Comparison of Leukocytes Labeled with Indium-111-2-Mercaptopyridine-*N*-Oxide and Indium-111 Oxine for Abscess Detection

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Indium-111 leukocyte scanning has evolved into a practical and highly accurate method for the identification of infectious and inflammatory processes. The most commonly used agent for labeling leukocytes has been [^{111}In]oxine. We have investigated a newer agent, 2-mercaptopyridine-*N*-oxide (Merc) at our institution which unlike oxine, allows us to label leukocytes in plasma, using a simple kit procedure. Of the 92 consecutive patients referred for detection or localization of an infectious process, autologous leukocytes of 55 patients were labeled with [^{111}In]Merc, while those of the remaining 37 patients were labeled with [^{111}In]oxine. The sensitivities for Merc and oxine procedures were 87% and 92%, respectively, while the respective specificities were 100% and 92%. We conclude that the [^{111}In]Merc-labeled leukocytes are equally effective as [^{111}In]oxine-labeled leukocytes in detecting infectious processes. The use of [^{111}In]Merc is advantageous over [^{111}In]oxine for white blood cell labeling because of its easier preparation.

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Over the past few years, indium-111 (^{111}In) oxine-labeled leukocytes have been used extensively for the detection and localization of infectious and inflammatory processes in humans (*1*). Labeling with oxine requires that the white blood cells be removed and washed free from plasma because of the high affinity of ^{111}In to plasma transferrin. The removal of plasma increases cell manipulation and decreases the cell protection and viability. Labeling leukocytes in plasma is therefore desirable (*2*). We have developed a new agent namely indium-111-2-mercaptopyridine-*N*-oxide (Merc) that allows us to label leukocytes in plasma by a kit procedure (*3*). Since the plasma is not removed during preparation, the procedure requires fewer steps as compared to [^{111}In]oxine cell labeling and preserves cell viability. This study was undertaken in order to compare the results of leukocyte scanning using autologous leukocytes labeled with [^{111}In]Merc and [^{111}In] oxine.

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MATERIALS AND METHODS

Cell Separation and Labeling Technique

Indium-111 Merc leukocytes were prepared using the method of Thakur et al. (*3*). Approximately 30 ml of whole blood were collected in a syringe containing 300 IU heparin. This was placed in a laminar flow hood for 1 hr, to allow the erythrocytes to sediment and the leukocytes to concentrate in the supernatant plasma. The supernatant was separated, centrifuged at 450 *g* for 5 min and the leukocyte button was suspended in 0.5 ml of plasma. These were then incubated for 5 min at 22°C with 20 μg of Na-Merc (1 mg/ml) in 0.05*M* phosphate buffer at pH 7.4 and transferred to a sterile test tube containing 0.5 mCi ^{111}In* in 0.25*M* citrate buffer, pH 6.0. After incubation for 20 min at room temperature, cells were centrifuged and supernatant removed. The cell button was then washed with 0.5 ml of plasma and suspended in 4 ml of fresh plasma. The activity within the supernatant and cell suspension was measured in a calibrated ionization chamber and the labeling efficiency was calculated. The labeled leukocytes were then reinjected into the patient. The average binding efficiency was 62 ± 13%.

Indium-111 oxine leukocytes were also prepared by the method of Thakur (*4*). In this preparation, autologous leukocytes from heparinized blood were isolated using the sedimentation technique similar to that used in the Merc labeling procedure except that cells were washed free of plasma with isotonic phosphate buffered saline (PBS) pH 7.4 and were

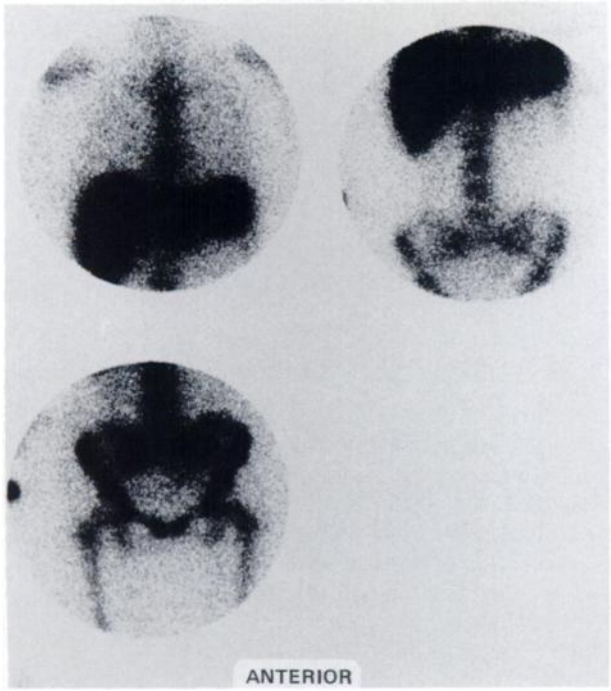


FIGURE 1
Normal [^{111}In]Merc leukocyte scan.

resuspended in 4 ml of PBS. Cells were then incubated with ~500 μCi of [^{111}In]oxine† for 15 min (22°), centrifuged (450 *g* × 5 min), and radioactivity associated with the cells and supernatant was measured. The average cell-labeling efficiency was 85 ± 11%. Labeled cells were resuspended in 4 ml of autologous plasma for injection.

Imaging Technique

All patients were scanned 18 to 24 hr after injection of the labeled leukocytes and five of 92 patients were also imaged at 4 hr following injection. Images were obtained using a LFOV camera with a medium-energy collimator, at a minimum of 200,000 counts per view. Twenty percent windows were set on the 174 keV and 247 keV photopeaks of ^{111}In. Routinely, images of the neck, chest, abdomen, and pelvis were obtained (Fig. 1), and if clinically warranted, the extremities were imaged as well.

Patient Population

A total of 92 consecutive patients (51 males, 41 females) suspected of harboring an occult infectious process were evaluated. Their ages ranged from 3 to 81 yr. The Merc compound was used in 55 patients, while oxine was employed in the remaining 37. Scan interpretation was done without knowledge of the type of agent used for leukocyte labeling.

In each case, the diagnosis was confirmed retrospectively by review of each patient's chart or medical record, with regard to subsequent clinical course, correlation with ultrasound, CT scanning, or other radiological studies. In addition, surgical, pathological, or autopsy reports were evaluated when available. A definitive diagnosis (i.e., infectious condition versus no infectious condition) was established in 84 of the 92 patients referred. The remaining eight cases were considered indeterminate because follow-up was not possible.

RESULTS

Of the 55 patients in whom the Merc compound was employed, 20 demonstrated positive scans and were subsequently proven to have an infectious process (Figs. 2–4). There were 29 true negative cases, as well as three false-negative examinations. The latter included chronic osteomyelitis, pneumocystic pneumonia, and chronic pyelonephritis. There were no false-positive scans. Three of the cases were classified as indeterminate because of inadequate follow-up. Four patients were scanned both at 4 and 24 hr after injection. Two of these patients had true-positive scans, demonstrating pyelonephritis. Abnormal renal uptake was present on both the 4-hr and 24-hr images, but more apparent on the latter. The other two patients both had true-negative examinations.

Oxine-labeled leukocytes were utilized in 37 patients. Of these, an infectious process was demonstrated in 12 of 13 patients. One patient with a negative scan was found to have a lesser sac abscess, proven at surgery. This patient was scanned at 4 and 24 hr after injection, and both scans were false-negative. There were 17 true-negative cases and two false-positive cases. One of the false-positive cases was most probably due to early heterotopic bone formation in a quadriplegic patient. The other false-positive scan demonstrated increased activity in the nasopharyngeal area. However, otorhinolaryngoscopic examination of the patient was unremarkable. Five cases were indeterminate, again because of lack of follow-up.

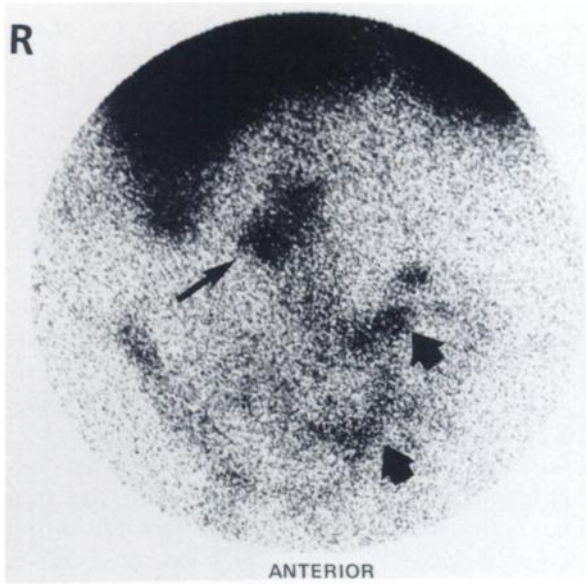
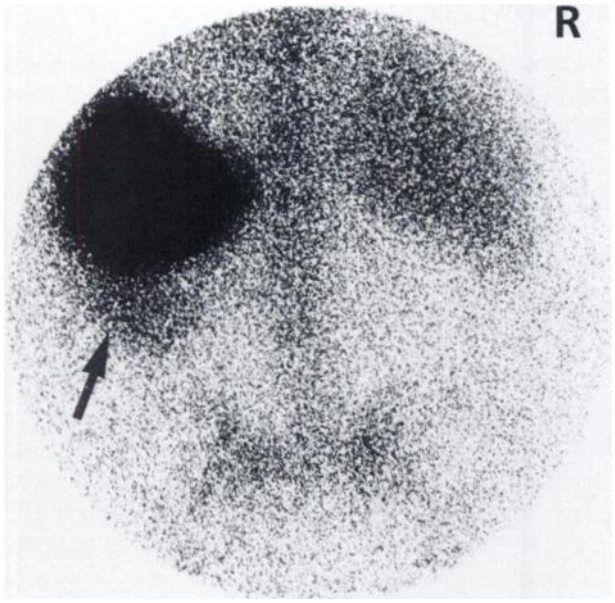


FIGURE 2
42-yr-old man with abdominal pain and *E. coli* sepsis, whose [^{111}In]Merc leukocyte scan demonstrates activity within the lower mid abdomen (arrow) and pelvis (arrowhead), proven at surgery to represent an abdominal and pelvic abscess, respectively.



POSTERIOR

FIGURE 3
69-yr-old woman complaining of fever, left flank pain, and dysuria. [^{111}In]Merc leukocyte scan demonstrates abnormal activity in left kidney (arrow). At surgery, an inflamed left kidney was removed. Final diagnosis: chronic calculous pyelonephritis.

A statistical comparison of the two agents is provided in Table 1. Although the cell labeling efficiency for Merc is lower than that for oxine, (62 ± 13% and 85 ± 11%, respectively), there was no significant difference in overall accuracy. Coleman et al. also observed that low white cell labeling efficiencies did not reduce sensitivity and accuracy (*5*).

DISCUSSION

Indium-111 leukocyte scintigraphy has gained wide acceptance for use in abscess and infectious process detection. Cells labeled with oxine were employed initially (*1*). The ^{111}In from the oxine chelate binds to transferrin in plasma. This necessitates the removal of plasma from the leukocytes during the labeling process. However, due to evidence that plasma protects the white blood cells, its removal is undesirable and could result in their physical or physiological impairment (*2*). Because of this, many investigators have employed other agents such as [^{111}In]acetylacetone (*6*) and tropolone (*7–10*). Some authors, however, have found tropolone to decrease the chemotactic properties of the leukocytes (*11*) and to be more toxic to the cell than oxine (*12*).

Indium-111-2-Mercaptopyridine-*N*-oxide is a new lipid-soluble compound which allows efficient labeling of leukocytes in plasma by a kit procedure (*3*). The results indicate that there is no significant difference in the sensitivity or accuracy between the two compounds ($p = 0.3$).

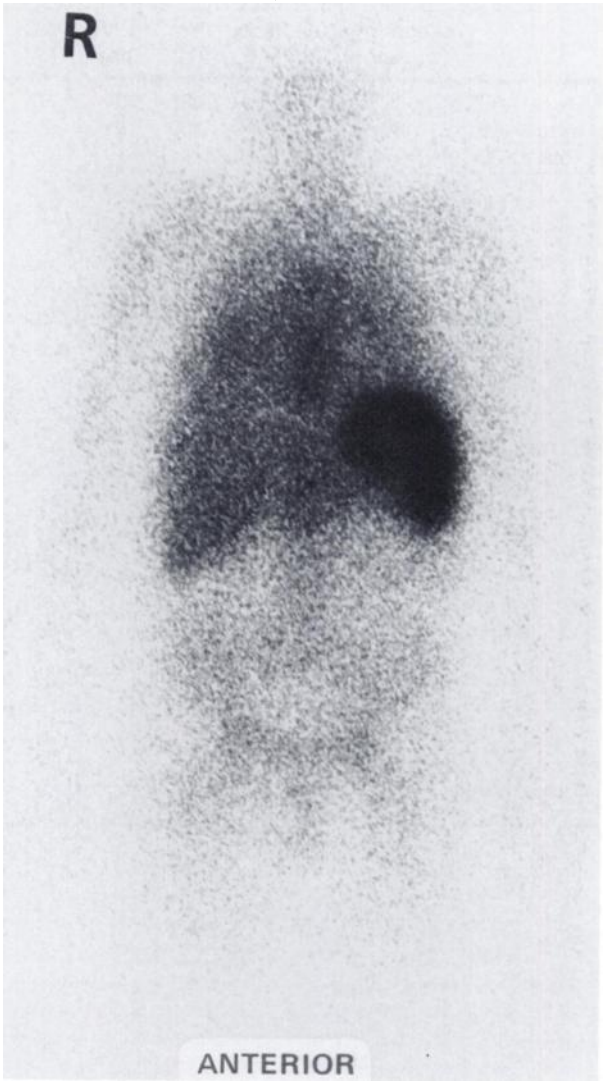


FIGURE 4
55-yr-old renal transplant recipient with fevers and persistent cough. [^{111}In]Merc leukocyte scan demonstrates bilateral lung uptake, with focally increased activity within the right mid-lung field. A biopsy specimen during thoracotomy revealed cytomegalovirus pneumonitis.

Four patients of the group studied with Merc and one patient of the oxine group were scanned at 4 hr after injection in addition to the routine image obtained on the following day. The results were similar at both time intervals with both groups.

A total of 24 patients in our series were also evaluated with ultrasound and computed tomographic (CT) scanning. Of these, four had true-positive radiolabeled leukocyte scans, but false-negative CT and ultrasound examinations. The [^{111}In]oxine leukocyte scan of one patient demonstrated abnormal activity within the abdomen, later proven at surgery to represent a mesenteric

TABLE 1
Statistical Analysis: Merc Versus Oxine-WBCs

	Sensitivity (%)	Specificity (%)	PPV* (%)	NPV† (%)	ACC‡ (%)
Oxine n = 32	92	90	86	94	91
Merc n = 52	87	100	100	91	94
Total n = 84					

* Positive predictive value.
† Negative predictive value.
‡ Accuracy.

abscess. The [^{111}In]Merc WBC scan of another patient revealed abnormal pelvic activity, also surgically proven to be an abscess. The CT scans and ultrasounds of both patients were negative. In addition, two patients presenting with fever and flank pain were referred to our department for indium abscess scanning. Scans were obtained with the Merc compound and revealed renal uptake of the radiolabeled white blood cells. One patient, a renal transplant recipient, was treated for a urinary tract infection and subsequently improved (her ultrasound and CT scan were both normal prior to therapy). The second patient's CT scan was also normal, and her ultrasound was unremarkable except for renal calculi. A laparotomy was then performed, which demonstrated an inflamed left kidney. These two cases of pyelonephritis illustrate the advantage of an imaging modality that reflects a physiological process (i.e., leukocyte localization within an infectious focus) over those that are based simply on anatomic abnormalities.

When leukocytes are labeled with ^{111}In compounds, some lymphocytes are also inadvertently labeled. Although theoretically this could result in their malignant transformation these lymphocytes are killed by the radiation exposure, and therefore there is no potential long-term risk (*13*).

In summary, there is no significant difference in the ability of Merc- and oxine-labeled [^{111}In]leukocytes to detect and localize within abscesses and other infectious processes. Due to the ease of preparation of [^{111}In]Merc-labeled leukocytes compared with that of [^{111}In]oxine-labeled leukocytes, Merc should be the preferable compound for routine clinical use.

NOTES

*Indium-111 chloride (50 m Ci/ml 0.01*M* HCL) was obtained either from Medi-Physics, Inc., Richmond, CA or from Atomic Energy Canada.

†Medi-Physics, Inc., Richmond, CA.

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