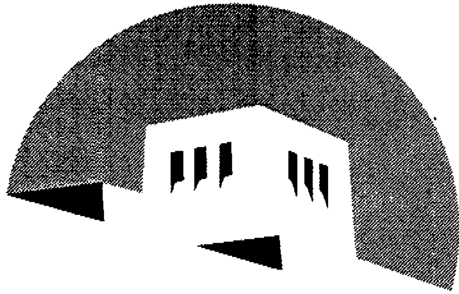

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Correspondence Continuing Education Courses for Nuclear Pharmacists and Nuclear Medicine Professionals

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Radiopharmaceuticals for Imaging of Infectious and Inflammatory Lesions

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RADIOPHARMACEUTICALS FOR IMAGING OF INFECTIOUS AND INFLAMMATORY LESIONS

STATEMENT OF OBJECTIVES

The primary goal of this correspondence course is to increase the reader's knowledge and understanding of the various radiopharmaceuticals used for localizing infectious and inflammatory lesions. In order to achieve this goal, this continuing education lesson presents pertinent information for understanding methods of production, the mechanisms for labeling and localization, and the physical, chemical, and biological properties of different imaging radiopharmaceuticals for infection and/or inflammation imaging. In addition, advantages and disadvantages of each infection/inflammation imaging radiopharmaceutical as well as comparisons among different radiopharmaceuticals are also discussed. The conclusion will include a discussion of clinical application of the radiopharmaceuticals used in detecting and evaluating infectious and inflammatory lesions.

Upon successful completion of this material, the reader should be able to:

1. Identify the differences between infection and inflammation.
2. Discuss the advantages and disadvantages of different modalities used for localizing infectious and inflammatory lesions.
3. List the required characteristics of an ideal imaging radiopharmaceutical for detecting infectious and inflammatory lesions.
4. Describe the currently accepted mechanisms of localization for gallium-67 (^{67}Ga) citrate.
5. Discuss the distinctive characteristics and functions of five major types of white blood cells (WBCs).
6. Describe the general steps involved in the method for radiolabeling leukocytes.
7. Compare the differences between using pure granulocytes and mixed leukocytes in infection/inflammation imaging studies.
8. State the various factors that may affect the labeling efficiency of radiolabeled WBCs.
9. Describe the *in vitro* and *in vivo* quality control procedures for radiolabeled leukocytes.
10. List several drawbacks associated with technetium-99m (^{99m}Tc)-labeled leukocytes using both the phagocytic and pretinning methods.
11. List the advantages and disadvantages of using indium-111 (^{111}In)-labeled leukocytes as an imaging agent for the detection of infectious and/or inflammatory disease.
12. Present the proposed mechanism involved in the labeling process for ^{111}In-oxine-labeled leukocytes.
13. Explain the circumstances which necessitate a search for other new complexing agents of indium other than oxine.
14. Compare and contrast the biologic properties between ^{99m}Tc hexamethylpropylene amineoxime (HMPAO)-labeled leukocytes and ^{111}In-oxine labeled leukocytes.
15. Describe the clinical advantages and potential limitations associated with the use of radiolabeled leukocytes for imaging infection/inflammation sites.
16. List five major difficulties related to the use of radiolabeled antibodies for the detection of infection and/or inflammation.
17. Describe the advantages and disadvantages involved with the clinical use of ^{111}In-labeled nonspecific polyclonal human immunoglobulin (IgG) for the detection of infectious and/or inflammatory foci.
18. List the underlying principles for ^{99m}Tc-labeled BW 250/183 when used as an infection/inflammation imaging agent.
19. Compare the size of ^{99m}Tc nanocolloids with other particulate imaging radiopharmaceuticals for infection and/or inflammation detection.
20. Discuss the approach for improvement of the target-to-nontarget ratios in the clinical use of ^{111}In-labeled avidin-biotin.

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VI. CONCLUSIONS

RADIOPHARMACEUTICALS FOR IMAGING OF INFECTIOUS AND INFLAMMATORY LESIONS

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INTRODUCTION

Pathophysiology of Infection and Inflammation

Infection involves the invasion and multiplication of microorganisms in body tissues which may result in local tissue injury or destruction. In order to destroy, dilute, or sequester the invading microorganisms and injured tissue, the body's defense mechanism responds with a complex series of events. It is normally associated in the acute stage with signs of inflammation: heat (calor), redness (rubor), swelling (tumor), and pain (dolor). The heat and redness results from dilatation of arterioles, capillaries, and venules with increased permeability and blood flow; the swelling is formed by the accumulation of fluid, plasma proteins, and leukocytes which migrate into the site of infection; and the pain is caused by tissue tension from the edema.

Inflammation may be caused by a local protective reaction of vascularized tissue to injury resulting from either a noxious or innocuous stimulus. The main function of the inflammatory response is to destroy, dilute, or "wall off" (sequester) both the injurious agent and the injured area. Noninfected inflammation is normally associated with the same series of responses (i.e., local heat, redness, swelling, and pain) as

infection. In fact, the most important cause of inflammation is bacterial infection, although other types of noxious injuries, such as the death of tissue and thermal, chemical, or physical trauma may also induce a similar pattern of inflammatory reaction. Rheumatic and allergic diseases are caused by innocuous stimulus in which the inflammatory response itself is the cause of tissue damage. The exact details of cellular cytosine radiation of the inflammatory response are beyond the scope of this continuing education (CE) lesson.

Modalities for Localization of Infection and Inflammation

Computed tomography (CT), ultrasound, magnetic resonance imaging (MRI), and radionuclide scintigraphy have all been used in the workup of patients with suspected infectious and inflammatory disorders. CT has the advantage of precise anatomical localization of abscess and is not hindered by tubes, sites of drains, or the presence of open wounds. CT is a relatively fast procedure that can be performed with a minimum of patient discomfort. However, false positive diagnosis of abscess formation may be seen in tumor necrosis, thick-walled cysts, and unopacified bowel loops. In addition, if there are no localizing signs or symptoms present, a whole-body CT scan is time-consuming and results in a high radiation dose to the patient.

Ultrasound has some advantages over CT, MRI, and radionuclide scintigraphy as it is the fastest and least expensive mode of abscess imaging. It also has the advantage of imaging in multiple planes, which may allow more accurate lesion localization. However, ultrasound evaluation is highly dependent upon the operator's expertise, and its ability to obtain images may be limited when open wounds, drain lines, or tubes exist. Gas and bone also interfere with the performance of ultrasound. It also demonstrates low specificity since some conditions, such as hematoma, lymphocele, cyst, seroma, and fluid filled bowel loop, may mimic the sonographic appearance of an abscess.

MRI is a new modality that is also useful in the workup of a patient with infection/inflammation. It has some of the advantages of CT as it is not affected by open wounds or drains. Like ultrasound, MRI can image in multiple planes. Like all the imaging modalities, MRI also has limitations as to the specificity of a positive finding when any cause of increased intercellular fluid (edema) may mimic infection/inflammation. It is also costly and very time consuming to do a whole body MRI study.

Both CT and ultrasound require the formation of abscess for accurate diagnosis, whereas radionuclide scans and MRI can detect the site of infection/inflammation even prior to abscess formation. The major advantage of radionuclide imaging is its ability to survey the entire body with a single examination.

The question of which modality to use for the evaluation of infection and when to use it can be best answered by examining the clinical condition of the patient. Patients who are not critically ill or who have no localizing signs should be studied first with a radionuclide scintigraphy study (e.g., ^{111}In-labeled leukocytes). If, however, patients require prompt intervention or have localizing signs, CT, ultrasound, or MRI should be used initially.

Many radiopharmaceuticals have been used in the evaluation of patients with suspected infectious and/or inflammatory process. Before the advent of CT scanning, brain scintigraphy was used in the evaluation of brain abscess and cerebral inflammation (1-4). Liver/spleen scintigraphy was used prior to the days of ultrasound and CT in the evaluation of hepatic abscess (5,6). Currently, bone scintigraphy remains a very important part of the diagnostic armamentarium in the evaluation of musculoskeletal sepsis (7). Upon review of data compiled from a series of articles, bone scanning shows a sensitivity of 94% and a specificity of 95% in bone that has not been complicated by previous infections, trauma, or surgery. However, the specificity of bone scanning drops significantly to a range of 33% when the disorder is complicated by remodeled bone (7). In the case of bone remodeling, additional imaging with radiolabeled white blood cells (WBCs) or ^{67}Ga-citrate is necessary for further delineation. The remainder of this CE lesson will be limited to the radiopharmaceuticals whose primary role involves the general evaluation of infection and inflammation.

Ideal Characteristics of Radiopharmaceuticals for Imaging of Infectious and Inflammatory Lesions

The value of radionuclide scintigraphy is well established. The following represents a "wish list" for the characteristics of an ideal radiopharmaceutical in localizing infectious and/or inflammatory lesions:

1. High sensitivity and specificity in different disease sites to help ensure that an accurate diagnosis can be made. Biological properties of the imaging radiopharmaceutical, such as rapid localization in the focus, a high target-to-nontarget ratio, and rapid blood and background clearance, are all critical in contributing to high sensitivity. The agent should localize only in sites of infection/inflammation for ideal specificity.

2. Easy availability and simple preparation.

3. Inexpensive cost.

4. Acceptable radiation dosimetry without toxicity so that serial studies can be performed when needed.

Radiopharmaceuticals for the Detection of Infectious and Inflammatory Lesions

A number of radiopharmaceuticals are available for diagnosing infection and inflammation (Table 1). Each radiopharmaceutical for use in imaging infectious and inflammatory lesions (listed in Table 1) will be discussed in detail in respective sections which follow:

Table 1. Available Radiopharmaceuticals for Infection and Inflammation Imaging

Radionuclides
• ^{67}Ga-citrate
• $^{111}InCl_3$
Radiolabeled Leukocytes
• ^{111}In-labeled leukocytes
• ^{99m}Tc-labeled leukocytes
Radiolabeled Antibodies
• ^{111}In-labeled nonspecific polyclonal human immunoglobulin-G
• ^{99m}Tc-labeled monoclonal antigranulocyte antibody
Other Agents
• ^{99m}Tc-labeled nanocolloids
• ^{111}In-labeled avidin-biotin

RADIONUCLIDES

^{67}Ga-Citrate

Gallium-67 citrate (^{67}Ga-citrate) (Figure 1) was first demonstrated to accumulate in inflammatory lesions in 1971 (8), and since then this radiopharmaceutical has been used routinely for the detection of infection and inflammation. ^{67}Ga has the advantage of not requiring any special preparation before administration. However, after two decades of clinical use, the mechanism of ^{67}Ga localization in abscess and other inflammatory sites is not yet fully elucidated. Localization of ^{67}Ga has been attributed to [a] binding to intracellular lactoferrin of leukocytes (9,10), [b] direct binding to microorganisms (10-12), and [c] abnormal passive diffusion of the ^{67}Ga-transferrin complex through the altered vascular endothelium of inflamed tissue (13).

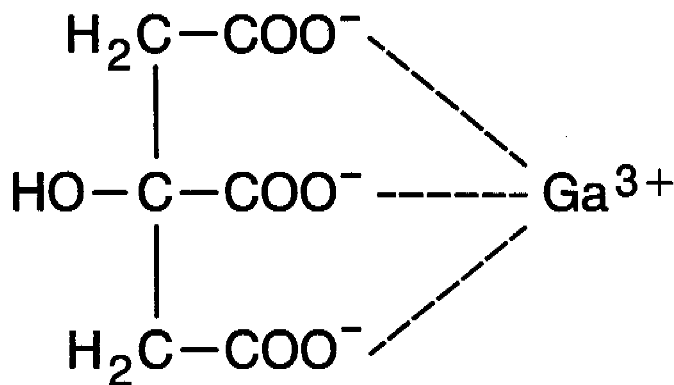

Figure 1. Chemical structure of ^{67}Ga-citrate.

Although ^{67}Ga has been shown to be useful in the evaluation of patients with infectious and inflammatory lesions, ^{67}Ga has a number of disadvantages. The high radiation absorbed dose to the patient limits the maximum amount of ^{67}Ga which can be administered. The physiological localization of ^{67}Ga in the liver and its normal renal and bowel excretion may obscure lesions and complicate the diagnosis of abdominal abscess. Even with bowel cleansing prior to imaging, activity in the gut is usually seen on the ^{67}Ga scan and often necessitates delayed imaging (14). The lack of specificity of ^{67}Ga uptake is especially a problem with cancer patients, since ^{67}Ga is taken up by a variety of tumors. In addition, areas of recent surgery and normally healing wounds are visualized with ^{67}Ga; therefore, ^{67}Ga is less useful in the postoperative period when detection or exclusion of an abscess may be extremely important. All of these reasons make ^{67}Ga-citrate a less than optimal radiopharmaceutical for delineating infectious and inflammatory lesions. However, there are areas where ^{67}Ga is the agent of choice. When a patient presents with a fever of unknown origin (FUO), the cause may be infection, malignancy, or other chronic inflammatory or granulomatous process. Nonspecific ^{67}Ga localization in these types of lesions can actually be a benefit in this circumstance since detection and localization is more important than a specific diagnosis. Gallium is also better than other agents (i.e., ^{111}In-labeled leukocytes) for the diagnosis of disc space infection (15,16) and for infection foci of more than two-weeks duration (17).

$^{111}InCl_3$

Indium-111 chloride ($^{111}InCl_3$) was first suggested in 1976 for use in patients with inflammatory disease (18). Sayle et al. (19) have demonstrated its potential usefulness in the detection of suspected abscess. However, the accumulation of $^{111}InCl_3$ activity in the kidneys and bladder makes the interpretation of

infectious and inflammatory disease at these sites difficult. As with ^{67}Ga, $^{111}InCl_3$ also localizes in patients with tumor, and this may be misinterpreted as acute abscess. However, unlike ^{67}Ga, $^{111}InCl_3$ is not excreted through the gastrointestinal tract. This is particularly advantageous in patients with abdominal inflammatory disease who cannot undergo an adequate bowel cleansing procedure. Due to the limited clinical experience with $^{111}InCl_3$ in detection of infectious and inflammatory lesions, more extensive investigation and confirmation of its clinical usefulness is needed in this application. $^{111}InCl_3$ has been approved recently by the U.S. Food and Drug Administration (FDA); however, its indication for usage as stated in the package insert (20) is for radiolabeling OncoScint® preparations used for the detection and staging of colorectal cancer (21).

RADIOLABELED LEUKOCYTES

Biological Functions of Leukocytes

WBCs (leukocytes) play a major role in the body's defense mechanism in which they immobilize, phagocytize, and kill organisms involved in infections. The usual range of leukocytes in the normal adult is 5,000 to 10,000 WBCs/mm³. These WBCs include granulocytes (50% to 83%), monocytes (0% to 7%), and lymphocytes (20% to 40%). The granulocytes are made up of three subclasses--neutrophils [polymorphonuclear leukocytes (PMNs)], basophils, and eosinophils, whose names are derived from their staining characteristics. PMNs, which phagocytize and degrade many types of particles, serve as the body's first line of defense when tissue is damaged or foreign material gains entry. PMNs congregate at sites in response to a specific stimulus through a process known as chemotaxis. The function of basophils in the circulation is not clearly understood; eosinophils and lymphocytes are classically associated with immune reaction, and lymphocytes are involved with chronic infection. Monocytes are phagocytic cells and serve as a secondary defense mechanism.

Since leukocytes comprise the primary defense mechanism against infection, researchers have attempted to image abscesses and other sites of infection by labeling these cells with a variety of gamma-emitting radionuclides. The use of radiolabeled cells has certain advantages when compared with conventional radiopharmaceuticals; first, the normal "homing" functions of cells are exploited to carry the radiopharmaceuticals to specific sites in the body and, second, it allows the biodistribution of normal and diseased blood cells to be studied noninvasively.

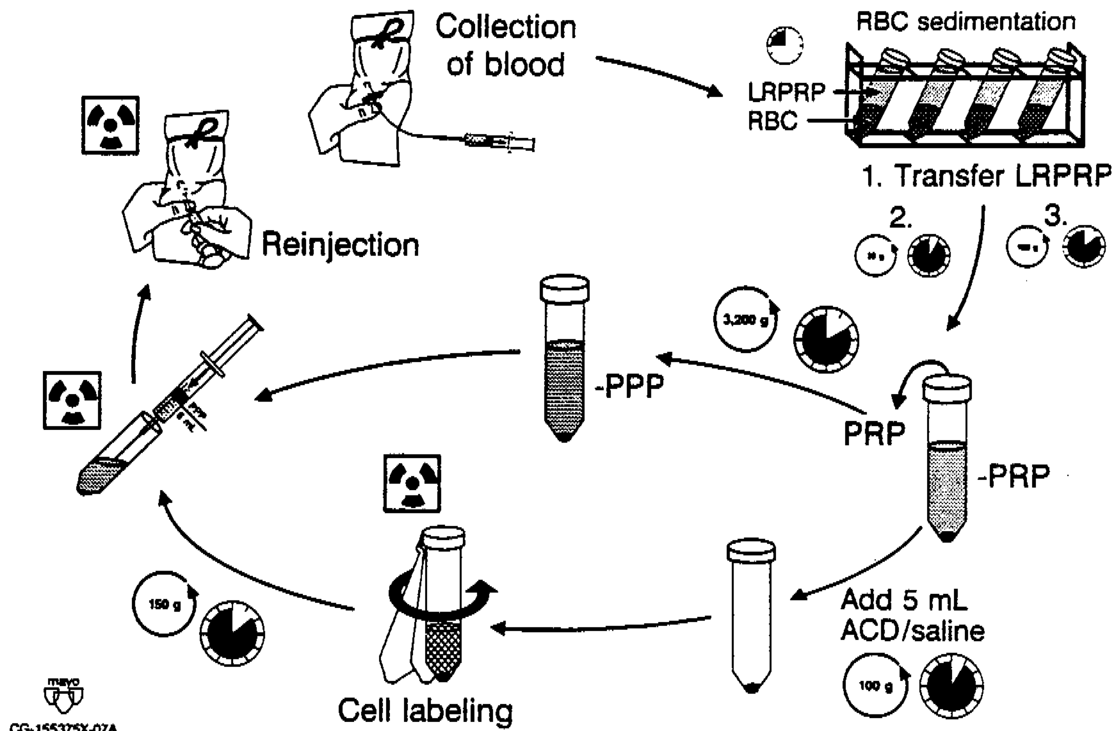

Figure 2. **Basic schematic illustration depicting the various steps involved in the radiolabeling of leukocytes. Please refer to text for detailed description. LRPRP: leukocyte-rich platelet-rich plasma, PRP: platelet-rich plasma, PPP: platelet-poor plasma.**

General Steps for Radiolabeling Leukocytes

Since most of the radioactive chelating agents used for leukocyte labeling are nonselective (i.e., they tag all cells), it is necessary to isolate the leukocytes from the remainder of the cellular components of blood (erythrocytes, lymphocytes, monocytes, and platelets) before radiolabeling can be performed. The general steps involved in the radiolabeling of leukocytes are described above in Figure 2.

Collection of Blood. In order to obtain a sufficiently large population of leukocytes, approximately 50-100 ml of venous blood is withdrawn from the patient. However, the requirement for a large volume of blood may present problems with pediatric patients. For this reason, the Childrens Hospital of Los Angeles, Los Angeles, California, has established a guideline in which the volume of blood withdrawn for a radiolabeled leukocyte study is 1 ml/kg for a pediatric patient age 2 to 18 (22). The minimum and maximum volumes of withdrawn blood are 20 ml and 50 ml, respectively (22). Another guideline for blood volume to be withdrawn in children is 15-30 ml for body weights less than 100 lb, and 30-60 ml for body weights greater than 100 lb (23).

Both acid citrate dextrose (ACD) and heparin have been used as an anticoagulant with the collection of patient blood; however, there is a consensus that ACD is a better anticoagulant than heparin for this purpose because leukocytes show less tendency to adhere to plasticware and to each other with ACD (24,25). However, Peters et al. (26) have found that ACD promotes microaggregation during labeling.

Separation of "Crude" Leukocytes. To accelerate the rate of sedimentation of the erythrocytes, polysaccharide (e.g., dextrose, methylcellulose, or hydroxyethyl starch) is added to the anticoagulated blood. These agents speed the sedimentation process by causing the red blood cells (RBCs) to clump together, thereby reducing the required sedimentation time by one-half. Hydroxyethyl starch (HES) [6% hetastarch in 0.9% NaCl solution (Volex®, American Critical Care, McGaw Park, IL; Hespan®, Du Pont Pharmaceuticals, Wilmington, DE)] is an effective settling agent when used one part HES to five parts blood (24). Roy et al. (27) have shown that HES increases the RBC sedimentation rate and the leukocyte recovery is greater than with dextrose. In addition, HES is an FDA-approved drug and is not associated with allergic reaction (28), whereas dextrose and methylcellulose have been reported to produce adverse reaction in some patients. Special care is required for storage as the 6% HES is available in 500 ml containers only and does not contain any bacteriostatic agent. Since HES readily supports growth of organisms, the commercially available 6% HES solution is, therefore, designed for single use only.

Following approximately 30 to 45 minutes of gravity sedimentation, the supernatant is rich in leukocytes (approximately 70%) and platelets, and is known as leukocyte-rich platelet-rich plasma (LRPRP). However, one to two red cells per leukocyte as well as the majority of platelets are still present in the supernatant (29). To eliminate RBC contamination of the LRPRP, hypotonic saline or ammonium chloride (29,30) has been used to lyse RBC. However, decreased circulation half-times and increased hepatic uptake of leukocytes have been found following the hypertonic or hypotonic RBC lysis, possibly due to WBC damage (31). In our institution, we use a centrifugation method (30 g for five minutes on LRPRP layer) (Figure 2, sub-step 2) to remove any remaining RBCs contained in the plasma.

To remove the smaller and lighter platelets in the LRPRP layer, the supernatant is carefully separated from the RBC pellet and is then centrifuged at 100 g for 8 minutes to obtain the leukocyte pellet (Figure 2, sub-step 3). A "rocking" step has been developed by Kaminsky (32) to be used in order to deplete RBC contamination (if this has not already been accomplished by other means). Following isolation of the leukocyte button, the leukocytes are resuspended in 4 ml of normal saline and then allowed to rock upon a specially modified rocker arm for 15 minutes (32). Kaminsky has demonstrated that this patented method results in a 1% to 5% residual RBC contamination as compared to 20% contamination of tagged RBC without the RBC depletion steps (32).

The platelet-rich plasma (PRP) is drawn off and transferred to a fresh centrifuge tube. This tube is then centrifuged at 3,200 g for 10 minutes to form platelet-poor plasma (PPP) (Figure 2). Following the removal of the PRP, the leukocyte pellet is resuspended in ACD/saline solution and labeled by the addition of a radioactive complexing agent (Figure 2).

Separation of "Pure" Granulocytes. When "pure" granulocyte preparations are requested, granulocytes cannot be separated from monocytes and lymphocytes by differential centrifugation. However, because of slight differences in cell density, they can be separated by the density gradient centrifugation method. In centrifuging with discontinuous density gradients, the cells migrate until they reach the surface of a solution whose density is equal to or greater than their own; hence, cells of varying densities come to rest at different depths. By using a multiple-density solution (e.g., the widely-used Ficoll-Hypaque discontinuous density gradient), granulocytes, monocytes, and lymphocytes can be separated with less than 10% cross-contamination (24,33) (Figure 3). Other discontinuous density

gradients that have been used include Percoll (34) and Metrizamide (35). Although Ficoll-Hypaque gradients have been widely used for separation of neutrophils, some studies have shown that this mixture may have an adverse effect upon leukocyte viability (36). Percoll has been recommended as a more innocuous substitute (24,36) since Percoll is a colloidal silica coated with pyrophosphate which renders it nontoxic. Leukocytes separated by the use of Percoll gradients have been shown to have normal chemotaxis and bactericidal activity (36,37). Other cell-separation techniques that have been used for obtaining pure granulocytes include column separation (37,38), phagocytosis (29,39), centrifugal elutination (24), and flow cytometry (24,40).

Pure Granulocytes vs. Crude Leukocytes. Schauwecker et al. (41) have conducted a study to compare the advantages and disadvantages of purified ^{111}In-labeled granulocytes with ^{111}In-labeled mixed leukocytes. They have found that the crude leukocyte preparation is probably the agent of choice for imaging of infection because:

1. cell separation and labeling of a standard mixed cell preparation is easier.

2. in acute infection, the uptake sensitivity of either pure granulocytes or mixed cells is high and not statistically different.

3. mixed cell preparations have a slightly superior sensitivity when compared with pure granulocytes for chronic infections; this is likely due to the presence of labeled lymphocytes.

In addition, Peters (42) has demonstrated that, in his experience, pure granulocytes are markedly activated *in vivo* and show prolonged retention in the lung. However, pure granulocytes are useful in certain specific circumstances:

1. When patients are without neutrophilia.

2. When granulocyte deposition may be obscured against a high blood background resulting from RBC and platelet-associated activity as in cardiovascular inflammation when mixed cells are used (42).

3. When granulocyte quantitation or kinetic information is required.

Cell Labeling. Cell labeling is a very simple and rapid procedure involving the addition of the radioactive agent (e.g., ^{111}In-oxine, ^{111}In-tropolone, ^{99m}Tc-sulfur colloid, ^{99m}Tc hexamethylpropyleneamineoxime [^{99m}Tc-HMPAO]) to the leukocytes and incubation for five to fifteen minutes at room temperature or 37°C.

It is desirable to incubate the leukocytes in plasma in order to maintain cell viability. The granulocytes labeled in plasma clear rapidly from normal lungs, migrate to sites of abscess more rapidly, and demonstrate a reduced quantity of radioactivity absorbed by the liver (43). However, certain ligands such as oxine have less affinity for ^{111}In than plasma protein; therefore, leukocytes must be removed from the plasma for labeling.

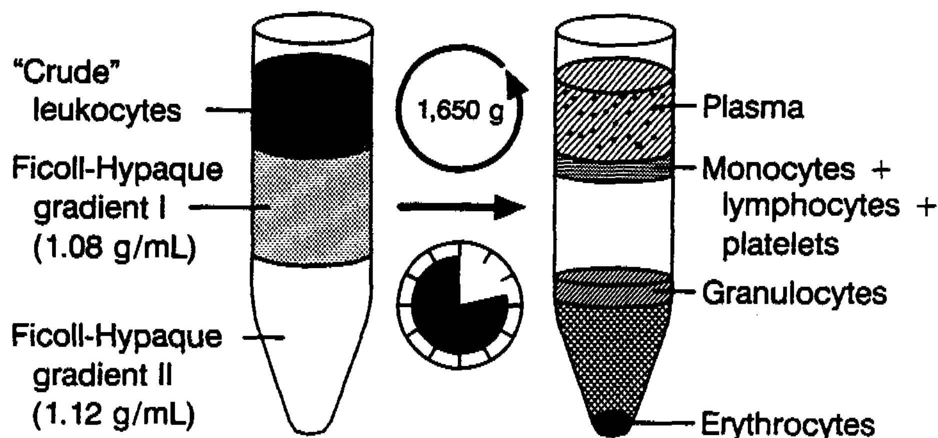

Figure 3. Different cell components of leukocytes separated by using two Ficoll-Hypaque discontinuous density gradients.

Factors Which Affect Labeling Efficiency

The ability of a radioactive ligand to label leukocytes is usually assessed by measuring the labeling efficiency (LE). However, it is often difficult to compare values quoted in the literature since the labeling conditions in different studies are rarely the same.

The LE of radiolabeled leukocytes is affected by the following factors:

1. **pH of the medium**. Cells are usually labeled at a pH between 6.5 and 7.5 to maintain the viability of the cells.

2. **Concentration of ligand**. Approximately 10^2 to 10^6 times more ligand than ^{111}In is required before cell labeling will occur. With physiological saline, the maximum LE is obtained over a much wider concentration range than in plasma. Thus, it is very important to use the optimum concentration of ligand, especially when labeling cells in plasma.

3. **Cell concentration**. In general, the LE increases with cell concentration, although higher cell concentrations are needed when labeling cells in plasma (44-47). This is due to a portion of the radionuclide (especially ^{111}In) being diverted to plasma components.

4. **Incubation time and temperature**. Cell labeling is usually rapid, reaching a plateau after 5 to 30 minutes incubation at 25°C or 37°C (44,48,49). However, a much slower rate is observed when the labeling is performed at 4°C (46,50).

Quality Control of Radiolabeled Leukocytes

Ideally, one wants to obtain a high LE; however, it is much more important to maintain the viability and function of the labeled leukocytes. Certain degrees of labeling injury to the leukocytes may occur during *in vitro* manipulation; therefore, it is essential to perform quality control on radiolabeled leukocytes to ensure that viability and function of the labeled granulocytes are well preserved. Cell viability may be assessed either *in vitro* or *in vivo*.

In Vitro Tests. Various *in vitro* methods have been used; these include quality control Ficoll-Hypaque distribution analysis which shows the percentage of radioactivity in each cellular component (51) (Figure 3), trypan blue exclusion test which evaluates cell death (52), dual-filter radiochemotaxis assay (53), phagocytosis and intracellular killing capacity of granulocytes (54), and others. However, the *in vitro* tests are not practical on a routine basis as they are time-consuming and have failed to show damage which is clearly seen *in vivo* (55).

In Vivo Tests. The real test of the integrity of the labeled cells is upon reinjection into the patient. Cell recovery, as measured by the percentage of radiolabeled WBCs circulating in the blood after reinjection and disappearance half-time of labeled leukocytes, gives some measurement of *in vivo* behavior of the radiolabeled granulocytes. The leukocytes with lower viability are irreversibly sequestered in the liver, whereas samples contaminated with RBCs and lymphocytes result in greater splenic activity (35,36). In addition, damaged granulocytes are reversibly retained in the lung (35). Datz et al. (57), however, could not find any significant correlation between the early transient lung uptake of radiolabeled leukocytes and the decrease in sensitivity for detecting infection at early imaging times (< 6 hours). Weisberger et al. (54) have suggested that the early lung localization may represent only the natural physiologic margination of PMN leukocytes in the lung capillaries.

Considering the drawbacks associated with the quality control methods mentioned above, it has been suggested that routine complete blood count (CBC), white cell differential count, microscopic examination, and determination of LE are adequate to maintain quality control of radiolabeled leukocytes (25).

Leukocyte Count. In order to obtain adequate imaging, approximately 1×10^8 leukocytes are required when autologous granulocytes are used (59). For reinjection, when non-autologous cells must be used, McCullough et al. (60) have suggested that no fewer than 2-4 $\times 10^8$ cells must be given since histocompatibility factors present in some patients may reduce the intravascular survival of the non-autologous cells.

^{111}In-labeled Leukocytes

In 1976, McAfee and Thakur (29,39) demonstrated that ^{111}In oxine could be used to label leukocytes. The need for a radiopharmaceutical such as ^{111}In labeled leukocytes can probably be best measured by the more than 700 investigational new drug applications, sponsored by the Amersham Corporation, on file with the FDA, prior to official approval of ^{111}In oxine on October 17, 1985 (61).

Although ^{99m}Tc-oxine was also investigated and found to be useful, ^{111}In was preferred to ^{99m}Tc because its longer physical half-life (67.2 hours) allowed imaging at 24 hours following administration of the labeled WBCs when blood and background activities were low.

Repeated investigations up to one week post-injection are also possible with ^{111}In. ^{111}In has a high abundance of two gamma photons at 171 keV (90% abundance) and 245 keV (94% abundance) which are suitable for nuclear medicine imaging. However, since ^{111}In is a cyclotron-produced radionuclide, it is relatively expensive and not readily available. In addition, because of high radiation dosimetry, especially to the spleen, the dose and resultant count activities are limited.

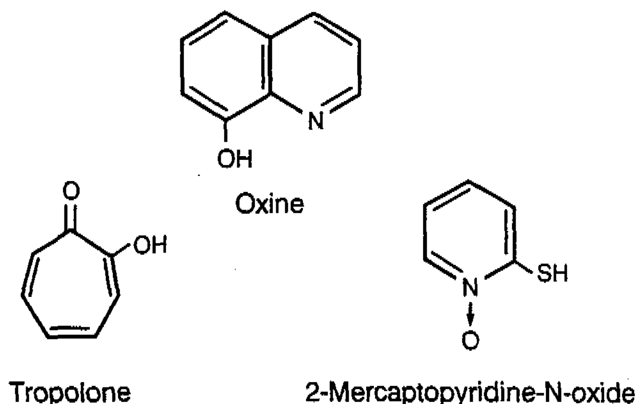

Figure 4. **Chemical structures of the ligands used to form indium complexes for leukocyte labeling.**

Mechanism of Leukocyte Labeling with ^{111}In-Oxine. Oxine (8-hydroxyquinoline) is a lipophilic chelating agent that has been used as a topical bacteriostatic and fungistatic solution, antiperspirant, and spermicide (Figure 4). Indium forms a saturated (1:3) complex (Figure 5) (the precise chemical structure of ^{111}In-oxine

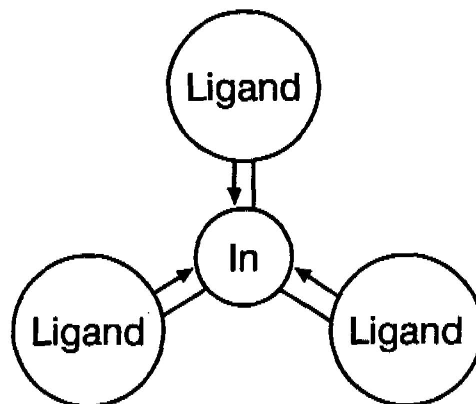

Figure 5. **Possible chemical structure configuration of indium-ligand complexes.**

is unclear at this time) with oxine that is neutral and lipid-soluble. This complex is able to penetrate the cell membrane of leukocytes. Once within the cell, the ^{111}In-oxine complex dissociates, and the ^{111}In binds to nuclear and cytoplasmic proteins which have higher binding affinities for the indium (44) (Figure 6). Some of the released oxine readily diffuses from the cells, and some may chelate with other cell ions (62).

Biological Distribution of ^{111}In-Oxine-Labeled Leukocytes. Usually in a mixed-cell population, with the cell pellet containing approximately 3-4 $\times$ 10^8 WBCs after following the recommended cell labeling procedure, the cell-bound activity distribution in neutrophils, lymphocytes, and RBCs is 80%, 15%, and 5%, respectively, whereas pure granulocyte preparations obtained by Ficoll-Hypaque separation have 98% radioactivity associated with neutrophils (63,64). Goodwin et al. (65) have achieved an LE of 84% ±9%,

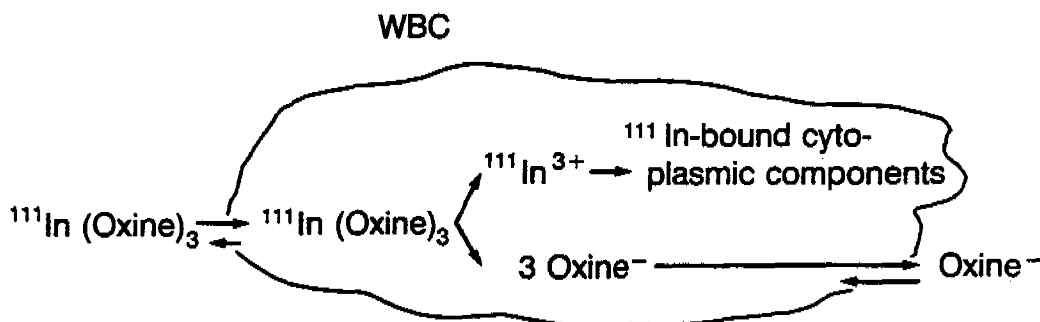

Figure 6. **Schematic diagram of the proposed mechanism for leukocyte labeling with ^{111}In-oxine.**

and in patients with WBC counts above 15,000 mm^3 in peripheral blood, it was usually $\geq 90\%$.

The package insert for Indium In 111 Oxyquinoline Solution (66) recommends that the maximum time between drawing patient blood and reinjection of ^{111}In-labeled leukocytes should not exceed five hours. It is also recommended that the labeled WBCs should be reinjected within one to three hours after the radiolabeling procedure (66,59).

Immediately after reinjection of the labeled leukocytes, activity is distributed throughout the lung. One-half of this lung activity clears within 15 minutes, and it is no longer present by four hours (56). Activity is distributed subsequently to the spleen (30%), liver (30%), and bone marrow (35%) (66). The concentration in the liver and spleen remains unchanged for up to 72 hours (66). Accumulation of approximately 60% of the injected dose in the liver and spleen appears to represent uptake of viable cells as well as removal of damaged cells or cell fragments. The radioactivity found in bone marrow may represent transferrin-free ^{111}In carrier or migration of cellular components. Between 9.5% to 24.4% of the injected dose remains in whole blood and clears with a disappearance half-time of 5 to 7.5 hours (65,56,67). The recovery of ^{111}In-labeled leukocytes has been measured to be 50% to 75% using a mixed leukocyte suspension which was contaminated with RBCs (44,56). Release of radioactivity from the labeled cells is about 3% at one hour and 24% at 24 hours (66).

Dosage. The standard adult (70 kg) dose for ^{111}In labeled leukocytes is 18.5 MBq (500 μCi), whereas the pediatric dose can be adjusted by using a standard table (68) based upon the ⅔ power of a child's body weight with a minimum dose of $\sim$3.7 MBq ($\sim$100 μCi) as suggested by Marcus et al. (69)

Imaging Protocol. The localization of cells at infectious and inflammatory sites is not usually apparent during the first hour post-injection but may be visible at four hours. The relative accumulation of radioactivity in inflammatory lesions reaches a maximum at 24 hours which also coincides with available activity considering a blood disappearance half-time of 5 to 7 hours. Images obtained at 48 hours may be beneficial to monitor intestinal activity seen earlier. If the bowel activity persists at 48 hours, this is an indication of infections and/or inflammatory lesion sites. If, on the other hand, this intestinal activity disappears at 48 hours, this would then indicate that the radioactivity originated from swallowed leukocytes caused by nasopharyngeal or pulmonary infection rather than inflammatory lesion. In normal volunteers, only negligible amounts of ^{111}In (less than 1%) are lost from the body in urine or feces. Therefore, the elimination of injected ^{111}In from the body is probably through physical decay to stable cadmium (66).

^{111}In and Other Chelating Complexes. Other chelating agents of indium have been studied for their ability to label cell components. A major reason for pursuing the use of other agents has been to achieve the labeling of leukocytes in plasma in order to preserve WBC viability and function. Two chelating agents that have been used for leukocyte labeling in plasma are tropolone (2-hydroxy-2,4,6-cycloheptamine-1-one) and mercaptopyridine-N-oxide (MERC) (Figure 4). Both tropolone and MERC are lipophilic chelating agents that label leukocytes using the same mechanisms as ^{111}In-oxine. During a four-month period in late 1981 and early 1982, three independent reports appeared in the literature on the use of ^{111}In-tropolone for labeling blood cells (70,71,34). MERC was first reported by Thakur and Barry in 1982 (49).

Both tropolone and MERC are water-soluble bidentate chelating agents which can be complexed with ^{111}In and will label blood cells in plasma. This seems to be an advantage when compared to oxine, which requires the removal of plasma during the labeling process and, therefore, subjects the cells to possible physical or physiological impairment. However, results of studies directly comparing the *in vitro* and *in vivo* behavior of leukocytes labeled with oxine, tropolone, or MERC are confusing. Some investigators have discovered that tropolone used in the labeling process may diminish chemotactic properties of the labeled leukocytes (72), and that the tropolone may be more toxic to the cell than oxine (73). Although ^{111}In-tropolone-labeled leukocytes have proved slightly more sensitive than leukocytes labeled with ^{111}In-oxine for the detection of abscess on images taken one to four hours post-injection, the difference in lesion detectability is not statistically significant between the two agents at 24 hours. Goedemans et al. (74) have conducted a study comparing the LE among oxine, tropolone, and MERC. They found that if the plasma residue is greater than 30%, neither ^{111}In-tropolone nor ^{111}In-MERC exceeds the LE of ^{111}In-oxine. This seems to suggest that neither tropolone nor MERC is completely free from the "plasma competition" effect with respect to cell LE. In animal studies (37,49), ^{111}In-MERC has been shown to have less uptake in muscle, liver, and spleen at 24 hours, suggesting that leukocyte function may be better preserved with this chelating agent. However, the results from a clinical comparison study (72) indicate that no significant difference in sensitivity was found between ^{111}In-oxine-labeled leukocytes and ^{111}In-MERC-labeled leukocytes.

The conflicting results mentioned above may be the

main reason that there is not yet a manufacturer who is willing to initiate clinical trials for commercialization of either tropolone or MERC. Currently, ^{111}In-oxine is the only chelating radiopharmaceutical approved in the United States for leukocyte labeling.

^{99m}Tc-Labeled Leukocytes

Leukocytes Labeled with ^{99m}Tc Using Phagocytic and Pretinning Methods. Prior to 1976, blood cells were labeled with one of three gamma-emitting radionuclides: ^{51}Cr, ^{67}Ga, or ^{99m}Tc. Neither ^{51}Cr nor ^{67}Ga has ideal physical characteristics for imaging. In contrast, ^{99m}Tc has excellent physical properties, and previous attempts have been made to label leukocytes with ^{99m}Tc by either phagocytic uptake of various ^{99m}Tc-sulfur colloid preparations (75-80) or by incubation of $^{99m}TcO_4^-$ with pretinned leukocytes (similar to the classic method used for RBC labeling) (81-84).

In theory, neither the phagocytic nor the pretinning method for leukocyte labeling requires cell separation. However, in practice, separation techniques are still performed due to the poor LEs *in vivo* and in non-separated cells. Drawbacks with these two methods continue to be poor LE, difficulties in complete separation of ^{99m}Tc products from the ^{99m}Tc-WBCs, significant activity rinsed from the leukocytes during the labeling process, and rapid elution of radioactivity from the cells.

The mechanism for phagocytic labeling appears to be surface adherence rather than phagocytosis, and is the cause for lung and liver ^{99m}Tc uptake following release of the colloidal products (75-80,85). Using WBCs labeled with ^{99m}Tc colloids, Hanna et al. (86) have found that prolonged lung transit time can be explained by aggregation or activation of the neutrophils. Although interest in ^{99m}Tc-colloid has recently been revived by demonstration that, when the particle size of the colloid is rigidly maintained at 2.1 μm, the technique does work (87,88). Many of the commercial sulfur colloid kits contain colloid in which the majority of the particles are between 0.1 and 1 μm, with only 5% greater than 1 μm (89). Marcus et al. (90) have reported success using ^{99m}Tc-microaggregated albumin colloid-labeled WBCs in acute infection. Acute infection was visualized by 30 min, while chronic or low-level infection and/or inflammation took several hours to identify (90). Pending further clinical testing by other investigators, this method promises substantial clinical usefulness.

The pretinning method for labeling ^{99m}Tc-leukocytes with different agents has been previously investigated (81,82), and the glucoheptonate method appears to be the best, demonstrating LEs of $48.3\% \pm 12.6\%$. A recent report indicates that consistent labeling yields of up to 90% have been achieved. However, *in vivo* recovery and biodistribution of ^{99m}Tc-WBCs (labeled using the stannous glucoheptonate pretinning method) in dogs were found to be low and variable unless buffered saline was used (91). Unfortunately, the use of buffered saline in the glucoheptonate method reduces the LE to 50% to 60% (91).

^{99m}Tc-HMPAO-Labeled Leukocytes. In 1986, Peters et al. (92) described the possibility for labeling leukocytes with ^{99m}Tc-HMPAO (Figure 7), with LEs ranging from 41% (93) to greater than 86% (94). This new radiopharmaceutical (i.e., ^{99m}Tc-HMPAO not labeled to WBCs) has been introduced for cerebral perfusion imaging. Due to its lipophilic character, like ^{111}In-oxine, this agent is able to diffuse through the cell membrane and is rapidly incorporated into the leukocytes to bind intracellularly. However, the application of ^{99m}Tc-HMPAO in labeling WBCs as an aid in detecting infectious/inflammatory lesions is not listed in the manufacturer's package insert (95).

Compared with ^{111}In-labeled leukocytes, ^{99m}Tc-HMPAO-labeled leukocytes offers all of the potential advantages of a ^{99m}Tc agent, such as convenience, better imaging characteristics, and reduced patient radiation absorbed dose.

The cost-effective advantage, which is a benefit typical of using ^{99m}Tc agents, is negated by the expense of the HMPAO cold kit. The concept for fractionating the HMPAO kit has been proposed recently (96-100) in order to utilize HMPAO in a more cost-effective manner. Other constraints imposed by the use of ^{99m}Tc-HMPAO include the very short shelf life of ^{99m}Tc-HMPAO (i.e., 30 minutes post-reconstitution) (95) and certain restrictions for the ^{99m}Tc-pertechnetate that must be used in the preparation of ^{99m}Tc-HMPAO (95).

The use of ^{99m}Tc-HMPAO to label leukocytes has resulted in a substantial reduction in radiation exposure to patients (e.g., approximately 30% reduction of radiation absorbed doses to liver, spleen, and bone marrow) with 185 MBq (5 mCi) ^{99m}Tc-labeled leukocytes compared to 18.5 MBq (0.5 mCi) ^{111}In-labeled leukocytes (101). However, there is a higher radiation dose to the person who performs the leukocyte radiolabeling procedure with the ^{99m}Tc agent. Ponto et al. (102) have demonstrated that personnel doses from the ^{99m}Tc-HMPAO leukocyte labeling procedures (starting activities: 518-588.3 MBq [14.0-15.9 mCi]) are 3.3 times higher for the whole body and 4.6 times higher for the hands than are the doses from ^{111}In leukocyte labeling procedures (starting activities: 24.1 to 25.9 MBq [0.65-0.70 mCi]). Therefore, it is important for nuclear pharmacists and nuclear medicine technologists to improve upon methods used for radiation protection in the labeling process of ^{99m}Tc-HMPAO leukocytes.

Biodistribution of ^{99m}Tc-HMPAO-Labeled Leukocytes The initial biodistribution of ^{99m}Tc-HMPAO-labeled leukocytes is similar to that with ^{111}In-labeled granulocytes, showing a rapid initial lung transit, prominent splenic activity, bone marrow activity, and hepatic activity. The blood disappearance curves and percent recovery of ^{99m}Tc and ^{111}In are also comparable (103,104). However, in the studies done by Mock et al. utilizing leukocytes labeled with ^{99m}Tc-HMPAO and ^{111}In-tropolone (105), they have discovered that the respective blood clearance half-times and percent recovery of the two agents were often comparable, but in some cases, the percent recovery of ^{99m}Tc in the blood was lower. This was probably due to different radiochemical purity of the ^{99m}Tc-HMPAO preparations from batch to batch.

Unlike ^{111}In, ^{99m}Tc activity is also seen in urine, occasionally in the gallbladder, and from about four hours, consistently in the bowel.

Elution of 99mTc-HMPAO Activity. Costa et al. (106) studied 47 patients without known inflammatory bowel disease (IBD) or abdominal infection, and labeled cells (which severely limits quantification and kinetic studies) may be the reason for the higher renal, biliary, and bowel excretion. The nonspecific bowel activity (including biliary activity) and urinary activity is probably due to a secondary hydrophilic ^{99m}Tc complex, and only to a small extent to the primary lipophilic complex or free [^{99m}Tc] pertechnetate, since no significant brain, thyroid, or stomach uptake is demonstrated on either the four hour or 24 hour images. The major chemical composition of spontaneous elution *in vitro* has also been confirmed to be a secondary complex of ^{99m}Tc-HMPAO (51.8% to 65.2%) (109).

The unbound ^{99m}Tc activity which localizes in the kidney and bladder may make the detection of urinary tract infection difficult. The nonspecific bowel activity associated with ^{99m}Tc-HMPAO-labeled leukocytes may also present difficulties in the detection of abdominal infection. Surgical management of acute intrabdominal sepsis requires that accurate localization be made as quickly as possible. It has been reported that four hours is the earliest time at which positive uptake in intraabdominal sepsis is routinely noted (92,110) in spite of the nonspecific bowel activity. A recent report by

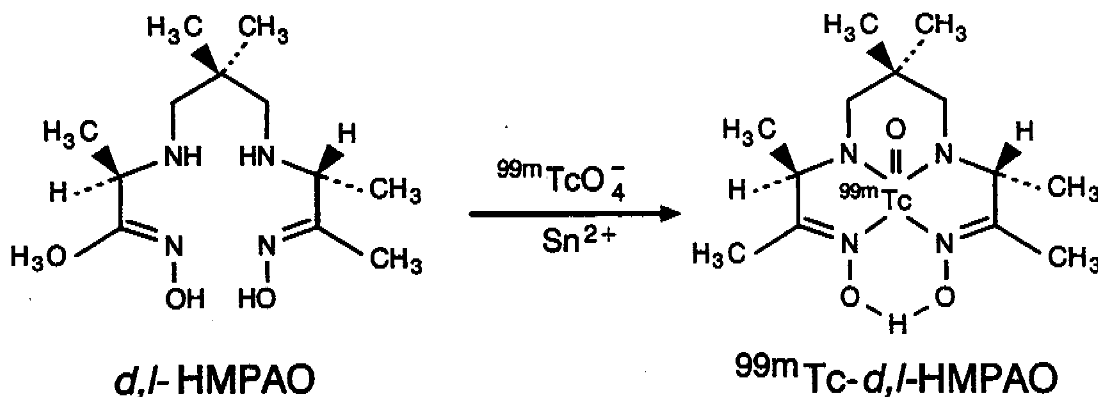

Figure 7. Schematic representation of chemical structures for d,l-HMPAO and ^{99m}Tc-d,l-HMPAO.

found that activity in the small bowel at 30 to 60 minutes post injection was seen in 16% of the ^{99m}Tc-labeled WBC and in 7% of the ^{111}In-labeled WBC studies. This percentage increased to 95% of the ^{99m}Tc-labeled WBC and 33% of the ^{111}In-labeled WBC studies at 18 to 24 hours post injection. In another study (107), the urinary bladder was consistently seen on the 30 to 60 minute study, with approximately 17% cumulative urine activity at 24 hours with ^{99m}Tc-labeled WBC. This was not the case with the ^{111}In studies. It has been found that the *in vitro* elution in saline from WBCs of ^{111}In and ^{99m}Tc was 2% and 9% per hour, respectively (104,108). The higher elution from ^{99m}Tc-

Lantto et al. (111) showed sensitivities for abdominal infection of 74%, 88%, 95% and 96% at two minutes, 30 minutes, two hours, and four hours, respectively. Allan et al. (112) have demonstrated that one-hour ^{99m}Tc-HMPAO leukocyte images for IBD are as informative as three-hour ^{111}In-oxine labeled leukocyte images. This may be related to better resolution associated with the higher photon flux and optimal imaging energy of ^{99m}Tc. The improved resolution is probably also due to the shorter imaging times, which minimize bowel motion and probably also minimize intraluminal movement of activity.

Selective Radiolabeling. Peters et al. (107) have suggested that a pure radiolabeled granulocyte preparation, without the necessity for granulocyte purification from a mixed leukocyte preparation, can be achieved with ^{99m}Tc-HMPAO. Their suggestion is based upon two distinctive features of ^{99m}Tc-HMPAO. First, leukocytes can be labeled in the presence of plasma proteins by using ^{99m}Tc-HMPAO (92); second, radioactivity elution occurs more rapidly from other cell types than from granulocytes (92,108) in a ^{99m}Tc-HMPAO-labeled leukocyte preparation. However, Kelbaek et al. (113) argue that since Peters et al. did not associate their findings with the cell differential counts in their final suspension of radiolabeled granulocytes, the statement regarding selective granulocyte labeling made by Peters et al. may not be supported. Kelbaek et al. (113) had noted that 11% of the ^{99m}Tc-HMPAO activity is attached to lymphocytes and monocytes, when both cell types constitute only 6% of the differential counts.

Potential Pitfalls With Radiolabeled Leukocytes
There has been concern that the sensitivity of ^{111}In-labeled leukocytes may be affected by antibiotic therapy and chemotherapeutic agents. For antibiotics, these factors could reduce leukocyte chemotaxis by killing or suppressing the bacteria and also directly affecting the leukocyte itself (114). In addition, *in vitro* studies have indicated that some chemotherapeutic agents decrease WBC chemotaxis (115,116). Other factors that may potentially cause false-negative WBC scans by affecting leukocyte chemotaxis include steroids, hyperalimentation, hemodialysis, and hyperglycemia. However, clinical studies of ^{111}In-labeled leukocytes in patients with the aforementioned factors show no significant decrease in the sensitivity of the leukocyte scan (114,117-119).

In addition to potential problems involving false-negative results, ^{111}In-labeled leukocytes may present occasional false-positive uptake in tumor. In a study of 249 patients, Fortner et al. (120) found that 12% of patients with known tumor at the time of imaging had false-positive studies. The mechanism of leukocyte uptake in tumor is not completely understood. It may be due to tumor necrosis, free indium bound to transferrin, labeling of lymphocytes in a mixed cell sample, or presence of a variable number of inflammatory cells in certain tumor types. Other causes of false-positive studies with labeled leukocytes include hematoma, early fracture, muscle contusion, and swallowed leukocytes.

However, the major concern, especially with the increasing acquired immune-deficiency syndrome (AIDS) incidence in the past few years, has to be either infection of health care workers with human immune-deficiency virus (HIV) from a needle stick during the labeling process, or the possibility of the misadministration of HIV-infected blood cells to a non-HIV patient. This latter concern has, in fact, recently occurred.

For these reasons, there have been numerous efforts to develop a "kit preparation" so that the laborious blood cell separation, labeling, and reinjection can be avoided. Other promising alternatives to radiolabeled autologous leukocytes have been used in clinical studies with some success, such as ^{111}In-labeled nonspecific polyclonal human immunoglobulin-G (IgG) and ^{99m}Tc-labeled antileukocyte monoclonal antibodies.

RADIOLABELED ANTIBODIES

Recently there has been a great deal of interest in the development of radiolabeled antibodies for the detection of infection and inflammation.

Two approaches have been described for the radiolabeled antibody imaging of infections:

1. Antibodies directed against a surface determinant of the infectious organism.

2. Antibodies directed against cells responding to the infection such as white blood cells.

Although the concept for application of radiolabeled antibodies to infection/inflammation imaging appears to be quite simple, there are some major difficulties which must be addressed prior to a wide clinical application of these non-neoplastic radioimmune imaging agents:

1. The antigen-antibody concentration at the lesion site should be maximized.

2. The target-to-nontarget ratios *in vivo* should be high.

3. The surface antigens should not be shed from the cell during the study.

4. Antibodies which cause lysis of cells, especially in the presence of complement, (i.e., complement-mediated lysis), should not be used.

5. Human directed response against murine antibody (HAMA) must be nil.

^{111}In-Labeled Nonspecific Polyclonal Human Immunoglobulin-G

In 1988, Reburn, Fischman, and coworkers at Massachusetts General Hospital developed IgG for the

scintigraphic detection of foci of infection (121-125). Milligram quantities (0.025 mg per kilogram of body weight) of Sandoz IgG modified for intravenous use were labeled with approximately 55.9 MBq (1.5 mCi) of ^{111}In via the cyclicanhydride diethylenetriamine-penta-acetic acid (DTPA) method.

When compared with the currently available infection and inflammation imaging agents, ^{111}In-IgG has a number of advantages:

1. Early localization at the site of focal inflammation (the study can often be completed within one day), which may lead to more rapid diagnosis.

2. Unlike ^{67}Ga and ^{99m}Tc-HMPAO-labeled WBCs, ^{111}In-IgG has minimal bowel excretion; therefore, abdominal infection can be more easily identified without the use of cathartics.

3. ^{111}In-IgG may detect nonbacterial infection (e.g., fungus-caused infection), AIDS, and malignancies, whereas radiolabeled leukocytes only occasionally delineate these abnormalities.

4. The radiation dosimetry of ^{111}In-IgG permits administration of 55.5 MBq (1.5 mCi) ^{111}In with a radiation burden of $<$ 40 mGy (4 rad) to the spleen.

5. IgG will be available as a kit, which is easier and quicker to prepare than labeling leukocytes.

6. Nonlabeled IgG has already been approved for human use.

7. Since there is no need to withdraw a large volume of blood for cell labeling, ^{111}In-IgG should be quite suitable for pediatric patient use.

8. No HAMA response is associated with the use of ^{111}In-IgG since the IgG is obtained from humans.

However, ^{111}In-IgG has four major limitations. First, ^{111}In-IgG has a higher background of normal uptake in the liver, spleen, and to a lesser extent, in the urinary bladder. This may interfere with the diagnosis of infection in these areas. Second, the sensitivity of the ^{111}In-IgG study is decreased in patients with azotemia. Third, ^{111}In-IgG may localize in noninfected tumor sites, as does ^{67}Ga-citrate, producing a false-positive result. Fourth, false-negative results may occur in infections with little associated inflammation (^{111}In-labeled WBCs may also miss these types of infections).

The exact mechanism of IgG uptake at sites of infection and inflammation is not fully understood. Many possible explanations have been proposed. First, nonspecific IgG binds to the enhanced expression of the Fc receptors in circulating macrophages, leukocytes, and lymphocytes in the presence of focal inflammation. This hypothesis was based on animal experiments showing good localization of intact IgG and Fc fragments, but minimal localization of Fab fragments at the site of inflammation (121). However, studies by Oyen et al. (126) and Juweid et al. (125) provide sufficient evidence to reject this hypothesis. Oyen et al. (126) have demonstrated that ^{111}In-IgA, an immunoglobulin that contains hardly any Fc receptors and which lacks Fc gamma receptor affinity, also accumulated in infectious foci. When IgG was treated with endoglycosidase-F to remove carbohydrates from IgG, *in vitro* Fc-receptor binding was markedly decreased, but localization at sites of infection was unaffected (125). Second, localization of IgG in sites of infection may be through the binding to bacterias (127). Although this may be a possible explanation for the mechanism of uptake, it does not explain IgG uptake at sites of fungal infection or sterile inflammation. Third, it is likely that increased capillary permeability, which is a hallmark of inflammation causing redness and swelling, probably plays the major role. However, studies comparing labeled ^{99m}Tc-human serum albumin and ^{67}Ga with IgG showed greater IgG accumulation as early as three hours post-injection (124). Therefore, this phenomenon may not be due solely to increased blood flow and permeability at sites of inflammation.

Although the mechanism for uptake of nonspecific IgG remains obscure, Fischman et al. (128) have suggested that the Fc portion of IgG is the imaging fragment of choice in detecting hepatic and splenic lesions and in imaging vascular infection since the Fc fragment has approximately 50% lower accumulation in the liver and spleen than IgG, and it also clears from the blood pool more rapidly than IgG. They further speculate that it is possible that other Fc fragments, such as mFc, sFc, or stFc (128), may be more effective imaging agents. As of this time, labeled nonspecific IgG is not an FDA-approved agent.

^{99m}Tc-Labeled Monoclonal Antigranulocyte Antibody (^{99m}Tc-Labeled BW 250/183)

A "neutrophil specific" monoclonal antibody would have a greater potential for clinical application than nonspecific ^{111}In-IgG because of higher specificity as well as the sensitivity associated with monoclonal antibodies when compared to polyclonal antibodies such

as IgG, which cannot discriminate inflammation from other diseases with increased capillary permeability. The drawbacks of the murine monoclonal antibody directed to a leukocyte antigen are the lack of FDA approval in the U.S. and the possible development of HAMA response in patients.

An antigranulocyte IgG monoclonal antibody (MAb BW 250/183) was developed by Behringwerke (Marburg, Germany). This antibody is directed at a nonspecific cross-reacting antigen (NCA-95), a 95 kD surface glycoprotein, present on almost all human granulocyte surfaces, which allows the antibody to interact with "almost 95% of all granulocytes" (129). Despite the strong affinity between MAb BW 250/183 and the granulocyte membrane, there is no occurence of either antibody-dependent or complement-dependent lysis of cells (130). Joseph et al. (131,132) labeled 75 to 400 mg of monoclonal antibody with 296 MBq (8 mCi) ^{99m}Tc using the Schwarz method. In order to achieve an efficient labeling of the antibody with ^{99m}Tc, the Schwarz method calls for the reduction of antibody disulfide bonds to free thiol groups, to which reduced ^{99m}Tc binds. The reduced antibody was lyophilized in the presence of the phosphate buffer (pH 7.2). The labeling of the antibody with ^{99m}Tc is similar to a kit procedure for preparing ^{99m}Tc-labeled radiopharmaceuticals. First, the reduction of $^{99m}TcO_4^-$ is achieved by using $SnCl_2$ obtained from any commercially available kits, such as PYP (pyrophosphate), DTPA, or MDP (medronate). The reduced ^{99m}Tc then binds to the antibody. The LE has been reported to be greater than 95% (133).

The half-life for disappearance of ^{99m}Tc-labeled BW 250/183 from the blood is approximately six hours. The initial uptake in the lungs clears by four hours. Approximately 17% of the injected dose remains in the liver, and approximately 10% of the activity is seen in the spleen at five hours after administration. Activity in bone marrow is always visible. At six hours post-injection, approximately 25% of the circulating radioactivity is bound to the granulocytes. This number is not high enough to be consistent with a monoclonal antibody that is supposed to be highly specific for the blood cells.

In their studies, Joseph et al. (131) have found that 10% to 20% of the patients showed a HAMA response with block potential, whereas about 20% to 30% of the patients had a detectable HAMA response without blocking potential. Joseph et al. (131) indicate that the HAMA response is mostly of the IgM type, which is very weak and is detectable two to ten months after the scan.

Becker et al. (134) have observed that the recovery rate of ^{99m}Tc-BW 250/183-labeled granulocytes was less than 15% at 90 minutes, which is much lower than that observed for ^{111}In-oxine-labeled leukocytes and ^{99m}Tc-HMPAO-labeled leukocytes. This may indicate that the localization of this antibody at foci of infection may not be primarily attributable to binding to the NCA-95 surface glycoprotein *in vivo*. McAfee et al. (135) suggest that the free MAb BW 250/183 may migrate to an infectious focus and then bind to viable or damaged granulocytes that are already localized there.

OTHER AGENTS

^{99m}Tc-Labeled Nanocolloid

In 1986, human albumin colloid particles with a mean diameter of 30 nm (Nanocoll, Solco Basel, Switzerland) were labeled with technetium-99m (^{99m}Tc-labeled nanocolloid) (Figure 8) and were reported to be effective in the detection of infection and inflammation (136). A possible mechanism for uptake of ^{99m}Tc-nanocolloid in foci of infection are the small colloidal particles which freely diffuse through the increased leaky vascular endothelium and basement membrane at the infectious/inflammatory site.

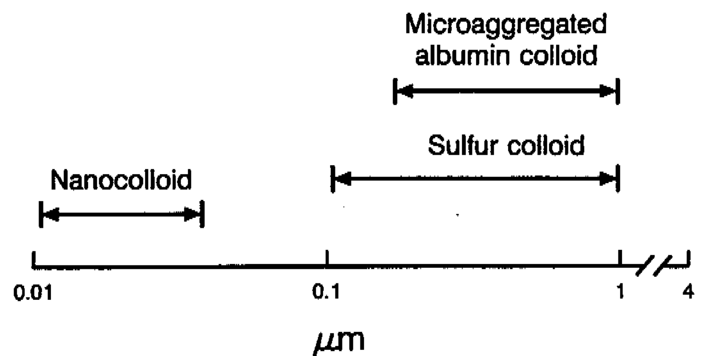

Figure 8. **Size ranges for three different particulate radiopharmaceuticals which are used for imaging of infectious and inflammatory lesions.**

This localization process is similar to microcolloids of serum albumin with a larger particle size (0.2-0.3 μm). Due to the smaller size of ^{99m}Tc-labeled nanocolloid, the blood clearance is much faster than with microcolloids. Thus, imaging of ^{99m}Tc-labeled nanocolloid can be completed within four hours. Thereafter, activity may appear in the gastrointestinal tract which may be attributed to spontaneous oxidation.

^{99m}Tc-labeled nanocolloid has been thought to be useful in the detection of osteomyelitis (137) and

suspected bone or joint disease (138,139), whereas this agent has been shown to be less sensitive than ^{111}In leukocytes for the assessment of IBD (140,141) and soft tissue abscess or prosthetic vascular graft infection (138). ^{99m}Tc-labeled nanocolloid may provide the quickest answer, but cannot differentiate between sterile and septic inflammatory lesions. This agent is not approved for use in the USA by the FDA.

^{111}In Labeled Avidin-Biotin

Recently streptavidin or avidin, both of which are xenoproteins, have been proposed by Rusckowski et al. (142-144) as alternatives to radiolabeled IgG for imaging infection. The nonspecific accumulation of IgG has been attributed to increased vascular permeability (145), and Rusckowski et al. (144) have reasoned that the protein streptavidin may exhibit the same nonspecific mechanism of uptake as IgG. The results in their study (144) using a mouse model with an E. coli infection show that both ^{111}In-labeled IgG and ^{111}In-labeled strepavidin have similar levels of localization at the site of infection. This observation strengthens the suggestion that the accumulation of radiolabeled IgG may result from nonspecific diffusion through leaky capillaries (145).

However, a common drawback in the use of both labeled IgG and streptavidin is the excess uptake in liver, spleen, and kidneys, and the slow clearance of activity from blood, both of which result in low target-to-background ratios. In order to further improve the target-to-background ratios, Rusckowski et al. (144) have used unlabeled streptavidin to "pretarget" the abscess before the administration of radiolabeled biotin, a relatively small molecule with high affinity (K_a is approximately 10^{15}) (146) for both avidin and streptavidin. This high affinity of biotin for streptavidin and the rapid blood clearance of unlabeled biotin improved the infected thigh-to-normal thigh ratio by three fold, and the infected thigh-to-liver and blood ratios by eight fold in comparison with labeled IgG or labeled streptavidin (144). If the pretargeting avidin-biotin concept for localizing infection can be successfully applied in humans, the lower background levels of radiolabeled avidin-biotin would allow an earlier imaging time. However, there is evidence that avidin and streptavidin are immunogenic in humans, even at low doses (147). These agents are not yet FDA approved.

CONCLUSIONS

A number of radiopharmaceuticals are available for diagnosing infectious and inflammatory lesions. In the past, the radionuclide of choice for the detection of occult infection has been ^{67}Ga. However, ^{67}Ga is not specific for abscess localization, as it also localizes in tumor. In addition, ^{67}Ga-citrate has other disadvantages (e.g., poor target-to-nontarget ratio, normal bowel activity). Therefore, the radiolabeled leukocyte technique has been developed and is increasingly applied to infection/inflammation imaging. The use of ^{111}In-labeled leukocytes has been well established in the field of nuclear medicine and has allowed a number of infectious and inflammatory processes to be investigated. In fact, the use of ^{111}In-labeled WBCs has become the gold standard for imaging infectious and inflammatory foci. Recently, a number of newer ^{99m}Tc-labeled radiopharmaceuticals (e.g., ^{99m}Tc-albumin colloid and ^{99m}Tc-HMPAO) have been used for leukocyte labeling. These ^{99m}Tc-labeled WBCs require less time between reinjection and imaging and have demonstrated good sensitivity in localization of lesions. Another new area of investigation is the use of radiolabeled antibodies (e.g., ^{111}In-IgG and ^{99m}Tc-labeled BW 250/183) for the detection of infection and/or inflammation, which has shown improved sensitivity and specificity. Finally, ^{99m}Tc-labeled nanocolloids and ^{111}In-labeled avidin-biotin are also being studied as potential infection/inflammation imaging agents.

The currently available agents which have FDA approval for the detection of infectious and/or inflammatory lesions are ^{67}Ga citrate and ^{111}In-labeled leukocytes. ^{67}Ga citrate is best used when an FUO is suspected, as it localizes not only in areas of acute and chronic infection but also in tumors and chronic granulomatous disease, all of which may cause an FUO. ^{67}Ga has also been shown to be superior to ^{111}In-labeled leukocytes in the evaluation of disc space infection. When single photon emission computed tomography is required, ^{67}Ga is also the agent of choice due to the larger amount of activity that can be administered. This is especially true when malignant external otitis is suspected. ^{111}In-labeled leukocytes are preferred when infection or inflammation is suspected in soft tissues or the musculoskeletal system. For musculoskeletal sepsis, a combination of bone scintigraphy and ^{111}In-labeled leukocytes is recommended for non-marrow containing bone, and a combination of ^{111}In-labeled leukocytes and a marrow imaging study (usually with ^{99m}Tc-sulfur colloid) is recommended in marrow-containing regions in order to help differentiate the normal leukocyte distribution within marrow from sites of infection or inflammation. Some investigators believe that a pure granulocyte preparation should be used when studying suspected vascular graft infection, as labeled platelets can localize on foreign surfaces, although work with mixed cell populations has shown good sensitivity and specificity. A purified granulocyte preparation has also

been recommended for the evaluation of inflammatory bowel disease, although again both the ^{111}In and ^{99m}Tc mixed cell preparations have shown good results clinically.

The use of ^{99m}Tc-HMPAO-labeled leukocytes has been increasing rapidly. The use of ^{99m}Tc-HMPAO to label leukocytes is not yet FDA approved. This agent will be especially useful in children, where radiation dosimetry is a concern with the ^{111}In-labeled leukocytes. With the ^{99m}Tc-HMPAO-labeled WBCs, special concern is needed in evaluating sites of abdominal infection and inflammation, as the normal distribution of ^{99m}Tc-HMPAO-labeled leukocytes includes a significant component of bowel activity after two to four hours. Once the newer indication for ^{99m}Tc HMPAO is approved, this agent may have significant benefit over our current radiopharmaceuticals. Some articles (148,149) have addressed the issue as to the use of approved drugs in a nonapproved way (e.g., unlisted indication, dosage, route of administration, or age group); it is generally believed that the unapproved use of approved drugs should be considered as part of the practice of medicine provided the physician is well informed regarding the drug and that the unapproved usage be based on firm scientific rationale.

Finally, it is important to remember that in both infectious and non-infectious inflammatory lesions the same pathophysiology is present. Therefore, it is unlikely, that with utilization of either currently approved agents or agents now under investigation for future use that one will be able to differentiate with any degree of accuracy infectious from non-infectious inflammatory processes.

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REFERENCES

1. Jordan CE, James AE Jr., Hodges FJ III. Comparison of the cerebral angiogram and the brain radionuclide image in brain abscess. *Radiology* 1972; 104:327-311.

2. Crocker EF, McLaughlin AF, Morris JG, Benn R, McLeod JG, Allsop JL. Technetium brain scanning in the diagnosis and management of cerebral abscess. *Am J Med* 1974; 56:192-201.

3. McKillop JH, Holtzman DS, McDougall IR. Detection of subdural empyema with radionuclides. *Clin Nucl Med* 1980; 5:263-267.

4. Gilday DL. Various radionuclide patterns of cerebral inflammation in infants and children. *AJR* 1974; 120:247-253.

5. Geslien, GE, Thrall JH, Johnson MC. Gallium scanning in acute hepatic amebic abscess. *J Nucl Med* 1974; 15:561-563.

6. Preimesberger KF, Goldberg ME. Acute liver abscess in chronic granulomatous disease of childhood. *Radiology* 1974; 110:147-150.

7. Schauwecker DS. The scintigraphic diagnosis of osteomyelitis. *AJR* 1992; 158:9-18.

8. Lavender JP, Lowe J, Barker JR, Burn JI, Chaudhri MA. Gallium 67 citrate scanning in neoplastic and inflammatory lesions. *Br J Radiol* 1971; 44:361-366.

9. Hoffer PB. Mechanisms of localization. In: Hoffer PB, Beckerman C, Henkin RE, editors. *Gallium-67 Imaging*. New York:Wiley, 1978:3-7.

10. Tsan MF. Studies of gallium accumulation in inflammatory lesions: III. Roles of polymorphonuclear leukocytes and bacteria. *J Nucl Med* 1978; 19:492-495.

11. Menon S, Wagner HN, Tsan M. Studies on gallium accumulation in inflammatory lesions. II. Uptake by staphylococcus aureus: Concise Communication. *J Nucl Med* 1978; 19:44-47.

12. Camargo EE, Wagner HN, Tsan M Jr. Gallium accumulation in inflammatory lesions: role of polymorphonuclear leukocytes in the distribution of gallium in experimental inflammatory exudates. *Nuklearmedizin* 1979; 18:147-150.

13. Tzen KY, Oster ZH, Wagner HN Jr, Tsam MF. Role of iron-binding proteins and enhanced capillary permeability on the accumulation of Gallium-67. *J Nucl Med* 1980; 21:31-35.

14. Vorne M, Soini I, Lantto T, Paakkinen S. Technetium-99m HM-PAO-labeled leukocytes in detection of inflammatory lesions: comparison with gallium-67 citrate. *J Nucl Med* 1989; 30:1332-1336.

15. Whalen JL, Brown ML, McLeod R, Fitzgerald RH. Limitations of Indium leukocyte imaging for the diagnosis of spine infections. *Spine* 1991; 16:193-197.

16. Bruschwein DA, Brown ML, McLeod RA. Gallium scintigraphy in the evaluation of disk-space infections: concise communication. *J Nucl Med* 1980; 21:925-927.

17. Sfakianakis GN, Al-Sheikh W, Heal A, Rodman G, Zeppa R, Serafini A. Comparisons of scintigraphy with In-111 leukocytes and Ga-67 in the diagnosis of occult sepsis. *J Nucl Med* 1982; 23:618-626.

18. Hussein MAD, Breen J, Leslie EV. Clinical experience with [^{111}In] indium chloride scanning in inflammatory diseases. *Clin Nucl Med* 1978; 3:196-201.

19. Sayle BA, Balachandran S, Rogers CA. Indium-111 chloride imaging in patients with suspected abscesses: concise communication. *J Nucl Med* 1983; 24:1114-1118.

20. Medi-Physics, Inc. INDICLOR™ High Purity Indium Chloride In-111 Sterile Solution package insert. Arlington Heights, IL: 1993 January.

21. Cytogen Corporation. OncoScint® CR/OV Kit (Satumomab Rendetide) package insert. Princeton, NJ: 1992 December.

22. John H. Miller, M.D. Division of Nuclear Radiology, Childrens Hospital of Los Angeles, Los Angeles, CA [personal commumication].

23. Robert F. Carretta, M.D. Nuclear Medicine Department, Roseville Community Hospital, Roseville, CA [personal communication].

24. McAfee JG, Subramanian G, Gagne G. Technique of leukoccyteharvesting and labeling: problems and perspectives. *Semin Nucl Med* 1984; 14:83-106.

25. Baker WJ, Datz FL. Preparation and clinical utility of In-111 labeled leukocytes. *J Nucl Med Technol* 1984; 12:131-136.

26. Peters AM, Saverymuttu SH. The value of Indium-labelled leucocytes in clinical practice. *Blood Reviews* 1987; 1:65-76.

27. Roy AJ, Franklin A, Simmons WB, et al. A method for separation of granulocytes from normal human blood using hydroxyethyl starch. *Prepar Biochem* 1971; 1:197-203.

28. Boyum A. Separation of blood leucocytes, granulocytes and lymphocytes. *Tissue Antigens* 1974; 24:269-274.

29. McAfee JG, Thakur ML. Survey of radioactive agents for in vitro labeling of phagocytic leukocytes. I. Soluble agents. *J Nucl Med* 1976; 17:480-487.

30. Colas-Linhart NC, Barbu M, Gougerot A, Bok B. Five leucocyte labeling techniques: a comparative study. *Br J Haematol* 1983; 53:31-41.

31. Dooley DC, Takahashi T. The effect of osmotic stress on the function of the human granulocyte. *Exp Hematol* 1981; 9:731-741.

32. Kaminsky D, inventor. Syncor International Corporation, assignee. *Method for labelling leukocytes with indium in-111 oxine*. U.S. Patent 5,093,104. 1992 March 3.

33. Loos H, Blok-Schut B, Van Doom R, Holsbergen B, delaRiviere AB, Meerhof L. A method for the recognition and separation of human blood monocytes in density gradients. *Blood* 1976; 48:731-742.

34. Danpure HJ, Osman S, Brady F. The labelling of blood cells in plasma with ^{111}In-tropolonate. *Br J Radiol* 1982; 55:247-249.

35. Saverymuttu SH, Peters AM, Danpure HJ, Reavy HJ, Osman S, Lavender JP. Lung transit of ^{111}Indium-labelled granulocytes. *Scan J Haematol* 1983; 30:151-160.

36. Dooley DC, Simpson JF, Meryman HT. Isolation of large numbers of fully viable human neutrophils: a preparation technique using Percoll density gradient centrifugation. *Exp Hematol* 1982; 10:591-599.

37. Thakur ML Seifert CL, Madsen MT, McKenney SM, Desai AG, Park CH. Neutrophil labeling: problems and pitfalls. *Semin Nucl Med* 1984; 14:107-117.

38. Rabinowitz Y. Separation of lymphocytes and polymorphonuclear leukocytes on glass columns. *Blood* 1964; 23:811-827.

39. McAfee JG, Thakur ML. Survey of radioactive agents for *in vitro* labeling of phagocytic leukocytes. II. Particles. *J Nucl Med* 1976; 17:488-492.

40. Loken MR, Stall AM. Flow cytometry as an analytical and preparative tool in immunology. *J Immunol Methods* 1982; 50:R85-R112.

41. Schauwecker DS, Burt RW, Park H-M, Mock BH, Tobolski MM, Yu P-I, et al. Comparison of purified Indium-111 granulocytes and Indium-111 mixed leukocytes for imaging of infections. *J Nucl Med* 1988; 29:23-25.

42. Peters AM. Recent advances in cell labelling [editorial]. *Nucl Med Commun* 1987; 8:313-316.

43. Thakur ML, McKenney SL, Park CH. Evaluation of indium-111-2-mercaptopyridine-N-oxide for labeling leukocytes in plasma: a kit preparation. *J Nucl Med* 1985; 26:518-523.

44. Thakur ML, Coleman RE, Welch MJ. Indium-111-labeled leukocytes for the localization of abscess: preparation, analysis, tissue distribution, and comparison with gallium-67 citrate in dogs. *J Lab Clin Med* 1977; 89:217-228.

45. Segal AW, Deteix P, Garcia R, Tooth P, Zanneli GD, Allison AC. Indium-111 labeling of leukocytes: a detrimental effect on neutrophil and lymphocyte function and an improved method of cell labelling. *J Nucl Med* 1978; 19:1238-1244.

46. Danpure HJ, Osman S. Cell labelling and cell damage with indium-111 acetylacetone--an alternative to indium 111 oxine. *Br J Radiol* 1981; 54:597-601.

47. Saverymuttu SH, Crofton ME, Peters AM, Lavender JP. Indium-111 tropolonate leucocyte scanning in the detection of intra-abdominal abscesses. *Clinical Radiology* 1983; 34:593-596.

48. Joist JH, Baker RK, Thakur ML, Welch MJ. Indium-111-labeled human platelets: uptake and loss of label and in vitro function of labeled platelets. *J Lab Clin Med* 1978; 92:829-836.

49. Thakur ML, Barry MJ. Preparation and evaluation of a new Indium-111 agent for efficient labeling of human platelets in plasma. Proceedings of the Fourth International Symposium on Radiopharmaceutical Chemistry. *J Label Comp Radiopharm* 1982; 19:1410-1412.

50. Sinzinger H, Angelberger P, Kolbe H, Ludwig H. Methodological study of In-111-oxine human-platelet and leukocyte labeling. *J Label Comp Radiopharm* 1981; 18:69-71.

51. Clay ME, McCullough J. Studies of the granulocyte cytotoxicity assay [abstract]. *Transfusion* 1978; 18:395.

52. Chilton HM, Burchiel SW, Watson NE Jr. Radiopharmaceuticals for imaging tumors and inflammatory processes: gallium, antibodies, and leukocytes. In: Swanson DP, Chilton HM, Thrall JH, editors. *Pharmaceuticals in Medical Imaging*. New York: MacMillan Publishing, 1990:587.

53. English D, Clanton JA. Evaluation of neutrophil labeling techniques using the chemotaxis radioassay. *J Nucl Med* 1984; 25:913-916.

54. Mortelmans L, Verbruggen A, Malbrain S, De Backker C, Heynen MJ, Boogaerts M, et al. Isolation of granulocytes. *Nuc Compact* 1988; 19:141-145.

55. Peters AM, Lavender JP. Platelet kinetics with indium-111 platelets: comparison with chromium-51 platelets. *Semin Thromb Hemostas* 1983; 9:100-114.

56. Thakur ML, Lavender PJ, Arnot RN, Silvester DJ, Segal AW. Indium-111-labeled autologous leukocytes in man. *J Nucl Med* 1977; 18:1014-1021.

57. Datz FL, Jacobs J, Baker W, Landrum W, Alazraki N, Taylor A Jr. Decreased sensitivity of early imaging with In-111 oxine-labeled leukocytes in detection of occult infection: concise communication. *J Nucl Med* 1984; 25:303-306.

58. Weisberger AS, Heinle RW, Storasli JP, Hannah R. Transfusion of leukocytes labeled with radioactive phosphorus. *J Clin Invest* 1950; 29:336-341.

59. Loken MK, Clay ME, Carpenter RT, Boudreau RJ, McCullough JJ. Clinical use of indium-111 labeled blood products. *Clin Nucl Med* 1985; 10:902-911.

60. McCullough J, Weiblen BJ, Clay ME, Forstrom LA. Effect of leukocyte antibodies on the fate in vivo of indium-111 labeled granulocytes. *Blood* 1981; 58:164-170.

61. Amersham receives "approvable letter" from FDA for indium-111 oxyquinoline [newsline]. *J Nucl Med* 1985; 26:1359.

62. Datz FL, Taylor AT Jr. Cell labelling techniques and clinical utility. Section I: radiolabeled leukocytes. In: Freeman LM, editor. *Freeman and Johnson's Clinical Radionuclide Imaging*. 3rd ed. Orlando: Grune & Stratton, 1986:1785-1847.

63. 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; 82:217-228.

64. Thakur ML, Gottschalk A. Experiences with indium-111 oxine. In: Thakur ML, Gottschalk A, editors. *Indium-111 labeled neutrophils, platelets, and lymphocytes*. New York: Triverum, 1981: 41-50.

65. Goodwin DA, Doherty PW, McDougall IR. Clinical use of indium-111 labeled white cells: an analysis of 312 cases. In: Thakur ML, Gottschalk A, editors. *Indium-111 labeled neutrophils, platelets and lymphocytes*. New York: Triverum, 1981:131.

66. Amersham Corporation. Indium In 111 Oxyquinoline Solution package insert. Arlington Heights, IL: 1989 May.

67. Weiblen BJ, Forstrom LA, McCullough J. Studies of the kinetics of Indium-111 labeled granulocytes. *J Lab Clin Med* 1979; 94:246-255.

68. NCRP. Protection in nuclear medicine and ultrasound diagnosis procedures in children. Bethesda (MD): NCRP 1983. NCRP Report No.73:10.

69. Marcus C, Stabin MG, Watson. Pediatric radiation dose from [^{111}In]leukocytes. [letter]. *J Nucl Med* 1986; 27:1220-1221.

70. Dewanjee MK, Rao SA, Didisheim P. Indium-111 tropolone, a new high-affinity platelet label: preparation and evaluation of labeling parameters. *J Nucl Med* 1981; 22:981-987.

71. Burke JET, Roath S, Ackery D, Wyeth P. The comparison of 8-Hydroxyquinolone, tropolone, and acetylacetone as mediators in the labelling of polymorphonuclear leucocytes with indium-111: a functional study. *Eur J Nucl Med* 1982; 7:73-76

72. Gunter KP, Lukens JN, Clanton JA, Morris PJ, Janco RL, English D. Neutrophil labeling with indium-111: tropolone vs. oxine. *Radiology* 1983:149:563-566.

73. Hall CE, Gunson BK, Hawkes JR. Indium-tropolone for cell labeling--a questionable advance [abstract]. *Haemostasis* 1982; 12:193.

74. Goedemans WT, T de Jong MM. Comparison of several Indium-111 ligands in labeling blood cells: effect of diethylpyrocarbonate and CO_2. *J Nucl Med* 1987; 28:1020-1026.

75. Fisher CH, Scheffel U, Tsan MF, Rhodes BA, Wagner HN. Leukocyte tagging by phagocytosis of Tc-99m human serum albumin microspheres. use in localization of experimental abscesses by external scanning [abstract]. *J Nucl Med* 1975; 16:527.

76. English D, Andersen BR. Labeling of phagocytes from human blood with Tc-99m sulfur colloid. *J Nucl Med* 1975; 16:5-10.

77. Bagwe BA, Sharma SM. Bone accumulation of Tc-99m labeled leukocytes. *J Nucl Med* 1976; 17:313-316.

78. Uchida T, Vincent PC. In vitro studies of leukocyte labeling with technetium-99m. *J Nucl Med* 1976; 17:730-736.

79. Papierniak CK, Bourey RE, Kretschmer RR, Gotoff SP, Colombetti LG. Technetium-99m labeling of human monocytes for chemotactic studies. *J Nucl Med* 1976; 17:988-992.

80. English D, Andersen BR. Organ distribution of canine leukocytes labeled with Tc-99m sulfur colloid. *J Nucl Med* 1977; 18:289-295.

81. Gerson B, Ballinger JR, Reid RH, Gulenchyn KY. Comparison of pretinning methods to label leukocytes with technetium-99m. *J Nucl Med Technol* 1988; 16:64-66.

82. Kelbaek H, Fogh J. Technetium-99m labeling of polymorphonuclear leukocytes: preparation with two different stannous agents. *J Nucl Med* 1985; 26:68-71.

83. Kelbaek H. Isolation and labelling of human leukocytes with ^{99m}Tc. *Eur J Nucl Med* 1986; 12:107-109.

84. Gil MC, Straub RF, Srivastava SC, Godoy N, Mena P, Lopez I. A new kit method for efficient labeling of leukocytes and platelets with Tc-99m [abstract]. *J Nucl Med* 1986; 27:946.

85. Mock BH, English D. Leukocyte labeling with technetium-99m tin colloids. *J Nucl Med* 1987; 28:1471-1477.

86. Hanna R, Braun T, Levendel A, Lomas F. Radiochemistry and biostability of autologous leukocytes labelled with Tc-99m-stannous colloid in whole blood. *Eur J Nucl Med* 1984; 9:216-219.

87. Pullman W, Hanna R, Sullivan P, Booth JA, Lomas F, Doe WF. Technetium-99m autologous phagocyte scanning: a new imaging technique for inflammatory bowel disease. *Br Med J* 1986; 293:171-174.

88. Hanna RW, Lomas FE. Identification of factors affecting Tc-99m leukocyte labelling by phagocytic engulfment and development of an optimal technique. *Eur J Nucl Med* 1986; 12:159-162.

89. Davis MA, Jones AG, Trundade H. A rapid and accurate method for sizing radiocolloids. *J Nucl Med* 1974; 15:923-928.

90. Marcus CS, Kuperus JH, Butler JA, Henneman PL, Salk RD, Chen BY, et al. Phagocytic labeling of leukocytes with ^{99m}Tc-albumin colloid for nuclear imaging. *Nucl Med Biol* 1988; 15:673-682.

91. Srivastava SC, Straub RF. Blood cell labeling with ^{99m}Tc: progress and perspectives. *Semin Nucl Med* 1990; 20:41-51.

92. Peters AM, Osman S, Henderson BL, Kelly JD, Danpure HJ, Hawker RJ, et al. Clinical experience with ^{99m}Tc-hexamethylpropylene-amine oxime for labelling leucocytes and imaging inflammation. *Lancet* 1986; 2:946-949.

93. Uno K, Imazeki K, Yoshikawa K, Arimizu N. Clinical use of technetium-99m HMPAO labeled leukocytes for inflammatory imaging [abstract]. *J Nucl Med* 1987; 28:648.

94. Rao SA, Aksut G, Trembath LA, Collier BD. Evaluation of Tc-99m HMPAO for leukocyte labeling. *J Nucl Med* 1987; 28:638-639.

95. Amersham Corporation. Technetium Tc-99m Exametazime Injection package insert. Arlington Heights, IL; 1990 November.

96. Piera C, Pavia A, Bassa P, Garcia J. Preparation of [^{99m}Tc] HM-PAO [letter]. *J Nucl Med* 1990; 31:127-128.

97. Ballinger J. Preparation of technetium-99m-HMPAO [letter]. *J Nucl Med* 1990; 31:1892.

98. Sampson CB, Solanki C, Barber RW. ^{99}Tc^{m}-exametazime-labelled leucocytes: effect of volume and concentration of exametazime on labelling efficiency, and clinical protocol for high efficiency multi-dose radiolabeling. *Nucl Med Commun* 1991; 12:719-723.

99. Hawkins T, Reeder A, Keavey PM, Thakker S, Hawker R. The long-term stability of reconstituted exametazime: a clinical and laboratory evaluation. *Nucl Med Commun* 1991; 12:1045-1055.

100. Morrissey GJ, Powe JE. Routine application of fractionated HMPAO stored at -70°C for WBC scintigraphy. *J Nucl Med* 1993; 34:151-155.

101. Roddie ME, Peters AM, Osman S, Danpure HJ, Lavender JP, Neirinckx RD. Osteomyelitis. *Nucl Med Commun* 1988; 9:713-717.

102. Ponto JA, Graves JM. Personnel radiation dose from leukocyte labeling procedures: indium-111 oxine versus technetium-99m HMPAO. *J Nucl Med Technol* 1991; 19:236-237.

103. McAfee JG, Subramanian G, Gagne G. ^{99m}Tc-HM-PAO for leukocyte labeling - experimental comparison with ^{111}In oxine in dogs. *Eur J Nucl Med* 1987; 13:353-357.

104. Becker W, Schomann E, Fischback W, Borner W, Gruner KR. Comparison of ^{99}Tc^{m}-HMPAO and ^{111}In-oxine labelled granulocytes in man: first clinical results. *Nucl Med Commun* 1988; 9:435-447.

105. Nock BH, Schauwecker DS, English D, Young KA, Wellman HN. In vivo kinetics of canine leukocytes labeled with technetium-99m HM-PAO and Indium-111 tropolone. *J Nucl Med* 1988; 29:1246-1251.

106. Costa DC, Lui D, Ell PJ. White cells radiolabelled with ^{111}In and ^{99}Tc^{m} - study of relative sensitivity and in vivo viability. *Nucl Med Commun* 1988; 9:725-731.

107. Peters AM, Roddie ME, Danpure HJ, Osman S, Zacharopoulos GP, George P, et al. ^{99}Tc^{m}-HMPAO labelled leucocytes: comparison with ^{111}In-tropolonate labelled granulocytes. *Nucl Med Commun* 1988; 9:449-463.

108. Danpure HJ, Osman S, Carroll MJ. The development of a clinical protocol for the radiolabelling of mixed leukocytes with ^{99}Tc^{m}-hexamethylpropyleneamine oxime. *Nucl Med Commun* 1988; 9:465-475.

109. Kao CH, Wang YL, Wang SJ. Elution analysis and normal biodistribution of technetium-99m HMPAO-labeled white blood cells. *J Nucl Med Technol* 1992; 20:224-227.

110. Roddie ME, Peters AM, Danpure HJ, Osman S, Henderson BL, Lavender JP, et al. Inflammation: imaging with Tc-99m HMPAO-labeled leukocytes. *Radiology* 1988; 166:767-772.

111. Lantto EH, Lantto TJ, Vorne M. Fast diagnosis of abdominal infections and inflammations with Technetium-99m-HMPAO labeled leukocytes. *J Nucl Med* 1991; 32:2029-2034.

112. Allan RA, Sladen GE, Bassingham S, Lazarus C, Clarke S. Comparison of Tc-99m HM-PAO and In-111 labelled white cell scans in inflammatory bowel disease. *J Nucl Med* 32 Suppl 5:924.

113. Kelbaek H, Linde J, Nielsen SL. Evaluation of a new leukocyte labeling procedure with ^{99m}Tc-HMPAO. *Eur J Nucl Med* 1988; 14:621-623.

114. Datz FL, Thorne DA. The effect of antibiotic therapy on the sensitivity of the In-111-labeled leukocyte scan. *J Nucl Med* 1986; 27:1849-1853.

115. Turner RA, Johnson JA, Mountz JD, Treadway WJ. Neutrophil migration in response to chemotactic factors: effects of generation conditions and chemotherapeutic agents. *Inflammation* 1983; 7:57-65.

116. MacFadden DK, Saito S, Pruzanski W. The effect of chemotherapeutic agents on chemotaxis and random migration of human leukocytes. *J Clin Oncol* 1985; 3:415-419.

117. Schell-Frederick E, Fruhling J, Van der Auwera P, Van Laethem Y, Klastersky J. Indium-111-oxine-labeled leukocytes in the diagnosis of localized infection in patients with neoplastic disease. *Cancer* 1984; 54:817-824.

118. Datz FL, Thorne DA, Taylor A. Evaluation of factors that could potentially decrease the sensitivity of the In-111-labeled leukocyte scan. *J Nucl Med* 1986; 27:914-915.

119. Magnuson JE, Brown ML, Hauser MF, Berquist TH, Fitzgerald RH, Klee GG. In-111-labeled leukocyte scintigraphy in suspected orthopedic prosthesis infection: comparison with other imaging modalities. *Radiology* 1988; 168:235-239.

120. Fortner A, Datz F, Taylor A, Alazraki N. Uptake of In-111-labeled leukocytes by tumor. *Am J Roentgenol* 1986; 146:621-625.

121. Fischman AJ, Rubin RH, Khaw B-A, Callahan RJ, Wilkinson R, Keech F, et al. Detection of acute inflammation with ^{111}In-labeled nonspecific polyclonal IgG. *Semin Nucl Med* 1988; 18:335-344.

122. Rubin, RH, Fischman AJ, Callahan RJ, Khaw B-A, Keech F, Ahmad M, et al. ^{111}In-labeled nonspecific immunoglobulin scanning in the detection of focal infection. *New Engl J Med* 1989; 321:935-940.

123. LaMuraglia GM, Fischman AJ, Strauss W, Keech F, Wilkinson R, Callahan RJ, et al. Utility of the Indium 111-labeled human immunoglobulin G scan for the detection of focal vascular graft infection. *J Vasc Surg* 1989; 10:20-27.

124. Rubin RH, Fischman AJ, Needleman M, Wilkinson R, Callahan RJ, Khaw Ban-An, et al. Radiolabeled, nonspecific, polyclonal human immunoglobulin in the detection of focal inflammation by scintigraphy: comparison with gallium-67 citrate and technetium-99m-labeled albumin. *J Nucl Med* 1989; 30:385-389.

125. Juweid M, Strauss HW, Yaoita H, Rubin RH, Fischman AJ. Accumulation of immunoglobulin G at focal sites of inflammation. *Eur J Nucl Med* 1992; 19:159-165.

126. Oyen WJG, Claessens RAMJ, Meer JWM van der, Corstens FHM. Biodistribution and kinetics of radiolabeled proteins in rats with focal infection. *J Nucl Med* 1992; 33:388-394.

127. Calame W, Feitsma HIJ, Ensing GJ, Ardnt J-W, Van Furth R, Pauwels EKJ. Binding of ^{99m}Tc-labeled polyclonal immunoglobulin to bacteria as a mechanism for scintigraphic detection of infection. *Eur J Nucl Med* 1991; 18:396-400.

128. Fischman AJ, Rubin RH, White JA, Locke E, Wilkinson RA, Nedelman M, et al. Localization of Fc and Fab fragments of nonspecific polyclonal IgG at focal sites of inflammation. *J Nucl Med* 1990; 31:1199-1205.

129. Bosslet K, Luben G, Schwarz A, Hundt E, Harthus HP, Seiler FR, et al. Immunohistochemical localization and molecular characteristics of three monoclonal antibody-defined epitopes detectable on carcinoembryonicantigen (CEA). *Int J Cancer* 1985; 36:75-84.

130. Bosslet K, Schorlemmer HU, Steinstraesser A, Schwarz A, Sedlacek HH. Molecular and functional properties of the granulocyte specific MAB BW 250/183 suited for the immunoscintigraphic localization of inflammatory processes. In: Höfer R, editor. *Radioactive Isotopes in Clinical Medicine and Research*. New York: Schattauer, 1988:15-20.

131. Joseph K, Höffken H, Bosslet K, Schorlemmer HU. In vivo labelling of granulocytes with ^{99m}Tc anti-NCA monoclonal antibodies for imaging inflammation. *Eur J Nucl Med* 1988; 14:367-373.

132. Joseph K, Höffken H, Bosslet K, Schorlemmer HU. Imaging of inflammation with granulocytes labelled in vivo. *Nucl Med Commun* 1988; 9:763-769.

133. Schwarz A, Steinsträßer A, Hoechst AG. A novel approach to Tc-99m-labeled monoclonal antibodies [abstract]. *J Nucl Med* 1987; 28:721.

134. Becker W, Borst U, Fischbach W, Pasurka B, Schafer R, Borner W, et al. Kinetic data of in-vivo labeled granulocytes in humans with a murine Tc-99m-labeled monoclonal antibody. *Eur J Nucl Med* 1989; 15:361-366.

135. McAfee JG, Gagne G, Subramanian G, Schneider RF. Reply to Scink J, Schober O, Becker W. The localization of indium-111-leukocyte, gallium-67-polyclonal IgG and other radioactive agents in acute focal inflammatory lesions [letter]. *J Nucl Med* 1992; 1428-1429.

136. De Schrijver M, Streule K, Senekowitsch R, Fridrich R. Scintigraphy of inflammation with nanometer-sized colloidal tracers. *Nucl Med Commun* 1987; 8:895-908.

137. Streule K, De Schrijver M, Fridrich R. $^{99}Tc^{m}$-labeled HSA-nanocolloid versus ^{111}In oxine-labeled granulocytes in detecting skeletal septic process. *Nucl Med Commun* 1988; 9:59-67.

138. Vorne M, Lantto T, Paakkinen S, Salo S, Soini I. Clinical comparison of $^{99}Tc^{m}$-HMPAO labeled leukocytes and $^{99}Tc^{m}$-nanocolloid in the detection of inflammation. *Acta Radiologica* 1989; 30:633-637.

139. Abramovici J, Rubinstein M. Tc-99m nanocolloids: an alternative approach to the diagnosis of inflammatory lesions of bones and joints [abstract]. *Eur J Nucl Med* 1988; 14:244.

140. Arndt JW, van der Sluys Veer A, Blok D, Griffioen G, Verspaget HW, Pena S, et al. Technetium-99m nanocolloid for the scintigraphic assessment of inflammatory bowel disease in the colon: its value in comparison with Indium-111-labelled granulocytes. *Eur J Radiol* 1991; 10:30-34.

141. Wheeler JG, Slack NF, Duncan A, Palmer M, Harvey RF. $^{99m}Tc^{m}$-nanocolloid imaging in inflammatory bowel disease. *Nucl Med Commun* 1990; 11:127-133.

142. Rusckowski M, Paganelli G, Hnatowich DJ, Virzi F, Fogarasi M, Fazio F. Imaging infection/inflammation in patients with streptavidin and radiolabeled biotin: preliminary observations [abstract]. *J Nucl Med* 1992; 33:924.

143. Hnatowich DJ, Fritz B, Virzi F, Mardirossian G, Rusckowski M. Tumor localization with (strept)avidin and labeled biotin as a substitute for antibody. *J Nucl Med* 1992; 5:934-935.

144. Rusckowski M, Fritz B, Hnatowich DJ. Localization of infection using streptavidin and biotin: An alternative to nonspecific polyclonal immunoglobulin. *J Nucl Med* 1992; 33:1810-1815.

145. Morrel EM, Tompkins RG, Fischman AJ, Wilkinson RA, Burke JF, Rubin RH et al. Autoradiographic method for quantitation of radiolabeled proteins in tissues using indium-111. *J Nucl Med* 1989; 30:1538-1545.

146. Hnatowich DJ, Virzi F, Rusckowski M. Investigations of avidin and biotin for imaging applications. *J Nucl Med* 1987; 28:1294-1302.

147. Paganelli G, Malcovati M, Fazio F. Monoclonal antibody pretargeting techniques for tumour localization: the avidin-biotin system. *Nucl Med Commun* 1991; 12:211-234.

148. Committee on Drugs. Unapproved uses of approved drugs: the physician, the package insert, and the FDA. *Pediatrics* 1978; 62:262-264.

149. Spector R. Use of approved drugs in a nonapproved way. *Iowa Medicine* 1987; October:513-515.

QUESTIONS

1. The oxidation state of ^{67}Ga in the chemical structure of ^{67}Ga-citrate is:

   A. 1+
   B. 2+
   C. 3+
   D. 4+

2. Which of the following modalities offers the fastest and least expensive mode for the detection of abscesses?

   A. computed tomography
   B. magnetic resonance imaging
   C. ultrasound
   D. radionuclde scintigraphy

3. What is the optimal pH of the labeling medium for radiolabeled leukocytes?

   A. 4.5
   B. 6.5
   C. 8.5
   D. 10.5

4. Why is hetastarch added to the whole blood in the process of radiolabeling leukocytes?

   A. to improve the labeling efficiency
   B. to reduce microaggregation of leukocytes during labeling
   C. to remove smaller and lighter platelets
   D. to increase the RBC sedimentation rate

5. Which of the following indium chelating agents has been approved by the FDA?

   A. ACD
   B. MERC
   C. oxine
   D. tropolone

6. What is the mean particle size for ^{99m}Tc-labeled nanocolloids?

   A. 3 nm
   B. 30 nm
   C. 300 nm
   D. 3000 nm

7. The pretarget approach used in the ^{111}In-labeled avidin-biotin is:

   A. to improve the target-to-nontarget ratios
   B. to increase the labeling efficiency
   C. to reduce HAMA responses
   D. to allow higher administration dose

8. Which of the following quality control methods is the *in vivo* test for radiolabeled leukocytes?

   A. chemotaxis
   B. Ficoll-Hypaque distribution
   C. CBC
   D. cell recovery

9. The standard dose of ^{111}In-labeled IgG for the scintigraphic detection of infection/inflammation foci is:

   A. 0.05 mCi
   B. 0.5 mCi
   C. 1.5 mCi
   D. 2.5 mCi

10. Which of the following structural formulas represents ^{111}In-oxine?

    A. ^{111}In (Oxine)
    B. ^{111}In $(Oxine)_2$
    C. ^{111}In $(Oxine)_3$
    D. ^{111}In $(Oxine)_4$

11. Which of the following is the reason for using Percoll in the radiolabeling process?

    A. to separate pure granulocytes
    B. to remove LRPRP
    C. to reduce plasma in the final preparation
    D. to promote aggregation during labeling

12. When radiolabeling leukocytes in vitro, the most suitable incubation temperature is:

    A. 4°C
    B. 10°C
    C. 25°C
    D. 41°C

13. In order to have an efficient radiolabeling of MAb BW 250/183 with ^{99m}Tc, the Schwarz method calls for which of the following ^{99m}Tc species to bind with the antibody?

    A. reduced ^{99m}Tc
    B. hydrolyzed reduced ^{99m}Tc
    C. ^{99m}Tc-PYP
    D. $^{99m}TcO_4$

14. Which of the following radiopharmaceuticals may develop a HAMA response in patients?

    A. ^{111}In-labeled IgG
    B. ^{111}In-labeled avidin-biotin
    C. ^{99m}Tc-labeled nanocolloids
    D. ^{99m}Tc-labeled BW 250/183

15. In WBCs radiolabeled with ^{99m}Tc-HMPAO, this agent is able to diffuse through cell membrane due to its ________________ character.

A. symmetric
B. lipophilic
C. hydrophilic
D. ionic

16. Polysaccharide helps to clump RBCs together and reduce the required sedimentation time by:

A. 1/5
B. 1/4
C. 1/3
D. 1/2

17. Which statement best describes the outcome of the final product if leukocytes are labeled with ^{111}In-oxine in the presence of plasma?

A. increased urinary excretion
B. improved sedimentation rate
C. decreased labeling efficiency
D. diminished chemotactic properties

18. The blood disappearance $t_{1/2}$ for ^{111}In oxine-labeled leukocytes in humans is:

A. 2 - 3.5 hr
B. 5 - 7.5 hr
C. 10 - 14.5 hr
D. 22 - 25.5 hr

19. Which of the following is the major composition of ^{99m}Tc-HMPAO in the spontaneous elution from labeled WBCs?

A. primary ^{99m}Tc-HMPAO complex
B. secondary ^{99m}Tc-HMPAO complex
C. hydrolyzed-reduced ^{99m}Tc
D. ^{99m}Tc-pertechnetate

20. Both streptavidin and avidin are:

A. proteins
B. lipids
C. steroids
D. carbohydrates

21. Approximately ______________ times more complexing agent than ^{111}In is required before cell labeling will occur.

A. 5
B. 10
C. 50
D. 100

22. The chemical name for oxine is:

A. 8-hydroxyquinoline
B. 8-deoxyquinoline
C. 8-methoxyquinoline
D. 8-xenoxyquinoline

23. Which of the following radiopharmaceuticals has a shelf life of only 30 minutes?

A. ^{99m}Tc-nanocolloid
B. ^{111}In-tropolone
C. ^{99m}Tc-HMPAO
D. ^{111}In-IgG

24. Hydroxyethyl starch is commercially available in _______ % solution?

A. 2%
B. 4%
C. 6%
D. 8%

25. Which of the following is not considered an advantage when using ^{111}In-IgG for the localization of infection and/or inflammation?

A. ^{111}In-IgG can detect nonbacterial infection
B. ^{111}In-IgG may localize in tumor sites
C. ^{111}In-IgG has minimal bowel excretion
D. IgG is available as a kit