Prospective Comparative Study of Technetium-99m-WBCs and Indium-111-Granulocytes for the Examination of Patients with Inflammatory Bowel Disease

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In a prospective comparative study of 14 patients with inflammatory bowel disease (IBD), the abilities of ^{99m}Tc-HMPAO labeled white blood cells (WBCs) and ^{111}In-granulocytes to assess the presence and location of active disease were compared. The two examinations were carried out within 2 wk of each other. Scintigraphically concordant positive or discordant segments were evaluated by radiologic or endoscopic examination performed within 14 days. When bowel segments were compared, concordance was found for 102/111 (91.8%) segments between ^{99m}Tc-WBC images obtained at 1 hr after injection and 3-hr ^{111}In-granulocyte images. For five of five ^{99m}Tc-WBCs positive/^{111}In-granulocyte negative segments, it could be proven that the ^{99m}Tc-WBC result was caused by active disease. For patients, ^{99m}Tc-WBC scintigraphy detected four more patients with active disease than ^{111}In-granulocytes (11 and 7 patients, respectively). Technetium-WBCs was superior in the assessment of active disease, especially for small bowel segments. We conclude that early imaging 1 hr after the injection of ^{99m}Tc-WBCs can reliably replace ^{111}In-granulocyte scintigraphy in IBD patients because the radiopharmaceutical is available on a daily basis. Thus, there is less radiation burden to the patient and cell separation is simpler and less time-consuming.

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For the assessment of the presence and location of active inflammation in patients with inflammatory bowel disease (IBD), scintigraphic examination with ^{111}In-labeled granulocytes has been shown to be suitable (*1–7*). Ex vivo measurement of the excretion of ^{111}In-radioactivity in stools collected from 24 hr to 4 days after reinjection of the labeled cells proved to yield a reliable measurement of the degree of inflammation (*8,9*). However, the time-consuming and involved procedure of separating and labeling granulocytes has led to the search for a radiopharmaceutical that is easier to prepare.

Recently, patients with IBD have been examined with ^{99m}Tc-sucralfate (*10,11*), ^{99m}Tc-nanocolloids (*12–15*), ^{99m}Tc and ^{111}In-labeled polyclonal human immunoglobulin (IgG) (*16–20*) and ^{99m}Tc-labeled monoclonal antibodies against granulocytes (*21*). Our experience with most of these radiopharmaceuticals was disappointing (*10,12,20*).

In 1986, the labeling of white blood cells (WBCs) with ^{99m}Tc-HMPAO was introduced (*22*). Soon afterwards reports on optimizing this procedure (*23*) and the suitability of ^{99m}Tc-HMPAO-WBC in the detection of inflammation were published (*24–28*). However, only a few authors have compared ^{111}In-leukocytes with ^{99m}Tc-WBCs (*24,25,29,30*). There was a comparative study of ^{111}In-leukocytes and ^{99m}Tc-WBCs performed using the same patients in two instances (*25,30*). Because of scanty information in these reports, however, the exact merits of the two radiopharmaceuticals for IBD patients cannot be defined. Detailed information on each patient group is mandatory (*31*), especially because excretion of ^{99m}Tc activity into the bowel can potentially introduce false-positive results (*24,27,30*). Although the preparation of ^{99m}Tc-WBCs is not as simple as that for the ^{99m}Tc-labeled radiopharmaceuticals mentioned earlier, we started a prospective comparative study of ^{99m}Tc-WBCs versus ^{111}In-granulocytes as part of our ongoing search for a ^{99m}Tc-labeled radiopharmaceutical to replace ^{111}In-granulocytes in the scintigraphic examination of IBD patients.

PATIENTS AND METHODS

Twenty-two consecutive patients with active IBD diagnosed clinically who were referred for ^{111}In-granulocyte scintigraphy to assess the presence and location of active disease were also examined with ^{99m}Tc-WBC scintigraphy. The study was approved by the medical ethics committee of the hospital and all patients gave verbal informed consent.

Conditions for inclusion in the study were: (a) ^{111}In-granulocyte and ^{99m}Tc-WBC scintigraphy within 14 days of each other

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and (b) evaluation of scintigraphically abnormal or discrepant segments with other diagnostic techniques (enteroclysis, barium enema, endoscopy or surgery) within 14 days of ^{99m}Tc-WBC scintigraphy. These other diagnostic modalities, however, were performed only if required by the treating physician during clinical work-up. This evaluation step was considered especially important to exclude the possibility of false-positive results of ^{99m}Tc-WBCs caused by excretion of (degradation products of) ^{99m}Tc-HMPAO into the gut (*24*).

Eight patients were excluded from analysis because other diagnostic modalities were not available within 14 days to scintigraphically evaluate abnormal segments. Therefore, the study was comprised of 14 patients: 7 females and 7 males, mean age 39 yr (range 21–82 yr). Two patients had ulcerative colitis (UC) and 12 had Crohn's disease (CD). For the comparison, the bowel was divided into nine segments. Before the study, 15 large bowel segments had been resected. In the six patients who underwent ileocecal resection, the ileal segment was assumed to be partly present. Therefore, 111 segments were available for scoring.

Cell Separation and Labeling

Technetium-HMPAO-WBCs were prepared by drawing 46 ml of blood into syringes containing 10 ml acid citrate dextrose (ACD) and 4 ml hydroxyethyl-starch (Plasmasteril). After sedimentation for 60 min and centrifugation of the erythrocyte-poor supernatant to remove thrombocytes, the mixed leukocytes were recovered. Technetium-99m-HMPAO (Ceretec, Amersham, U.K.) was freshly prepared according to the manufacturer's instructions. The cell pellet then was incubated with 1000 MBq ^{99m}Tc-HMPAO for 10 min at room temperature, washed with thrombocyte-free autologous plasma (TFP) and resuspended in 4 ml of TFP. This procedure took 2 hr.

Autologous granulocytes were separated and labeled with ^{111}In-tropolonate as previously described (*32*). In short, mononuclear cells were separated from 50 ml heparinized blood by Ficoll-Hypaque density-gradient centrifugation. The cell pellet containing erythrocytes and granulocytes was treated with buffered ammonium chloride at 0°C to lyse the erythrocytes. Subsequently, the granulocytes were labeled with ^{111}In-tropolonate (Mallinkrodt Medical, Petten, The Netherlands) by incubation for 15 min at room temperature. The labeled cells were washed twice with 50% autologous plasma in Hank's balanced saline solution and resuspended in 4 ml of pure plasma for 3 hr. The mean ^{111}In dose was 7.01 MBq (range 1.30–10.30 MBq) and the mean ^{99m}Tc dose was 297 MBq (range 225–360 MBq). The mean labeling efficiency for ^{99m}Tc-WBC was 46% (range 25%–61%) and 69% (range 29%–91%) for ^{111}In-granulocytes.

Imaging

At 1 and 3 hr after injection of ^{99m}Tc-WBCs, total body anterior and posterior images (scan speed 30 cm/min) and anterior, posterior and lateral spot views of the abdomen (preset time 5 min) were obtained on a large field of view (30 × 50 cm) gamma camera fitted with a low-energy, all-purpose collimator (Toshiba 90B, Tokyo, Japan). Four hours after injection of ^{111}In-granulocytes, an anterior image of the abdomen was acquired on a round-field (diameter 35 cm) gamma camera fitted with a medium-energy collimator (Toshiba 501, Tokyo, Japan). There were at least 48 hr between the two investigations to prevent crosstalk of counts.

For 11 of the patients, ^{111}In-granulocyte scintigraphy was performed first. Within the whole group, ^{111}In-granulocyte scintigraphy was performed from 13 days before to 7 days after (mean 5 days before) ^{99m}Tc-WBC scintigraphy.

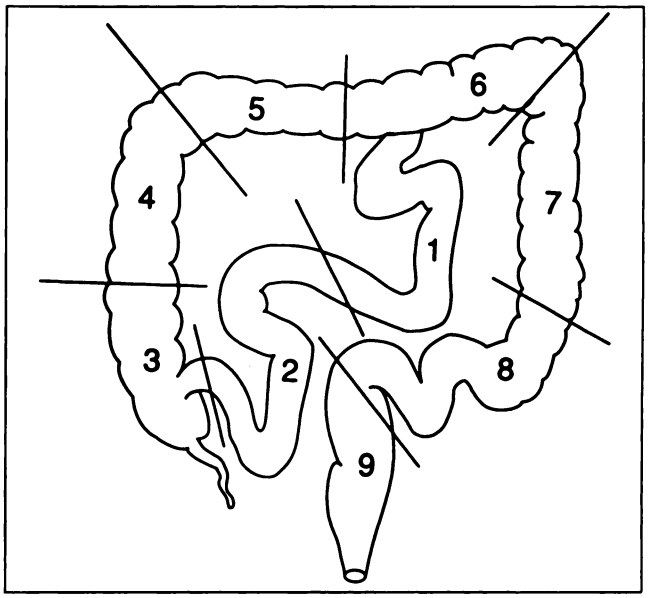

FIGURE 1. Division of the bowel into nine segments to score scintigrams.

Interpretation

All scintigraphic studies were independently analyzed by two observers without prior knowledge of either the patient's history, except for previous surgical resections, or the results of other diagnostic tests. Disagreements in interpretation were resolved at a later stage by consensus. Reading of the ^{111}In-granulocyte and 1 hour ^{99m}Tc-WBC studies also occurred independently. The bowel was divided into 9 segments (2 for the small bowel and 7 for the large bowel, Fig. 1) and each segment was scored for the presence or absence of pathologic activity.

RESULTS

Interpretation

A comparison of ^{99m}Tc-WBC scintigraphy 1 hr after injection with ^{111}In-granulocyte scintigraphy revealed normal studies in three patients for both studies and active disease in four patients (four segments) with ^{99m}Tc-WBC scintigraphy only (Table 1). Of the 111 segments, 16 (14.4%) had a concordant positive (Figs. 2 and 3) and 86 (77.5%) had a concordant negative score. Total concordance was 102/111 (91.8%). Five (4.5%) segments appeared pathologic only on ^{99m}Tc-WBC images and four (3.6%) were pathologic only on ^{111}In images.

For the small bowel (28 segments), there were three positive concordant and three discordant segments (all ^{99m}Tc-WBC positive, Figs. 4 and 5). Active disease in these ileal segments was confirmed with diagnostic procedures. In another patient (Patient 7), a small spot of increased ^{99m}Tc activity in the left lower abdomen was erroneously attributed to the ileum, but should have been attributed to the sigmoid, where active ulcerative lesions were demonstrated with a barium enema. For this patient, we found a discrepancy in the ^{111}In-granulocyte scintigram, which was completely normal.

For large bowel segments, a fifth discordant ^{99m}Tc-positive segment was the cecum in Patient 5. This

patient's ileum was positive according to both examinations. Active disease, also of the cecum, was demonstrated with enteroclysis.

Of the four segments positive only on ^{111}In-granulocyte scintigrams (all in Patient 14), endoscopy revealed that two segments were true-positive (cecum and ascending colon), but other two (descending and sigmoideal colon) contained barely any inflammatory lesions. The discrepancies between the three imaging techniques in this patient are possibly related to the time intervals between them: ^{111}In-granulocyte scintigraphy was performed 13 days before ^{99m}Tc-WBC scintigraphy and 19 days before endoscopy.

The 1-hr images did not yield any false-positive results for ^{99m}Tc-WBCs. On the 3-hr ^{99m}Tc-WBC images (scored by one other interpreter, JWA) several segments already visible at 1 hr were visualized better and 11 additional segments exhibited abnormal accumulation. Three of these proved to be true-positives. For eight segments, however, no supporting data were available to clarify the real nature of the accumulation.

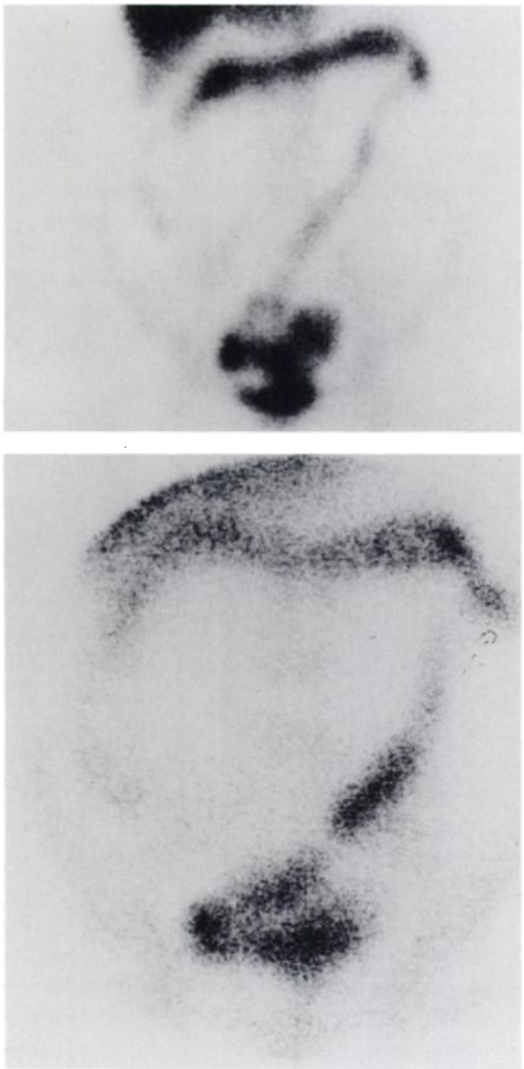

FIGURE 2. Patient 12. Ulcerative colitis with active disease from the cecum to the sigmoid and rectum. Concordant images. Technetium-99m-WBC 1 hr (top) and ^{111}In-granulocytes 3.5 hr after injection (bottom).

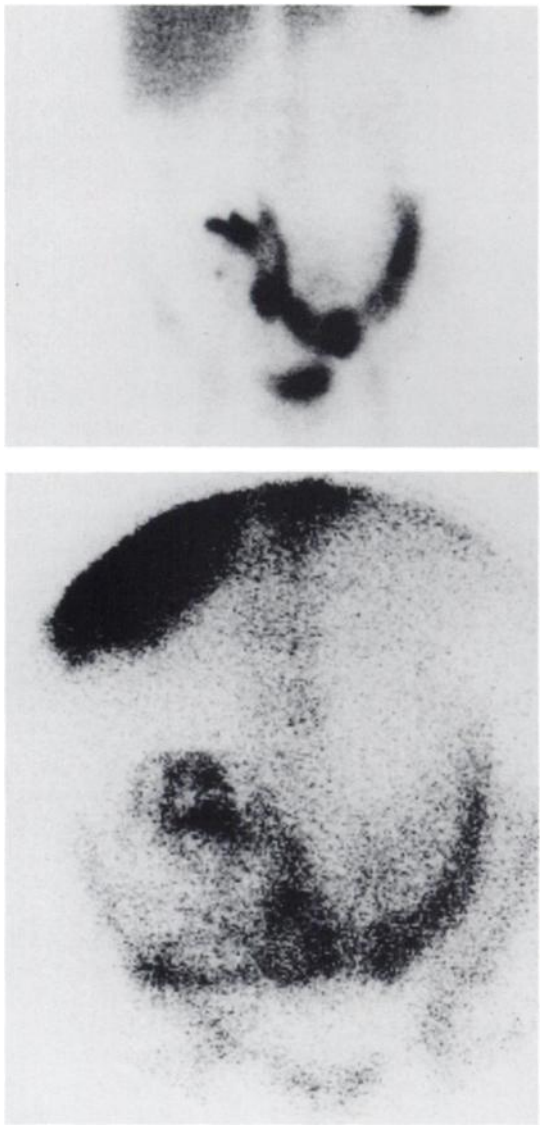

FIGURE 3. Patient 11. Status of Crohn's disease after resection of the whole large bowel and ileostoma. Concordant images. Intense pathologic activity in the ileum in the ^{99m}Tc-WBC image (top) and the ^{111}In-granulocyte scintigram (bottom).

DISCUSSION

This prospective study shows that early imaging with ^{99m}Tc-WBCs of patients with IBD is superior to imaging with ^{111}In-granulocytes. This study confirms a previously reported impression (*1,27,33,34*) that early imaging with ^{99m}Tc-WBCs gives reliable information about active disease. Costa et al. (*30*) who compared ^{111}In-WBCs and ^{99m}Tc-WBCs using a dual-isotope method (coinjection of ^{111}In and ^{99m}Tc-labeled WBCs) in 41 individuals with IBD reported an agreement rate of 71% (59% positives) and 29% discordances. Their results are probably based on the number of patients, but no mention is made of the imaging times on which these results were based, disagreement or agreement between segments or the accuracy of ^{99m}Tc-

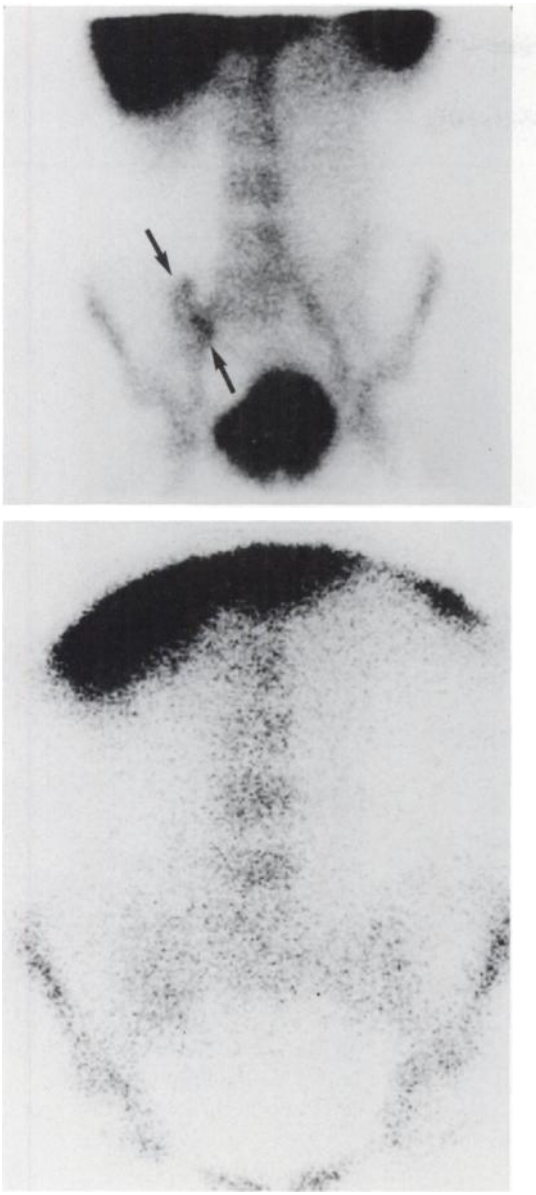

FIGURE 4. Patient 6. Crohn's disease of the small bowel. Discordant images. Clear pathologic activity in the terminal ileum (between the arrows) on the ^{99m}Tc-WBC image (top). Normal ^{111}In-granulocyte scintigram (bottom).

WBC images among the discordant patients. For patient data, our agreement rate (10/14 = 71%) is similar, but we have shown that disagreement (4/14 = 29%) was caused by true-positive ^{99m}Tc-WBC and false-negative ^{111}In-granulocyte images. No other reports on comparative studies with detailed information on discordant results could be found in the literature.

Because of the higher dose and the better imaging characteristics of ^{99m}Tc in comparison with ^{111}In, images take less time to acquire, can be obtained from different views and have a higher spatial resolution. The fact that the ^{111}In-granulocyte dose was less than optimal for imaging in some patients may have attributed to the superiority of ^{99m}Tc-WBC images (*32*). In clinical practice, we have found that granulocyte recovery and labeling efficiency with ^{111}In-tropolonate can give very low yields. Six ileal segments with active inflammation were detected with ^{99m}Tc-WBCs in comparison to only three segments detected with ^{111}In-granulocytes. This is probably attributable to advantageous imaging characteristics of ^{99m}Tc over ^{111}In as the radiolabel.

When this prospective study was started, we had two aims in mind:

1. To investigate whether ^{99m}Tc-WBCs can replace ^{111}In-granulocytes in the assessment of active disease in patients with IBD. This would mean that a radiopharmaceutical would be available daily that is less time-consuming to prepare and yields a faster diagnosis at a lower radiation burden to the patient.
2. To investigate whether ^{99m}Tc-WBC, due to the higher dose of activity that can be administered, would have a higher sensitivity for diseased segments of the small bowel than ^{111}In-granulocytes. As reported earlier, ^{111}In-granulocytes are somewhat less accurate in detecting active disease in the small bowel in compari-

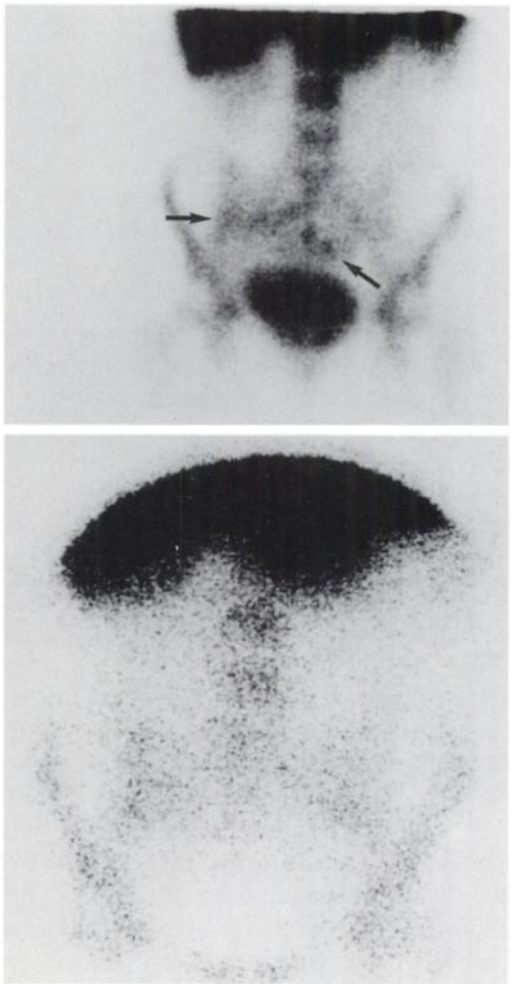

FIGURE 5. Patient 1. Crohn's disease of the small bowel. Discordant images. Clear pathologic activity in the ileum (between the arrows) on the ^{99m}Tc-WBC image (top). Normal ^{111}In-granulocyte scintigram (bottom).

TABLE 1
Scintigraphic Results per Patient

Patient no.	Tc + In − Segments*	Tc − In + Segments*	Both positive segments*	Both negative (no. of segments)	Resection segments*
1	2			7	3
2	2			8	
3			8–9	7	
4			4–5	3	6–9
5	3		2	7	
6	2			7	3
7	8†			8	
8				9	
9				8	3
10				8	3
11			2	2	3–8
12			3–9	2	
13			8	7	3
14		3–4, 7–8‡	2, 6	3	
Total	5	4	16	86	15

*Number refers to segment numbers: 1 = jejunum, 2 = ileum, 3 = cecum, 4 = ascending colon, 5 = right-sided transverse colon, 6 = left-sided transverse colon, 7 = descending colon, 8 = sigmoid, 9 = rectum (see Fig. 1)
†Corrected for location (see text).
‡Positive ^{111}In-granulocyte result for segments 7 and 8 are not in concordance with endoscopy (see text).

son to the large bowel (diagnostic accuracy 77% and 95%, respectively) (*3*). For the small bowel, only one alternative diagnostic modality is available, i.e. enteroclysis, which is not very pleasant for the patient.

Only a small number of patients were included in this study. From past experience we know that ^{111}In-granulocytes, although very specific, are not the perfect gold standard because of their limited sensitivity, especially in the small bowel (*3*). To prove therefore that ^{99m}Tc-WBCs are at least as good, we had to rely on other diagnostic modalities, which were only used when clinically needed. The results from these other methods were used only if the study had been performed within 2 wk of the scintigraphic examinations to eliminate changes in the patient's condition.

Because of the nonexistence of a gold standard, we were unable to calculate sensitivity and specificity for the two scintigraphic modalities. The results of this comparative study, however, have convinced us that ^{99m}Tc-WBC can reliably replace ^{111}In-granulocytes in the scintigraphic assessment of IBD patients with suspected exacerbation of their disease if imaging is finished within 2 hr of the injection. The only disadvantage of ^{99m}Tc-WBCs in comparison to ^{111}In-granulocytes is that the radioactivity excreted in the stools cannot be used to measure the degree of disease activity. As a result of this investigation, we no longer use ^{111}In-granulocytes in the detection of (exacerbation of) IBD in our clinical practice.

CONCLUSIONS

We have shown that early imaging with ^{99m}Tc-WBCs can reliably replace ^{111}In-granulocyte scintigraphy for the assessment of presence and location of active disease in patients with suspected exacerbation of IBD. Technetium-99m-WBCs show particular superiority for small bowel segments. Use of ^{99m}Tc-WBCs is also advantageous because it is available daily, results in lower radiation burden to the patient and cell separation is less time-consuming and simpler.

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