
Scintigraphic Assessment of Indium-111-Labeled Granulocyte Splenic Pooling: A New Approach to Inflammatory Bowel Disease Activity

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We have conducted a prospective study into the sensitivity and the specificity of the fall in splenic activity (FSA) as an index of activity in inflammatory bowel disease (IBD). FSA was measured on scintiscans obtained at 3 and 24 hr postinjection of indium-111-labeled granulocytes. One hundred and twenty-two scans were acquired in 96 patients who were divided into six groups: Gr. I = normal volunteers (n = 10); Gr. II = inflammatory rheumatism (n = 10); Gr. III = abscesses (n = 17); Gr. IV = ulcerative colitis (UC: n = 23); Gr. V = colonic Crohn's disease (CCD: n = 22); and Gr. VI = ileal Crohn's disease (ICD: n = 14). FSA for Groups I and II was constantly below 10%, but it was increased in the other four groups (abscesses: 39% ± 12%; UC: 35% ± 13.5%; CCD: 23.7% ± 14.7%; ICD: 21.5% ± 11.7%). There was a significant correlation between fecal excretion of ¹¹¹In (FEI) and FSA in patients with IBD (UC: r = 0.71, p < 0.001; CCD: r = 0.74, p < 0.001; ICD: r = 0.43, p < 0.001). FSA was followed in 16 patients with IBD after medical treatment and there was a significant correlation between variations in FSA and in FEI (r = 0.879, p < 0.001). FSA is a very sensitive although nonspecific index of disease activity in IBD and may replace FEI in the assessment of IBD activity.

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The contribution of indium-111- (¹¹¹In) labeled autologous granulocytes to the diagnosis of inflammatory bowel disease (IBD) is now well established. The extent of disease can be evaluated by scintiscans (1-5) and disease activity by fecal excretion of the ¹¹¹In (FEI) (3,5-10). FEI would appear to be a good index as it correlates well with clinico-biologic indices (3,6,9,10)

such as Crohn's disease activity index (11) and Harvey index (12). Its measurement, however, requires careful collection of stools over a period of 4 days and it is for this reason that other activity indices such as the morphologic scintigraphic index, have been proposed (9). However, scintigraphic investigation of Crohn's disease in comparison to ulcerative colitis is now known to be less sensitive (13) and may compromise the diagnostic value of an activity index calculated from scintiscans. Furthermore, we have observed in some patients with IBD that there is a rapid fall in splenic activity (FSA) between the 3rd and 24th hr following administration of ¹¹¹In-granulocytes (3). We conducted a prospective study to assess the correlation between the FSA and IBD activity indexes.

PATIENTS AND METHODS

Patients

Table 1 summarizes the characteristics of the six different groups of patients studied. The first three groups made up the control populations: normal volunteers (n = 10), 10 patients with inflammatory rheumatism (IR) (8 rheumatic polyarthritides and 2 rheumatoid spondylitis) (n = 10) and with deep abscesses (DA) (n = 17). The other patients with IBD (n = 59) were divided into ulcerative colitis (UC = 23), colonic Crohn's disease (CCD = 22), and ileal Crohn's disease (ICD = 14). Sixty-nine studies were performed in the 59 patients on their admission into the hospital for different attacks of IBD. Furthermore, 16 patients (9 UC and 7 CCD) had a second exploration after two to three weeks of medical treatment.

The diagnosis of UC, CCD, or ICD was confirmed in all patients on the basis of their clinical history, endoscopic examination, and histology. IBD activity was evaluated by the Crohn's disease activity index (CDAI) (12), which is known to correlate well with the Truelove index for UC (3,14).

Methods

Following MacAfee et al. (15) and Thakur et al. (16) works on granulocytes labeling with ¹¹¹In complex oxinate, we have

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TABLE 1
Clinical and Scintigraphic Findings in Six Groups of Patients

Groups	Patients (n = 96)	Sex	Age (yr)	CDAI	FEI (%)	FSA (%)
Normal (n = 10)	10	F: 7 M: 3	35* 25-45†		<0.6 —	3.9 ± 3
IR (n = 10)	10	F: 5 M: 5	55 45-68		— —	3 ± 2.8
Abscess (n = 17)	17	F: 8 M: 9	48 20-71		— —	39 ± 12
UC (n = 26)	23	F: 11 M: 12	36 20-61	276 ± 83 114 - 423	19.8 ± 12.4 1.9 - 46	35 ± 13.5 8 - 63
CCD (n = 24)	22	F: 16 M: 6	36 21-64	240 ± 87 94 - 349	8.6 ± 7.5 0.3 - 37	23.7 ± 14.7 0 - 66
ICD (n = 19)	14	F: 9 M: 5	29 17-73	193 ± 75 107 - 335	3 ± 2.8 0.8 - 11.6	21.5 ± 11.7 0 - 48

* Mean value.

† Range.

Normal = normal volunteers; IR = inflammatory rheumatism; DA = deep abscesses; UC = ulcerative colitis; CCD = chronic Crohn's disease; and ICD = ileal Crohn's disease.

developed our own labeling procedure (17). The granulocytes were isolated from 40 ml of blood drawn onto heparin. Sedimentation gave a plasma rich in cells, which was diluted to 1.5 times its initial volume with a culture medium (RPMI). The granulocyte button was obtained by centrifugation (200 g × 15 min) of leukocyte-rich plasma over a Ficoll-Metrizoate gradient. The granulocytes were resuspended in the RPMI and labeled with 100-150 μCi (3.7 to 5.5 MBq) of ¹¹¹In-oxine (Mallinckrodt). Following incubation at 20°C for 5 min, the suspension was washed with 10 ml of RPMI and centrifuged at 150 g and 20°C for 2 min. A pellet of labeled granulocytes was obtained and resuspended in autologous plasma for reinjection. Labeling efficiency averaged 95%. Red blood cell contamination was less than 10%. No platelets and mononuclear cells were visualized. For all preparations, blue trypan test exclusion was performed before reinjection. Viability was always superior to 90%. Bactericidal and chemotactic tests were performed in 10 preparations. Electronic microscopy examination of labeled and unlabeled cells had not shown any alteration in the cells (five cases). The validity of our method has been previously reported in vitro (17).

FEI was measured in stools collected over 24 hr on four consecutive days and was expressed as the percentage of the injected dose (%ID). Scans were performed with a gamma camera which was fitted with a medium-energy parallel-hole collimator (Acticamera, CGR) set at both peaks for ¹¹¹In (173 and 245 keV, 20% window), and interfaced with a data processing system (A2 MDS).

FSA was calculated on the basis of two measures of splenic activity (SA) by scintiscans (anterior view) centered on the splenic region over a period of 5 min at 3 and 24 hr postinjection (Figs. 1 and 2). FSA was calculated as follows after a correction for ¹¹¹In decay:

$$FSA = ((SA_{3h} - SA_{24h})/SA_{3h}) \times 100.$$

Results were expressed as a mean ± 1 s.d. The correlation coefficient (r) was used for statistical analysis.

RESULTS

Mean values for CDAI, FEI, and FSA are summarized in Table 1.

FSA Values. These were <10% (M + 2DS = 10%) for all the normal volunteers and were not significantly different from those for the patients with IR. On the other hand, these were greatly increased in the four other groups of patients.

FSA and FEI Correlation. A significant correlation was found between the FSA and the FEI in the patients with UC (r = 0.71, p < 0.001) and those with CCD (r = 0.74, p < 0.001).

Ten of the ICD patients had an FEI >2% [upper limit of normal range (8)] and in this case the FSA was always >10%. The remaining four patients with an FEI <2% had an FSA >10%.

FSA and CDAI Correlation. A significant correlation existed between FSA and CDAI for the UC patients (r = 0.52, p < 0.01) and the CCD patients (r = 0.54, p < 0.01). There was no correlation between these two parameters for the ICD cases (r = 0.15, ns).

FEI and CDAI Correlation. FEI and CDAI also correlated well: r = 0.58, p < 0.01 (UC patients); r = 0.67, p < 0.01 (CCD patients). No correlation was found for ICD patients (r = 0.38, ns).

Follow-up. Explorations in 16 patients after treatment showed a significant correlation between the variations in FSA and in FEI (r = 0.79, p < 0.001).

DISCUSSION

Rapid splenic pooling following the injection of ¹¹¹In-labeled granulocytes is a well-reported observation. The FSA has not been widely studied (8,18-20).

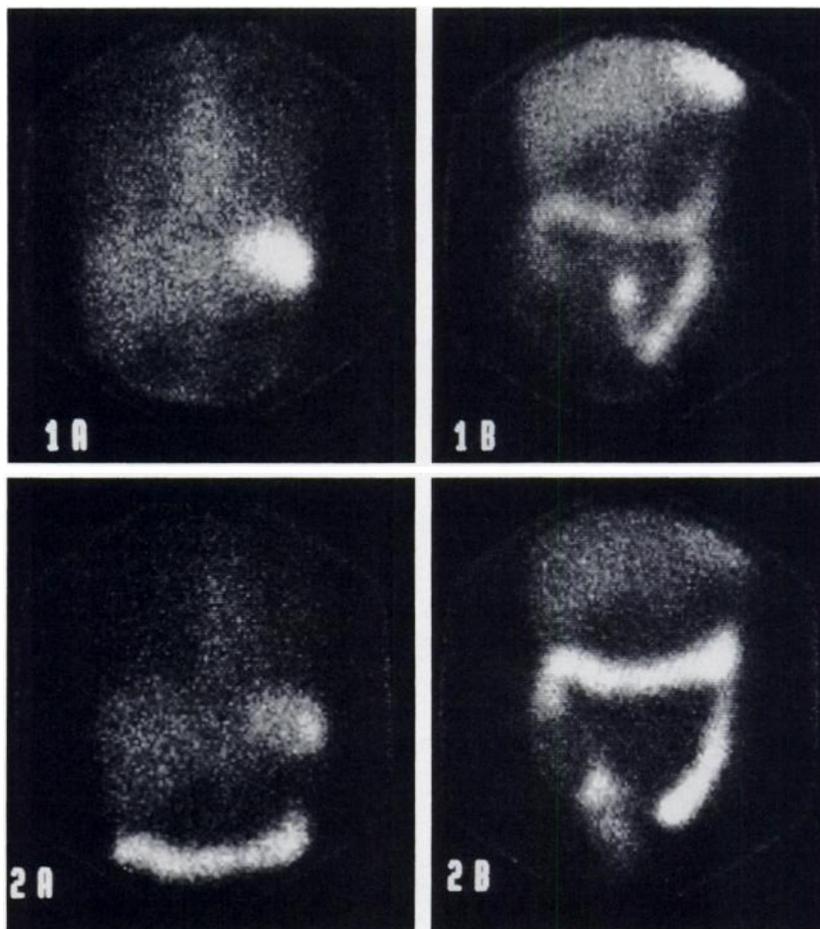


FIGURE 1 and FIGURE 2
 Scintigraphic decrease of ^{111}In -granulocyte splenic pool between 3 (1A) and 24 hr (2A) in a patient with UC (FSA = 52%). Abdominal scans at 3 hr (1B) and 24 hr (2B) of same patient.

The present study demonstrates that in normal volunteers, normal FSA value is $<10\%$. This is inferior to the value reported by Saverymuttu et al. (20). The difference is probably related to the count rate being carried out at 3 hr instead of 40 min. We have also found that FSA is very important during attacks of IBD (means: UC $35\% \pm 13.5\%$, CCD $23.7\% \pm 14.7\%$, ICD $21.5\% \pm 11.7\%$). This was first reported by Segal et al. (18) and then confirmed by Peters and Saverymuttu et al. (19,20).

To the best of our knowledge, no prospective studies on the measurement of FSA in cases of IBD have been conducted. Our results show that FSA is a sensitive index since it correlates well with the FEI, which is considered the best criterion of activity in IBD. In patients with clinical remission in Crohn's disease, the FEI may still be increased when the other inflammatory parameters have returned to normal (21). We found a significant but mediocre correlation between the FSA and the CDAI as well as between the FEI and the CDAI for the patients with UC and CCD. This result shows the less than perfect relationship between a clinical index (calculated in part on the basis of subjective elements) and indirect measurement of the degree of bowel inflammation. We noted that FSA correlated less

well (and was even not significant) with FEI or CDAI in ICD than in UC or CCD patients. This is probably due to lower values for FEI in cases of ICD that we have already reported (3,5). In the present study, we found that FSA was a more sensitive index than FEI in patients with Crohn's ileitis, as FSA was abnormal in five of the nine patients with a normal FEI value. Further proof of index sensitivity is given by the good correlation between the variations in FSA and FEI during treatment in patients who had follow-up studies (22).

Increased FSA is not specific to IBD. Our findings in cases of abscesses concur with those of Saverymuttu et al. (20). This is not surprising, since FSA expresses the migration of splenic granulocytes towards sites of infection.

Our study also demonstrates that FSA does not vary in inflammatory rheumatism. This result suggests that inflammatory rheumatism associated with IBD should not influence FSA values.

In conclusion, our results show that FSA is a sensitive but nonspecific index of IBD activity. FSA is rapidly calculated on scans; it correlated well with FEI and so could replace FEI in the assessment of IBD activity and be used in monitoring patient response to treatment.

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