# Three-Phase White Blood Cell Scan: Diagnostic Validity in Abdominal Inflammatory Diseases

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Indium-111-oxine saline-labeled "mixed" leukocyte (n = 16) and "pure" granulocyte (n = 66) scans were prospectively performed as "three-phase" white blood cell (WBC) scans (imaging: 30 min, 4 hr, and 24 hr after reinjection of the cells) in 82 patients suspected of having abdominal or retroperitoneal abscesses or inflammatory lesions. Inflammation was verified histologically, endoscopically, radiologically or by autopsy in 51 and excluded in 31 patients. Sensitivity, specificity, and diagnostic accuracy of the 30-min scan (90%, 56%, 72%) were statistically significantly lower than the 4-hr scan (96%, 97%, 98%). Of the 24-hr scan sensitivity, specificity, and diagnostic accuracy were only 84%, 98%, and 89% because many patients with chronic inflammatory bowel diseases had excreted a portion of intestinal $^{111}$In activity by 24 hr. The overall sensitivity, specificity, and accuracy of the "three-phase" WBC scan were 98%, 97%, and 98%, respectively. Only one female patient showed a false-positive scan with granulocyte uptake in an ulcerating adenocarcinoma of the colon. The 4-hr scan or the three-phase study are recommended because of their high sensitivity, specificity, and excellent diagnostic accuracy (98%). The 30-min scan is less specific (56%); the 24-hr scan less sensitive (84%). The three-phase study additionally allows the differentiation between inflammatory bowel diseases and abscesses because it allows observation of granulocyte kinetics for 24 hr.

Most authors (*1–3*) recommend 4 hr and 24 hr after reinjection of the labeled cells as the best scanning time in leukocyte scans, because of an unpredictable localization of inflammations in the first 4 hr. Recent studies (*4–6*), however, demonstrate that indium-111-($^{111}$In) labeled leukocytes can be visible in the site of an abscess 30–40 min after injection. Other groups (*7*) found a very low sensitivity (33%) of early scans (1–4 hr p.i.). We performed a prospective study in patients suspected to have abdominal abscesses or inflammatory diseases. We scanned them 30–40 min, 3–4 hr, and 18–24 hr after reinjection of the cells to determine the diagnostic value of such a "three-phase" white blood cell (WBC) scan and to determine the best scanning time for a given disease.

## MATERIALS AND METHODS

### Cell Separation and Labeling

All procedures of cell separation and $^{111}$In-labeling were carried out aseptically in a laminar flow cabinet at room temperature using sterile one way material. All centrifugation steps were completed without use of the centrifuge brake.

Eighty milliliters of venous blood were drawn into two sterile 50-ml plastic syringes, each containing 2,500 IU heparin-sodium (Liquemin) to prevent coagulation and 8 ml hydroxyethylstarch (Plasmasteril) to accelerate sedimentation.

*Preparation of "Mixed" Leukocytes.* After gentle rotation of the syringes, 10 ml blood of each were drawn into 15-ml plastic tubes and centrifuged (1,000 *g*/5 min) to obtain platelet-rich plasma (PRP). PRP was stored aseptically. The other 40 ml of venous blood was allowed to sediment for 45 min with the conus of the syringe upright. The supernant (leukocyte rich plasma)

was centrifuged (90 ml/7 min) and washed twice with 5 ml of 0.9% sodium chloride. Five hundred microcuries (8.5 MBq) [$^{111}$In]oxine (t=67 hr, 173 keV-89%, 247 keV-94%) of a saturated complex (3:1) of 8-chinolinol (oxine) were added and incubated for 15 min after centrifugation (90 ml/7 min) and resuspension of the pellet with the stored PRP. The labeling efficiency (50-80%) was determined and 1 ml of resuspended separated cells was taken for differential blood cell counting. The reinjected dose was 250-300 $\mu$Ci (9.4 MBq) [$^{111}$In] oxine.

*Preparation of "Pure" Granulocytes.* Mixed leukocytes were isolated from 80 ml whole blood as described above. The granulocytes were then isolated by sedimentation on discontinuous density-gradient columns made up of mixtures of autologous plasma and Percoll. To prepare a stock solution, 9 ml iso-osmolar Percoll and 0.9 ml of 10% sodium chloride were mixed. Three densities were prepared by mixing stock solution (ss) and PRP to obtain 50%, 60%, and 65% Percoll plasma solutions (50%: 1 ml PRP/1 ml ss; 60%: 0.8 ml PRP/ 1 l, 2 l, ml ss; 65%: 0.7 ml PRP/1 l, 3 ml ss).

Two milliliters of the 60% and 50% solutions were then carefully layered on the 65% solution. Finally, the mixed leukocyte pellet was layered on top of the gradient, and spun at 400 $g$/7 min. The majority of the granulocytes were isolated from the interface between the 60% and 65% bands. The isolated granulocytes were washed twice (90 $g$/7 min) with a plasma/sodium chloride mixture (1 vol. plasma/5 vol. 0.9% sodium chloride) to eliminate Percoll totally. The resuspension in 10 ml of 0.9% sodium chlorate was followed by incubation for 15 min with 500 $\mu$Ci (8.5 MBq) [$^{111}$In] oxine. A new centrifugation (90 $g$/7 min) preceded the resuspension of the granulocyte pellet in PRP. Labeling efficiency was 50-80%. One milliliter cell suspension was taken for differential cell counting. The preparation of pure granulocytes required ½ hr more than the preparation of mixed leukocytes.

### Imaging

Without special preparation, patients were scanned with a gamma camera fitted with a parallel hole, high resolution, low-energy, collimator using only the 173 keV peak. Static images were obtained 30-40 min, 3-4 hr and 18-24 hr after reinjection.

### Patients

Eighty-two patients (45 M, 37 F) suspected of having either inflammatory bowel diseases or an intra-abdominal or retroperitoneal abscess were studied. Sixteen studies were performed with mixed leukocyte preparations and 66 studies with pure granulocyte preparations. Diagnoses were verified by standard methods (Table 1). In patients with inflammatory bowel diseases we used endoscopy, biopsy, or radiography, clinical indices, and laboratory investigations; in patients with abscesses, histologic evaluation of surgical specimens, and in two patients autopsy served as reference methods.

## RESULTS

### Three-Phase White Blood Cell Scan

Fifty out of 82 patients showed a positive scan; 31 demonstrated a negative scan (Table 2). Only one female patient with a necrotising adenocarcinoma of the sigmoid showed a false-positive result. She had intensive, abscess-like accumulation of radioactivity in the tumor but had no abscess. The other 49 positive scans had inflammatory disease as could be shown surgically, endoscopically, or by autopsy. None of the 31 patients with a negative scan had active inflammatory diseases or an abscess at the time of scanning. Thus, the overall sensitivity and specificity of the three-phase WBC scan was 98% (95% confidence interval (c.i.: 92.8-100%) and 97% (c.i.: 83.3-99.9%) (Table 2), respectively, and the diagnostic accuracy 98% (c.i.: 91.3-100%) (Table 2). The specificity and diagnostic accuracy of the 4 hr and 24 hr scan and the three-phase scan were significantly higher than the 30-min scan. The differences between the 4 hr-, 24 hr-, and three-phase scans were statistically not significant.

### Thirty-Minute Scan

Only 80 of 82 patients could be scanned 30 min after reinjection of the cells; two patients from the intensive care unit were scanned only 4 hr and 24 hr after

**TABLE 1**
Diagnosis of 82 Patients Examined

| 50 Patients with acute or chronic inflammatory diseases | | 32 Patients without inflammatory diseases | |
| --- | --- | --- | --- |
| Diagnosis | No. | Diagnosis | No. |
| Active Crohn's disease | 35 | Inactive Crohn's disease | 11 |
| Active ulcerative colitis | 4 | Inactive ulcerative colitis | 3 |
| Abscesses (abdominal or retroperitoneal) | 7 | Inactive Whipple's disease | 1 |
| | | Irritable bowel syndrome | 6 |
| Unspecific enteritis | 1 | Suspected unspecific enteritis | 2 |
| Yersinia enteritis | 1 | Suspected abdominal abscesses | 6 |
| Gold colitis | 1 | Treated Yersinia enteritis | 1 |
| Pancreatitis | 1 | Treasted unspecific colitis | 1 |
| Total | 50 | Sigmacarcinoma | 1 |
| | | Total | 32 |

TABLE 2
Sensitivity, Specificity, and Diagnostic Accuracy of Three-Phase WBC Scans

| Scan time (p.i.): | 30 min | 4 hr | 24 hr | Three-phase |
|---|---|---|---|---|
| Sensitivity | n* = 49 | n = 50 | n = 49 | n = 49 |
|  | 90% | 96% | 84% | 98% |
|  | (77.8–96.6%)† | (86.3–99.5%) | (70.3–92.7%) | (92.8–100%) |
| Specificity | n = 31 | n = 32 | n = 31 | n = 31 |
|  | 56% | 97% | 98% | 97% |
|  | (36.0–72.7%) | (83.8–99.9%) | (83.3–99.9%) | (83.3–99.9%) |
| Diagnostic accuracy | n = 80 | n = 82 | n = 80 | n = 80 |
|  | 72% | 98% | 89% | 98% |
|  | (63.6–83.8%) | (92.5–99.7%) | (79.7–94.7%) | (91.3–100%) |

* n = Number of patients.
† ( ) = 95% Confidence intervals.

reinjection. Of the other 49 patients with active inflammatory diseases, 44 already showed ^{111}In accumulation at the site of the disease in these early scans. The sensitivity of the early scan was 90% (c.i.: 77.8–96.6%) (Table 2).

All abscesses in seven seriously ill patients could be localized 30 min after reinjection of the cells (Fig. 1) with high ^{111}In uptake. Five patients with negative 30-min WBC scans had chronic inflammatory diseases: gold colitis (n–1); chronic pancreatitis (n–1); Crohn's disease (n–2); and severe reactive monocytosis (n–1). In one patient with Crohn's disease, the 24-hr scan gave only a positive imaging of the colonic lumen. In one female with reactive monocytosis, only the 24-hr scan showed slight ^{111}In activity in the right pelvic region.

Forty-three of the patients examined early were male and 37 female. Thirty-nine (90%) of the 43 male patients showed a positive penile imaging due to blood-pool activity that disappeared in nearly all cases in the 4-hr scan (Fig. 1). None of the female patients showed genital blood-pool activity. It was not difficult in most of the patients to differentiate an inflammatory infiltration of the rectum in Crohn's disease or ulcerative colitis from genital hyperemia in men when performing lateral views. Furthermore, in most cases with abscesses or rectal inflammation, the activity was approximately circular, whereas penile blood-pool activity is band-like, thus both entities could be differentiated.

There may be another reason for a positive scan 30 min, but negative scan 4 hr, after reinjection of the cells in patients with short-term but successfully treated inflammatory bowel diseases. It always presented as minimal early accumulation less than or comparable to bone marrow activity in projection on the small or large bowel. We observed this phenomenon in follow-up of three patients with severe Crohn's disease under successful treatment (Fig. 2) and three other patients with successfully treated nonspecific colitis, inactive Whipple's disease, and Yersinia infection of the terminal ileum, respectively. The specificity of the 30-min scan
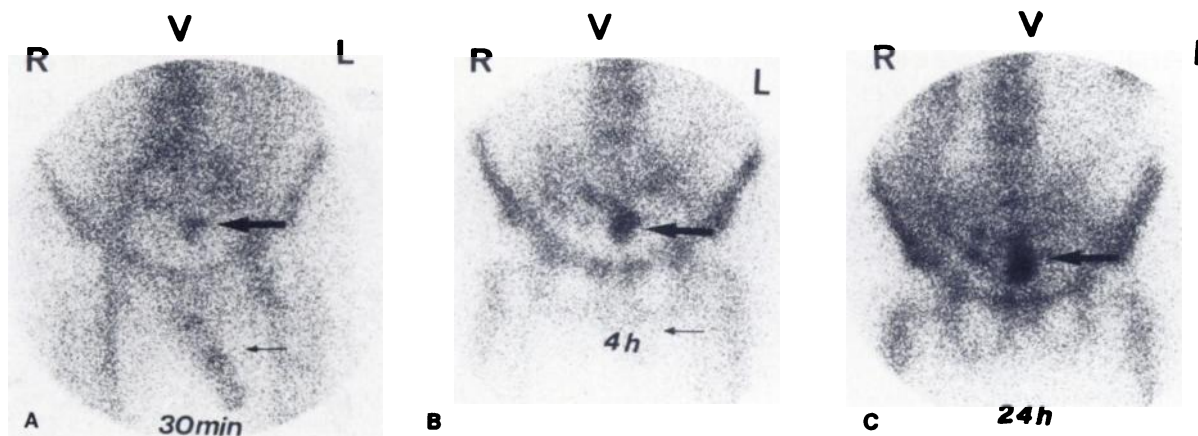


**FIGURE 1**
Active Crohn's disease of terminal ileum (see bowel activity) with pelvic abscess in 78-yr-old male patient. Note intense granulocyte accumulation in A: 30-min, B: 4-hr, and C: 24-hr scan. Remarkable intensive penile ^{111}In uptake in the 30-min scan but absent from 4-hr scan
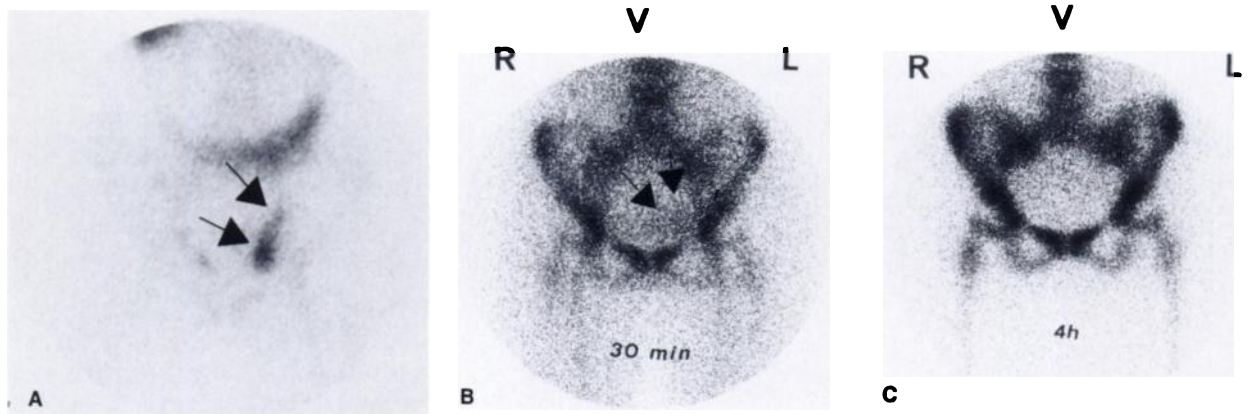
**FIGURE 2**
A: Pancolitis in 21-yr-old female with untreated Crohn's disease (4-hr scan) and maximum of $^{111}$In uptake in the sigmoid colon. B: Hyperemia of sigmoid 30 min p.i. in clinically inactive disease after 3-mo corticosteroid treatment; and C: Negative 4-hr-scan in same patient

is 56% (c.i.: 36–72.7%), the accuracy 72% (c.i.: 63.6–83.8%) (Table 2) when performing anterior views.

## Four-Hour Scan

The 4-hr scan was the most important scan in patients with inflammatory bowel diseases. We found that the exact localization of diseased bowel segments had to be evaluated 3–4 hr after reinjection of the cells (Fig. 3). In earlier scans, especially in the 30-min scan, although activity could be seen at the site of inflammation, the exact extent of diseased bowel segments could be assessed correctly only in the 4-hr scans (Fig. 4).

Forty-nine of the 51 patients with positive scans showed the correct site of inflammation in the 4-hr scans. Thus, the sensitivity of the 4-hr scan is 96% (c.i.: 86.3–99.5%) (Table 2). The other two patients suffered from chronic inflammatory diseases. The specificity of the 4-hr scan was 97% (c.i.: 83.8–99.9%), and the accuracy 98% (c.i.: 92.5–99.7%) (Table 2).

## Twenty-Four-Hour Scan

In one female patient with reactive monocytosis, only the 24-hr scan showed inflammation in the pelvic region. Surgery and histology revealed a hemorrhagic infarction of the right ovary with an additional surrounding chronic inflammation of the ovary, the tube, and the ileum. Therefore, it might be possible that the positive imaging was not only due to the inflammation, but also to the infarction of the ovary. In one male patient with moderately active Crohn's disease of the terminal ileum, no abdominal accumulation could be seen in the 4-hr scan. At 24-hr, there was an activity band, comparable to bowel lumen accumulation in the descending colon. In one patient with an infected iliac bypass, only the 24-hr scan showed the exact localization and drainage of a fistula (Fig. 5). All the abscesses seen in the 4-hr scan could also be seen in the 24-hr scan.

The 24-hr scan serves only to diagnose abscesses and, sometimes, to recognize the presence of infection. In
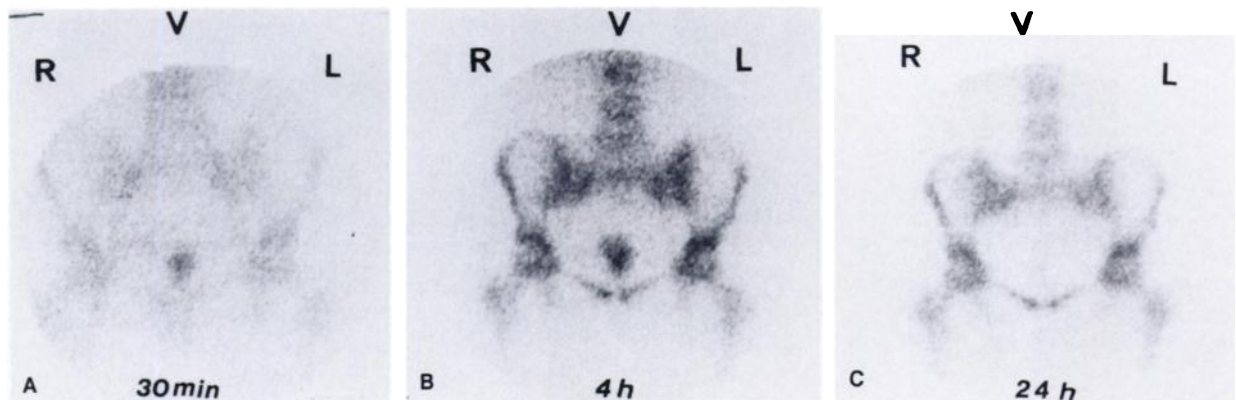


**FIGURE 3**
Circumscribed rectal $^{111}$In granulocyte infiltration in ulcerative colitis in 22-yr-old white female. A: 30 min, and B: 4 hr p.i. C: Total fecal excreted intestinal activity 24 hr p.i.
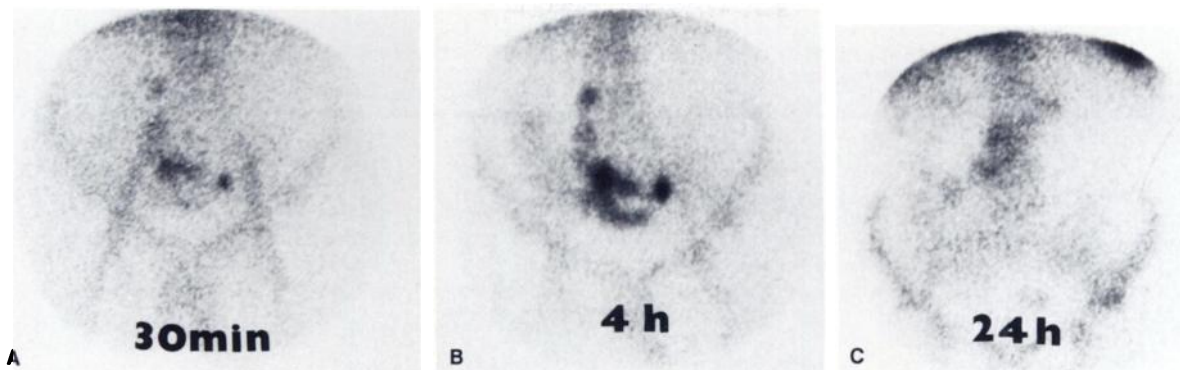
**FIGURE 4**
Active Crohn's disease (47-yr-old male) of terminal ileum, ascending colon, and fistula with intensive $^{111}$In accumulation A: 30 min p.i. Exact assessment of diseased bowel segments only possible in B: 4 hr scan. C: Visualization of drainage of fistula 24 hr p.i. into transverse colon

inflammatory bowel disease, the scan may already be negative again at 24-hr or the bowel lumen distal to the inflamed segment may be visualized. The sensitivity of the 24-hr scan for detection of abscesses (n = 7) is 100%. In eight patients with Crohn's disease and ulcerative colitis, the 24-hr scan was negative (Fig. 3). The overall sensitivity of the 24-hr scan for diagnosing inflammatory disease is 84% (c.i.: 70.3–92.7%) (Table 2). The specificity is 98% (c.i.: 83.3–99.9%), and the accuracy 89% (c.i.: 79.7–94.7%) (Table 2). In none of the patients with bowel disease could the diseased segment be identified correctly in the 24-hr scan.

## DISCUSSION

White blood cell scans, with a 98% sensitivity and 97% specificity (Table 2), should be considered as the primary procedure for locating inflammatory disease. Because of a delayed accumulation of labeled cells as reported in a number of studies (7–9) less specific methods, e.g., ultrasound (4) have been preferred because the results are more immediately available. Recent studies report early abscess detection 30 min after a reinjection of the cells (4–6,10). However, these results are not uniformly experienced. Datz and co-workers (7), for example, reported a sensitivity of 33% for the 30-min leukocyte scan. For this reason, we determined the diagnostic validity of the WBC scan at different times after reinjection of the labeled cells in patients with suspected abdominal or retroperitoneal inflammatory diseases. In our experience, the sensitivity of the 30-min scan (90%) is as high as that of the 3–4-hr scan (96%). The overall sensitivity of the three-phase scan is the best (98%). The difference between the sensitivity of the 4-hr scan and the three-phase scan is statistically not significant. The sensitivity of the 24-hr scan alone is the lowest (84%) because of the predominance of inflammatory bowel diseases in our patient population. In these diseases, the 4-hr scan is best in
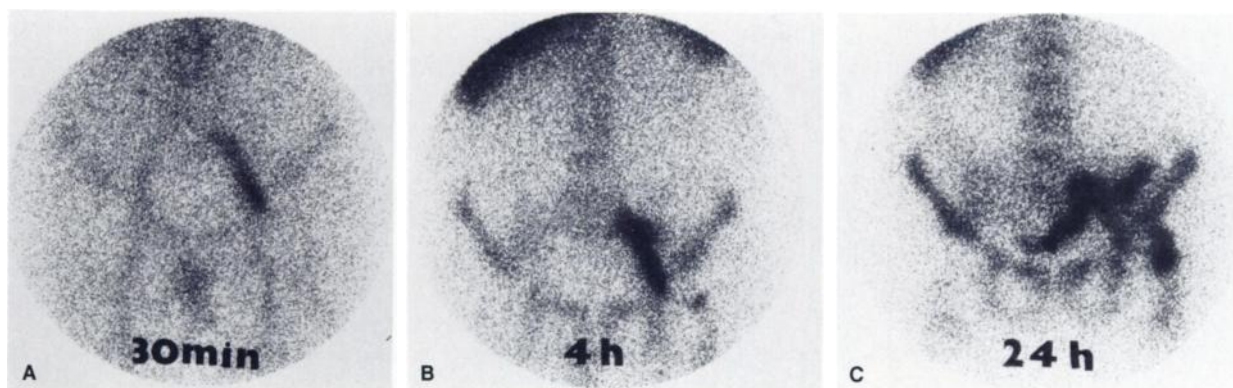


**FIGURE 5**
Iliacal graft infection in left graft branch (71-yr-old male) A: 30 min, B: 4 hr, and C: 24 hr p.i. Late scan (24 hr scan) only demonstrates surgically proven perforation to the sigma. Note circumscribed activity of left upper thigh demonstrating external pus drainage

**TABLE 3**
Diagnostic Accuracy of 30-min, 4-hr, 24-hr, and Three-Phase WBC Scans

| Item | 30-min scan | 4-hr scan | 24-hr scan | Three-phase scan |
|---|---|---|---|---|
| Localization of: | | | | |
| 1. Abscesses, inflammations | ++ | ++ | ++ | ++ |
| 2. Inflamed bowel segments | + | ++ | – | ++ |
| 3. Fistulas, chronic inflammations | – | – | ++ | ++ |
| Differentiation of 1, 2, 3 | – | – | – | ++ |

determining the exact location of the inflammation ( *11,12*). The 30-min scan also allows some localization of the site of inflammation, but its main purpose is to recognize the presence or absence of active disease rather than its extent. The 24-hr scan shows the bowel lumen distal to the site of inflammatory bowel disease. Eight cases in our study show no activity at all. This is the reason for the low sensitivity of the 24-hr scan. Thus, the combination of the three phases allows for the optimum diagnostic accuracy in our experience (Table 3).

The visualization of inflammatory sites 30 min after reinjection often allows for a decision whether there is an inflammatory process or not. It is not possible to differentiate between an abscess or another inflammatory lesion. This is only possible if the location of radioactivity does not change between 30 min and 24 hr. The intensity of uptake is not a suitable criterion for differentiation of abscesses (*4*) from inflammatory bowel lesions (Figs. 4 and 5) although abscesses show more intense ^{111}In uptake than the liver uptake (*4*).

An uptake less than liver uptake does not always indicate an inflammatory lesion. Most of our male patients (86%) had penile blood-pool activity (*13*), which disappeared completely by 4 hr.

Another pattern of activity in the early scan, usually comparable to bone marrow ^{111}In uptake, can be seen in patients with successfully treated bowel diseases (Fig. 2). It also disappears completely in the 4-hr scan. We believe this is a hyperemia of the previously inflamed bowel. The specificity of the 30-min scan, therefore, is 56% (c.i.: 36–72.7%) (Table 2). If additional lateral views are performed to differentiate penile hyperemias, the specificity increases. The early 30-min scan allows the diagnosis of inflammatory abdominal or retroperitoneal disease only if ^{111}In uptake is comparable to or higher than liver uptake. The diagnosis of an abscess is possible only if the presence of an intra-abdominal mass can be confirmed by other methods (e.g., sonography, computed tomography). Severe inflammatory bowel diseases can also be shown in the early scan (Fig. 4). Accumulations less than liver activity can be diagnosed only as inflammation, if slight or treated bowel disease can be ruled out. We believe that the reported lower sensitivity (*7*) of 33% of the 30-min scan may be due to patient selection. Datz and co-workers (*7*) also examined patients with osteomyelitis in whom the WBC

scan seems not to be as sensitive as in soft-tissue infections (*14*).

Gamma camera images 3–4 hr after reinjection of the labeled cells are not disturbed by hyperemia of the bowel or the penis. Any abnormal activity must be due to inflammation or to an abscess. Therefore, the 4-hr scan is essential in patients with inflammatory bowel disease. At this time, the results of localization of inflamed bowel segments at 4 hr correlate best with endoscopic and radiologic examinations (*11,15,16*). The labeled cells later pass through the bowel wall and distal bowel lumen affecting imaging results. At 4 hr, abscesses can be suspected but not proven, because patients with Crohn's disease or ulcerative colitis can also have an early ^{111}In uptake higher than the liver uptake (Fig. 5). Only the additional 18–24-hr scan allows certain differentiation between abscesses with "fixed" activity and inflammatory bowel diseases with "mobile" bowel lumen activity. In two out of 39 patients with active inflammatory bowel disease without an abscess we found an unchanged abscess-like activity in the 4-hr and 24-hr scan. The reason for this was a severe stenosis after the diseased segment causing delayed bowel emptying. Thus, the 24-hr scan is essential in diagnosing an abscess and the complete extent of an inflammatory lesion. Fistulas are often later filled up with labeled cells (Fig. 5). In some patients (three of 51), an inflammatory lesion could be seen only in the 24-hr scan.

Regarding sensitivity, specificity, and diagnostic accuracy, the 4-hr scan yields the same results as a three-phase scan. The question arises: Why perform a three-phase scan? Using a 30-min or 4-hr scan, only abscesses, inflammations, and inflamed bowel segments could be seen. The combination of 30-min, 4-hr, and 24-hr scans additionally allows for the differentiation of an abscess, inflamed bowel segments, the drainage of fistulas and chronic inflammations.

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