VOID SHEET

TO: License Fee Management Branch, C. Fleming

FROM: W. ADAM

SUBJECT: VOIDED APPLICATION

Control Number: 389917

Applicant: Syncor - Kansas City, MO

Date Voided: 8-1-90

Reason for Void: Licensing action unnecessary, already authorized on license. Voided after review.

W. J. Adam, 8-1-90.
Signature Date

Attachment:
Official Record Copy of
Voided Action

FOR LFMB USE ONLY

Final Review of VOID Completed:

- ☐ Refund Authorized and processed
- ☒ No Refund Due after rev
- ☐ Fee Exempt or Fee Not Required

Comments:

Log completed ☒

Processed by:

ML30

BETWEEN:

LICENSE FEE MANAGEMENT BRANCH, ARM
AND
REGIONAL LICENSING SECTIONS

(FOR LFMS USE)
INFORMATION FROM LTS

PROGRAM CODE: 02500
STATUS CODE: 0
FEE CATEGORY: 3C 3N_EX 2C
EXP. DATE: 19940930
FEE COMMENTS: 3N_NOTE _ON_5/5/87_

LICENSE FEE TRANSMITTAL

A. REGION

1. APPLICATION ATTACHED
APPLICANT/LICENSEE: SYNCOR CORP.
RECEIVED DATE: 900726
DOCKET NO: 3011339
CONTROL NO.: 389917
LICENSE NO.: 24-16617-01MD
ACTION TYPE: AMENDMENT

2. FEE ATTACHED
AMOUNT: $370.00
CHECK NO.: 223933

3. COMMENTS

SIGNED P. Lutloff
DATE 7-27-90

B. LICENSE FEE MANAGEMENT BRANCH (CHECK WHEN MILESTONE 03 IS ENTERED /V/)

1. FEE CATEGORY AND AMOUNT: 3C 3NEX2C

2. CORRECT FEE PAID. APPLICATION MAY BE PROCESSED FOR:
AMENDMENT ✓
RENEWAL
LICENSE

3. OTHER

SIGNED
DATE 8/1/90

syncor

NRCTADM

July 22, 1990

William J. Adam, Ph.D.
Material Licensing Section
U. S. Nuclear Regulatory Commission
Region III
799 Roosevelt Road
Glen Ellyn, Illinois 60137

RE: Amendment Request for NRC Materials License Number 24-16617-01MD Kansas City, MO. This is an amendment request that we have been discussing with Dr. Bill Adam. I would appreciate it very much if he was assigned this amendment request.

RECEIVED AUG 10 1990

Dear Dr. Adam:

Please amend our NRC Materials License Number 24-16617-01MD, Kansas City, for authorization to label white blood cells (WBCs) with Tc-99m Ceretec and to dispense this material upon prescription to authorized recipients. In support of this request please see the attached documents relative to the use of this NDA drug for a use not listed in the package insert.

This material will be administered to the patient using the same route of administration and in the same recommended quantities as stated in the package insert. The radiation dose to the critical organs is about the same for Tc-99m and In-111 since the amount of activity is larger using Tc99m. The images thus the diagnostic information is superior using Tc99m since better statistics are available for imaging.

I have asked Dr. Hoogland to include a letter to you concerning his conversation with the FDA contact. He has also listed the information contained in the attached documentation.

Your consideration in this matter is appreciated.

Log Aug 4 III
Remitter
Check No. 223933
Amount $320
Fee Category 3C
Type of Fee amend
Date Check Rec'd.
Date Completed 8/1/90
By

Sincerely,

Frank M Comer

Frank M Comer
Manager, Regulatory Compliance

cc. Deborah Kaminsky
Dr Hoogland
License file

Enclosure: Amendment fee $320.00

RECEIVED
JUL 26 1990
REGION III

RECEIVED '90 JUL 26 P2:30

JUL 26 1990

CONTROL NO. 89917

syncor

CHECK REQUEST

CO. NO. SYN

14166

VENDOR CODE

PAY TO

EXPENSE MONTH

DATE

DUE DATE

AMOUNT $

REQ. # _____ **P.O. #** _____

FOR

ACCOUNTING DISTRIBUTION		
Account	Cost Center	Amount

SPECIAL INSTRUCTIONS

Requested By _____

Approved By _____

Please Check One:

- ☐ C.O.D.
- ☐ ADVANCE PAYMENT
- ☐ DOWN PAYMENT
- ☐ NO INVOICE RECEIVED
- ☐ OTHER _____

PURCHASING

syncor®

July 20, 1990

William J. Adam, Ph.D.
Material Licensing Section
U.S. Regulatory Commission
Region III
799 Roosevelt Road
Glen Ellyn, Illinois 60137

Dear Dr. Adam:

With regard to Syncor Nuclear Pharmacies providing nuclear medicine departments Tc-99m Cereted (HMPAO) labeled White Blood Cells (WBCs), the following data are provided for your records:

1. Letter: Syncor to FDA requesting definition that the Syncor method for preparing Tc-99m WBCs is considered the dispensing of an approved drug for an unapproved use, and therefore, does not require an IND status.

2. Letter: FDA to Syncor confirming IND status is not required for the approved drug (Tc-99m Ceretec) being used to label WBCs for patient administration (unapproved use of an approved drug in physicians practice of medicine).

3. ICRP Publication 53 Reference
   A. Radiation dosimetry In-111 WBCs
      (Current commercial WBC labeling product)
   B. Radiation Dosimetry Tc-99m WBCs
      (Proposed product)

4. Package insert for Tc-99m Ceretec which indicates radiation dosimetry for any drug which may leach from WBCs which is minimal.

5. Journal Article: Inflammation: Imaging with Tc-99m HMPAO-labeled leukocytes (Ceretec = HMPAO) which also contains data relating to radiation dosimetry and clinical efficacy.

6. Journal Article: In Vitro and In Vivo Evaluation of Granulocyte Labeling with Tc-99m HMPAO which describes a labeling procedure, Tc-99m wash out values, biodistribution, and clinical efficacy.

7. Journal Article: Tc-99m Ceretec-Labeled Leukocytes in Detection of Inflammatory Lesions: Comparison with Ga-67 Citrate.

Syncor International Corporation • 20001 Prairie Street • P.O. Box 2185 • Chatsworth, California 91313-2185
(818) 886-7400 • FAX (818) 993-3507 • Telex MCI 67-18642 Syncor CHATS

CONTROL NO. 89917

Mr. Mark Anderson of the FDA (301/443-5963) has verbally verified on July 13, 1990 that dispensing Tc-99m Ceretec WBCs instead of In-111 WBCs upon a physicians prescription for patient diagnostic studies is recognized as an unapproved clinical use of an approved radiopharmaceutical. This clinical application is outside the requirements of 21 CFR Parts 50 and 56, respectively. Neither IRB approval, nor patient consent is required when Tc-99m Ceretec WBCs are administered to a patients upon a physician prescription for the purpose of diagnosing their condition.

This diagnostic clinical application of Tc-99m Ceretec WBCs is identical to the use of Tc-99m DTPA as a lung aerosol, Tc-99m sulfur colloid for evaluating gastric emptying times, In-111 DTPA for evaluating gastric emptying times, Tc-99m MAA for radionuclide venography, Tc-99m and Tl-201 for parathyroid evaluation, Tc-99m sulfur colloid or Tc-99m MAA for Levine shunt patency, Tc-99m red blood cells for radionuclide venography, etc. These clinical diagnostic procedures require neither IRB approval nor patient consent before accomplishing diagnostic studies not listed in the package insert of an NDA approved radiopharmaceutical.

Syncor requests that its FDA communications contained in this letter should be kept confidential.

If I can be of any additional service, please ask.

Sincerely,

Dennis R. Hoogland, Ph.D., BCNP
Manager, Technical Training and Development

cc: Frank Comer, Syncor RSO

Enclosures

syncor®

May 1, 1989

Mark D. Anderson
Robert L. West, M.S.
Consumer Safety Officers
United States Food and Drug Administration
Center Drugs and Biologics
Office Drug Research and Review
Radiopharmaceutical Drug Products
5600 Fishers Lane
Rockville, Maryland 20857

Dear Messrs. Anderson and West:

Syncor nuclear pharmacy service centers have received physician requests to label white blood cells (WBCs) with technetium-99m Ceretec based on clinical literature. Technetium-99m Ceretec, which is approved as an adjunct in the detection of altered regional cerebral perfusion in stroke, will be administered to patients as Tc-99m labeled white blood cells to diagnose inflammatory processes.

Based on past responses from FDA, filling prescriptions for Tc-99m labeled WBCs using an approved radiopharmaceutical Tc-99m Ceretec for an "unapproved use" is within FDA's position relative to the use of approved drugs (21 CFR 312.2(b); April 1982 FDA Drug Bulletin).

Before approving the dispensing of Tc-99m labeled WBCs, Syncor would like a reply from your office that FDA's position has not changed relative to previous communications citing the above references.

Thank you in advance for your attention to our request.

Sincerely,

Dennis R. Hoogland, Ph.D.
Manager, Technical Training and Development

CONTROL NO. 89917

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

MAY 17 1989

Syncor International Corporation
20001 Prairie Street
P.O. Box 2185
Chatsworth, California 91313-2185

Attention: Dennis R. Hoogland, Ph.D.
Manager, Technical Training and Development

Dear Dr. Hoogland:

Reference is made to your May 1, 1989 letter regarding clarification of FDA's position stated in the April 1982 FDA Drug Bulletin and IND regulations concerning the use of approved drugs for unapproved indications. Specifically, you have inquired about the acceptability, under the practice of medicine and/or pharmacy, for a licensed nuclear pharmacy to radiolabel a patient's white blood cells (WBCs) with Ceretec, a radiopharmaceutical approved by FDA for the detection of altered regional perfusion in patients with stroke. Once labeled with Ceretec, the WBCs are to be used for an indication which is not approved, i.e., localization of inflammatory processes.

As you have mentioned, FDA published revised New Drug, Antibiotic, and Biologic Drug Product Regulations in the Federal Register (52 FR 8798-8847) on March 19, 1987, which became effective on June 19, 1987. Those regulations [21 CFR 312.2 (b)(1)] state that the clinical investigation of a drug product that is lawfully marketed in the United States is exempt from the IND requirements if all of the following apply:

1. The study does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the product.

2. The study is not intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling or advertising for the drug;

3. Informed patient consent is to be obtained and the study has been reviewed and approved by an Institutional Review Board (IRB) in accordance with the requirements of 21 CFR Parts 50 and 56, respectively.

4. The investigation is conducted in compliance with the requirements of 21 CFR 312.7, i.e., the investigational drug may not be represented as safe or effective for the purposes for which it is under investigation nor may it be commercially distributed, test marketed or sold.

In addition to FDA's recent actions, on April 1, 1987 the United States Nuclear Regulatory Commission (NRC) revised their regulations and published them as a final rule in the Federal Register of October 16, 1986 (51 FR 36932-36951). These regulations state that the NRC will no longer require a physician to obtain an IND in order to administer an approved radiopharmaceutical by a route or for an indication other than that provided for in the approved labeling for the product.

Like the NRC's regulation, FDA's new regulation is not intended necessarily to tie the investigator to the dose, route of administration and patient population(s) described in the drug product's approved labeling, but rather is designed to permit deviation from the approved labeling to the extent that such changes are supported by the scientific literature and generally known clinical experiences. As noted in the April 1982 FDA Drug Bulletin, FDA does not regulate the practice of pharmacy or medicine, which the Agency has consistently viewed as including the use of marketed drugs for unlabeled indications in the "day to day" treatment of patients.

Upon review of the information provided in your May 1, 1989 communication, we conclude that your proposal to label WBCs with Ceretec meets the requisites of 21 CFR 312.2(b)(1) and may be performed under the practice of medicine and/or pharmacy without benefit of an IND. However, as noted in the regulation cited above and if clinical studies are to be conducted, both patient informed consent and internal review and approval by the appropriate committee within the referring institution are required. In addition, no data have been submitted to the Agency to support the safety and effectiveness of using WBCs labeled with Ceretec for localization of imflammatory processes. Therefore, you may not actively promote the use of Ceretec for the unlabeled indication.

Sincerely yours,

Robert L. West, for

A. Eric Jones, M.D.
Group Leader
Division of Radiopharmaceutical,
Surgical and Dental Drug Products
Office of Drug Research and Review
Center for Drug Evaluation and Research

Code N.159Z

Ceretec™ kit for the preparation of Technetium Tc99m Exametazime Injection

Diagnostic radiopharmaceutical-
For intravenous single use only

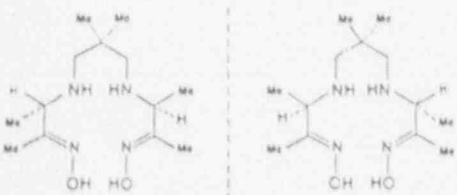
DESCRIPTION

The Amersham Ceretec™ kit is supplied as packs of 5 single dose vial units for use in the preparation of a technetium Tc99m exametazime intravenous injection as a diagnostic radiopharmaceutical for use as an adjunct in the detection of altered regional cerebral perfusion. Each single dose vial unit contains a pre-dispensed sterile, non-pyrogenic, lyophilized mixture of 0.5 mg exametazime [(RR,SS)-4,8-diaza-3,6,6,9-tetramethylundecane-2, 10-dione bisoxime], 7.6 µg stannous chloride dihydrate (minimum stannous tin 0.6 µg, maximum total stannous and stannic tin 4.0 µg per vial) and 4.5 mg sodium chloride, sealed under nitrogen atmosphere with a rubber closure. The product contains no antimicrobial preservative.

Caution: Federal (U.S.A.) Law prohibits dispensing without a prescription.

Prior to publication of the USAN, exametazime was formerly known as hexamethylpropylene amine oxime (HM-PAO). The name HM-PAO appears in many publications.

The structural formula of exametazime is



$C_{13}H_{28}N_4O_2$

When sterile pyrogen-free sodium pertechnetate Tc99m in isotonic saline is added to the vial, a Tc99m complex of exametazime is formed.

Administration is by intravenous injection for diagnostic use.

Physical Characteristics

Technetium Tc99m decays by isomeric transition with a physical half-life of 6.03 hours.[1] Photons that are useful for imaging studies are listed in Table 1.

Table 1. Principal radiation emission data—technetium Tc99m

Radiation	Mean %/ disintegration	Mean energy (keV)
Gamma 2	87.87	140.5

1) Dillman, L.T. and Von der Lage, F.C. Radionuclide Decay schemes and nuclear parameters for use in radiation-dose estimation. MIRD Pamphlet No. 10, p62, 1975.

External radiation

The specific gamma ray constant for technetium Tc99m is 206 microCoulomb $kg^{-1}/37$ MBq-h, (0.8 R/millicure-h) at 1 cm. The first half-value thickness of lead (Pb) for technetium Tc99m is 0.2 mm. A range of values for the relative attenuation of the radiation emitted by this radionuclide that results from interposition of various thicknesses of Pb is shown in Table 2. For example, the use of a 2.7 mm thickness of Pb will decrease the external radiation exposure by a factor of 1,000.

Table 2. Radiation attenuation by lead shielding

Shield thickness (Pb) mm	Coefficient of attenuation
0.2	0.5
0.95	10^{-1}
1.8	10^{-2}
2.7	10^{-3}
3.6	10^{-4}
4.5	10^{-5}

To correct for physical decay of this radionuclide, the fractions that remain at selected intervals relative to the time of calibration are shown in Table 3.

Table 3. Physical decay chart—Tc99m half-life 6.03 hours

Hours	Fraction remaining	Hours	Fraction remaining
0*	1.000	7	0.447
1	0.891	8	0.399
2	0.795	9	0.355
3	0.708	10	0.317
4	0.631	11	0.282
5	0.563	12	0.252
6	0.502	24	0.063

*Calibration time (time of preparation)

CLINICAL PHARMACOLOGY

When technetium Tc99m pertechnetate is added to exametazime in the presence of stannous reductant, a lipophilic technetium Tc99m complex is formed. This lipophilic complex is the active moiety. It converts with time to a secondary complex which is less lipophilic. When the secondary complex is isolated from the lipophilic species, it has been shown to be unable to cross the blood-brain-barrier. **A consequence of the conversion of lipophilic to secondary complex is that the useful life of the reconstituted agent is restricted to 30 minutes.**

Studies in normal volunteers have shown that the technetium Tc99m complex of the RR,SS(d,l) diastereoisomer of exametazime is rapidly cleared from the blood after intravenous injection. Uptake in the brain reaches a maximum of 2.5-7.0% of the injected dose within one minute of injection. Up to 15% of the activity is eliminated from the brain by 2 minutes post injection, after which little activity is lost for the following 24 hours except by physical decay of technetium Tc99m. The activity not associated with the brain is widely distributed throughout the body particularly in muscle and soft tissue. About 30% of the injected dose is found in the gastrointestinal tract immediately after injection and about 50% of this is excreted through the intestinal tract over 48 hours. About 40% of the injected dose is excreted through the kidneys and urine over the 48 hours after injection resulting in a reduction in general muscle and soft tissue background.

INDICATIONS AND USAGE

Technetium Tc99m exametazime scintigraphy may be used as an adjunct in the detection of altered regional cerebral perfusion in stroke.

CONTRAINDICATIONS

None known.

PRECAUTIONS

The contents of the Ceretec vial are not radioactive. However, after the sodium pertechnetate Tc99m is added, adequate shielding of the final preparation must be maintained.

The contents of the Ceretec™ vial are intended only for use in preparation of technetium Tc99m exametazime injection and are NOT to be administered directly to the patient.

A thorough knowledge of the normal distribution of intravenously administered technetium Tc99m exametazime injection is essential in order to interpret pathologic studies accurately.

The technetium Tc99m labeling reaction involved in preparing technetium Tc99m exametazime injection depends on maintaining tin in the divalent (reduced) state. Any oxidant present in the sodium pertechnetate Tc99m employed may adversely affect the quality of the preparation. Sodium pertechnetate Tc99m containing oxidants should not be used for the preparation of the labeled product. **To meet the last requirement, a generator must be eluted within 24 hours prior to obtaining any eluate for reconstitution with the Ceretec kit.**

Sodium Chloride Injection, USP must be used as the diluent. Do not use bacteriostatic sodium chloride as a diluent for sodium pertechnetate Tc99m injection because it will increase the oxidation products and adversely affect the biological distribution of Ceretec.

GENERAL

The contents of the Ceretec vial are sterile and pyrogen free. The vial contains no bacteriostatic preservative. It is essential that the user follow the directions carefully and adhere to strict aseptic procedures during preparation of the radiopharmaceutical.

Technetium Tc99m exametazime injection, like other radioactive drugs, must be handled with care and appropriate safety measures should be used to minimize radiation exposure to clinical personnel. Care should also be taken to minimize radiation exposure to the patient consistent with proper patient management.

Radiopharmaceuticals should be used only by or under the control of physicians who are qualified by training and experience in the safe use and handling of radionuclides and whose experience and training have been approved by the appropriate governmental agency authorized to license the use of radionuclides.

To minimize radiation dose to the bladder, the patient should be encouraged to void when the examination is completed and as often thereafter as possible. Adequate hydration should be encouraged to permit frequent voiding.

Carcinogenesis, Mutagenesis, Impairment of Fertility

No long term animal studies have been performed to evaluate carcinogenic potential or whether technetium Tc99m exametazime affects fertility in males or females. Studies in rats did not demonstrate mutagenic potential following intraperitoneal administration at doses of 70, 140 and 280 mg/kg.

Pregnancy Category C

Since adequate reproduction studies with technetium Tc99m exametazime have not been performed in animals to determine whether this drug affects fertility in males and females, has teratogenic potential, or has other adverse effects on the fetus, this radiopharmaceutical preparation should not be administered to pregnant or nursing women unless it is considered that the benefits to be gained outweigh the potential hazards.

Ideally, examinations using radiopharmaceuticals, especially those which are elective in nature, in women of childbearing capability should be performed during the first few (approximately 10) days following the onset of menses.

Nursing Mothers

Technetium Tc99m is excreted in human milk during lactation. It is not known whether exametazime is excreted in human milk. Therefore, formula feedings should be substituted for breast feeding.

Pediatric Use

Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

Rash with generalized erythema, facial edema and fever has been reported. A transient increase in blood pressure was seen in 8% of patients.

DOSAGE AND ADMINISTRATION

The user should wear waterproof gloves and use shielding at all times when handling the vial and syringes.

The recommended dose range for i.v. administration, after reconstitution with sodium pertechnetate Tc99m, to be used in the average adult (70 kg) is 370-740 MBq (10-20 mCi).

Do not use the final radiopharmaceutical preparation after 30 minutes from the time of reconstitution. Discard any unused material.

Dynamic imaging may be performed between 0 to 10 minutes following injection. Static imaging may be performed from 15 minutes up to 6 hours after injection.

Although gross abnormalities of regional cerebral perfusion may be visualized by planar imaging, it is strongly recommended that SPECT imaging is carried out to maximize the value of the study.

RADIATION DOSIMETRY

Based on human data, the absorbed radiation dose to an average human adult (70 kg) from an intravenous injection of this product are estimated below. The values are listed as µGy/MBq (rads/mCi) with urination every 2 hours. Bladder wall dose is 19 µGy/MBq (0.07 rads/mCi) with 4 hour urination and 89 µGy/MBq (0.33 rads/mCi) with no urination.

Table 4. Estimated Absorbed Radiation Dose*

Target organ	Absorbed radiation dose Tc99m exametazine injection			
	µGy/MBq	rads/mCi	mGy/740 MBq	rads/20 mCi
Lachrymal glands	69.4	0.258	51.36	5.16
Gallbladder wall	51.0	0.19	37.74	3.80
Kidney	35.0	0.13	25.90	2.60
Thyroid	27.0	0.10	19.93	2.00
Upper large intestine wall	21.0	0.079	15.54	1.58
Liver	15.0	0.054	11.10	1.08
Small intestine wall	12.0	0.044	8.88	0.88
Lower large intestine wall	15.0	0.054	11.10	1.08
Urinary bladder wall	13.0	0.047	9.62	0.94
Brain	6.9	0.026	5.11	0.52
Ovaries	6.3	0.023	4.66	0.46
Testes	1.8	0.007	1.33	0.14
Whole body	3.6	0.013	2.66	0.26
Red Marrow	3.4	0.013	2.52	0.26
Bone Surfaces	4.8	0.018	3.55	0.36
Eyes	6.9	0.026	5.11	0.52

*Data supplied by Oak Ridge Associated Universities, Radiopharmaceutical Internal Dose Information Center.

ANIMAL TOXICOLOGY SUMMARY

Acute toxicity studies have been performed on intravenously administered Ceretec in male and female rats and rabbits. No adverse reactions or mortality were observed at a dose equivalent to the single injection of 1200 times the maximum human equivalent dose. Fourteen day repeat-dose studies in rats and rabbits at a cumulative dose of up to 14,000 times the maximum human equivalent dose did not reveal adverse reactions, abnormalities, or mortality. At termination, thorough histopathology, hematology and blood chemistry revealed no abnormalities.

HOW SUPPLIED

The kit comprises five individual vials of sterile, non-pyrogenic, freeze-dried mixture of exametazime stannous chloride dihydrate and sodium chloride, ten radiation labels, five sterile alcohol swabs, five radiochemical purity worksheets, and one package insert. The vial and contents are sealed under a nitrogen atmosphere with a rubber stopper.

PROCEDURE

For the Preparation of Technetium Tc99m Exametazime Injection

Use aseptic technique throughout.

1) Place one of the vials in a suitable shielding container and swab the rubber septum with the sterile swab provided.

2) Using a 10 ml syringe, inject into the shielded vial 5 ml of sterile eluate from a technetium Tc99m generator (see notes 1-4). Before withdrawing the syringe from the vial withdraw 5 ml of gas from the space above the solution to normalize the pressure in the vial. Shake the shielded vial for 10 seconds to ensure complete dissolution of the powder.

3) Assay the total activity and calculate the volume to be injected. The patient dose should be measured in a suitable radioactivity calibration system immediately prior to administration.

4) Complete the label provided and attach to the vial shield. The technetium Tc99m exametazime injection is ready for quality control.

5) Maintain adequate shielding of the radioactive preparation.

6) **Do not use the preparation after 30 minutes from time of formulation. Discard any unused material.**

7) Visually inspect the reconstituted material at a safe distance behind leaded glass, and do not use if there is evidence of foreign matter.

Cautionary Notes

1) 0.37-1.11 GBq (10-30 mCi) technetium Tc99m may be added to the vial.

2) Before reconstitution the generator eluate may be adjusted to the correct radioactive concentration (0.37-1.11 GBq [10-30 mCi] in 5 ml) by dilution with preservative-free saline for injection.

3) Generator eluate more than 2 hours old should not be used. **For the highest radiochemical purity reconstitute with freshly eluted technetium Tc99m generator eluate.**

4) **Use only eluate from a technetium Tc99m generator which was previously eluted within 24 hours.**

5) The pH of the prepared injection is in the range 9.0-9.8.

Quality Control

Radiochemical purity determination must be performed before administration to the patient. Three potential radiochemical impurities may be present in the prepared injection of the lipophilic complex technetium Tc99m exametazime. These are a secondary technetium Tc99m exametazime complex, free pertechnetate and reduced-hydrolyzed-technetium Tc99m. A combination of 3 chromatographic systems is necessary for the complete definition of the radiochemical composition of the injection.

The following protocol has been designed to enable analysis of the radiochemical purity of Ceretec (^{99m}Tc-exametazime) to be performed within 2 minutes of reconstitution taking approximately 15 minutes.

Equipment and Eluents

Gelman ITLC/SG strips 6 cm x 0.7 cm (available from Atomic Products—Black Strip)
Whatman strips 6 cm x 0.7 cm (available from Atomic Products—Red Strip)
MEK (methyl ethyl ketone [butanone]) (Aldridge Cat #27069-5 99.9 + % HPLC Grade)
0.9% aqueous sodium chloride (without bacteriostat)
50% aqueous acetonitrile (Aldridge Cat #27071-7 99.9 + % HPLC Grade)
Dilute with water for injection without bacteriostat
Glass test tubes (12 x 75 mm)
1 ml syringes with 25 gauge needles
Suitable counting equipment

Method

1) Prepare three chromatography tubes containing 0.9% sodium chloride, 0.2-0.3 ml of fresh MEK and 50% acetonitrile respectively. Identify the solvent in each tube.

2) Prepare two ITLC/SG (black) strips and one paper (red) strip. Each are marked by the manufacturer 1.0 cm from the bottom as the point of origin.

3) Reconstitute a Ceretec vial according to the package insert.

4) Apply 5 µl samples of Ceretec to the origin of the three strips within 15 minutes of reconstitution. Immediately place one ITLC/SG (black) strip into the saline tube, the second ITLC/SG (black) strip into the MEK tube and the paper (red) strip into the 50% acetonitrile tube. Make sure strips are not adhering to the side of the test tube.

5) The ITLC/SG MEK (black) strip takes approximately 45 seconds to run. When the eluate has reached the solvent front remove the strip from the tube with forceps and immediately cut 1.0 cm above the origin.

6) The ITLC/SG saline (black) strip takes approximately 45 seconds to run. When the eluate has reached the solvent front remove the strip from the tube with forceps and immediately cut 2.5 cm above the origin.

7) The Whatman paper CH_3CN (red) strip takes approximately 100 seconds to run. When the eluate has reached the solvent front remove the strip from the tube with forceps and immediately cut 0.5 cm above the origin.

8) Count the separate sections of each strip to determine the activity distribution. Make sure proper counting geometry is maintained attempting to reduce any interference from equipment dead time.

9) Determine:
% top of saline strip (= % pertechnetate)
% bottom of saline strip -% bottom of MEK strip (= % lipophilic exam... me complex)
% bottom of Whatman paper strip (= % reduced-hydrolyzed-Tc)

A radiochemical purity of >80% may be expected provided the measurement has been carried out within 30 minutes of reconstitution.

Interpretation of Chromatograms

System 1 (ITLC: MEK (butanone))
Secondary Tc exametazime complex and reduced-hydrolyzed-Tc remain at the origin.

Lipophilic Tc exametazime complex and pertechnetate migrate at R_f 0.8-1.0.

System 2 (ITLC: 0.9% sodium chloride)
Lipophilic Tc exametazime complex, secondary Tc exametazime complex and reduced-hydrolyzed-Tc remain at the origin.

Pertechnetate migrates at R_f 0.8-1.0.

System 3 (Whatman No. 1: 50% aqueous acetonitrile)
Reduced-hydrolyzed-Tc remains at the origin.

Lipophilic Tc exametazime complex, secondary Tc exametazime complex and pertechnetate migrate at R_f 0.8-1.0.

Storage

Store the kit at 2-25 °C.

Store the formulated drug at room temperature (15-25 °C) using appropriate radiation shielding.

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TECHNETIUM-LABELLED WHITE BLOOD CELLS (LEUKOCYTES)
^{99m}Tc

Biokinetic Model

The same model is used as for indium-labelled leukocytes (see p. 255), with the exception that, in view of the short physical half-life, the retention half-times are set to infinity.

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Biokinetic Data

Organ (S)	F_S	T	a	$\tilde{A}_S/A_0$
Blood	1.0	0	0.60	1.87 hr
		7 hr	0.40	
Liver	0.20	0	−0.60	1.36 hr
		7 hr	−0.40	
		∞	1.0	
Red marrow	0.30	0	−0.60	2.05 hr
		7 hr	−0.40	
		∞	1.0	
Spleen	0.25	0	−0.60	1.70 hr
		7 hr	−0.40	
		∞	1.0	
Remaining tissues	0.25	0	−0.60	1.70 hr
		7 hr	−0.40	
		∞	1.0	

Tc-LABELLED WHITE BLOOD CELLS (LEUKOCYTES)

^{99m}Tc 6.02 hours

Organ	Absorbed dose per unit activity administered (mGy/MBq)				
	Adult	15 year	10 year	5 year	1 year
Adrenals	8.9E-03	1.2E-02	1.8E-02	2.5E-02	4.2E-02
Bladder wall	2.6E-03	3.7E-03	5.6E-03	8.0E-03	1.4E-02
Bone surfaces	1.3E-02	1.7E-02	2.8E-02	5.0E-02	1.0E-01
Breast	3.1E-03	3.1E-03	5.1E-03	7.8E-03	1.3E-02
GI-tract					
Stomach wall	8.0E-03	9.6E-03	1.4E-02	2.0E-02	3.1E-02
Small intest	4.9E-03	5.8E-03	8.8E-03	1.3E-02	2.2E-02
ULI wall	4.9E-03	6.0E-03	9.3E-03	1.4E-02	2.3E-02
LLI wall	3.9E-03	5.0E-03	7.6E-03	1.0E-02	1.8E-02
* Heart	9.0E-03	1.1E-02	1.6E-02	2.3E-02	3.9E-02
* Kidneys	9.9E-03	1.2E-02	1.8E-02	2.5E-02	4.0E-02
* Liver	2.0E-02	2.4E-02	3.6E-02	5.2E-02	9.2E-02
Lungs	6.9E-03	9.0E-03	1.3E-02	2.0E-02	3.6E-02
Ovaries	4.2E-03	5.2E-03	7.5E-03	1.1E-02	1.8E-02
* Pancreas	1.4E-02	1.6E-02	2.4E-02	3.3E-02	5.2E-02
Red marrow	2.2E-02	2.9E-02	4.5E-02	7.8E-02	1.5E-01
* Spleen	1.5E-01	2.1E-01	3.2E-01	4.8E-01	8.7E-01
Testes	1.7E-03	2.3E-03	3.4E-03	5.2E-03	9.7E-03
Thyroid	2.4E-03	3.7E-03	5.7E-03	9.1E-03	1.7E-02
Uterus	3.8E-03	4.5E-03	6.8E-03	9.9E-03	1.7E-02
Other tissue	3.4E-03	4.2E-03	6.0E-03	9.0E-03	1.6E-02
Effective dose equivalent (mSv/MBq)	1.7E-02	2.3E-02	3.5E-02	5.4E-02	9.8E-02

In
49
WBC

INDIUM-LABELLED WHITE BLOOD CELLS (LEUKOCYTES)
^{111}In

Biokinetic Model

The fate of intravenously administered leukocytes depends to a great extent on details in the preliminary isolation and *in vitro* labelling of the cells. The cells may become activated and damaged to a varying degree, resulting in an immediate uptake predominantly in the lungs and liver. When modern, more innocuous methods are used, there is only a very short transient holdup in the lungs, and the initial uptake in organs is effected by equilibration with the pools of marginating leukocytes. Cells initially remaining in the circulation show a blood clearance which is exponential with a half-life between 5 and 10 hr. There is a very slow excretion of label in the urine.

For absorbed dose calculations a model is proposed, where 60% of the cells are immediately distributed in liver, spleen, bone marrow and other tissues, and 40% circulate in the blood with a half-time of 7 hr, after which they are taken up in the same organs and tissues and in the same proportions as for the early uptake. The total uptake is taken to be 20% in the liver, 25% in the spleen, 30% in red bone marrow and 25% in other tissues. From all sites the activity is assumed to be eliminated with a half-time of 70 d, in analogy with the model proposed for ionic indium.

The model chiefly refers to granulocytes, which normally form the majority of cells in a preparation of mixed leukocytes. It may be inappropriate for other types of white blood cells, such as lymphocytes, having somewhat different biokinetics.

The actual white blood cell suspension used for labelling may also contain erythrocytes and thrombocytes, which become labelled at the same time, and there may also be some unbound activity. The dose contributions from these other fractions of activity thus have to be added appropriately.

The ^{111}In-preparation may be contaminated with ^{114m}In and its daughter ^{114}In. The effective dose equivalent per unit activity of these radionuclides is therefore presented in the dosimetric table.

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Biokinetic Data

Organ (S)	F_s	T	a	$\tilde{A}_s/A_0$
Blood	1.0	0	0.60	3.66 hr
		7 hr	0.40	
Liver	0.20	0	−0.60	18.1 hr
		7 hr	−0.40	
		70 d	1.0	
Red marrow	0.30	0	−0.60	27.2 hr
		7 hr	−0.40	
		70 d	1.0	
Spleen	0.25	0	−0.60	22.7 hr
		7 hr	−0.40	
		70 d	1.0	
Remaining tissues	0.25	0	−0.60	22.7 hr
		7 hr	−0.40	
		70 d	1.0	

In-LABELLED WHITE BLOOD CELLS (LEUKOCYTES)

^{111}In 2.83 days

Organ	Absorbed dose per unit activity administered (mGy/MBq)				
	Adult	15 year	10 year	5 year	1 year
* Adrenals	3.1E-01	4.0E-01	5.9E-01	8.2E-01	1.4E+00
Bladder wall	7.2E-02	1.0E-01	1.6E-01	2.4E-01	4.1E-01
Bone surfaces	3.5E-01	5.0E-01	8.0E-01	1.4E+00	2.9E+00
Breast	9.0E-02	9.0E-02	1.5E-01	2.3E-01	3.9E-01
GI-tract					
Stomach wall	2.8E-01	3.3E-01	4.9E-01	6.8E-01	1.1E+00
Small intest	1.6E-01	1.9E-01	2.9E-01	4.3E-01	7.1E-01
ULI wall	1.6E-01	1.9E-01	3.0E-01	4.7E-01	7.8E-01
LLI wall	1.3E-01	1.6E-01	2.4E-01	3.3E-01	5.4E-01
Heart	1.7E-01	2.1E-01	3.0E-01	4.3E-01	7.3E-01
* Kidneys	3.3E-01	3.9E-01	6.0E-01	8.7E-01	1.4E+00
* Liver	7.1E-01	8.8E-01	1.3E+00	1.8E+00	3.2E+00
Lungs	1.6E-01	2.1E-01	3.1E-01	4.6E-01	8.1E-01
Ovaries	1.2E-01	1.7E-01	2.4E-01	3.5E-01	5.6E-01
* Pancreas	5.2E-01	6.1E-01	9.1E-01	1.3E+00	2.1E+00
Red marrow	6.9E-01	8.8E-01	1.3E+00	2.3E+00	4.5E+00
* Spleen	5.5E+00	7.6E+00	1.1E+01	1.7E+01	3.0E+01
Testes	4.5E-02	6.4E-02	9.9E-02	1.5E-01	2.8E-01
Thyroid	6.1E-02	9.0E-02	1.3E-01	2.1E-01	3.8E-01
Uterus	1.2E-01	1.4E-01	2.1E-01	3.0E-01	5.0E-01
Other tissue	1.1E-01	1.4E-01	2.0E-01	3.0E-01	5.3E-01
Effective dose equivalent (mSv/MBq)	5.9E-01	7.9E-01	1.2E+00	1.8E+00	3.2E+00
Impurities: Effective dose equivalent (mSv/MBq of the inputity)					
^{114m}In (49.51 d)	6.9E+01	9.3E+01	1.5E+02	2.5E+02	4.9E+02

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Inflammation: Imaging with Tc-99m HMPAO–labeled Leukocytes[1]

Leukocytes labeled with technetium-99m hexamethylpropyleneamine oxime (HMPAO) were used in 100 patients: 32 with suspected inflammatory bowel disease, 17 with fever of unknown origin, 21 with suspected abdominal sepsis, 20 with suspected bone sepsis, seven with bronchiectasis, and three with recent myocardial infarction. The distribution of activity in patients subsequently shown not to have inflammatory bowel disease was similar to that previously described for indium-111-labeled leukocytes. However, in this study, activity was also seen in the kidneys and bladder and occasionally the gallbladder on both early (1–3 hours) and late (24 hours) views, and in the colon in late views. Migration of Tc-99m-labeled granulocytes was seen in inflammatory disease as early as 30 minutes after injection, while normal bowel activity was not seen before 4 hours. The sensitivity of Tc-99m-labeled leukocytes in the detection of inflammation was 100%, the specificity was 95%.

Index terms: Hexamethylpropyleneamine oxime • Inflammation, radionuclide studies, 70.125 • Leukocytes • Radionuclide imaging, radiation dose, 70.125 • Technetium, radioactive

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THE use of indium-111–labeled leukocytes has become established as a noninvasive and accurate means of diagnosing a variety of inflammatory conditions in which granulocyte migration is a prominent pathologic feature (1–4). For reasons of convenience, radiation dosimetry, expense, and image resolution, there have been many attempts to replace In-111 with technetium-99m in the form of lipid-soluble complexes—such as Tc-99m oxine (5), and with reducing agents such as Tc-99m stannous pyrophosphate (6, 7), Tc-99m porphyrin complexes (8), and Tc-99m tin colloid for phagocytic labeling (9, 10). None of these approaches has had lasting success, either because the complexes and/or labeling have proved unstable or, in the case of phagocytic labeling, because the cells have become activated and sequestered in the lungs immediately following injection (11).

A new agent, Tc-99m hexamethylpropyleneamine oxime (HMPAO), has recently been introduced for brain imaging (Amersham, Arlington Heights, Ill.) (12). Because it is lipophilic, it was thought that this agent could bind to blood cells; we have shown that it does label leukocytes and is more stable with granulocytes than with mononuclear cells (13). We used Tc-99m HMPAO as a leukocyte-labeling agent in a series of 100 patients suspected of having various inflammatory conditions.

PATIENTS AND METHODS

The 100 patients comprised six clinical groups. Thirty-two had known or suspected inflammatory bowel disease, 17 had fever of unknown origin, 21 had suspected intraabdominal sepsis, 20 had suspected osteomyelitis, seven had bronchiectasis, and three had had a recent large myocardial infarction (Table 1). The clinical diagnosis was confirmed with surgery in 18 patients, with histologic and/or microbiologic findings in 46, and by means of computed tomography (CT) in 17. The diagnosis was based on clinical findings in 13 patients. In three patients, no final diagnosis was made, and in the three patients with myocardial infarction, no final diagnosis was made since no alternate techniques were available to confirm granulocyte migration into the myocardium.

First, 102 mL of fresh venous blood was collected into 18 mL of acid citrate dextrose (ACD, National Institutes of Health formula A). Then, 20 mL was immediately centrifuged at 2,000 *g* for 10 minutes to give plasma, which was used to suspend cells for labeling and reinjection. The remaining 100 mL of anticoagulated blood was used to isolate mixed leukocytes, as previously described (3). A 6% wt/vol solution of hydroxyethyl starch was added to the whole blood in a ratio of one volume hydroxyethyl starch to ten volumes blood to promote red blood cell sedimentation.

Tc-99m HMPAO was formed by adding 450–600 MBq Tc-99m in 5 or 6 mL isotonic saline to a vial of Ceretec (Amersham) containing 0.5 mg HMPAO, 7.6 μg stannous chloride dihydrate, and 4.5 mg sodium chloride with nitrogen. After the vial was shaken to dissolve the contents, 4 or 5 mL of the Tc-99m complex was *immediately* added to the mixed leukocytes. Aliquots of the remaining 1 mL were used to determine the extent of complex formation.

With method A, used in 18 patients, 5 mL Tc-99m complex was added to the mixed leukocytes, which were suspended in 5 mL phosphate-buffered saline enriched with 1 mL autologous plasma; that is, the cells were labeled in 10% plasma at an HMPAO concentration of 42 μg/mL.

With method B, used in 82 patients, 4 mL Tc-99m complex was added to mixed leukocytes in 1 mL plasma; that is, the cells were labeled in 20% plasma at an HMPAO concentration of 80 μg/mL.

With both methods, the cells were incubated for 10 minutes at room temperature, washed with 10–15 mL plasma containing hydroxyethyl starch, and centrifuged at 150 *g* for 5 minutes. The cell pellet was resuspended in 8–10 mL plasma without hydroxyethyl starch and reinjected. The dose was about 200 MBq (5.5 mCi).

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1a. 1b. 2a. 2b.

Figures 1, 2. (1) Normal distribution of activity 3 hours after injection of Tc-99m HMPAO-labeled leukocytes. (a) Anterior view. (b) Posterior view. S = spleen, L = liver. (2) Normal activity in the kidneys. (a) Three hours after injection, there was prominent urinary activity (arrows) in both collecting systems and minimal renal parenchymal activity in this thin male patient with suspected inflammatory bowel disease. (b) Prominent renal parenchymal activity (arrow), but no collecting system activity in a patient with a negative image.

Table 1
Results of Imaging with Tc-99m-labeled Leukocytes

	Results*					Confirmation of Diagnosis				
Initial Diagnosis	TP	FP	TN	FN	No Diagnosis	Surgical	Pathologic	CT	Clinical	No Diagnosis
Inflammatory bowel disease (n = 32)	25	0	7	0	0	1	30	1	0	0
Fever of unknown origin (n = 17)	4	0	12	0	1	2	1	7	6	1
Intraabdominal abscess (n = 21)	15	2	3	0	1	6	9	4	1	1
Osteomyelitis (n = 20)	4	1	14	0	1	9	4	0	6	1
Bronchiectasis (n = 7)	6	0	1	0	0	0	2	5	0	0
Myocardial infarction (n = 3)	0	0	0	0	3	0	0	0	0	3
Total (n = 100)	54	3	37	0	6	18	46	17	13	6

* The sensitivity was 100%, the specificity 95%. TP = true positive, FP = false positive, TN = true negative, FN = false negative.

The reconstitution of a vial of Ceretec with Tc-99m resulted in the formation of three Tc-99m-labeled products: lipophilic Tc-99m HMPAO, which labeled the cells; secondary Tc-99m complex; and reduced, hydrolyzed Tc-99m. The amount of primary lipophilic Tc-99m HMPAO formed was measured by means of three ascending chromatographic systems according to the manufacturers' recommended methods.

An intravenous blood sample was taken about 45 minutes after injection for the calculation of labeled granulocyte recovery. For recovery to have any useful meaning, about 40 minutes must be allowed for complete equilibration between circulating and marginating granulocyte pools (14–16). The cells in 1 mL of the blood sample and those in a known small fraction of the injected cell suspension were separated on a three-step discontinuous density gradient of Percoll (Pharmacia, New Jersey) in saline, and the granulocyte-associated activity was measured (17). Recovery was calculated as the granulocyte-associated activity present in circulating blood at 45 minutes as a percentage of the injected granulocyte-associated activity. The percentage of whole blood activity present as non-cell-bound activity in plasma was also calculated in blood samples taken up to 4 hours after injection.

Patients underwent imaging at about 1, 4, and 24 hours after injection, with a gamma camera (400A or 400T; General Electric, Milwaukee) fitted with a low-energy, general-purpose or high-resolution collimator. The three patients with myocardial infarction received Tc-99m HMPAO-labeled cells in the late afternoon and underwent imaging at the beginning of the following day, or at about 16 hours. Because of this timing, they were given higher doses, about 500 MBq (13.5 mCi).

Generally, 500,000 counts per image were obtained, and images were recorded in analog and digital form. Oblique and pelvic outlet views were obtained when considered necessary, the latter in particular being used to clearly identify bladder activity.

We assumed, on the basis of the images, that the quantitative biodistribution of Tc-99m HMPAO-labeled leukocytes was the same as that of In-111-labeled pure granulocytes. Since quantitative data on urinary and fecal excretion of free Tc-99m HMPAO (or derivatives) are not currently available, to our knowledge, we did not calculate the radiation doses to the urinary tract and colon.

RESULTS

At the time of labeling, 84% ± 6% (mean ± standard deviation, $n = 34$) of the Tc-99m HMPAO was present, 10% ± 5% was secondary complex, and 6% ± 4% was reduced technetium. No free pertechnetate was found.

3a. 3b. 4.

Figures 3, 4. (3) Gallbladder activity (arrow) at 3 hours in a patient with ulcerative colitis, as seen on (a) anterior and (b) right lateral views. Note abnormal accumulation in the entire colon. (4) Normal image shows colonic activity at 24 hours. Note gallbladder (arrow) and bladder activity.

5a. 5b. 6.

Figures 5, 6. (5) Abnormal uptake in the left iliac fossa (arrow) in a patient with ulcerative colitis. Views obtained (a) 30 minutes and (b) 3 hours after injection confirm the presence of inflammatory bowel disease. *b* = bladder. (6) Improved resolution with Tc-99m is demonstrated in a 10-year-old boy with Crohn disease involving the terminal ileum and cecum, on an image obtained 1 hour after injection.

The labeling efficiency was slightly but significantly ($P < .05$) higher with method B (56% ± 13%, $n = 82$) than with method A (49% ± 10%, $n = 18$). The distribution of cell-bound activity with method A was 78%, 15%, and 7% for the granulocytes, mononuclear leukocytes, and platelets or red blood cells, respectively; with method B, the corresponding values were 77%, 17%, and 6%.

The granulocyte recovery at 45 minutes was 40% ± 13% for method A ($n = 10$) and 34% ± 15% for method B ($n = 16$). The difference was not significant, and the overall mean recovery was 37% ± 14% ($n = 26$).

Normal distribution.—During the first hour, activity was seen in the lungs, liver, spleen, and bone marrow. This distribution of activity (Fig. 1) is very similar to that seen with pure granulocytes separated and labeled in plasma with In-111 tropolonate. In addition, bladder activity was seen in all patients. The kidney and renal pelvis were also occasionally seen (66%), especially in thin patients. (Fig. 2). Gallbladder activity was seen in 4%. At 4 hours, lung activity was minimal and bone marrow uptake was more marked. The activity in liver, spleen, kidneys, and bladder remained unchanged. The gallbladder was still rarely visualized (10%) (Fig. 3). However, faint bowel activity (in the proximal colon) was visible in five patients (5%) without evidence of inflammatory bowel disease or pulmonary sepsis. At 24 hours, the normal distribution of activity remained the same as that at 4 hours, except that activity was also seen within the colon in all patients (Fig. 4).

Inflammatory disease.—All patients with inflammatory bowel disease (ulcerative colitis, Crohn disease, or radiation-induced colitis) showed intense activity in the affected bowel during the first hour, with a further increase in activity by 4 hours (Figs. 5, 6). One patient with Whipple disease and another with Behçet syndrome, both of whom had bowel involvement, did not show intense bowel activity until 4 hours. The normal bowel excretion of Tc-99m HMPAO made the 24-hour images not helpful in this group of patients unless they also had an associated abdominal abscess without enteric communication, in which case there was a further focal increase in activity (four patients). No false-positive gut activity was seen at 1 hour. Abnormal bowel activity was therefore easily distinguishable from normal bowel activity, which probably represents

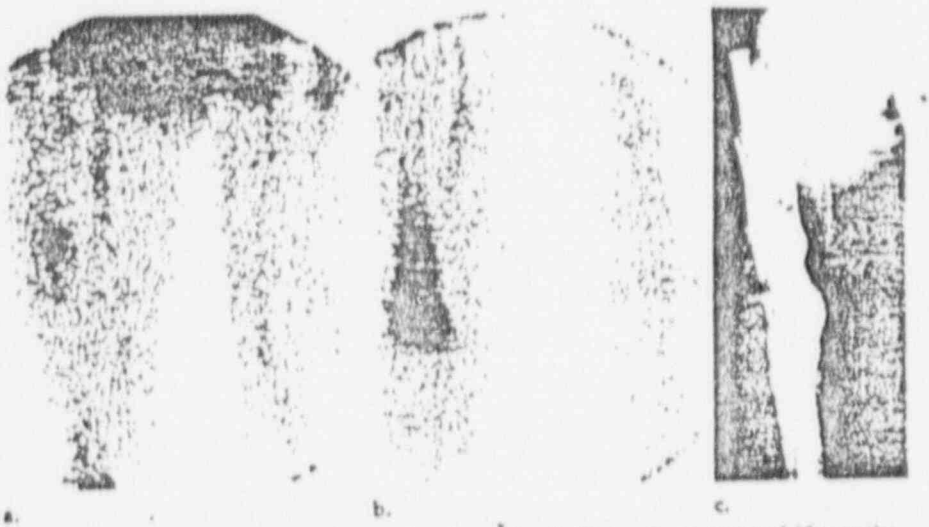
7a. 7b. 8a. 8b.

Figures 7, 8. (7) Cholecystitis in a 60-year-old man, manifested by fever of unknown origin. (a) Tc-99m HMPAO image at 1 hour shows uptake in the gallbladder wall (arrow) but not in the lumen. (b) Tc-99m *HIDA* image at 1 hour (obtained 2 days later) demonstrates a nonfilling gallbladder, indicating that the activity seen after Tc-99m labeling represented abnormal migration of leukocytes. (8) Intraabdominal collection complicating acute pancreatitis in a 40-year-old man. (a) Early view (at 3 hours) shows the collection in the left paracolic gutter. (b) Late view (at 24 hours) again demonstrates the collection, but bowel activity is also seen in the ascending and transverse colon. This activity could be normal or could represent enteric drainage of the abdominal collection.

early biliary excretion of Tc-99m HMPAO. Of 17 patients with fever of unknown origin, 12 showed no abnormal activity. Of the five who did, two with graft-versus-host disease following bone marrow transplantation showed intense early (1-hour) uptake in loops of the small bowel. One patient showed intense uptake at the site of a gastrojejunal anastomosis that had broken down. Of the remaining two patients, one had activity in the wall of a distended gallbladder (Fig. 7). No activity was seen within the lumen of the gallbladder, which was shown with Tc-99m HIDA scanning to be obstructed. Acute cholecystitis was diagnosed. The fifth patient showed early (3-hour) activity in loops of the small bowel, but no final diagnosis was reached and this patient was not included in the accuracy analysis.

Of the 21 patients with suspected intraabdominal sepsis (Fig. 8), 17 had positive images with uptake in the abscess by 4 hours and a further increase at 24 hours. At laparotomy, one patient was found to have a bleeding splenic artery corresponding to the abnormal focus on the images, while in another a focus of intense uptake seen anterior to the spleen could not be confirmed with CT. These two patients therefore had false-positive results.

Twelve of the 20 patients with suspected bone sepsis had prosthetic joints or fixation plates (Fig. 9). Two of them showed abnormal activity around the prosthesis, and infection was confirmed during surgery. One patient whose right knee was swollen and painful showed several areas of focal activity around both knees, and bilateral septic arthritis was initially suspected. A sulfur colloid scan, however, showed normal bone marrow, and this case was classified as a false-positive result. Seven patients with known bronchiectasis were imaged. All showed activity in areas of bronchiectatic lung at 24 hours, but the intensity was not great. Furthermore, the 4-hour images were normal in three of these patients. One patient who received labeled cells 24 hours after a large inferior myocardial infarction showed abnormal activity in the region of the infarct 24 hours after injection, but the results were negative in two patients with documented myocardial infarctions who received Tc-99m HMPAO-labeled cells 48–72 hours after infarction.



a. b. c.

Figure 9. Osteomyelitis in a patient with a femoral nail. The nail was inserted 12 months previously, after the shaft of the femur was fractured. Images obtained 2 hours after injection show two foci of migrating cells, in the midshaft at the site of the fracture (a) and at the lower end of the nail (b). Normal circulating activity is visible in the blood pool of the limbs. (c) Radiographic appearance.

The doses received by the target organs—spleen, liver, and bone marrow—were 21.3 mGy/100 MBq (0.79 rad/mCi), 2.5 mGy/100 MBq (0.093 rad/mCi), and 2.3 mGy/100 MBq (0.085 rad/mCi), respectively. The corresponding doses for In-111-labeled granulocytes were 716 mGy/100 MBq (26.5 rad/mCi), 86 mGy/100 MBq (3.2 rad/mCi), and 70 mGy/100 MBq (2.6 rad/mCi), respectively. The effective dose equivalents were 0.017 mSv/100 MBq (0.063 rem/mCi) and 0.57 mSv/100 MBq (2.1 rem/mCi), respectively. The imaging dose for Tc-99m HMPAO, however, is higher than that for In-111—200 MBq (5.4 mCi) versus 12 MBq (320 μCi), giving dose ratios (Tc-99m to In-111) per imaging dose of 0.5, 0.48, and 0.55 for spleen, liver, and bone marrow, respectively.

DISCUSSION

In-111 labeling of leukocytes is useful in the detection of certain inflammatory conditions (18). However, although it has a suitable half-life for abscess imaging, In-111 is expensive and gives a high radiation dose to the target organs (bone marrow and spleen). Tc-99m is more convenient to use and gives better resolution than In-111, but no Tc-99m agents have been established for leukocyte labeling. The rationale for testing Tc-99m HMPAO is that it is highly lipophilic, which is why it is used in imaging cerebral perfusion (12).

In preliminary work (13) with method A, we found that Tc-99m HMPAO labeled cells with reasonable efficiency. Like the In-111 complexes, Tc-99m HMPAO selectively labels the leukocytes, but unlike In-111 it fortuitously labels the granulocytes with significantly more stability than the mononuclear cells. Furthermore, labeling can be performed in the presence of plasma, which we have previously shown to be beneficial to the ultimate functional integrity of the granulocytes (3).

We were unable to observe any differences in activity distribution or granulocyte recovery between methods A and B, but we have adopted method B because we prefer to maintain the cells in the highest possible plasma concentration compatible with efficient labeling. Although 102 mL of blood is routinely obtained (giving a total volume with anticoagulant of 120 mL), a smaller volume can be obtained from children and patients with difficult venous access or high neutrophil counts (>20,000/mm^3 [20.0×10^9/L]).

Chromatography demonstrated that, at the time of labeling, about 85% of the Tc-99m HMPAO was lipophilic. Because of the early appearance of bladder activity and absence of thyroid activity, free activity eluting from the cells is probably in the form of a hydrophilic complex. Thus, at no stage do the images resemble those seen after direct Tc-99m HMPAO injection as for brain imaging; for instance, there is no suggestion of cerebral activity.

Activity circulating in the blood remained predominately cell bound (90%), suggesting that eluted activity is rapidly cleared from blood. Because this eluted activity originates predominately from the mononuclear cells (13), and because the agent has a high degree of selectivity for leukocytes in the first place, Tc-99m HMPAO provides an essentially pure granulocyte label. A potential disadvantage of the low level of stability in lymphocytes is the possibility of a higher frequency of chromosomal aberrations resulting from a nonlethal dose. Whether this represents a significant problem remains controversial and has been the subject of much discussion (19).

Early images suggest that the labeled leukocytes are functionally optimal, as suggested by minimal sequestration in the lungs and minimal hepatic activity, criteria that we have previously shown to be related to leukocyte activation and/or damage (20). Splenic activity is intense, but this is the result of physiologic granulocyte pooling in the spleen (14). Further evidence that the granulocytes are intact is the high recovery of granulocyte-associated activity in the blood 45 minutes after injection. This recovery was 37%, very similar to that of pure granulocytes separated on plasma-enriched, density gradient columns and labeled in plasma with In-111 tropolonate (15) and only slightly lower than the 45% reported for granulocytes labeled with phosphorus-32 diisopropyl fluorophosphonate (21).

The normal distribution of activity is similar to that seen with In-111 labeling, except that bowel activity is also present. This activity probably arises from biliary excretion, but we do not know whether it is in the form of the primary lipophilic complex, which when used for cerebral imaging does undergo significant hepatic uptake and ultimately appears in the bowel (22), or in the form of a secondary metabolite. As with the bladder activity, we initially thought that this normal bowel activity would make it difficult to interpret abnormal abdominal activity, but this has not been the case. Thus, in patients without inflammatory bowel disease, activity does not appear in the bowel until about 4 hours after injection, whereas activity on migrating cells is usually visible in inflamed bowel much earlier than this, sometimes as early as 15 minutes after injection. Furthermore, it is much more intense than the normal activity, and in the two patients (with Whipple disease and Behçet syndrome) in whom migrated activity was not seen until 4 hours, this intensity enabled us to make a positive diagnosis of complicating inflammatory bowel disease. Bladder activity in the collecting system is cleared early, leaving only faint parenchymal activity, and has also not caused any diagnostic confusion. It is usually identified as bladder activity by comparison of anterior and posterior images. However, it is possible that an element of urinary stasis in the kidney or collecting system could obscure, or be misinterpreted as, sepsis, or, conversely, that migrated neutrophil activity could be misinterpreted as bladder activity.

Only four of 17 patients with fever of unknown origin had true-positive findings with Tc-99m-labeled leukocyte imaging, a proportion consistent with the known low frequency of infection in this clinical setting (23). The majority of patients with suspected intraabdominal sepsis had positive findings, although there were false-positive results in two. In spite of the short half-life of Tc-99m, 24-hour views may still be helpful for confirming abscess by showing a further increase in activity. Late imaging is made possible by the stability of the Tc-99m in the abscess.

Osteomyelitis has always been thought of as difficult to demonstrate with labeled leukocytes, and gallium-67 has been proposed as a superior radionuclide (24). However, we have had good results with labeled leukocytes in osteomyelitis (25), and our success may be due to the use of pure granulocytes and maintenance of the cells in plasma throughout labeling. Abundant bone marrow activity is seen with these cells, probably as the result of reticuloendothelial destruction in bone marrow (16); this may interfere with the interpretation of the images in bone infections. Thus, we had false-positive results in one case in this clinical category. Imaging the bone marrow with Tc-99m sulfur colloid after leukocyte labeling is therefore often helpful. Bone marrow activity seemed more intense than after In-111 labeling of granulocytes, possibly because of the higher photon flux.

Bronchiectasis has previously been reported to appear markedly abnormal on In-111-labeled leukocyte images (26), and so our finding of only minimally positive images was somewhat disappointing and puzzling. This may partly be explained by the normal pulmonary uptake of free amine (22), which elevates the lung background activity.

In-111 labeling of leukocytes has been proposed as a means of monitoring myocardial inflammation after infarction but has not been successful (27). One explanation is the relative-

ly poor resolution with In-111. Because of improved resolution and ;h photon flux, Tc-99m labeling ..ay offer an exciting new approach to the study of myocardial inflammation. Of the three patients with myocardial infarction in our series, the results were positive only in the one patient who was studied within 24 hours of infarction. Although they had elevated enzyme levels and electrocardiographic changes indicative of infarction, all three patients were classified as having no final diagnosis, since we had no alternate method to confirm granulocyte migration into the myocardium.

As expected, the radiation dose is less with Tc-99m labeling than with In-111 labeling. Our calculated values for th : effective dose equivalents are very similar to previously reported values for both agents (28).

In conclusion, Tc-99m HMPAO-labeled leukocytes have been successfully used for imaging a wide variety of inflammatory processes, with an accuracy comparable to that achieved with In-111 labeling. In addition to greater convenience and a reduced radiation dose, Tc-99m labeling en- les the imaging of low-grade in- nmatory processes not readily im- ged with In-111. We believe that Tc-99m will soon be the preferred agent for routine abscess imaging. ■

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In Vitro and In Vivo Evaluation of Granulocyte Labeling with [^{99m}Tc]d,l-HM-PAO

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The functional integrity of white blood cells labeled with [^{99m}Tc]d,l-HM-PAO containing variable amounts of the ligand or of the ^{99m}Tc activity was evaluated by enzymatic tests and by measuring random migration, chemotaxis, phagocytosis, killing, and adhesion. The ultrastructure of labeled cells was studied by electron microscopy. The tracer dose and the HM-PAO concentration did not significantly affect phagocytosis and killing. The results of the other tests remained normal. A maximum labeling efficiency of 80% was reached by incubating the granulocytes for 20 min with 10–20 mCi of [^{99m}Tc]d,l-HM-PAO containing 50 μg of the ligand in 1 ml of saline. There was only a slow washout of 20% of activity from the labeled cells in 24 hr. The ultrastructure was not influenced by the labeling technique. Proven infection sites of 17 orthopedic patients were clearly visualized. After a short transient lung uptake, there was a clear spleen and moderate liver uptake with early bladder and late prominent colon visualization. Because of the lower cost, favorable radiation dose and more suitable tracer characteristics, this technique is a promising alternative for ^{111}In labeling of white blood cells.

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Indium-111- (^{111}In) labeled white blood cells have been used for years in the detection of infection. This technique combines both high sensitivity and specificity but the nonideal physical characteristics of the radionuclide are a disadvantage. As technetium-99m (^{99m}Tc) is the preferred radioisotope in view of its availability, ideal physical characteristics and low cost, many attempts have been made for more than 15 years to replace indium by technetium (*1,2*).

The most successful approaches to incorporate ^{99m}Tc in granulocytes have been the reduction of pertechnetate by stannous compounds diffused in the WBC in a similar way as used in red blood cell labeling (*3–5*) and the incorporation of ^{99m}Tc colloids by phagocytosis (*6,7*). In an animal study with ^{99m}Tc-labeled granulocytes obtained by the stannous pyrophosphate reduction method, Harper et al. mentioned the same radiation dose and less of a tendency to accumulate in the pus as compared to ^{111}In-labeled cells (*3*). Using WBC labeled with ^{99m}Tc colloids, Hanna and coworkers found a prolonged lung transit time that can be explained by aggregation or activation of the neutrophils (*6*). The results reported by Gil et al. when using stannous glucoheptonate as reducing agent could not be confirmed in our laboratory (*8*).

Recently, Peters et al. (*9*) described the possibility of labeling granulocytes with [^{99m}Tc]hexamethylpropylene amineoxime ([^{99m}Tc]-d,l-HM-PAO), a new radiopharmaceutical introduced for brain imaging (*10,11*). Because of its lipophilic character this agent is able to diffuse through the cell membrane and apparently binds intracellularly. The underlying mechanism is not fully understood.

The aim of the present study is the evaluation of this new labeling technique by checking the in-vitro and in vivo behavior of the technetium-labeled cells. The integrity of the cells was tested by means of electron

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microscopy and enzymatic and functional tests. Clinical studies have been performed in orthopedic patients with proven infections to demonstrate the clinical usefulness of the labeled granulocytes.

MATERIALS AND METHODS

Separation of White Blood Cells

The separation method is schematically shown in Figure 1.

Sedimentation of red blood cells for 1 hr to obtain leukocyte and platelet-rich plasma (LRP) was facilitated by the addition of 2 ml of 2% (w/v) methylcellulose to a mixture of 10 ml ACD and 50 ml whole blood. After centrifugation (150 *g*, 5 min) of the LRP, a second centrifugation (2500 *g*, 7 min) of the supernatant (platelet-rich plasma or PRP) was performed follows by filtration of the supernatant (platelet-poor plasma or PPP) through a 0.45 μm filter resulting in cell-free plasma (CFP).

The leukocyte-rich pellet was resuspended in 1 ml of Hanks' balanced salt solution (HBSS) containing 0.1% albumin, and the remaining red blood cells (RBCs) were eliminated by hypotonic lysis for 20 sec. The debris of the RBC on the surface of the pellet obtained after a subsequent centrifugation (150 *g*, 5 min) was eliminated by gently pipetting 1 ml of HBSS and aspirating the fluid. The remaining pellet was resuspended in 2 ml of cell-free plasma.

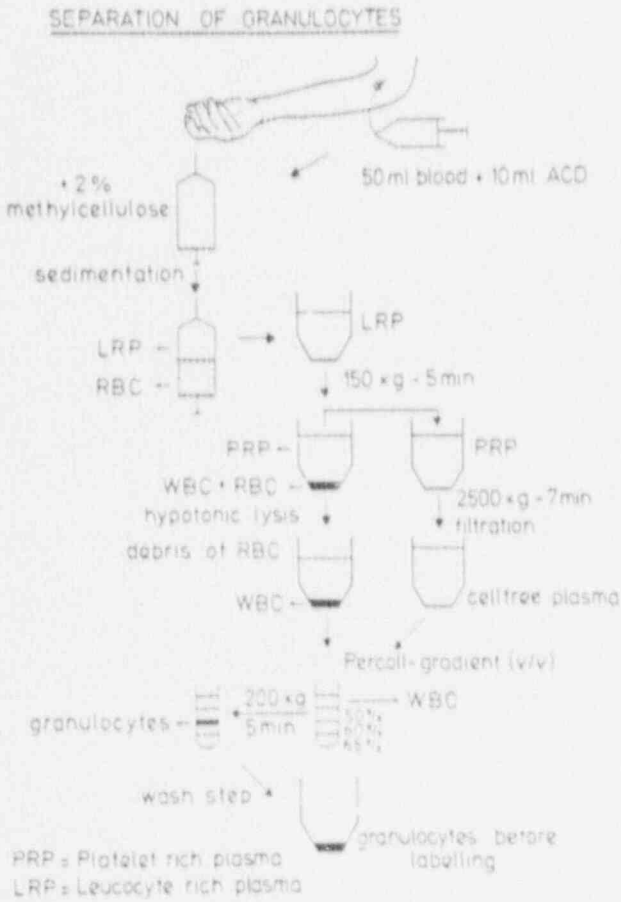

FIGURE 1
Schematic survey of separation procedure.

Further purification was performed by discontinous gradient centrifugation of the mixed WBC pellet applied on the top of three layers of Percoll-plasma mixtures of increasing density (65%, 60%, 50% (v/v) Percoll with a density of 1.129 g/ml). The bottom layer was washed with 4 ml of 0.9% (w/v) NaCl and centrifuged (150 *g*, 5 min) to obtain a WBC pellet.

Labeling Method

d,l-HM-PAO was synthesized following a published procedure (*11*). Five micrograms of $SnCl_2 \cdot 2H_2O$ in 2.5 μl HCl 0.05 N was added to 0.5 mg HM-PAO dissolved in 1 ml of water. Neutralization of the very small amount of HCl during labeling was not necessary as the final pH was still slightly alkaline. 120 mCi of [^{99m}Tc]-$NaTeO_4$ dissolved in 4 ml saline was added. The preparation was diluted with saline to a concentration of 20 mCi/ml. The pellet of granulocytes after Percoll separation was resuspended immediately in 20 mCi of [^{99m}Tc]d,l-HM-PAO in saline (1 ml) or was first resuspended in 1 ml of 0.9% NaCl and then added to 20 mCi of [^{99m}Tc] d,l-HM-PAO in 1 ml. The incubation time was 20 min. After centrifugation, the radioactivity associated with the cells was determined. Before use, each preparation of [^{99m}Tc]-d,l-HMPAO was analyzed by thin-layer chromatography on ITLC SG sheets with acetone and saline as mobile phases to check for "polar" [^{99m}Tc]HM-PAO and pertechnetate, respectively. Paper chromatography with acetonitrile-water (1:1) as the mobile phase was used to determine the content of colloidal ^{99m}Tc. Only preparations with a radiochemical purity over 88% were used for WBC labeling.

Evaluation of the Incorporation and the Washout of the Tracer

Aliquots of the incubation mixture were withdrawn at different time intervals (5, 10, 15, 20, 25, 30, 40, 50, and 60 min), and the radioactivity in the cells and the supernatant was measured after centrifugation.

After labeling, the cells were resuspended in plasma and preserved at 37° C for 24 hr after labeling. Aliquots were taken at 1, 2, 3, 4, 5, and 24 hr, and the radioactivity associated with the cells was measured after centrifugation.

In Vitro Tests of Granulocyte Function

1. In the first experiment (n = 3), the cells (30×10^6) were incubated for 20 min with different concentrations of HM-PAO (0.05, 0.25, and 0.5 mg HM-PAO/ml). The added amount of tracer varied between 1 and 15 mCi.

2. In a second experiment (n = 3), the tracer dose, dissolved in a constant volume, was varied (0, 1, 5, and 15 mCi) while the concentration of HM-PAO (0.25 mg/ml), the incubation volume (1 ml), and the number of cells (40×10^6) were kept constant.

The labeled cells obtained in these experiments were evaluated with the following enzymatic and functional tests.

The myeloperoxidase (MPO) content of granulocytes was assayed spectrophotometrically using a horseradish peroxidase standard. Enzyme activity is expressed in units of MPO/$2 \cdot 10^6$ PMN. Normal values: 11.28 ± 2.91 units/2×10^6 granulocytes.

Superoxide generation (SO) was quantified spectrophotometrically at 550 nm by the superoxide dismutase-sensitive reduction of ferricytochrome C. Zymosan activated by autologous serum (SAZ, 0.5 ml/10^7 PMN) was used as standard stimulant in a concentration of 2.5 ng/dl. The results are

expressed as nmol of cytochrome C reduced per 5.10^5 PMN in 15 minutes. Normal values: 26.91 ± 3.31.

Chemotaxis was evaluated by measuring the migration (μm) under agarose (0.9% w/v agarose). ZAS (zymosan activated serum) and FMLP (formyl-methionyl-leucyl-phenylaline) were used as chemoattractants. Normal values: random migration 430 ± 90 μm; chemotaxis ZAS 1040 ± 310 μm; FMLP 1340 ± 270 μm.

Phagocytosis and intracellular killing capacity of granulocytes were assayed using a pour-plating technique. The results are expressed as a percentage of *Staphylococcus aureus* phagocytosed and of *Staphylococcus aureus* killed after 20 min. The killing/phagocytosis index is an accurate measure of granulocyte antibacterial efficiency. Normal values are: phagocytosis: 83.03 ± 5.07%; killing: 72.7 ± 6.26%; killing/phagocytosis index: 0.86 ± 0.04.

Adherence was assayed by the ability of PMN to adhere to gelatin-coated plastic surfaces (overnight coating of culture plates with 1% gelatin solution). Labeled PMN and a free chelator were added to each well and preincubated at 37° C; after 10 min, the stimulant (FMLP $10^{-7}M$) was added and the plates incubated for another 30 min. The supernatant was then carefully removed, and the pellet was washed twice with warm HBSS-ALB, and then lysed twice with NaOH 0.1 N. The radioactivity in the supernatant and pellet was counted and results expressed as percent adherence. Each assay was performed in quadruplicate, and the results were calculated as the mean percentage of adherence or the mean percentage of control.

Electron Microscopy

Cell pellets were resuspended in 1.5% v/v glutaraldehyde in 0.1*M* cacodylate buffer (pH 7.3; 370 mOsm/l) for 30 min at room temperature. After washing in 0.1*M* cacodylate buffer containing 4% w/v sucrose (pH 7.3; 320 mOsm/l), the cells were postfixed for 1 hr in 1% w/v OsO_4 in Sörensen phosphate buffer (pH 7.3; 350 mOsm/l). Cells were washed in cacodylate buffer containing sucrose and stored overnight in 70% v/v alcohol in water. After dehydration in a graded series of ethanol and washing in propylene oxide, the cells were resuspended in a mixture of 1 volume of propylene oxide and 4 volumes of epon for 1 hr. After centrifugation, the propylene-oxide-epon mixture was replaced by epon. The cells were embedded in Beem polyethylene capsules and centrifuged at 9,000 *g* for 30 min before polymerization.

The cell pellets were sectioned with a diamond knife on an LKB III microtome. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined with an electron microscope (Zeiss EM 10).

Patient Studies

Seventeen orthopedic patients with proven infections were injected with leukocytes labeled with 10–17 mCi of [^{99m}Tc]-d,l-HM-PAO: eight patients with an infected fracture, seven with an infected total hip prothesis, one with a malum perforans, and one with an infected joint after an intra-articular injection. All the infections were confirmed by cultures, clinical evaluation, radiology, or surgical findings. Eleven patients were injected under a double-headed camera (SELO GAMMA CAT) interfaced to a computer system (PDP 11/34 (DEC)) and were continuously monitored to evaluate lung, liver, and spleen uptake. Delayed images were made 3–4 hr and 21 hr postinjection. Two experienced readers graded the images as follows: 0: negative; 1: possibly positive; 2: suspect; 3: positive.

RESULTS

The incorporation of [^{99m}Tc]d,l-HM-PAO in the white blood cells and the washout of the radionuclide in function of time is shown in Figures 2 and 3. The results of the functional tests of granulocytes labeled with varying amounts of HM-PAO are presented in Table 1. The results of the same tests obtained with a constant amount of HM-PAO but varying tracer activities are shown in Table 2.

The ultrastructure of a neutrophil granulocyte cell after labeling is shown in Figure 4.

Figure 5 shows the time activity curves during the first hour in the region of the lungs, the spleen and the liver of one of the patient studies. A clinical example of uptake of ^{99m}Tc-labeled WBC in an infected fracture of the left ankle of a patient is illustrated in Figure 6. Eleven patients had the highest score; two patients were scored as highly suspect; three were interpreted as possibly infected; and one had a negative scan.

DISCUSSION

The most important reasons for investigation of a labeling technique with ^{99m}Tc are the better physical characteristics of the tracer, the lower radiation dose,

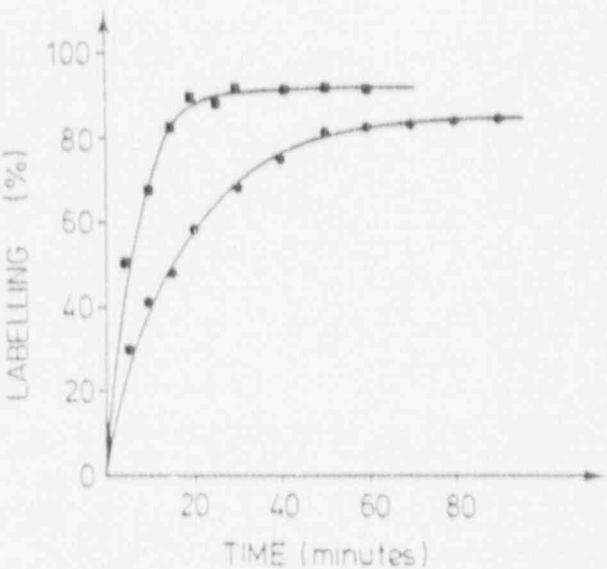

FIGURE 2
Incorporation of [^{99m}Tc]HM-PAO in the leukocytes (%) versus time for two different incubation volumes. "●": The granulocytes (60 × 10^6) are resuspended in 1 ml of 0.9% NaCl and 1 ml [^{99m}Tc]HM-PAO (54 μg HM-PAO; 11.1 mCi of ^{99m}Tc). "■": The granulocytes (60 × 10^6) are resuspended in 1 ml [^{99m}Tc]HM-PAO (54 μg HM-PAO; 12.7 mCi of ^{99m}Tc).

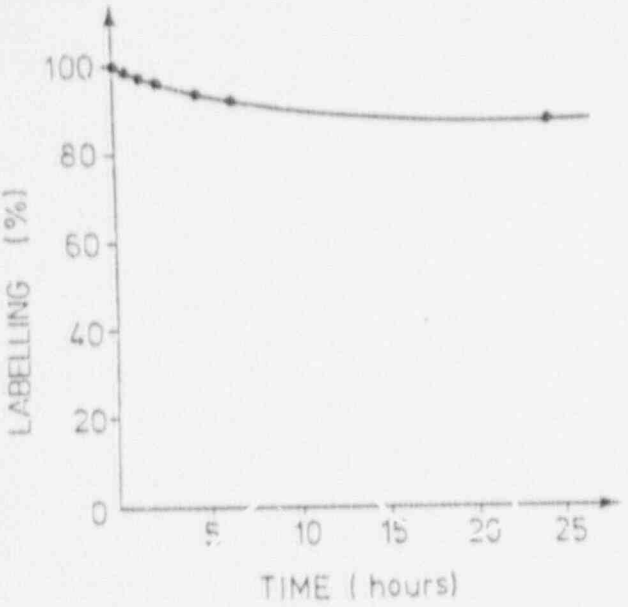

FIGURE 3
Percentage of the radioactivity retained in the cells versus time. After labeling (cfr. Figure 2; method 2), the granulocytes were resuspended in plasma and kept at 37°C for 24 hr. Sequentially the radioactivity remaining in the cells was measured.

the lower cost, and the ready availability of a ^{99}Mo-^{99m}Tc generator. Peters et al. (*9*) recently introduced [^{99m}Tc]d,l-HM-PAO labeling of WBC as a new alternative to currently used methods of labeling with ^{111}In or ^{99m}Tc. Many papers have since described the use of this technique to replace ^{111}In labeling of granulocytes (*12-22*). The success and usefulness of this labeling method will greatly depend on the functional integrity of the white blood cells after labeling, which is necessary for their localization at the inflammation site.

As illustrated by a previous study, it is essential to perform granulocyte functional tests in vitro on the purest possible cell population because contaminating cells (platelets, monocytes) definitely influence the results of the very sensitive granulocyte tests to a varying extent (*23*). For this reason, further steps like hypotonic lysis and percoll gradient were added to the separation procedure. Although it is well known that some activation of cells certainly occurs and although there may be some phagocytosis of silica particles present, sufficient granulocyte functional capacity is preserved to perform experiments in controlled conditions. Phagocytosis, in particular, is still sufficient, as is illustrated by its control value of 93% and 86% (Tables 1 and 2). The labeling of RBC fragments by lipid soluble tracers after the hypotonic lysis is prevented by three extra separation steps: (a) centrifugation of the isotonic mixture, (b) gently pipetting and aspiration of 1 ml of HBSS for the elimination of the debris of the RBC, and (c) further purification by discontinuous gradient centrifugation. Labeling of the isolated cells in saline was performed to enhance the labeling yield since the results of functional and morphologic tests could not demonstrate a deleterious influence of short incubation of the granulocytes in a medium without plasma.

As published previously (*24*), the separation technique developed in our laboratory delivers functionally and morphologically intact cells. In this study, normal values of lung transit time and spleen and liver uptake were measured, which is an argument that the leukocytes were not affected by the labeling procedure (Fig. 5).

TABLE 1
Functional and Enzymatic Test of Leukocytes Labeled with Different HM-PAO Concentrations

Test	Blanc	HM-PAO 0.05 mg/ml	HM-PAO 0.25 mg/ml	HM-PAO 0.5 mg/ml
MPO	11.9 ± 1.3	11.2 ± 2.0	11.9 ± 1.8	11.7 ± 1.5
S.O.*	27.1 ± 0.7	26.7 ± 1.5	27.1 ± 1.7	26.7 ± 2.9
Phagocytosis (%)	93.3 ± 1.8	84.7 ± 9.3	89.2 ± 1.7	83.2 ± 10.5
Killing (%)	83.6 ± 4.9	69.4 ± 14.7	60.6 ± 17.4	61.7 ± 22.8
K-Index	0.90 ± 0.04	0.81 ± 0.09	0.68 ± 0.19	0.73 ± 0.19
Chemotaxis (μm)				
FMLP†	1930 ± 352	2040 ± 393	2080 ± 335	2060 ± 330
ZAS‡	1650 ± 260	1440 ± 453	1780 ± 195	1720 ± 200
RM§	600 ± 165	670 ± 165	660 ± 137	627 ± 139
Adhesion				
Basal	27.4	16.1 ± 7.5	20.5 ± 8.4	28.9 ± 7.2
FMLP	60.7	56.4 ± 7.9	50.0 ± 5.2	53.7 ± 9.0

* Myeloperoxidase content (units MPO/2·10^6 PMN).
† Superoxide generation (nmol of cytochrome C reduced per 4·10^6 PMN in 15 min.
‡ Formyl methionyl leucyl phenylaline.
§ Zymosan activated serum.
¶ Random migration.

TABLE 2
Functional and Enzymatic Test of Leukocytes Labeled with a Constant Amount of HM-PAO and Increasing ^{99m}Tc Activities*

Test	Blanc	mCi ^{99m}Tc			
		0	1	5	15
S.O.		20.5	25.0	25.6	25.0
Chemotaxis (μm)					
FMLP	1790 ± 288	1598	1875	1643 ± 169	1640 ± 148
ZAS	1517 ± 90	1453	1500	1430 ± 62	1437 ± 76
RM	375 ± 93	345	440	350 ± 46	367 ± 38
Phagocytosis (%)	86.5 ± 9.2	85.0	96.2	86.9 ± 8.2	89.2 ± 6.1
Killing (%)	75.1 ± 15.6	70.9	85.2	75.0 ± 11.0	79.2 ± 17.4
K/F-Index	0.86 ± 0.10	0.83	0.88	0.86 ± 0.09	0.85 ± 0.10
Adhesion (%)					
Basal	42.6		43.0	37.6 ± 4.5	38.6 ± 6.0
FMLP	61.2		66.0	60.0 ± 5.6	58.4 ± 7.8

*For definitions of abbreviations, see Table 1.

From the results in Tables 1 and 2, it is clear that random migration, chemotaxis (FMLP and ZAS), and adhesion of the labeled granulocytes are not influenced by labeling with different amounts of tracer and/or HM-PAO. The results in Table 1 suggest that phagocytosis and killing are possibly slightly affected, but there is no clear correlation with the HM-PAO concentration. To exclude the potential influence of the amount of radioactivity, the same tests were repeated after labeling with a constant amount of HM-PAO but with increasing activities of ^{99m}Tc. The normal results of these tests indicate that the results of the first experiments were probably within the limits of experimental error. Normal in vitro results have also been reported by other authors (*12,15,18*).

Figure 2 shows that during the labeling step an equilibrium is reached 20 min after resuspending the white blood cell pellet in 1 ml of the [^{99m}Tc]d,l-HM-PAO solution. Resuspension of the white blood cells in 1 ml of physiologic saline before addition of the ^{99m}Tc complex results in a slower and less efficient tracer uptake, probably due to the dilution effect. The mean separation efficiency expressed as a percentage of the number of leukocytes present in the patients' blood was 40%

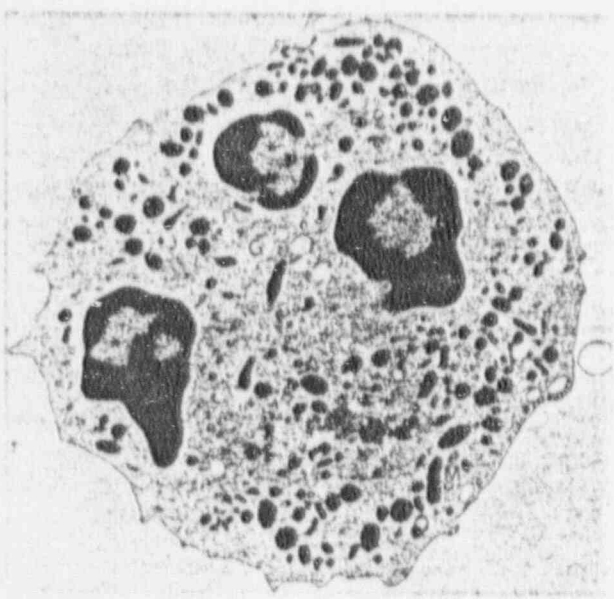

FIGURE 4
Electron micrograph of a ^{99m}Tc-labeled neutrophil granulocyte. The cell profile has a slightly irregular outline. Azurophil (A) and neutrophil (N) granules, mitochondria (M), and polyribosomes (arrow) are dispersed in the cytoplasm. Three nuclear profiles (*) are visible. The Golgi apparatus (G) is localized in the nuclear region. The shape, organization, and ultrastructure of the labeled cells do not appear to differ from that of the control cells (× 20,000).

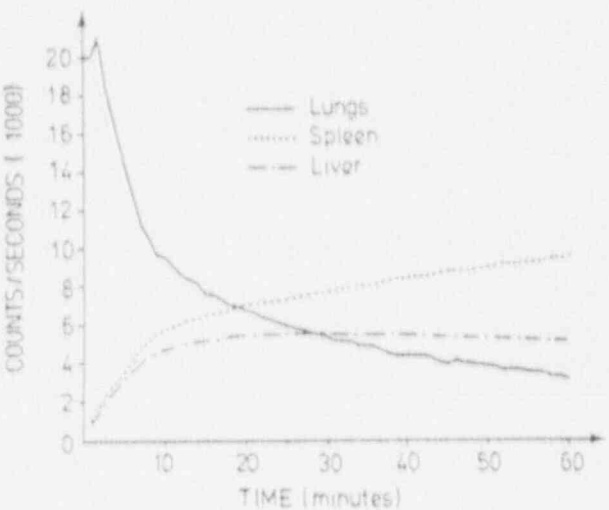

FIGURE 5
Time-activity curves in the region of the lungs, spleen, and liver during the first hour after injection of 15 mCi of autologous ^{99m}Tc-labeled granulocytes.

FIGURE 6
WBC scan is shown of a patient with a left bimalleolar ankle fracture. He was treated 6 mo ago with an open reduction and was admitted to the hospital with complaints of pain and inflammation. On the upper ray: the anterior projection of both ankles and the left lateral and medial projection of the left ankle 1 hr postinjection are shown. There is a clear uptake of labeled cells in the distal tibia and the left ankle. The same projections 3 hr postinjection are shown on the lower ray. A more focal accumulation in the medial malleolus, distal tibia and ankle joint is shown. The three-phase skeletal scintigraphy was positive in all phases. The x-rays of the left ankle were compatible with a septic arthritis. The cultures of the exudate showed the presence of *Staphylococcus aureus*.

(range 18–78). This means that, with an average leukocyte count of 7000 cells per μl, 8 to 26 × 10^7 cells were used for labeling. The average labeling efficiency in the case of the 17 patients was 69.3% (range 50–81), thus demonstrating the effectiveness of our labeling method as compared to the lower results of other authors (*19*). As shown in Figure 3 the labeling is quite stable, and only 20% of the bound tracer activity is eluted from the cells during the first 24 hr. It is not known in which chemical form ^{99m}Tc is released from the cells, but free pertechnetate is excluded because no thyroid uptake has been demonstrated.

Some authors (*9,19*) have reported that in vitro the activity elutes less from granulocytes (6.1% over 2 hr) than from mononuclear cells (30% over 2 hr). Based on these observations, one may conclude that the white blood cell suspension obtained after the hemolysis step (Fig. 1) does not have to be purified any further by supplementary steps (gradient centrifugation, etc.). As only one-fifth of the [^{99m}Tc]d,l-HM-PAO preparation is used during the labeling step, the granulocytes were incubated with no more than 0.1 mg of HM-PAO and 1 μg of $SnCl_2 \cdot 2H_2O$. The addition of such small amounts of these substances undoubtedly reduces the risk of alteration in the cell structure (Fig. 4).

Transmission electron microscopy (TEM) is a valuable tool in the evaluation of labeled cells, as was demonstrated in our previous study on the ^{111}In labeling of white blood cells with three different chelators (*25*). In this article, the deleterious effect of oxine and Merc on the morphology of cells was clearly demonstrated by TEM. As shown in Figure 4, the TEM image of the technetium-labeled white blood cells was completely normal, which is another argument in favor of this method.

The scintigraphic images of the patient studies have some special features. The bladder is visualized early whereas the thyroid gland was never seen. Bone marrow uptake is prominent, and there is some bowel visualization without previous hepatic secretion after 4 to 6 hr. In case of bowel disease, the early timing of the images and the intensity of the tracer uptake can be helpful in the final diagnosis (*9,17*). A 24-hr study with regular acquisition of the abdomen of normal persons could provide more information of the normal sequence of the intestine uptake.

The scintigraphic score was 2 (suspect) or 3 (positive) in 13 of the 17 patients. These lesions were already clearly visible at 3 hr and no extra lesion was found at 24 hr (Fig. 6). Some authors mention that most lesions become positive already 30–60 min after injection (*9,10,13–15*). This finding is also demonstrated in the clinical example showing a clear hot zone in the distal tibia. A more localized uptake is found on the images 3 hr p.i. There was one false-negative result with a patient with an infected fracture of the tibia. The lung transit of this study was prolonged, so this negative result could be explained by an improper separation or labeling step.

The images are as good or better than those obtained with indium-labeled WBC. This is in agreement with the findings of other authors who already used HM-PAO labeling (*26,27*). Comparing the images of simultaneously injected [^{99m}Tc]d,l-HM-PAO and [^{111}In]oxinate labeled white cells in 12 patients, Lui found complete agreement in lesion detectability but a superior quality (better spatial and contrast resolution) when using the ^{99m}Tc-labeled agent (*15*). An additional important advantage of the ^{99m}Tc-labeled granulocytes is the lower radiation dose (*9*), which permits the use of higher activities.

It is concluded that labeling of leukocytes with [^{99m}Tc]d,l-HM-PAO results in functionally and morphologically intact cells. The cost, availability, and physical characteristics of the tracer and the final clinical results justify this cell-labeling technique as a valuable alternative for ^{111}In labeling.

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Technetium-99m HM-PAO-Labeled Leukocytes in Detection of Inflammatory Lesions: Comparison with Gallium-67 Citrate

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Forty-three patients with suspected benign, inflammatory, or infectious diseases were imaged with [^{99m}Tc]HM-PAO-labeled leukocytes and [^{67}Ga]citrate. Technetium-99m leukocytes showed 22 true-positive, no false-positive, 19 true-negative, and two false-negative findings and [^{67}Ga]citrate 23, 7, 12 and 1, respectively. The sensitivity, specificity, and accuracy values with ^{99m}Tc leukocytes were 92%, 100%, and 95%, and with [^{67}Ga]citrate 96%, 63%, and 81%. Technetium-99m leukocyte scintigraphy has a promising future in comparison with [^{67}Ga]citrate because of the ready availability of [^{99m}Tc]HM-PAO, the good image quality, more rapid results (within few hours), and the lower radiation exposure to the patient with ^{99m}Tc leukocytes. The usefulness of ^{99m}Tc leukocytes in chronic osteomyelitis needs further evaluation.

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Gallium-67 (^{67}Ga) citrate was first shown to accumulate in inflammatory lesions in 1971 (*1*) and thereafter it has been used routinely in the detection of inflammation. In 1976 McAfee and Thakur (*2*) first investigated the use of indium-111 (^{111}In) oxine for the labeling of leukocytes and since then ^{111}In leukocyte scans have been shown to be useful in detection of infections and inflammatory lesions and successfully applied to the evaluation of a variety of pathologic inflammatory conditions (*3,4*). The sensitivity of [^{67}Ga] citrate and ^{111}In leukocytes to detect inflammation is relatively equivalent but increased specificity of ^{111}In leukocytes has made it the procedure of choice (*4*). However, [^{67}Ga]citrate has a greater sensitivity in the identification of prolonged infections of more than 2 wk duration (*5*). Technetium-99m (^{99m}Tc) hexamethylpropyleneamine oxime (HM-PAO) has recently been shown to radiolabel leukocytes in vitro (*6*) and promising results of its clinical use in the identification of inflammatory lesions have been published (*7–11*). Technetium-99m HM-PAO leukocyte method has many advantages over [^{111}In]oxine with respect to image quality, acquisition time, and radiation dose to the patient and it has a promising future as an imaging agent in inflammatory diseases (*9,10,12,13*).

In the present study we have compared [^{99m}Tc]HM-PAO leukocyte and [^{67}Ga]citrate scan findings in a series of 43 patients suspected of having various benign inflammatory conditions.

MATERIALS AND METHODS

Patients

Forty-three patients (27 men, 16 women, age range 25–78 yr, mean age 61 yr) were imaged with [^{99m}Tc]HM-PAO-labeled leukocytes and [^{67}Ga]citrate within 10 days (mean 4 days). The first study was ^{99m}Tc leukocyte scan in 40 cases and ^{67}Ga scan with three patients.

Seventeen patients had suspected bone or joint infection, 11 had suspected prosthetic vascular graft infection, four had suspected inflammatory bowel disease, five had suspected intra-abdominal abscess, five had fever of unknown origin, and one had suspected pulmonary infection. The final diagnosis was confirmed in every case by one or more of the following diagnostic procedures: bacteriologic culture, laboratory tests, histology, standard x-ray procedures, computed tomography, ultrasonography, endoscopy, and bone scan, in combination with clinically relevant signs and favorable response to antimicrobic therapy.

Leukocyte Labeling with [^{99m}Tc]HM-PAO

Mixed leukocytes were isolated and labeled as described previously (*11*). Forty milliliters venous blood was drawn into

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TABLE 1
Results of Imaging with ^{99m}Tc-Labeled Leukocytes and [^{67}Ga]Citrate

Initial diagnosis	^{99m}Tc leukocytes				[^{67}Ga]citrate			
	TP	FP	TN	FN	TP	FP	TN	FN
Bone or joint disease (n = 17)	8	0	7	2	9	2	5	1
Prosthetic vascular graft infection (n = 11)	8	0	3	0	8	1	2	0
Inflammatory bowel disease (n = 4)	2	0	2	0	2	1	1	0
Intra-abdominal abscess (n = 5)	3	0	2	0	3	2	0	0
Fever of unknown origin (n = 5)	0	0	5	0	0	1	4	0
Pulmonary infection (n = 1)	1	0	0	0	1	0	0	0
Total (n = 43)	22	0	19	2	23	7	12	1

a 60-ml plastic syringe containing 10 ml acid citrate dextrose and 10 ml 6% hydroxy ethyl starch to fasten the sedimentation. The erythrocytes were allowed to sediment at room temperature for 1 hr. The supernatant was centrifuged in sterile tubes at 100 *g* for 5 min. The platelet-rich supernatant was separated and centrifuged at 2,000 *g* for 5 min to obtain cell free-plasma. Leukocytes were suspended into 1 ml cell-free plasma. Technetium-99m HM-PAO was formed by adding 600 MBq ^{99m}Tc in 6 ml isotonic saline to a vial containing HM-PAO (Ceretec, Amersham International). Five milliliter (500 MBq) of [^{99m}Tc]HM-PAO-complex was added to the leukocyte suspension which was left for 10 min at room temperature. The cells were repelletted at 100 g for 5 min, resuspended in 5 ml cell-free plasma, and reinjected intravenously. The cell labeling efficiency was 39% (range 18–72%).

Scintigraphy and Analysis of Scintigrams

Planar images were obtained at 0.5 hr, 2 hr, 4–6 hr, and 18–24 hr after the injection of ^{99m}Tc leukocytes, and 24–72 hr after the administration of 110–185 MBq of [^{67}Ga]citrate. Two nuclear physicians interpreted the images without the knowledge of clinical diagnosis. The intensity of uptake was graded as weak, moderate, or strong by subjective evaluation. On rare occasions when the readings differed, the final result was obtained by consensus. A scintigram was considered "true positive" when the pathologic uptake was caused by a benign inflammatory or infectious disease confirmed by other diagnostic procedures mentioned earlier. A positive scintigram was considered "false positive" when the finding was verified to be noninfectious and noninflammatory by other diagnostic modalities. A negative scintigram was considered "true negative" when no focal infectious or inflammatory benign process was found, and "false negative" when a focal infectious or inflammatory process was found by other diagnostic procedures.

RESULTS

The results of the scintigraphic examinations are presented in Tables 1 and 2. The sensitivity of [^{67}Ga] citrate was a little better than that of ^{99m}Tc leukocytes but ^{99m}Tc leukocyte scan showed much greater specificity, accuracy, and positive predictive value.

The normal distribution of the activity in ^{99m}Tc leukocyte scan was similar to that seen with ^{111}In-labeling with exception of urinary bladder visualization in all patients. The normal distribution has been described in detail previously (*6,8,9,12–14*).

All patients with true positive findings in inflammatory or infectious disease, showed abnormal uptake even at 0.5 hr after injection of ^{99m}Tc leukocytes and the intensity of uptake increased in later images. The final diagnoses of the 17 patients with suspected bone or joint infection were four acute, two subacute, and three chronic osteitis or prosthetic joint infection; two metastatic malignancies, one reactive arthritis, and five with no inflammatory or malignant bone disease. Eight were true positive with ^{99m}Tc leukocytes and nine with [^{67}Ga]citrate. Results in acute and subacute infections were comparable. Two patients with bone metastases, one from lung carcinoma and the other from malignant melanoma had true-negative ^{99m}Tc leukocyte scans but false-positive [^{67}Ga]citrate scans. Two patients with chronic osteomyelitis showed false-negative ^{99m}Tc leukocyte scans but positive [^{67}Ga]citrate scans, however, one patient with chronic osteomyelitis was negative with [^{67}Ga]citrate but positive with ^{99m}Tc leukocytes. One patient with bacteriologically verified subacute prosthetic hip joint infection was moderately positive with ^{99m}Tc leukocytes but only weakly positive with ^{67}Ga citrate (Fig. 1).

All prosthetic vascular graft infections were positive with both agents and the information obtained was relatively comparable (Fig. 2). In addition there was

TABLE 2
Sensitivity, Specificity, Accuracy, and Predictive Values of ^{99m}Tc Leukocyte and [^{67}Ga]Citrate Scintigraphies in the Detection of Benign Infectious or Inflammatory Diseases

	^{99m}Tc leukocyte	[^{67}Ga]citrate
Sensitivity (%)	92	96
Specificity (%)	100	63
Accuracy (%)	95	81
Positive predictive value (%)	100	77
Negative predictive value (%)	90	92

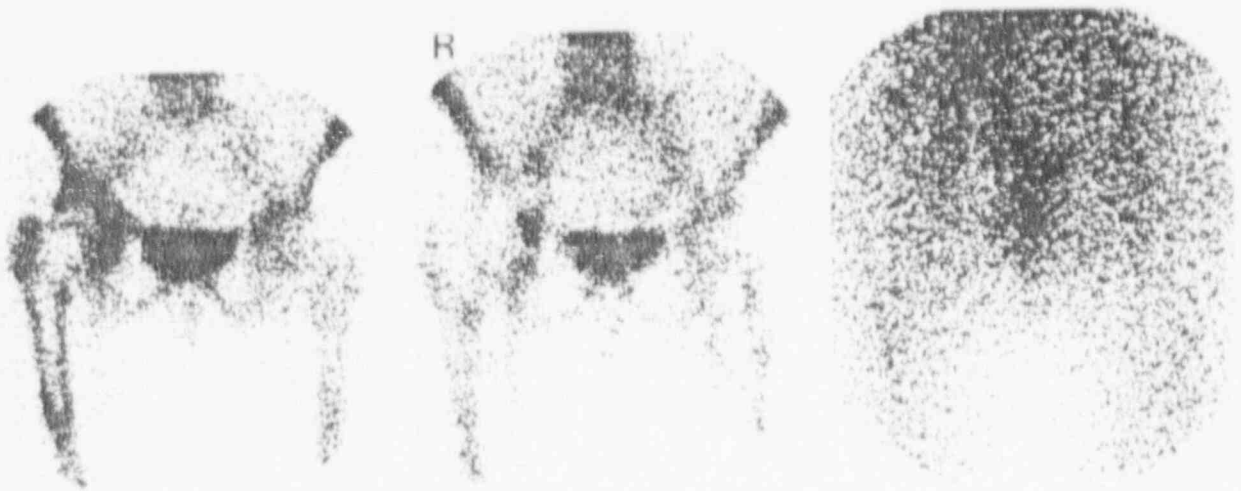

FIGURE 1
Infected prosthetic hip joint shows marked abnormal activity in bone scan (A) and ^{99m}Tc leukocyte scan (B) and weak abnormal activity in [^{67}Ga]citrate scan (C).

one false-positive aortic graft prosthesis with [^{67}Ga] citrate but ^{99m}Tc leukocyte scan showed a true-negative result.

Two patients with suspected inflammatory bowel disease had colonic diverticulitis somewhat more positive with ^{99m}Tc leukocytes than with [^{67}Ga]citrate. The para-aortic pathologic nodes of a patient with Hodgkin's disease were weakly false positive with [^{67}Ga] citrate but negative with ^{99m}Tc leukocytes. Three intra-abdominal abscesses were true positive with both agents (Fig. 3). Two patients with suspected abdominal abscesses had malignant diseases: splenic lymphoma and abdominal metastases of an adenocarcinoma of unknown origin, which showed uptake of [^{67}Ga]citrate but were negative with ^{99m}Tc leukocytes.

All patients with fever of unknown origin were true negative with ^{99m}Tc leukocyte scan but one patient with multiple bone metastases of lymphoma showed strong uptake of [^{67}Ga]citrate (Fig. 4). The pulmonary infection was bacteriologically verified tuberculosis and showed focal uptake in the left lung with both agents but the ^{99m}Tc leukocyte uptake was greater.

DISCUSSION

The sensitivity, specificity, and accuracy of ^{99m}Tc leukocyte scan were 92%, 100%, and 95%. The values are similar to a recent article by Roddie and collegues (9). In their study 100 patients suspected of having various inflammatory diseases were imaged with [^{99m}Tc] HM-PAO-labeled leukocytes and 100% sensitivity and 95% specificity were found. In the present study [^{67}Ga] citrate showed 96% sensitivity, but the specificity and accuracy values were lower, 63% and 81%, respectively, which agree with other studies with [^{67}Ga]citrate in various inflammatory conditions reviewed by Froelich (4).

The image quality of ^{99m}Tc leukocyte scans was better than the quality of [^{67}Ga]citrate scans and most, although not all, pathologic processes were better deline-

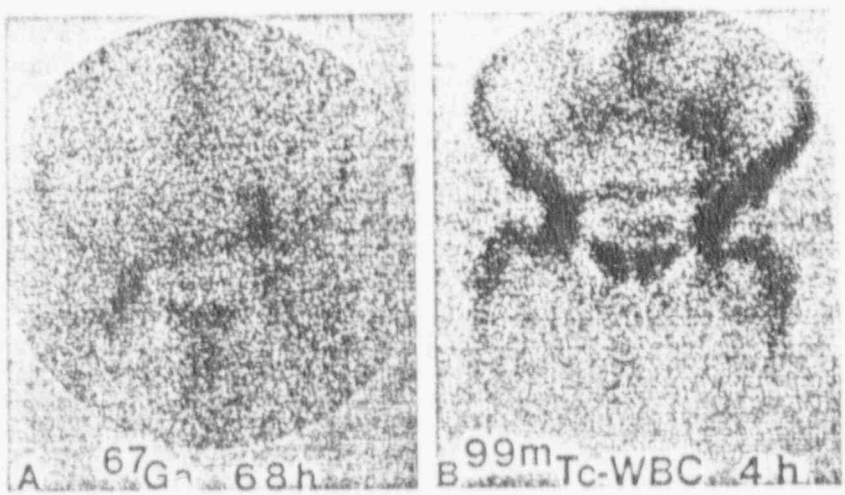

FIGURE 2
Iliofemoral prosthetic vascular graft infection is somewhat better visualized with [^{67}Ga]citrate (A) than with ^{99m}Tc leukocytes (B).

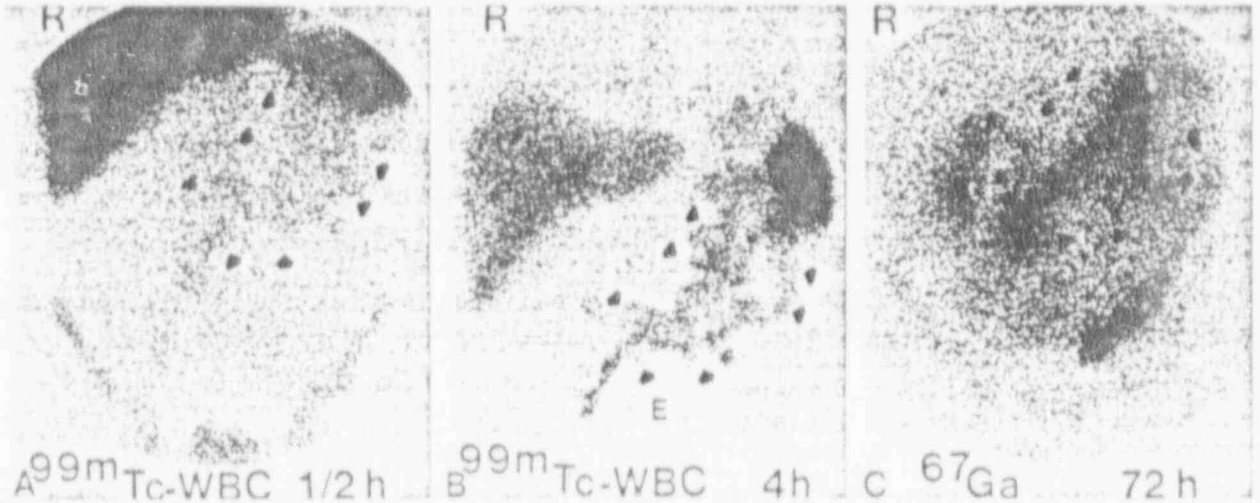

FIGURE 3
Abdominal abscess with multiple cavities is visualized with ^{99m}Tc leukocytes at 0.5 hr after injection (A), the uptake is increased at 4 hr and the abscess is sharply delineated from the background activity (B). The quality of the [^{67}Ga]citrate image at 72 hr is much poorer (C).

lated with ^{99m}Tc leukocytes. The difference in the image quality between these two agents was most conspicuous in the abdominal region. Weak to moderate activity in the gut was always seen in the [^{67}Ga]citrate scan in spite of bowel cleansing before imaging. There was only minimal background activity in the bowel up to 4–6 hr with ^{99m}Tc leukocytes but nonspecific bowel activity was seen in some patients in the 6 hr image and large bowel was always at least weakly visualized at 18–24 hr. We suggest that patients with suspected inflammation in abdominal region should be imaged during the first 4 hr after the administration of ^{99m}Tc leukocytes to avoid false-positive finding as was also suggested by others (7,9,12).

Technetium-99m leukocytes showed no false-positive finding whereas there were seven false-positive [^{67}Ga]citrate scans. One of these patients had an old noninflammatory and noninfected resolving para-aortic hematoma. The other six were various malignancies, which were negative on ^{99m}Tc leukocyte scan. Most bone metastases were seen as defects on ^{99m}Tc leukocyte scan. Such a defect in skeleton is not a specific sign of malignancy, however, because two false-negative chronic osteomyelitis showed similar defects. Although all malignancies were true negative with ^{99m}Tc leukocytes and no false-positive finding has been described so far in the literature, it is probable that some malignancies will accumulate ^{99m}Tc leukocytes since some positive findings have been described using ^{111}In leukocytes (13–20).

The only false-negative finding with [^{67}Ga]citrate was found in chronic osteomyelitis. Gallium-67 citrate has been described to be very useful and superior to ^{111}In leukocytes in chronic bone infections (14,15,21), although good results with ^{111}In leukocytes have also been reported (22,23). Our results with ^{99m}Tc leukocytes were good in suspected bone or joint infection except with two of three patients having chronic disease. More evaluations with ^{99m}Tc-labeled leukocytes are needed to make conclusions of the usefulness of this agent in chronic bone infections. In acute and subacute bone and joint diseases ^{99m}Tc leukocytes gave reliable inform-

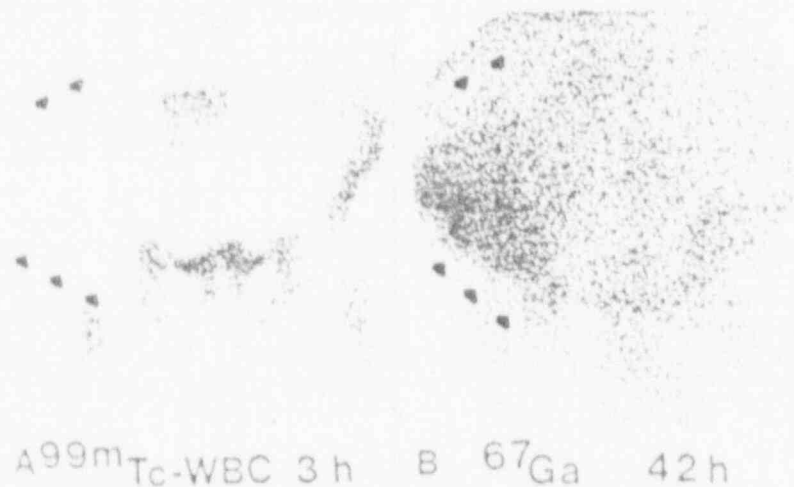

FIGURE 4
Anterior images of pelvis in a patient with metastatic lymphoma in right iliac bone. Large defect is seen in ^{99m}Tc leukocyte scan at 3 hr (A) and strong uptake in [^{67}Ga]citrate image at 42 hr (B).

mation which is in agreement with some previous studies with ^{111}In leukocytes (*15,21*).

Both ^{99m}Tc leukocytes and [^{67}Ga]citrate scan were found to be very reliable in detecting prosthetic vascular graft infection, which agrees with results described previously (*24–26*). All five patients with fever of unknown origin were true negative with ^{99m}Tc-labeled leukocytes. It has been shown previously that there is very low frequency of detectable infection in this clinical situation (*27*).

Recent comparisons of ^{99m}Tc leukocytes and ^{111}In leukocytes have shown the ^{99m}Tc method equal or better than the ^{111}In method and the quality of images has been better in ^{99m}Tc-labeled leukocyte scan (*6,7,10,12, 13*). Technetium-99m leukocytes were also superior to iodine-123-labeled monoclonal antibodies against granulocytes in a recent study (*14*).

In conclusion, [^{99m}Tc]HM-PAO-labeled leukocyte scintigraphy seems a very useful tool in the diagnosis of various infectious and inflammatory processes. Both the ^{99m}Tc and HM-PAO kit are readily available even for emergency use in many countries. The quality of ^{99m}Tc leukocyte image is superior to that of [^{67}Ga]citrate and ^{111}In leukocytes, the result is available more rapidly, and the radiation dose to the patient is lower (*8,12*). The usefulness of this method in chronic osteomyelitis and possibly in other chronic diseases needs further evaluation.

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