

## Technetium-99m HMPAO labeled leukocytes in inflammation imaging

Kimiichi UNO,\* Kyousan YOSHIKAWA,\* Keiko IMAZEKI,\*  
Satoshi MINOSHIMA\* and Noboru ARIMIZU\*

*\*Department of Radiology, Chiba University School of Medicine*

Technetium-99m-HMPAO (Tc-99m-HMPAO) labeled leukocyte imaging was carried out in 19 patients at 3-5 hr after reinjection. There were no side effects noted. Tc-99m leukocyte images showed gall bladder, colon, kidney, and urinary bladder activity in normal distribution as a result of excretion of the eluted Tc-99m complex. They yielded a sensitivity of 93%, a specificity of 100% and an accuracy of 95%. They were correctly positive in 14 out of 19 cases. But one false negative case was seen in a patient with pyonephrosis showing a lack of renal function with decreased renal blood flow. It was concluded that they have some advantages over In-111 leukocyte images, but we have to consider the fact that the ureteral obstruction or the lack of renal function with decreased renal blood flow may result in a false positive or a false negative case.

**Key words:** Tc-99m-HMPAO, labeled leukocytes, inflammation

### INTRODUCTION

In-111 labeled leukocyte imaging has evolved into a practical and highly accurate method for the detection of infectious and inflammatory processes.<sup>1-7</sup> In-111 leukocyte labeling may result in cell damage that is primarily radiation-induced.<sup>8</sup> This has led to the need for the labeling of leukocytes with Tc-99m. Tc-99m is an optimal labeling agent since it has ideal physical properties, is readily available and its cost is low. Although attempts to prepare Tc-99m labeled leukocytes have been performed for more than ten years, no satisfactory agent was found. Tc-99m-d,l-hexa-methylpro-pyleneamine oxime (HMPAO) was introduced as a new agent for brain imaging.<sup>9</sup> This compound is lipophilic and it was reported that it might be able to label leukocytes and used be clinically.<sup>10-14</sup> This study was undertaken to evaluate leukocyte labeling with Tc-99m-HMPAO for inflammatory imaging.

### MATERIALS AND METHODS

Nineteen patients including 12 men and 7 women, whose ages ranged from 19 to 82 years, were studied and three of them had the study performed twice (Table 1).

The indications for scanning these patients were fever, suspected post-operative infection or abscess, or follow up of known infection. Tc-99m-HMPAO was prepared by adding 1.11 GBq Tc-99m in 5 ml of isotonic saline to a vial containing a freeze-dried mixture of 0.5 mg d,l-HMPAO, 7.6 g stannous chloride dihydrate and 4.5 mg sodium chloride under nitrogen from Amersham Int. plc. Tc-99m complex was used for cell labeling within 30 min of reconstitution. The percentage of the lipophilic complex was calculated by three chromatographic systems. Forty milliliters of heparinized whole blood was obtained from each patient. The red blood cells were allowed to sediment for 0.5 to 1 hour. The resultant leukocyte-rich plasma was removed and centrifuged at 450 G for 5 min. The leukocyte pellet was washed twice in saline, resuspended and incubated with Tc-99m-HMPAO for 10 min at room temperature. Then the labeled cells were washed twice in saline. Fifty to 230 million leukocytes without plasma

Received April 1, 1991, revision accepted May 20, 1991.

For reprints contact: Kimiichi Uno, Department of Radiology, Chiba University School of Medicine, 1-8-1 Inohana, Chiba-city, 280 JAPAN.

labeled with 400 MBq of Tc-99m-HMPAO were reinjected intravenously. Dynamic images, whole-body images and spot views were obtained at 3 to 5 hrs after i.v. injection with a scintillation camera using equipped with a high resolution collimator and a 20% window centered at the 140 keV gamma photon peak.

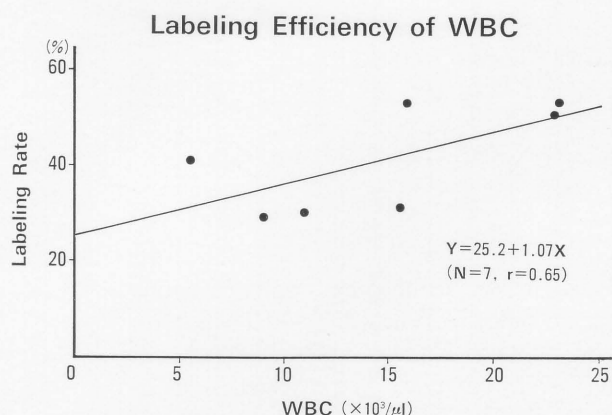


Fig. 1 Effect of cell concentration on labeling efficiency of mixed leukocytes with Tc-99m-HMPAO.

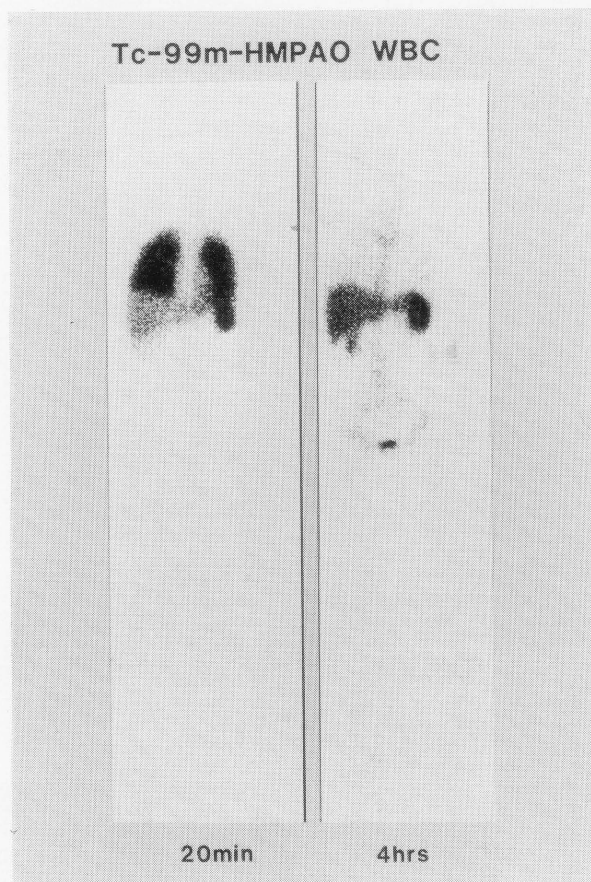


Fig. 2 Normal leukocytes distribution without plasma at 20 min and 4 hr after reinjection. At 20 min, lung, liver, spleen and faint bone marrow uptakes were seen. At 4 hr, lung activity was decreased and bone marrow uptake increased.

## RESULTS

The percentages of the primary lipophilic complex, the secondary lipophilic complex, the reduced hydrolysed technetium, and free pertechnetate were 85.2% (3.0 S.D.), 10.1% (3.4 S.D.), 3.0% (0.9 S.D.) and 1.2% (1.1 S.D.) by three chromatographic systems, respectively ( $n=9$ ). The mean labeling efficiency was 41% (29–53%) and there was a correlation between the labeling rate and leukocyte counts with  $r=0.65$  ( $n=7$ ) (Fig. 1).

After the injection of Tc-HMPAO leukocytes no side effects were noted. Normal leukocyte distribution without plasma at 20 minutes after injection was seen in the lungs, liver, spleen and faintly in the bone marrow. At 4 hours, lung activity was decreased and bone marrow uptake was further increased. The gall bladder and urinary bladder were also seen in whole body images (Fig. 2). The kidneys were faintly seen in spot images. Faint bowel activity was seen in some patients. The findings in our series of patients are summarized in Table 1. Tc-99m-HMPAO leukocyte scans were positive in 14 of 15 confirmed sites in inflammatory or infectious disease, representing a sensitivity of 93%, a specificity of 100% and an accuracy of 95% (Fig. 3, 4). One false negative was seen in a patient (#3) with pyonephrosis proved by US guided nephrostomy. The lack of renal function and the decreased renal blood flow resulted in the absence of uptake in the left kidney. All patients with negative Tc-99m leukocytescan were confirmed by bacteriologic culture, laboratory tests, X-ray procedures and US in combination with clinically relevant signs and long-term follow up.

## DISCUSSION

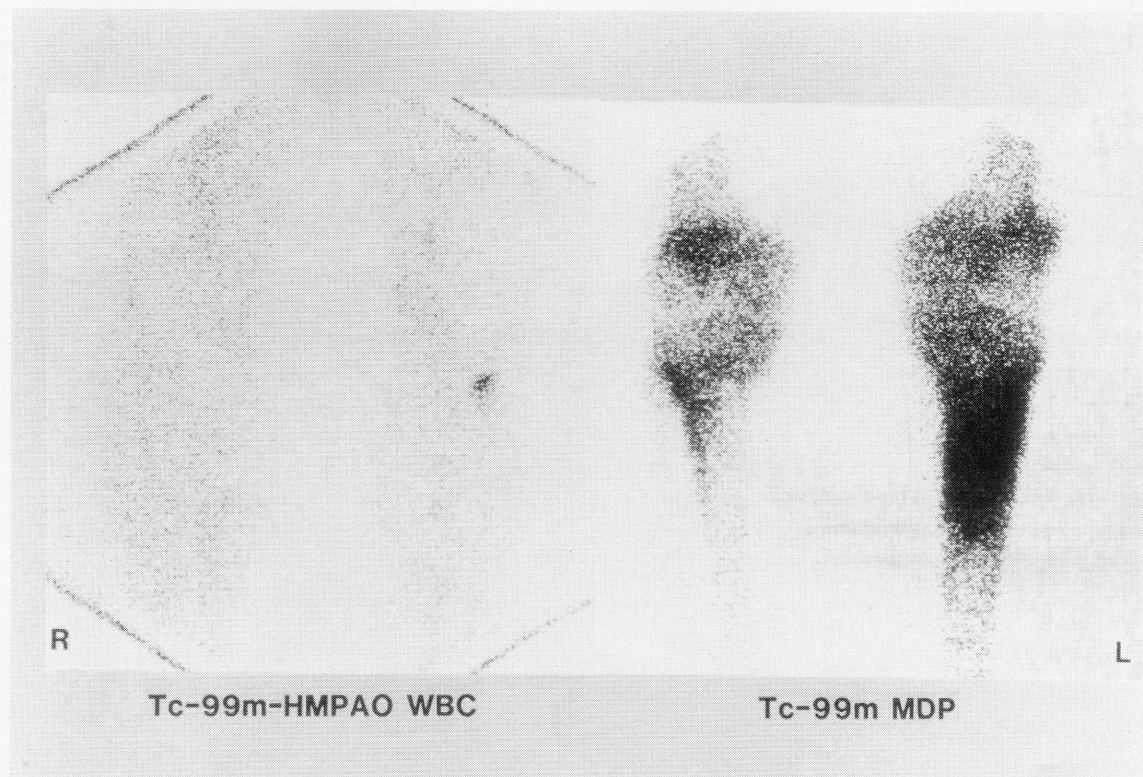
The results of this study indicate that we could demonstrate inflammatory or infected sites with Tc-99m-HMPAO labeled leukocyte imaging. A Tc-99m agent which irreversibly labels leukocytes without affecting their migration would be preferable to the In-111 lipophilic chelates now in use, because of the superior physical characteristics of Tc-99m for imaging and radiation doses received by the target organs.<sup>15,16</sup> Our average labeling efficiency of 41% corresponds well with other reports.<sup>10,15,17–20</sup> But some reports have shown higher labeling efficiency than this.<sup>14,21–23</sup> These differences may be related to the number of cells used, the pH, the presence of plasma, the HMPAO concentration, reconstitution volume, the incubation time, the radiochemical purity of Tc-99m-HMPAO, and the time of labeling. Our labeling method mostly covered these conditions except for shorter incubation time and slight lower radiochemical purity of Tc-99m-HMPAO. We will

**Table 1** Summary of patients with suspected infection or sites of inflammation

Scan No.	Patients No.	Sex/Age	Fever	WBC	CRP	Tc-99m WBC scan	Diagnosis
1	1	F/66	H	12800	6+	++	Abscess
2			H	7800	5+	++	Abscess
3	2	M/40	M	5400	1+	+	Infection
4			—	6700	—	—	Follow-up
5	3	M/26	H	7200	6+	—	Abscess
6			H	6000	6+	—	Abscess
7	4	M/82	M	6700	—	+	Infection
8	5	M/19	H	15900	6+	++	Infection
9	6	M/79	H	12200	6+	++	Infection
10	7	F/60	H	10590	6+	++	Cholangitis
11	8	M/62	M	15100	6+	++	Epididymitis
12	9	F/58	H	17200	6+	++	Abscess
13	10	M/62	H	12500	6+	++	Abscess
14	11	M/77	M	9300	3+	+	Infection
15	12	F/57	M	5400	1+	+	Infection
16	13	M/39	M	7800	1+	+	Sinusitis
17	14	M/48	M	8900	1+	+	Infection
18	15	F/45	M	3300	6+	—	Follow-up
19	16	F/73	M	4400	—	—	Follow-up
20	17	F/68	M	6800	—	—	Follow-up
21	18	M/50	M	6700	—	—	Follow-up
22	19	M/74	M	7900	—	—	Follow-up

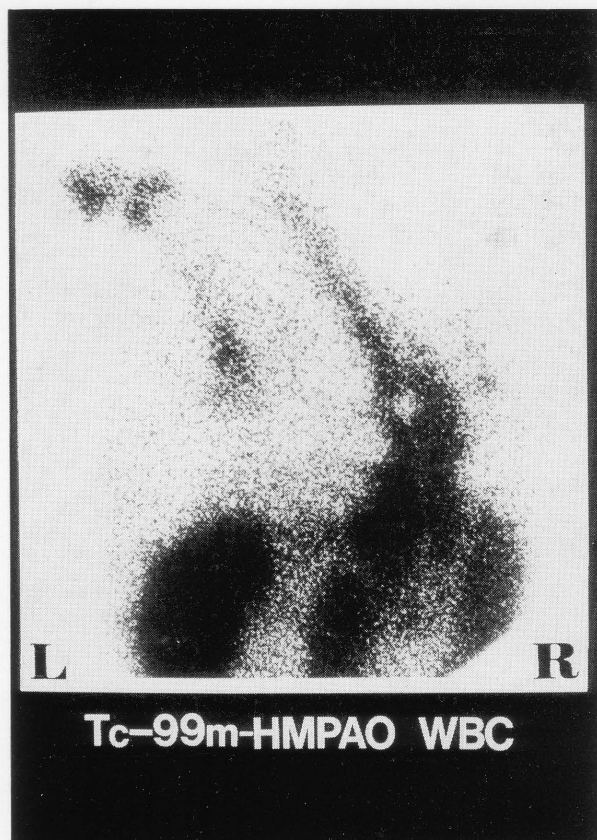
Fever as: H  $\geq 37^{\circ}\text{C}$ ; M  $< 37^{\circ}\text{C}$

Tc-99m WBC scan as: ++  $\geq$  liver; +  $<$  liver



**Fig. 3** A 77 year old male (patient #11) had a recurrence of chronic osteomyelitis in the region of the left proximal tibia that was associated with an old injury. Bone scan on the right side showed a diffuse increased uptake in that lesion. Tc-99m leukocyte scan on the left side showed a focal area of increased uptake that discharged pus. At surgery, the bone scan abnormality was not the result of abscess formation.





**Fig. 4** A 19 year old male (patient #5) with instrumentation surgery for kyphoscoliosis caused by neurofibromatosis showed a remarkable uptake of Tc-99m leukocytes parallel to the thoracic spine. Fistula formation was confirmed by second surgery.

need to change these conditions in future studies, if needed.

The scintigraphic images are as good as or better than those obtained with In-111 labeled leukocyte. But the interpretation of images with Tc-99m leukocytes is not easier than in the case of In-111 leukocytes, because the gall bladder, bowel, kidneys and urinary bladder are visualized normally. In the case of bowel disease, the early timing of the images and the intensity of the tracer uptake can be helpful in the final diagnosis.<sup>10</sup> In our experience, renal visualization gives false positives or negatives depending on the renal function. The former is caused by the ureteral obstruction and the latter is caused by the lack of renal function and the decreased renal blood flow.<sup>13</sup> Indium-111 leukocytes can be more helpful in this situation, because there is no excretion from the kidneys. Important advantages of the Tc-99m labeled leukocytes are the lower radiation doses and superior image quality, and the Tc-99m and HMPAO kit are readily available even for emergency use.

## ACKNOWLEDGEMENTS

The authors thank Dr. L. Krishna, Department of Radiation Oncology and Nuclear Medicine, Hahnemann University, for her valuable advice in the preparation of this manuscript. HMPAO was supplied for this study by Amersham International plc Japan, and their support is gratefully acknowledged.

## REFERENCES

1. Thakur ML, Lavender JP, Arnot RN, et al: Indium-111 labeled autologous leukocytes in man. *J Nucl Med* 18: 1014-1021, 1977
2. Uno K: Basic and clinical study of In-111-oxine labeled leukocytes for detection of inflammatory foci. *Nippon Act Radiol* 42: 565-575, 1982
3. Uno K, Yamaura A, Uchiyama G, et al: Evaluation of In-111-leukocyte imaging in patients with intracranial lesions. In *Radiolabeled Cellular Blood Elements* Thakur M, (ed.), New York, Plenum Press, pp 417-418, 1985
4. Uno K, Matsui N, Nohira K, et al: Indium-111 leukocyte imaging in patient with rheumatoid arthritis. *J Nucl Med* 27: 339-344, 1986
5. Uno K, Imazeki K, Arimizu N, et al: The complementary role of Indium-111 labeled leukocyte imaging, ultrasonography and computed tomography in the evaluation of postoperative infection or abscess. *Ann Nucl Med* 1: 27-31, 1987
6. Uno K, Imazeki K, Arimizu N, et al: Indium-111 labeled leukocytes for an inflammatory scintigraphy. *Jpn J Clin Radiol* 33: 249-255, 1988
7. Terauchi T, Uno K, Yuyama T, et al: Clinical usage of Indium-111 labeled leukocyte scintigraphy and Technetium-99m Methylene Diphosphonate scintigraphy in patients with total hip replacement. *Kaku Igaku* 25: 1103-1109, 1988
8. Segal AW, Deteix P, Gareia R, et al: Indium-111 labeling of leukocytes: A detrimental effect on neutrophil and lymphocyte function and an improved method of cell labeling. *J Nucl Med* 19: 1238-1244, 1978
9. Ell PJ, Hocknell JML, Jarritt PH, et al: A technetium-99m labelled radiotracer for the investigation of cerebral vascular disease. *Nucl Med Common* 6: 437-441, 1985
10. Peters AM, Danpure HJ, Osman S, et al: Preliminary clinical experience with Tc-99m-HM-PAO for labelling leukocytes and imaging infection. *Lancet* 2: 946-949, 1986
11. Danpure HJ, Osman S, Carroll MJ, et al: *In vitro* studies to develop a clinical protocol for radio-labeling mixed leukocytes with Tc-99m HMPAO. *J Nucl Med* 28: 694, 1987
12. Roddie ME, Peters AM, Henderson BL, et al: Imaging inflammation with Tc-99m HMPAO labelled with cells. *J Nucl Med* 28: 648, 1987
13. Uno K, Imazeki K, Yoshikawa K, et al: Clinical use of technetium-99m HMPAO labeled leukocytes for inflammatory imaging. *J Nucl Med* 28: 648, 1987

14. Rao SA, Aksut G, Trembath LA, et al: Evaluation of Tc-99m HMPAO for leukocyte labeling. *J Nucl Med* 28: 638, 1987
15. Roddie ME, Peters AM, Danpure HJ, et al: Imaging inflammation with Tc-99m hexamethyl propylene amine oxime (HM-PAO). *Radiol* 166: 767-772, 1988
16. McAfee JG, Subramanian G, Gagne G, et al: Tc-99m-HM-PAO for labeling experimental comparison with In-111-oxine in dogs. *Eur J Nucl Med* 13: 353-357, 1987
17. Danpure HJ, Osman S, Carroll MJ. The development of a clinical protocol for the radiolabelling of mixed leukocytes with Tc-99m hexamethylpropyleneamine oxime. *Nucl Med Commun* 9: 465-475, 1988
18. Solanki KK, Mather SJ, Janabi MAL, et al: A rapid method for the preparation of Tc-99m hexametzime labelled leukocytes. *Nucl Med Commun* 9: 753-761, 1988
19. Vorne M, Soini I, Lantto T, et al: Technetium-99m HM-PAO-labeled leukocytes in detection of inflammatory lesions: Comparison with Gallium-67 citrate. *J Nucl Med* 30: 1332-1336, 1989
20. Mountford PJ, Kettle AG, O'Doherty MJ, et al: Comparison of Tc-99m-HM-PAO leukocytes with Indium-111-oxine leukocytes for localizing intra-abdominal sepsis. *J Nucl Med* 31: 311-315, 1990
21. Mock BH, Schauwecker DS, English D, et al: *In vivo* kinetics of canine leukocytes labeled with technetium-99m HM-PAO and Indium-111 tropolonate. *J Nucl Med* 29: 1246-1451, 1988
22. Mortelmans L, Malbrain S, Stuyck J, et al: *In vitro* and *in vivo* evaluation of granulocyte labeling with Tc-99m d,l-HM-PAO. *J Nucl Med* 30: 2022-2028, 1989
23. Ecclestone M, Proulx A, Ballinger JR, et al: *In vitro* comparison of HMPAO and gentisic acid for labelling leukocytes with Tc-99m. *Eur J Nucl Med* 16: 299-302, 1990