Teaching Editorial

Technologic, Clinical, and Basic Science Considerations for In-111-Oxine-Labeled Leukocyte Studies

During its short history as a diagnostic specialty, nuclear medicine has experienced certain milestones that have helped shape the philosophy of its practitioners. It may still be too early to tell with certainty, but the introduction of In-111-oxine for labeling leukocytes may prove to be such a milestone. The following survey will point out many of the salient technologic, basic science, and clinical features of In-111-labeled leukocytes—which have recently made this imaging agent so useful and which promise to extend its use in the future.

The introduction of labeled leukocytes for diagnosing inflammation increased the specificity of such radionuclide studies. Previously, gallium-67 citrate was used. Its uptake into inflammatory lesions (including abscesses) occurs partially in response to the presence of lactoferrin from destroyed polymorphs (*1*), and partially in response to an increase in vascular permeability (*2*) over a period of 24 to 48 hr. By contrast, In-111-labeled leukocytes may be used to delineate the presence and extent of an abscess or acute osteomyelitis as soon as 2-hr postinjection. Rapid diagnosis in suspected abscess cases is especially important because the differential diagnosis dictates different therapeutic approaches.

Abscesses are defined as localized collections of pus in cavities, formed by disintegration of tissues. They should be differentiated from phlegmonae, which are inflammation of the tissues. The distinction is important, since surgical incision and drainage are often necessary for the resolution of an abscess, while phlegmonae usually heal spontaneously. The importance of early and accurate detection of abscesses need not be stressed, since the mortality rate of undrained abscesses may reach 35% (*3*).

The causes of abscesses are many. One study found that most occurred in postoperative patients (*4*). Abscesses also frequently occur in patients with bone-marrow depression where their location is often difficult to pinpoint, since these patients may present with fever and sepsis without signs indicating the abscess site.

Which Modality to Use—and When?

In most instances, x-rays of the abdomen in cases of suspected abscesses may be inconclusive or may even appear normal.

Computed tomography (CT), ultrasound, and radionuclide scintigraphy have all been used in the diagnosis of abscesses. In a study of 170 patients in whom one, two, or all three of these modalities was used, diagnostic accuracy for abdominal abscesses by CT was 96%, by ultrasound 90%, and by In-111 leukocyte scans 92% (*4*). Analysis of the different modalities resulted in a suggested sequence by which patients can be examined based upon their clinical condition. Patients who are not clinically ill or who have no localizing signs should be studied first with In-111-labeled leukocytes. If, however, localizing signs are present or the patient's condition necessitates prompt intervention, CT or ultrasound should be the first study performed.

The major advantage of radionuclide imaging is that the entire body is surveyed. Gallium-67 citrate has been used in the past for evaluation of suspected abcesses. However, because of colonic excretion and accumulation in certain tumors, as well as accumulation in postsurgical bed and healing wounds, Ga-67 has proved to be less than optimal. In addition, as noted previously, it

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frequently requires 48-72 hr before final interpretation is possible. Since leukocytes accumulate at the sites of inflammatory processes, several attempts have been made to label these white cells and use them as an imaging agent. McAfee et al. (*5*) found that the concentration of In-111-oxine leukocytes in 30 dogs with chemical and bacterial abscesses and acute joint inflammation was invariably much higher than that of Ga-67 injected simultaneously. For bacterial abscesses, the mean abscess-to-muscle concentration ratio was 3,000 for labeled leukocytes and 72 for Ga-67. It was concluded, therefore, that leukocytes properly labeled with In-111-oxine are more effective than Ga-67 for the detection of acute focal inflammatory lesions.

Of the three modalities mentioned, ultrasound is the fastest and least expensive. Sagittal and transverse gray scale ultrasound scans can be obtained for accurate localization and aspiration of suspected pus pockets. On the other hand, in many instances, interference from adjacent gas and bone precludes obtaining adequate images. Skin contact is essential, making it impossible to scan through drains, tubes, open wounds, and stomata. The advantages of CT over ultrasound include precise anatomic localization of the lesion. Wounds, tubes, and stomata do not interfere with scanning, but surgical clips may degrade CT images.

Table 1 lists most of the present clinical applications for In-111-lymphocytes and leukocytes. Although usually only one or two of the studies listed are performed routinely in any one institution, clinical experience with all of them continues to accumulate.

TABLE 1. Applications of In-111-Oxine-Labeled Leukocytes and Lymphocytes

Cell type	Application
Leukocytes	Abscess location
	Acute osteomyelitis
	Inflammatory bowel disease
	Myocardial infarction
	Bacterial endocarditis
Lymphocytes	Lymph node visualization
	Hodgkin's disease
	Cardiac antirejection monitoring

What Are Leukocytes?

Leukocytes are a dynamic and heterogenous group of nucleated cells that emerge from bone marrow through similar patterns of differentiation. They help protect the host from hazards of the external environment. They usually spend a small fraction of their life span in the vascular space; movements into and out of this compartment serve to transport them to sites at which they perform their role in defense mechanisms. The mature cell types are neutrophils (about 59% of the leukocyte population in 21-year-old humans) and their tissue forms are called macrophages, lymphocytes (34%), monocytes (4%), eosinophils (3%), and basophils (0.5%). Neutrophils, eosinophils, and basophils, which contain nuclei usually with finely granular, evenly distributed chromatin, are also collectively called granulocytes. There are several other leukocyte subtypes (*6*) whose names and histologic descriptions make more than a cursory understanding of their structure and function a challenge.

Neutrophils and monocytes are motile. They engage in random movement and chemotaxis (directed movement usually regulated by many endogenous and exogenous substrates) whereby they play an important role in the genesis of the inflammatory response. The other white cell types exhibit predominantly chemotactic activity. Another attractive and important attribute common to these cells is their ability to engage in pinocytosis and phagocytosis, processes which are performed in conjunction with B-lymphocytes (*7*). Specific cells perform various types of endocytosis. For example, macrophages are capable of engaging in pinocytosis and phagocytosis; neutrophils, monocytes, and eosinophils may engage in phagocytosis. Substances thus engulfed are used as energy sources. Beneficial effects to the host result when these substances are harmful microorganisms or toxic chemicals in particulate form. It is the heterogeneity of the physical properties and functions of these cells, the interest that their "scavenging" ability has generated, and their ability to interact synergistically with each other that has made them objects of interest in medicine in general and nuclear medicine in particular.

Success in isolating the various cell types from whole human blood (*8*) has made it possible

to systematically investigate techniques for labeling them with radionuclides. Reports of surveys of radionuclides used to label leukocytes soon appeared (*9*). The successes using indium-111 chelates of the lipid soluble agent oxine (*10*) and later the water soluble agent tropolone (*11*) generated a degree of enthusiasm not experienced since the first clinical use of Tc-99m phosphate bone scanning agents in the early 1970s. However, no completely satisfactory procedure for obtaining unmodified neutrophils free of other cellular elements and plasma protein yet exists. This remains a hindrance in the basic science developments in this area of nuclear medicine.

Indium-111: Physical Characteristics and Cell Effects

In some ways the physical characteristics of In-111 are well suited to the performance of these studies. A physical half-life of 67 hr and emission of 173 keV (89%) and 247 keV (94%) photons make In-111 useful with either a rectilinear scanner or a scintillation camera. It is a heavy metal and forms moderately stable coordination complexes with oxine (8-hydroxy-quinoline), tropolone, and acetoacetic acid, which facilitate its transport across the cell membrane into the cytoplasm where it binds with much greater stability to cytosolular proteins. However, for other reasons, indium-111 is much less than ideal. Since it is a nonspecific cell labeling agent, the cells one desires to label must be removed from plasma for about 40 min. Additionally, the labeling process subjects the cells to the effects of a high photon flux in a restricted volume during the balance of their life cycle. Finally, the cells are subjected to the toxic effects of cadmium, the end product of the In-111 decay scheme, and to the potentially toxic effects of the ligands with which indium forms coordination complexes before entering cells (*20*). These processes probably contribute to some loss of cell viability and thus may adversely alter the kinetic properties of the final product.

Labeling Variations

During the last few years a poorly documented dichotomy in labeling procedures appears to have developed as a result of the proliferation and increasing clinical importance of these studies. While efforts to achieve ever more homogenous populations of cell types for research purposes continue, a more permissive approach to isolating and labeling these cells (appendix) is apparent. The newer approach accepts a level of erythrocyte and platelet contamination in the final product that its proponents consider too low to interfere with a nuclear physician's ability to diagnose a marginal true positive in cases of suspected abscesses. These products may thus exhibit a very slight pink hue due to the presence of 2–4% erythrocytes. This degree of contamination is tolerated because the alternative, lysing the remaining erythrocytes using Sterile Water for Injection, USP, may compromise some physiologic parameter of leukocyte function. The rationale given for this permissiveness is that since leukocytes are present in vastly greater numbers than erythrocytes in these preparations, they have a competitive advantage during the labeling process. The appendix offers an alternative method of lysing erythrocytes, which is used at our institutions.

Technologic preparation for leukocyte imaging procedures should include a review of the patient's chart and an interview to insure that no interfering radionuclide such as Ga-67, I-131, In-111, or Yb-169 has been administered. All forms of Tc-99m may also interfere with the procedure if administered within 24 hr prior to leukocyte imaging. The leukocyte count should be high enough (above 5,000/mm^3) to permit adequate labeling efficiency. Although under certain circumstances donor leukocytes may be used after proper crossmatching, unless a prior arrangement has been made to obtain such a product, this procedure may cause excessive delays, and in addition the resulting study may not accurately reflect whole body localization of the patient's own cells.

At our institutions, excellent results have been obtained using a large field scintillation camera that has a three-channel analyzer capable of handling multiple peak energy radionuclides such as In-111, and a 360-keV medium energy collimator to accept both the 173 keV and 247 keV photons.

Patient positioning for the whole body views depends upon the area of interest, if known. Otherwise multiple anterior static images are taken. Four and 24-hr postinjection images have been most useful. When 500 μCi of In-111 has been administered, enough activity remains to obtain 300,000 count images with scanning times of 5 min each or less. Images are also recorded on a dedicated computer for later contrast enhancement. Hard copy films are obtained as part of these studies.

In a recent prospective study involving 32 patients with clinical suspicion of focal infection, In-111 leukocyte scintigraphy showed 73% true-positive and 2.5% false-positive rates (*12*). Gallium-67 had 81% true-positive and 9% false-positive rates. The 27% false-negative In-111 leukocyte scans involved infection foci of more than two weeks' duration, and the 19% false negative Ga-67 studies represented patients having infections of less than one week's duration.

Exceptions to the Rule

The distribution of labeled leukocytes may be altered by splenectomy or bone marrow radiation. Hyperalimentation, hemodialysis, and hyperglycemia may alter the function of leukocytes and prevent their accumulation at sites of inflammation (*13*). Chronic processes may have a well-defined wall without significant inflammatory response, and antibiotic therapies may adversely affect the accumulation of leukocytes.

Dutcher et al. (*14*) have studied 14 granulocytopenic patients with known sites of infection. They used homologous, ABO-matched granulocytes labeled with In-111. Thirteen of the 14 patients had accumulation of radioactivity at the site of infection by 30 min, and all 14 patients had abnormal studies by 24-hr after administration. More recently, In-111-labeled donor leukocytes were used for the detection of foci of suppuration in eight severely leukopenic patients with marrow suppression (*15*). In three patients, good correlation was found between the results of imaging and clinical signs or subsequent proof of inflammation. In the other five patients in whom no evidence of localized suppuration occurred, no abnormal accumulations of radioactivity were demonstrable.

After inducing osteomyelitis in 18 rabbits by injecting *S. aureus* into a proximal pretibial metaphysis, Raptopoulos et al. (*16*) reported that In-111 leukocyte scans were positive in 83% of the 18 rabbits during the first week after injection of the microorganisms. By contrast, Tc-99m MDP bone scans were positive in only 22% of the animals ($p > 0.005$). They suggest that in patients with suspected acute osteomyelitis, leukocyte scans can detect the disease earlier than Tc-99m bone scans. The early diagnosis of acute osteomyelitis is extremely important so that appropriate treatment with antibiotics can be initiated before extensive bone necrosis leading to chronic disease has occurred.

The Challenges That Remain

As this area of nuclear medicine continues to evolve, so too will attempts to answer the associated clinical and basic science questions. For instance there should be extensive clinical confirmation of the specificity and predictive value of test results of In-111 chloride used to image patients with suspected abscesses (*19*). There is not yet a rapid or convenient method for evaluating the changes that may have taken place in cells during the labeling process, though several methods have been proposed for evaluating post-labeling cell viability (*17*). There are no reported studies that systematically measure the effects upon circulating labeled leukocytes of known plasma or tissue levels of antibiotics commonly used in patients with abscesses. It would be useful to have data that contrast the kinetics of leukocyte subpopulations obtained peripherally with those obtained centrally. Data showing which x-ray contrast studies interfere with the delineation of the extent and location of abdominal infections or abscesses would also be significant. In the absence of such data, we suggest that where possible, x-ray studies using barium should be performed after completion of the labeled leukocyte scan. Finally, it would be important to explore the clinical utility of labeled T-lymphocytes in studies of organ and tissue transplantation.

Other challenges exist, and have been elegantly discussed elsewhere (*18*). Their solution may have to await the development of more specific and effective cell labeling methods.

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APPENDIX:
Preparation of In-111 Leukocytes

1. Withdraw 35 ± 5 ml whole blood (patient or matched donor) into a syringe containing 1.5 ml hydroxyethyl starch 6% in sodium chloride injection (saline) and 1,000 I.U. preservative-free heparin.
2. Using a clamp and stand, place the syringe at an angle of 80°, needle facing up, inside a vertical laminar flow hood at room temperature.
3. When erythrocytes have settled enough to reveal at least 20 ± 5 ml of plasma (slight pink hue near the erythrocyte interface is unavoidable), attach a 19-gauge butterfly to the syringe, previously returned to an angle of 90°.
4. Express the plasma (slowly at first) equally into two sterile pyrogen-free, screw cap narrow-bottom test tubes. The first 1 ml of plasma may be discarded if there is excessive erythrocyte contamination. Clear straw-colored plasma should then begin to flow into the centrifuge tubes. However, slight erythrocyte contamination is acceptable (see step 7).
5. Centrifuge the plasma for 10 min at 180 × g.
6. Using a spinal needle (18-gauge) remove the supernate platelet rich plasma in equal amounts into two separate test tubes and save (see step 11). The leukocyte button usually coated with the few remaining erythrocytes will remain.
7. Most of the remaining erythrocytes may be lysed as follows: (*8*)
   a) Combine and disperse the leukocytes into a single test tube using 2-ml saline and a spinal needle.
   b) Add 4-ml sterile water for injection (USP) to the preparation. Agitate gently for 45 sec.
   c) Add 2-ml sterile, pyrogen-free sodium chloride solution, 36 mg/ml. Agitate gently to mix.
8. Centrifuge the preparation for 10 min at 180 × g.
9. Remove and discard the supernate. Disperse the leukocyte button using 3.5-ml saline and a spinal needle. Shield the preparation behind lead. Place one drop of the preparation on a microscope slide and perform a differential to identify erythrocytes and platelets that may be present.

10. Add 0.5–1.0 mCi of In-111 oxine in saline (0.5 ± 0.1 ml) to the dispersed leukocytes. Agitate gently. Incubate at 37 °C for 20 min agitating at the 10-min mark.
11. During the incubation period, centrifuge the platelet rich plasma for 10 min at 1,000 × g. Retain the supernate platelet poor plasma for use in step 12.
12. Centrifuge the labeled leukocyte preparation for 10 min at 180 × g. Remove and discard the supernate. To reconstitute the labeled leukocyte button:
    a) Disperse the leukocytes using 4-ml autologous patient plasma from step 11 and an 18-gauge spinal needle;
    b) If donor blood was used, disperse the leukocytes in 4-ml saline.
13. The leukocyte product will contain at least 80% of original radioactivity. Follow established recommendations as to the amount of radioactivity to be injected into the patient.
14. Subject 1 ml of the product to these additional quality control procedures before patient injection.
    a) *Leukocyte viability:* Place a drop of the preparation on a microscope slide followed by a drop of trypan blue suspension in saline (0.1%). Viable cells will exclude the dye. A control positive for dye uptake may be prepared by incubating 0.5 ml of the product for 30 min at 48 ± 1 °C;
    b) *Labeling efficiency and stability:* Add 4-ml saline to 0.5 ml of the product, disperse, then centrifuge at 450 × g for 10 min;
    c) *An acceptable preparation for 100 leukocytes:* not more than 5% erythrocytes; not more than 10% platelets; not more than 3% nonviable cells; and not less than 95% labeling efficiency after 15 min at 37 °C.