A New Method for Granulocyte Labeling with Technetium-99m: Preliminary Results in Abscess Detection

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A new method for ^{99m}Tc-labeling of granulocytes for clinical routine use has been developed. The labeling is simple to perform by means of a kit of radiopharmaceutical quality, utilizing dihydroxy-benzoic acid. Pretinning techniques are avoided. The technique has been applied clinically in 15 patients with indications of intra-abdominal abscess. In six patients, [^{99m}Tc]granulocyte scintigraphy at 3 hr and/or 24 hr after i.v. administration, correctly depicted the abscess, as verified by subsequent surgery. In the remaining patients, who were negative at surgery or recovered without operation, all scans were negative.

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The development of the indium-111 (^{111}In) oxinate method (*1*) for radiolabeling of leukocytes represents great progress in utilizing nuclear medicine techniques for abscess detection and localization. However, the nonideal physical properties, the cost and limited availability of ^{111}In have been major drawbacks of the technique, therefore an alternative technique using technetium-99m (^{99m}Tc) has been sought. Such a technique would combine the superior physical properties of ^{99m}Tc with the diagnostic potential of labeled granulocytes and leukocytes. Also, since ^{99m}Tc is readily available in nuclear medicine departments, the use of radiolabeled cells in acute situations is made easier.

Technetium-99m labeling of leukocytes has previously been performed with [^{99m}Tc]oxinate. (*2*), [^{99m}Tc]-tropolonate (*3*), or with pretinning techniques with stannous pyrophosphate (*4,5*). Phagocytic cells in whole blood have also been labeled by means of ^{99m}Tc-labeled colloid (*6,7*). However, none of these techniques have gained widespread clinical application. Shortcomings such as rapid elution of the radionuclide from the cells, low labeling yields, labeling of monocytes or reduced cell viability (8), have restricted the usefulness of these techniques.

Another approach, based upon pretreatment of ^{99m}Tc with concentrated hydrochloric acid and vacuum evaporation followed by cell labeling in the presence of dihydroxy-benzoic acid, has been successfully applied in vitro (*9*). The technical complexity of this method, however, has been an obstacle for clinical routine use. A second promising method, utilizing pretinning of the cells with the stannous salt of medronic acid and p-amino-benzoic-acid was recently reported (*10*).

We have also used different pretinning techniques resulting in good labeling efficiencies but inferior diagnostic results in our patients. Therefore we have sought a new method of labeling granulocytes. We present here a new, fast method for ^{99m}Tc-labeling of granulocytes by means of a kit of radiopharmaceutical quality, utilizing a balanced solution of di-hydroxy-benzoate and tartrate for labeling the cells without pretinning the cells. The technique has now been applied clinically for abscess detection in 15 patients with clinical signs of postoperative intra-abdominal abscess.

MATERIALS AND METHODS

Granulocyte Isolation

Thirty-five milliliters of venous blood was mixed with 5 ml of ACD-solution and allowed to settle for 1 hr. In patients

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who did not have a significantly elevated erythrocyte sedimentation rate (ESR), 10 ml of hydroxyethyl starch solution was added to the sample in order to increase the ESR.

After sedimentation, the leukocyte-rich plasma was removed and centrifuged at 300 *g* for 10 min. The cell pellet thus obtained was resuspended in a small volume of cell wash buffer (specified later) and deposited on top of a 4 ml density gradient solution, followed by centrifugation at 400 *g* for 15 min. The mononuclear cells remain at the top of the gradient while the granulocytes and contaminating erythrocytes are recovered at the bottom.

The unwanted contribution of erythrocytes was removed by resuspending the pellet in 8 ml of hemolyzing agent specified later and incubated at room temperature for 20 min. After centrifugation at 300 *g* for 5 min, the supernatant, containing the hemolyzed erythrocytes was removed. The granulocytes were resuspended in 8 ml of cell wash buffer and centrifuged at 300 *g* for 5 min, thus giving a pellet of purified granulocytes for subsequent labeling.

Kit Preparation

0.5 mg Dihydroxybenzoic acid, 3.75 μg stannous chloride and 9.75 μg disodium tartrate were dissolved in 0.5 ml sterile water which had been bubbled with nitrogen, and adjusted to neutral pH with sodium hydroxide solution and lyophylized.

Granulocyte Labeling

A sample of previously prepared and lyophilized material was redissolved by adding 1 ml [^{99m}Tc]pertechnetate solution and was added to the isolated cells. The cells were resuspended and gentle agitation was performed for 15 min. The labeled cells were washed finally with cell wash buffer, centrifuged at 300 *g* for 5 min and resuspended in about 5 ml of wash buffer. After measurement of total radioactivity, a desired amount was withdrawn in a syringe and injected into the patient.

REAGENTS

ACD solution. 8.0 g citric acid monohydrate, 22.0 g NaCl and 22.3 g glucose per liter aqueous solution.

Hydroxyethyl starch solution. 60.0 g hydroxy-ethyl-starch, 9.0 g NaCl and 5.0 mg NaOH per liter aqueous solution.

Density gradient centrifugation medium. 142.7 g Iohexol,* 0.6 g Trizma base,† 0.03 g $C_6H_5Na_3O_7 \cdot 2H_2O$, 0.22 g KCL, 3.6 g NaCl and 4.2 ml 1*M* HCL.

Hemolyzing agent. 8.0 g ammonium chloride per liter aqueous solution.

Washing buffer. 8.33 g NaCl, 0.29 g KCL, 1.66 mg $NaH_2PO_42H_2O$, 580 mg $Na_2HPO_4 \cdot 10H_2O$, 12.5 mg $CaCl_2 \cdot 2H_2O$, and 9.5 mg $MgCl_2 \cdot 6H_2O$ per liter aqueous solution, adjusted to pH = 7.2 with NaOH or HCL.

Granulocyte Yields and Labeling Efficiency

The yields of granulocytes and the labeling efficiencies were evaluated by labeling cells of five healthy volunteers.

The total yields and the fractional loss of granulocytes due to the isolation and labeling procedures were evaluated from cell number measurements of the initial and the labeled samples, respectively, using a Coulter counter (model S-plus) and differential counting in stained smears.

The labeling efficiency, which denotes the fraction of the added radioactivity that binds to the cells, was evaluated from comparative measurements (corrected for the radioactive decay) of the radioactivity in the eluate and in the labeled cell suspension, respectively, using a NaI-detector device.

Technetium-99m Release and Urinary Excretion

The release of the ^{99m}Tc from the labeled cells during a 24-hr period was analyzed in five healthy volunteers by suspending the labeled cells in 25 ml autologous serum which was rotated at room temperature. Aliquots were regularly removed and the radionuclides released from the cells were separated from the cells by centrifugation according to a published procedure (*9*). Urinary excretion of i.v. administered radioactivity was determined in three of the five volunteers during the first 24 hr after i.v. administration.

Patients

Fifteen consecutive patients exhibiting clinical signs and symptoms of intra-abdominal abscess after major abdominal surgery were examined. The results of the scintigraphic examinations were not allowed to influence the decision to operate on the patients for abscess treatment. This decision was based upon the development of the clinical picture and supporting results of computed tomography (CT) and/or ultrasonic examinations.

Scintigraphic Procedure

Scintigraphic examinations were performed at 0 hr, 3 hr, and at 24 hr after i.v. administrations of 5.4–10.8 mCi (200–400 MBq) ^{99m}Tc-labeled granulocytes. All studies were performed with a Maxicamera 400T‡, supplied with a low-energy, all-purpose (LEAP) collimator and connected on-line to a PDP 11/34, operating with gamma-11§ and SPETS tomographic software (*11*).

Lung Sequestration

The magnitude and the time course of early lung sequestration may reflect some malfunctioning of the granulocytes due to the labeling procedure.

Dynamic studies (20 sec/frame) of the lung uptake during the first 15 min after administration were performed in 11 of the 15 patients. The initial distribution phase was evaluated by plotting, as a function of time, the number of recorded events/sec from a small region of interest (ROI) over a central part of the right lung. A single semi-exponential function could be fitted to the results from frame 10 (3 min after administration) to frame 45, thus giving measures of the maximum uptake (extrapolated value at t = 0) and of the effective half-time ($T_{½}$) of the initial activity in the right lung.

RESULTS

Granulocyte Yields and Labeling Efficiency

The total number of granulocytes in the samples of the five volunteers varied from 9.1×10^7 to 2.56×10^8 with a median value of 1.95×10^8. After cell isolation and labeling, the remaining number of granulocytes was between 2.5×10^7 and 1.03×10^8 with a median value of 63×10^6. Differential counting of stained smears never demonstrated <90% granulocytes in the resulting suspension, usually 95–96% granulocytes. The labeling efficiency of these samples varied from 18 to 31% with a median value of 24%.

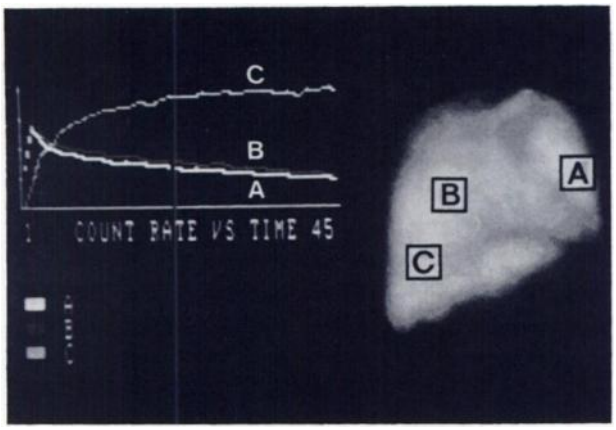


FIGURE 1
Sequestration in left (A) and right (B) lung and accumulation in liver (C) during first 15 min (45 frames) after i.v. injection of ^{99m}Tc-labeled granulocytes

Technetium-99m Release and Urinary Excretion

The release of ^{99m}Tc from the cells in the serum samples ranged from 20 to 31% with a median value of 24% after 24 hr (all values corrected for radioactive decay) and correspondingly, 25–32% of the administered radioactivity in the volunteers was excreted with the urine during the first 24 hr.

Lung Sequestration In Vivo

An initial accumulation of radioactivity in the lungs was observed in all of the 11 patients examined. The extrapolated uptake in the small ROI varied by about a factor of 4 (2.1–7.9 cpm/pixel in the ROI per μCi administered).

The release of radioactivity from the lungs (evaluated as the biologic half-time $T_{½}$ was 26.4 ± 14.4 min. Two patients had a biologic half-time of 39 and 66 min, respectively, and if they were excluded, $T_{½}$ became 20.7 ± 3.3 min. In four patients, hot spots appeared in the lung images, but they did not have a prolonged release time. The percentage of the injected radioactivity retained in the lungs was very similar to those obtained with indium-111 (^{111}In) (unpublished data). An example of the release of radiolabeled granulocytes is shown in Fig. 1. Although different interpretations of hot spots are possible, we think these hot spots are caused by cells aggregated in vitro, since the frequency of occurrence of such hot spots has declined when more practice had been gained.

Patients

Out of the 15 patients, seven had to be reoperated because of clinical deterioration. In six of these, the [^{99m}Tc]granulocyte scintigraphy correctly depicted the abscess; in five cases after three hours; in one case after 24 hr. Re-exploration of the seventh patient, in whom the scan was negative, revealed no abscess, but an enteroenteral fistula with small bowel obstruction. Two examples of pathologic accumulation in abscesses are presented in Figs. 2 and 3.

The remaining eight patients with a primary clinical picture suggestive of postoperative intra-abdominal abscess recovered without further surgical treatment. The scintigraphic 3- and 24-hr images did not demonstrate any signs of intra-abdominal abscesses in any of these subjects (Table 1).

DISCUSSION AND CONCLUSIONS

Prior work on ^{99m}Tc-labeling of granulocytes has been mainly based on pretinning of the cells before addition of pertechnetate solution. Very limited information on the clinical results of these techniques has been reported. In our hands labeling by pretinning techniques gave excellent in vitro but inferior scintigraphic results, the reasons for which we are presently investigating.

Indium-111 oxine and [^{111}In]tropolone techniques are based on a very different principle: The radionuclides are present as complexes in the suspension of the cells to be labeled. This has been a very fruitful strategy which we have expanded into the ^{99m}Tc-labeling field. The balanced solution of stannous benzoate and tartrate forms ^{99m}Tc complexes of moderate chemical stability allowing labeling of the granulocytes without any pretinning of the cells. The labeling efficiency of this method is slightly inferior to the pretinning techniques, but we find the clinical efficacy far better than that obtained by pretinning techniques. In vitro viability tests of granulocytes thus labeled have shown results which are comparable to those observed after labeling with [^{111}In]oxinate (*11*).

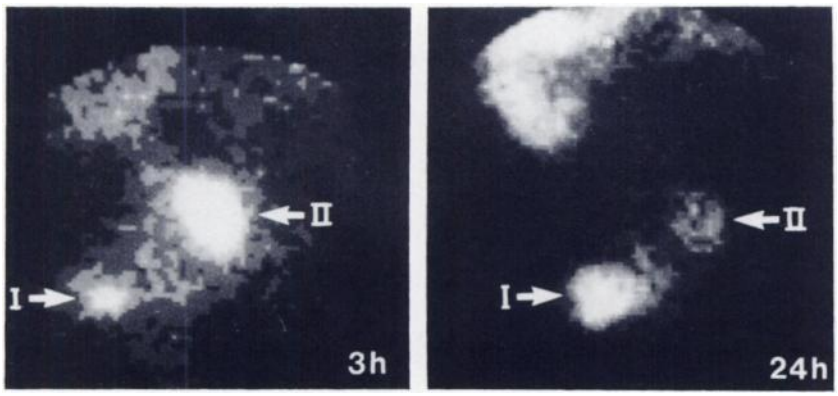
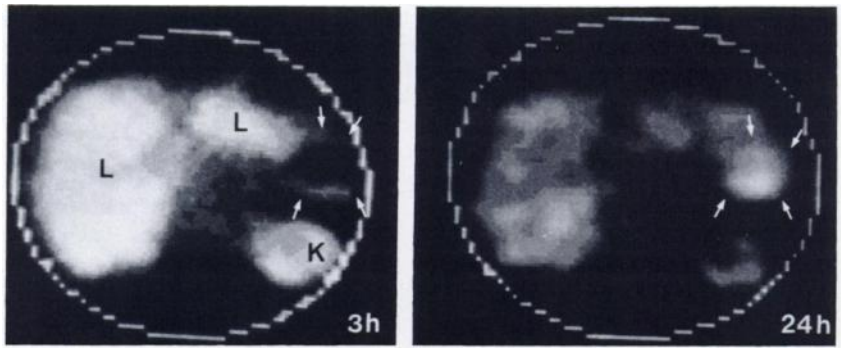


FIGURE 2
59-yr-old man with 2-yr history of complicated perineal abscess with fistulation. Condition had required several operations. Now 3-day history of abscess in right groin. Frontal views of the abdomen at 3 and 24 hr after administration of [^{99m}Tc]granulocytes demonstrate clinically obvious abscess in right groin (I) and previously unknown suprapubic abscess (II) which was verified by surgery on day after examination. Higher accumulation in abscess I at 24 hr may be due to drainage through fistula between two abscesses that was found at surgery on following day. Radioactivity in liver appears in upper left corner of images

FIGURE 3
SPECT examination of 67-yr-old male with clinical signs of intraabdominal abscess 8 days after total gastrectomy for gastric carcinoma. 3 and 24 hr transverse sections show increasing uptake of radiopharmaceutical in left upper quadrant of abdomen (arrows). This uptake (not caused by spleen which was removed during gastrectomy), is due to subphrenic abscess that was drained on following day. L and K denote accumulation of granulocytes in liver and in left kidney, respectively



Similar to ^{111}In-labeled granulocytes, cells labeled with ^{99m}Tc exhibit a considerable sequestration in the lungs during the first minutes after the injection of labeled cells. There is, however, a rapid decline of lung activity which is almost zero after 3 hr.

Accumulation of labeled granulocytes in the liver and the spleen, on the other hand, appear almost unaltered in the 3-hr and 24-hr scans. This accumulation makes difficult the diagnosis of abscesses in the vicinity of these organs, but SPECT examinations may help to overcome this problem (*12*).

According to our present clinical experiences, the release of ^{99m}Tc from the labeled cells may be a potential drawback of the method, especially when imaging is performed 24 hr after administration. The lack of thyroid uptake in our patients indicates that the released radioactivity was not in the pertechnetate form, as no thyroid-blocking agent was used. The urinary bladder, on the other hand, often exhibited various amounts of radioactivity. However, the patients were always asked to drain their bladders prior to the examinations.

Furthermore, some patients showed radioactivity in the colon, one on the 3-hr scan and six on the 24-hr scan. The patient where colonic uptake was seen after 3 hr was found to have pancolitis at colonoscopy, caused by *Clostridium difficile,* as revealed by subsequent stool culture and colonoscopy. There were no clinical signs of colitis in the six patients with colonic uptake on the late scans, this uptake probably represented normal excretion to the bowel of ^{99m}Tc complexes by some unknown mechanism. This observation questions the usefulness of the method in outlining colonic inflammatory lesions in inflammatory bowel diseases, an indication that has been suggested for scintigraphy with ^{111}In-labeled granulocytes.

Our initial experience with ^{99m}Tc-labeled autologous granulocytes for abscess detection suggests this method to be as effective as the [^{111}In]oxinate technique in diagnosing postoperative intra-abdominal abscesses that require surgical treatment. All six surgically verified abscesses were correctly depicted by the technique before operation. Among the patients who recovered without further surgical treatment all 3-hr scans were normal except in the case of *Clostridium difficile*-associated colitis, see above. Five out of eight patients in this group exhibited colonic activity on the 24-hr scans.

Abscess detection with scintigraphy 3 hr after the injection of ^{99m}Tc-labeled autologous granulocytes thus appears to be an alternative to the ^{111}In technique; an alternative that offers certain advantages. First, it may be used in acute situations such as in the differential diagnosis of acute abdominal disorders, since ^{99m}Tc is available in all nuclear medicine departments. Second, the administered activity may be diminished using 3-hr scans only, thus reducing the radiation dose to about one-tenth of that of a corresponding ^{111}In examination. The possible role of the technique as a diagnostic tool in various clinical situations such as in the differential diagnosis of acute abdominal disorders and in the diagnosis of infections around vascular and orthopedic prostheses is presently being evaluated.

TABLE 1
Surgical and Scintigraphic Findings in Patients who were Operated on for Suspected Intra-Abdominal Abscess and Scintigraphic Findings in Patients who Resolved Without Further Surgical Treatment.*

	Operation abscess		Abscess seen on scan	
Patients	Yes	No	Yes	No
Seven required surgery	6	1	6	1
Eight recovered without surgery	—		0	8

* Patient operated on after negative scan was found to have strangulated ileus and no abscess.

FOOTNOTES

* Nycodenz, Nyegaard, Oslo.
† Sigma Diagnostics, St. Louis, MO.
‡ General Electric Medical Systems, Milwaukee, WI.
§ Digital Equipment Corp., Maynard, MA.

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