HMPAO was 87% and specificity for both methods was 100%, in 25 cases.

The advantages and originality of our method for labeling leukocytes with ^{99m}Tc (SnTec) are: (i) the amount of Sn^{++} (6.2 μg) is less than that used by others,[22–25] (ii) the incubation temperature is a practical one (room temperature) while others prefer 37°C.[22–24] Our results show that the temperature does not interfere with labeling efficiency and/or stability (iii) the incubation time is minimal (10 minutes with $SnCl_2 \cdot 2H_2O$ plus 10 minutes with ^{99m}Tc, while others require up to 15 minutes for each incubation)[22,25] (iv) SnTec requires few steps and manipulations while other authors use unnecessary intermediate steps in order to wash the cells[22,25] (v) although stannous glucoheptonate (SnGH) is inexpensive, SnTec requires only stannous chloride that is cheaper and (vi) most of the results of similar SnTec techniques reported show only *in vitro* and/or animal studies.[22–25]

The analysis of various factors necessary for the labeling of WBC with ^{99m}Tc shows that the technique developed by us contributes to the elucidation of undiagnosed cases of infection. It is inexpensive and requires few manipulations and only small amounts of blood.

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