Localization of Indium-111 Leukocytes in Noninfected Neoplasms

Lamk M. Lamki, Leela P. Kasi, and Thomas P. Haynie

Department of Nuclear Medicine, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Indium-111-labeled autologous leukocyte studies in general carry a high sensitivity, specificity, and accuracy for the investigation of infections and abscesses. However, past studies have described sporadic cases in which ^{111}In leukocytes localized in tumors. Our experience using ^{111}In leukocytes for the investigation of fever of unknown origin in cancer patients, however, indicates a relatively high incidence of ^{111}In leukocyte localization in noninfected neoplasms. Out of the 61 patients studied for fever of unknown origin, 21 patients (34%) manifested abnormal localization of ^{111}In leukocytes in neoplasms without clinical evidence of infection. These included patients with abnormal localization in: (a) lymph nodes, (b) soft-tissue tumors, and (c) bone neoplasms. The tumors included both primary and secondary lesions, and hematologic as well as solid tumors. The mechanism of ^{111}In leukocyte localization in tumors is still not completely explained. Interpretations of ^{111}In leukocyte studies in cancer patients with fever should take into consideration the possibility that localization may occur in neoplastic tissue per se and does not always indicate the presence of infection.

J Nucl Med 29:1921–1926, 1988

Indium-111- (^{111}In) labeled autologous leukocytes are now well accepted for the investigation of infections and abscesses (*1–4*). There have been varying reports of the sensitivity, specificity, and accuracy of ^{111}In leukocyte studies but generally levels of 88%, 90%, and 89%, respectively, have been achieved (*2*), and as high as 98%, 97%, and 98%, respectively, when the three-phase white blood cell (WBC) study was employed (*5*). Despite the high specificity and accuracy for the diagnosis of infection, there have been sporadic cases (*5–16*) in which ^{111}In leukocytes localized in tumors. In two reports that specifically examined the use of ^{111}In leukocytes in patients with tumors (*17,18*) the findings varied widely. In one report (*17*) only one out of 117 cancer patients had a positive scan, whereas in the second study (*18*) six out of 51 patients had a positive scan (12%).

For several years we have used ^{111}In leukocytes for the investigation of fever in cancer patients. Here we describe our findings, regarding the relatively high incidence of ^{111}In leukocyte localization in neoplasms, without clinical evidence of infection

Received Jan. 14, 1988; revision accepted June 16, 1988.

For reprints contact: Lamk M. Lamki, MD, UT M.D. Anderson Cancer Center, Dept. of Nuclear Medicine, 1515 Holcombe Blvd., Houston, TX 77030.

MATERIALS AND METHODS

Patients

Using autologous mixed leukocytes 61 patients with various tumors were examined at The University of Texas M.D. Anderson Hospital from June 1985 to June 1987. Thirty-three patients had hematologic malignancies and 28 patients had solid tumors. There were 27 women and 34 men, and they were from 18 to 75 yr of age. The hematologic cases included 16 patients with lymphoma, 13 with leukemia, and four with multiple myeloma. The solid tumor cases encompassed a wider variety of diagnoses, but the majority of the patients had tumors in the pelvis and abdomen, including the prostate, bladder, pancreas, and colon. Five of these patients had soft tissue sarcomas. The remaining patients in this group had various other cancers (Table 1).

Cell Labeling

Leukocytes were isolated from 40 ml of autologous, heparinized whole blood, and labeled with [^{111}In]oxine (Amersham Corporation, Arlington, Il) using a modification of the technique described earlier by Thakur (*19*). Briefly, our method was as follows: The red blood cells were allowed to sediment in an upright syringe for 75–90 min. The supernatant containing the leukocyte-rich plasma was collected and centrifuged at 400 *g* for 15 min at room temperature to obtain a leukocyte pellet. The supernatant was separated and saved to obtain platelet-poor plasma (PPP) for washing and resuspension of the labeled cells. Any remaining traces of plasma was carefully removed from the leukocyte pellet which was then resus-

TABLE 1
Diagnoses of Patients Studied and Relative Incidence of Indium-111 Leukocyte Localization in Tumors

Primary site or neoplasm type	Total patients	(Patients with localization)
† Lymphoma	16	(4)
* Leukemia	13	(1)
Myeloma	4	(2)
Prostate	4	(1)
Bladder	4	(3)
‡ Soft Tissue Sarcoma	5	(3)
Pancreas	3	(1)
Pancreas Islet Cell	1	(0)
Colon	3	(1)
Cholangiocarcinoma/Bileduct	2	(2)
Breast	1	(0)
Seminoma	1	(1)
Ocular Melanoma	1	(0)
Larynx	1	(1)
Ovary	1	(0)
Osteosarcoma	1	(1)
Total	61	(21)

*Leukemia patients included myelogenous and lymphocytic, acute and chronic.

† Lymphoma patients included both Hodgkins and non-Hodgkins.

‡ Soft-tissue sarcoma included patients with angiosarcoma, epitheloid sarcoma and malignant fibrous histocytoma.

pended in 1 ml of normal saline, and 750–800 μCi of ^{111}In-labeled oxine was added. The suspension was incubated for 30 min with frequent shaking. The ^{111}In-labeled cell suspension thus obtained was centrifuged at 400 *g* for 10 min, and the supernatant removed. The cells were washed further with PPP and resuspended in PPP for reinjection. Total cell counts and cell-viability test (Trypan blue dye exclusion test) were performed in the sample both before and after labeling. The injected activity was 500 μCi ± 10%. An aliquot of the labeled material was also tested for sterility using Bactec (Bactec Sterility tester, Model 301, Johnston Labs., Div. of Becton Dickinson, Cockeysville, MD) sterility tester. The entire labeling procedure was carried out in a laminar flow hood using aseptic techniques.

Imaging Procedure

Following reinjection of the ^{111}In leukocytes, the patients were imaged at 4 hr and then again at 24 hr. In each imaging session, a minimum of six images using a large field-of-view gamma camera were obtained, including anterior and posterior images of the chest, abdomen, and pelvis. Each view was taken for 7 min in a digital format at a matrix of 128 × 128. Extra images were obtained in specific cases as appropriate, e.g., head and neck and the extremities. When an abnormality was suspected in the upper abdomen, a subtraction scan for the liver and spleen was performed using 1 mCi of [^{99m}Tc] sulfur colloid. Equalization of the counts in the region of interest (e.g., liver or spleen) was done prior to subtraction of the radioactivity in these organs.

Indium-111 leukocyte images in all patients were correlated with other procedures performed on that patient, e.g., plain radiography, computed tomography (CT) scanning, ultrasound, and biopsy or surgical specimen, if available. No one test was used as a standard.

RESULTS

Cell Labeling

The labeling yield was consistently between 70–75%. No more than 6% of radioactivity was eluted from the labeled cells by the platelet-poor plasma wash, indicating that the total activity was cell-associated at the time of injection. The viability of the labeled cells was always >98%. The final injected dose contained ~10^8 cells labeled with 500 μCi ± 10%. All 61 preparations were found to be negative for both aerobic and anaerobic growth when tested for sterility.

Imaging Analysis

Twenty-one of the 61 patients (34%) manifested abnormal localization of ^{111}In leukocytes in their neoplasms. These can be divided into three categories: (a) nine patients who had abnormal localization of indium-111 leukocytes in lymph nodes, (b) six patients who manifested abnormal localization in soft-tissue tumors, and (c) six patients with focal increased uptake of ^{111}In leukocytes in bone neoplasms including both solid tumors and other hematologic malignancies (Table 2).

Nine patients had localization in the lymph nodes, including the patients with Hodgkin's disease (Fig. 1), non-Hodgkin's lymphoma, leukemia, and metastatic lesions from solid tumors (Fig. 2). One patient with Hodgkin's disease also had a large soft-tissue mass in the chest that showed intense uptake of ^{111}In leukocytes. Histologic confirmation of tumor in areas of uptake was available in the lymph nodes of four patients. No evidence of infection was evident on these biopsies. CT scanning revealed that two other patients had lymphadenopathy with evidence of metastasis.

Six patients had uptake of ^{111}In leukocytes in soft-tissue tumors. Three of the six were anatomically related

TABLE 2
Distribution (by Diagnosis) of Indium-111 Leukocyte Localization

No. in lymph nodes		No. in soft tissue		No. in bone lesions	
Lymphoma	3	MFH*	1	Prostate	1
Leukemia	1	Lymphoma	1	Bladder	1
Bladder	2	Epitheloid sarcoma	1	Angiosarcoma	1
Colon	1	Cholangiocarcinoma	1	Larynx	1
Seminoma	1	Bile duct cancer	1	Myeloma	2
Osteosarcoma	1	Pancreas adenoCa	1		
Total	9		6		6

*Malignant fibrous histiocytoma

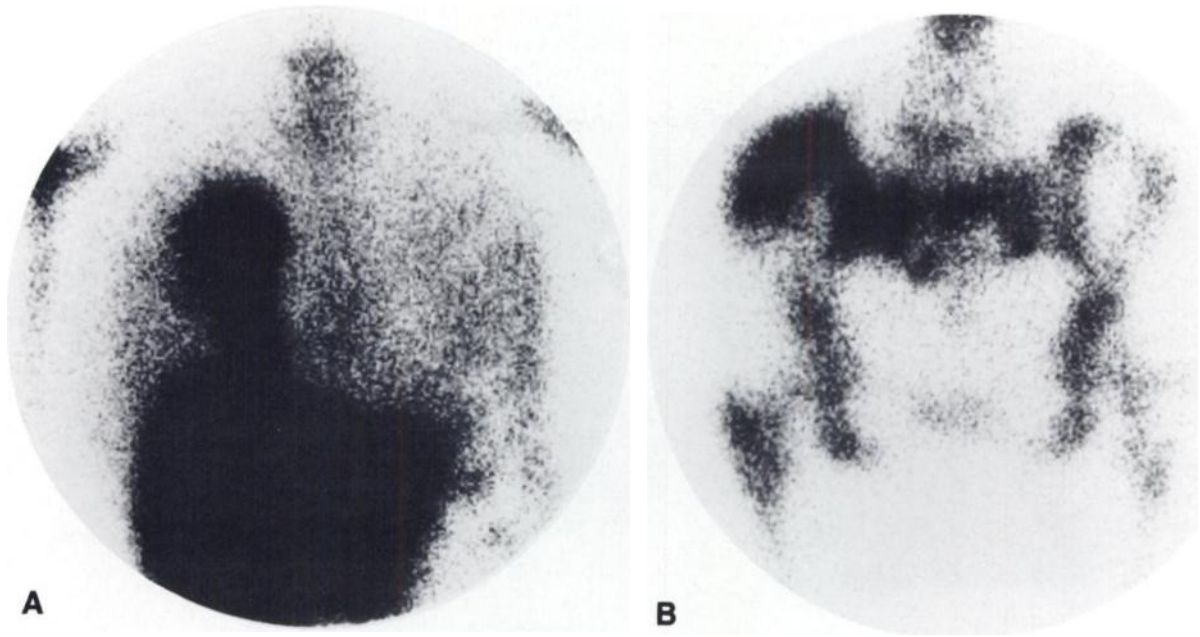

FIGURE 1
A: Anterior chest view of ^{111}In-labeled leukocyte imaging of a patient with lymphoma involving the right lung. There is intense localization of radioactivity in the right lung corresponding to a mass seen on chest x-ray (not shown). B: Posterior image of the pelvis in the same patient as 1A showing ^{111}In-labeled leukocyte localization in the bones with high concentration in the left ilium. A ^{99m}Tc methyl diphosphonate bone scan (not shown) showed an area of high concentration in the left ilium corresponding to the abnormal labeled leukocyte scan.

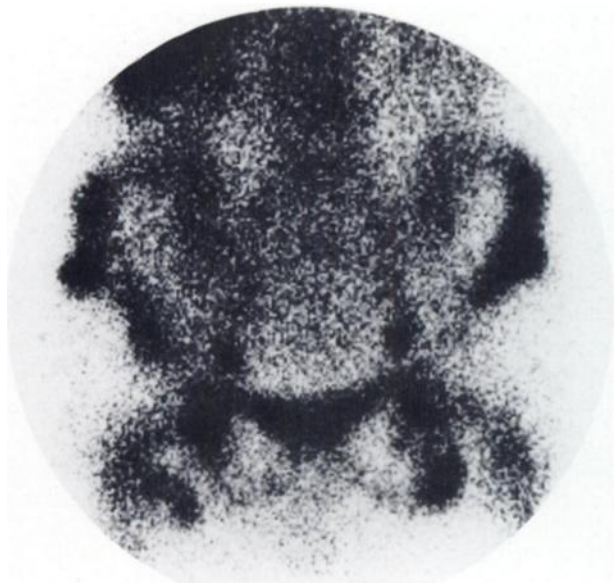

FIGURE 2
Indium-111-labeled leukocytes here localized in lymph nodes. Biopsy confirmed the presence of metastases from testicular cancer (this anterior pelvic image was taken 24 hr following injection of the label). A left anterior oblique view (not shown) clarified that the radioactivity is indeed in the lymph node in the groin.

to the liver (cholangiocarcinoma, bile duct carcinoma, and pancreatic adenocarcinoma). These patients required liver subtraction using technetium-99m sulfur colloid, to identify adequately the areas of abnormal localization. One patient had epitheloid sarcoma in the groin. Biopsy confirmed the nature of the tumor in the area of abnormal localization of ^{111}In leukocytes, but no evidence of infection. A patient with malignant fibrous histiocytoma had a tumor in the pelvis and secondary lesions in the adrenal areas, as identified in the scan.

The third category included six patients, four of whom had bony metastases (Fig. 3) from solid tumors, and two patients with lytic lesions in the bones from multiple myeloma (Fig. 4). None of these patients had evidence of bone infection.

DISCUSSION

Imaging with ^{111}In leukocytes has been favorably compared to CT, ultrasound, and ^{66}Ga scanning for detecting sites of infection or abscesses (*1–4*). Our experience suggests that a significant percentage (34%) of oncology patients may show ^{111}In leukocytes localization in solid or lymphatic tumors. In our study, all patients had malignancies and were being investigated for etiology of fever. By and large, the localization in most of our patients was obviously related to the tumor or lymph nodes and therefore did not constitute a true false-positive. The localization of ^{111}In leukocytes in tumors per se may be an explanation of the fever in some of the patients such as those with "aseptic pyrexia" or those with infection/inflammation of the tumor or necrotic tissue in the tumor.

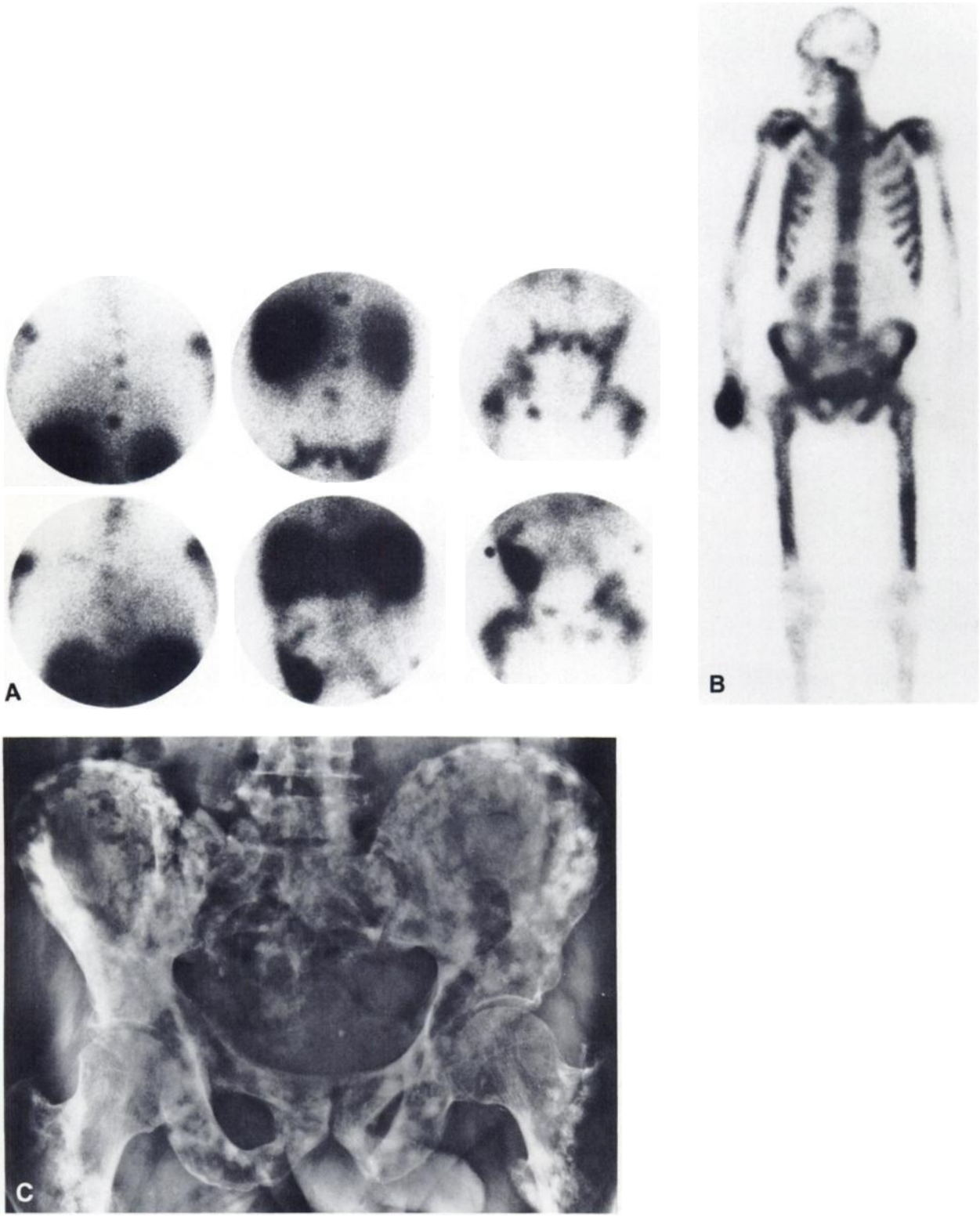

FIGURE 3
A: Multiple areas of increased uptake are noted corresponding to areas of metastatic prostatic carcinoma (Multiple spot views of ^{111}In-labeled leukocyte study taken at 24 hr following injection of the labeled cells). The top row includes posterior views of chest, abdomen and pelvis. The lower row are the corresponding anterior views. B: Technetium-99m methylene diphosphonate bone scan (anterior view) of the same patient showing diffuse metastasis. C: Pelvic radiograph of the patient showing areas of metastatic disease. However, some of the metastatic lesions are metabolically inactive, which may explain the irregular uptake of labeled cells.

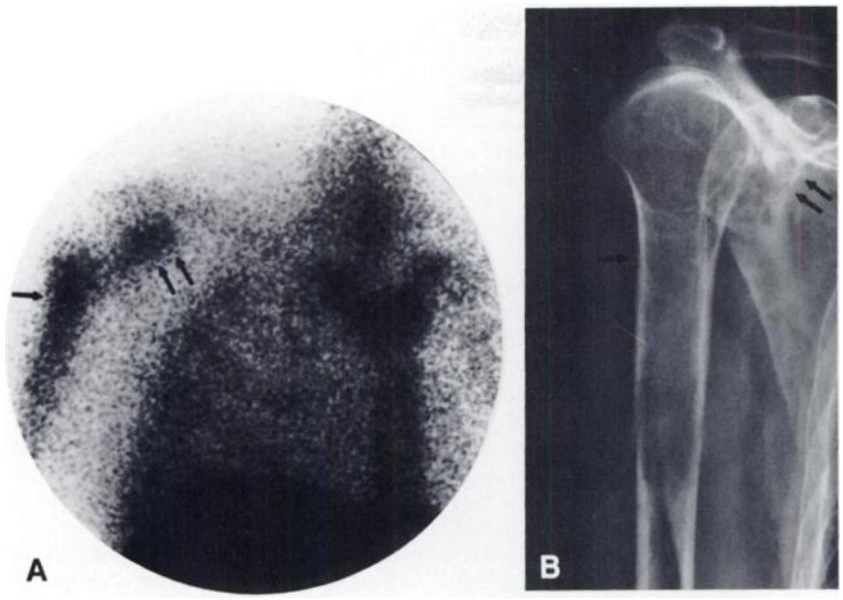

FIGURE 4
A:Indium-111-labeled leukocytes seen to localize in the right humerus neck (arrow), scapular (double arrow) and sternum in a patient with multiple myeloma. The mid shaft of the humerus is relatively spared (compare Fig. 4B radiograph) and takes up less ^{111}In leukocytes. The head of the humerus is also relatively spared and appears photo penic but without clear explanation. B: Corresponding radiograph of the same patient as Fig. 4A shows the myeloma lesions in the neck of the humerus (arrow) and scapular (double arrow) where the labeled leukocytes are localized in 4A.

Possibly, the high percentage of these patients showing indium-111 leukocyte localization in the tumors and lymph nodes is associated with the immunological activity caused by fever and associated stress. The leukocytes were perhaps playing a role in the nonmetastatic effects of malignancies, similar to the endocrinopathies, coagulopathies, and other paraneoplastic syndromes observed in some patients with solid tumors. We do not have evidence to support either one of the above possibilities, but future research may be directed towards further investigation of the factors involved.

Since mixed leukocytes were employed in this study, the labeled cells undoubtedly included lymphocytes and macrophages. It is possible that a greater proportion of these labeled mononuclear cells localized in the tumors and the lymph nodes (*20*). However, visualization of normal lymph nodes would be expected if lymphocytes formed a substantial part of the injected mixed leukocytes. Twenty-four-hour images show that ^{111}In lymphocytes normally localize in the lymph nodes (*21*). In our study, out of the 33 patients with hematologic neoplasms, we found only four patients with lymphoma or leukemia who had generalized lymph node uptake of ^{111}In leukocytes. The other five patients (of the nine with lymph node localization) (Table 2) had only sporadic focal uptake in lymph nodes rather than a whole chain of nodes. The possibility that the macrophages are responsible for the mixed leukocyte localization in tumors needs further investigation. Granulocyte enrichment of the leukocyte preparation however has not resulted in less neoplastic tissue uptake. Schmidt et al. (*22*) observed recently that ^{111}In granulocyte enriched preparation also localize in tumors of ten out of 25 patients with malignant neoplasms. The nature of the cell that localizes in these tumors is still not clear. Kwai and Kaplan (*23*) showed that when they used mixed cell preparations irradiated with 4,500 rad, they had very low incidence (2.2%) of ^{111}In-labeled cells localization in tumors. Further investigation is necessary to decipher the different variables and factors responsible for ^{111}In-labeled leukocytes localization in neoplasms. Consideration needs to be given to the cell type, the nature of the tumors, the immunologic state of the patient, and also the cell labeling procedure.

Our labeling is a modification of the procedure used by Schell-Frederick (*17*), who studied 117 cancer patients and found only one positive, in that we eliminated the additional cell washing step prior to the addition of [^{111}In]oxine. This was done in the interest of retaining higher cell viability and function in the final preparation. We did not find a significant difference in the labeling yield using either of the techniques. The washing of radiolabeled cells with autologous plasma prior to resuspension in plasma for injection removed any free ^{111}In activity, thus excluding the presence of any ^{111}In activity which is not cell bound in the injected material as the cause of the tumor uptake. We have also considered other misleading factors that are published in the literature(*24–26*) but none of them clearly explains our findings. Schmidt et al. (*22*) were able to establish a correlation between tumor granulocyte infiltration and positive ^{111}In granulocyte scintigraphy

Our results indicate that ^{111}In leukocyte scanning does not always differentiate an abscess from a malignant tumor. The mechanism of ^{111}In leukocyte localization in tumors is still not completely explained. Interpretation of ^{111}In leukocyte studies in cancer patients with fever should take into consideration the possibility that localization may occur in neoplastic tissue per se and does not always indicate the presence of infection.

REFERENCES

1. Carroll B, Silverman PM, Goodwin DA, et al. Ultrasonography and indium-111 white blood cell scanning

for the detection of intraabdominal abscesses. *Radiology* 1981; 140:155–160.
2. Seabold JE, Wilson DG, Lieberman LM, et al. Unsuspected extra-abdominal sites of infection: scintigraphic detection with indium-111 labeled leukocytes. *Radiology* 1984; 151:213–217.
3. Froelich JW, Swanson D.Imaging of inflammatory processes with labeled cells. *Semin Nucl Med* 1984; 14:128–140.
4. Marcus CS. The status of ^{111}In oxine leukocyte imaging studies. *Noninvasive Med Imag* 1984; 1:213–226.
5. Becker W, Fischbach W, Reiners C, et al. Three-phase white blood cell scan: diagnostic validity in abdominal inflammatory diseases. *J Nucl Med* 1986; 27:1109–1115.
6. McDougall IR, Baumert JE, Lantieri RL. Evaluation of Indium-111 leukocyte whole-body scanning. *Am J Roentgenol* 1979; 133:849–854.
7. McAfee JG, Samin A. Indium-111-labeled leukocytes: a review of problems in image interpretation. *Radiology* 1985; 155:221–229.
8. Rovekamp MH, van Royen EA, Reinders Folmer SCC, et al. Diagnosis of upper-abdominal infections by indium-111-labeled leukocytes with technetium-99m subtraction technique. *J Nucl Med* 1983; 24:212–216.
9. Becker W, Schaffer R, Borner W. Sigmoid carcinoma mimicking an intra-abdominal abscess in an indium-111-labeled white blood cell scan. *Eur J Nucl Med* 1985; 11:283–284.
10. Kipper MS, Basarab R, Kipper SA, et al. Positive medium-111 white cell scan in a patient with multiple metastases of the prostate. *Clin Nucl Med* 1985; 10:86–89.
11. Sfakianakis GN, Mnaymneh W, Ghandur-Mnaymneh L, et al. Positive indium-111 leukocyte scintigraphy in a skeletal metastasis. *Am J Roentgenol* 1982; 139:601–603.
12. Rehncrona S, Brismar J, Holtas S. Diagnosis of brain abscesses with indium-111-labeled leukocytes. *Neurosurgery* 1985; 16:23–26.
13. Bauman JM, Hartshorne MF, Cawthon MA, et al. False-positive leukocyte imaging in a case of colon carcinoma. *Clin Nucl Med* 1986; 11:211–212.
14. Saverymuttu SH, Maltby P, Batman P, et al. False positive localization of indium-111 granulocytes in colonic carcinoma. *Br J Radiol* 1986; 59:773–777.
15. Balachandran S, Husain MM, Adametz JR, et al. Uptake of indium-111-labeled leukocytes by brain metastasis. *Neurosurgery* 1987; 20:606–609.
16. McCarthy KE, Velchik MG. Indium-111 leukocyte uptake in a case of metastatic tumor. *Clin Nucl Med* 1986; 11:440–441.
17. Schell-Frederick E, Fruhling J, Vand Der Auwera P, et al. ^{111}In oxine labeled leukocytes in the diagnosis of localized infection in patients with neoplastic disease. *Cancer* 1984; 54:817–824.
18. Fortner A, Datz FL, Taylor A. Uptake of indium-111-labeled leukocytes by tumor. *Am J Roentgenol* 1986; 146:621–625.
19. Thakur ML, Coleman RE, Welch MJ. Indium-111-labeled leukocytes for the localization of abscesses: preparation analysis, tissue distribution, and comparison with gallium-67 citrate in dogs. *J Lab Clin Med* 1977; 89:217–228.
20. Wagstaff J, Gibson C, Thatcher N, et al. The migratory properties of ^{111}In oxine-labelled lymphocytes in patients with chronic lymphocytic leukemia. *Br J Haematol* 1981; 49:283–291.
21. Lavender JP, Goldman JM, Arnot RN, et al. Kinetics of indium-111-labelled lymphocytes in normal subjects and patients with Hodgkins's disease. *Br Med J* 1977; 2:797–799.
22. Schmidt KG, Rasmussen JW, Wedebye IM, et al. Accumulation of indium-111-labeled granulocytes in malignant tumors. *J Nucl Med* 1988; 29:479–484.
23. Kwai AH, Kaplan WD. "False positive" indium-111 (In-111) white blood cell (WBC) imaging in patients with malignancy: the Dana-Farber Cancer Institute Experience. *J Nucl Med* 1987; 28:562.
24. Goodgold HM, Samuels LD. Misleading findings on indium-111 leukocyte images. *Clin Nucl Med* 1986; 11:392–395.
25. Loken MK, Clay ME, Carpenter RT, et al. Clinical use of indium-111-labeled blood products. *Clin Nucl Med* 1985; 10:902– 911.
26. Carrier L, Picard D, Chartrand R, et al. Abdominal patterns of indium-111-labeled leukocyte imaging. *Clin Nucl Med* 1986; 11:153–157.