Gut, 1983, **24**, 293–299

^{111}Indium autologous leucocytes in inflammatory bowel disease

S H SAVERYMUTTU, A M PETERS, J P LAVENDER, H J HODGSON, AND V S CHADWICK

From the Departments of Medicine and Nuclear Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London

SUMMARY A non-invasive method of imaging and assessing inflammatory bowel disease is described. ^{111}Indium labelled leucocyte scans were performed on 33 patients with a wide variety of inflammatory bowel diseases and 25 control patients. All patients with moderate or severe inflammatory bowel disease had positive scans with localisation of abnormal activity corresponding to the sites assessed to be diseased by radiology in either small or large bowel. No false positives were recorded in the control patients. Faecal excretion of ^{111}In labelled leucocytes was increased in patients with inflammatory bowel disease according to disease severity and correlated with disease activity assessed by serum C-reactive protein levels ($r=0{\cdot}74$, $p<0{\cdot}001$) or in those patients with Crohn's disease by Crohn's Disease Activity Index ($r=0{\cdot}78$, $p<0{\cdot}001$). These data suggest that ^{111}In labelled leucocytes may be used to provide a safe, non-invasive method of imaging diseased bowel and objectively assessing disease activity.

Gamma camera scanning after intravenous injection of ^{111}Indium labelled autologous leucocytes is now established as an effective method for the localisation of abscesses.[1–6] The technique has also been applied with success to other conditions with an inflammatory component.[7] Leucocyte infiltration of the gut and the presence of a leucocyte-rich faecal exudate are characteristic features of acute inflammatory bowel disease and recently we[8 9] and others[10] have reported preliminary studies showing that inflamed bowel can be imaged using the ^{111}In labelled leucocyte technique. Labelled cells from inflamed gut, unlike those within abscesses, are rapidly excreted into the gut lumen, and gamma counting of faeces permits a quantitative assessment of the white cell excretion. The purpose of this prospective study was to use the technique in a series of patients with a variety of inflammatory bowel disorders to assess its value in localising inflamed bowel and to study the relationship between disease activity and labelled white cell excretion. Control studies were performed in patients with the irritable bowel syndrome and with a variety of non-inflammatory bowel disorders such as gut carcinomas.

Address for correspondence: Dr S H Saverymuttu, Gastroenterology Unit, Hammersmith Hospital, London W12.

Received for publication 1 July 1982

Methods

LEUCOCYTE LABELLING

Whole blood (42·5 ml) was drawn through a 19 gauge needle into a syringe containing 7·5 ml acid citrate dextrose (ACD) (NIH formula A). The red cells were allowed to sediment at 37°C and 1 *g* for 45 minutes. For those patients with low sedimentation rates hydroxyethyl starch (Fresenius) (10% by volume) was added to accelerate sedimentation. The supernatant cell rich plasma was then centrifuged at 150 *g* for five minutes. The cell pellet was resuspended in 8 ml 0·15M NaCl containing dextrose (1 part 5% dextrose to 10 parts NaCl), ACD (1 part to 100 parts NaCl), acetylacetone (0·19%) and 20 mM Hepes buffer (pH 7·6). About 200 μCi of ^{111}In in 0·04M HCl (The Radiochemical Centre, Amersham) of volume less than 0·25 ml was added and the mixture left under gentle agitation on a roller for 10 minutes. Five millilitres of cell free plasma were then added and the cells sedimented by centrifugation at 150 *g* for three minutes. The supernatant was discarded and the cells resuspended in 5 ml plasma for reinjection. The labelling efficiency averaged 90%.

PATIENTS STUDIED

A total of 58 patients were studied – the clinical details are summarised in Table 1. Four patients were studied on two occasions. All patients had routine biochemistry and haematology checked at the time of the study and all but one (with graft *vs* host disease) had recent bowel radiology or colonoscopy. The 33 patients with inflammatory bowel disease were classified into a mild, moderate, or severe category on the basis of a simple clinical grading system detailed below to enable comparison between the various disease groups. In most patients with Crohn's disease the Crohn's Disease Activity Index (CDAI) was calculated.

The control groups consisted of 11 patients with the irritable bowel syndrome and 14 patients with a variety of non-inflammatory bowel disorders – for example, gut carcinoma. All patients gave informed consent to the study. The radiation dose of ^{111}In labelled leucocytes is maximal to the spleen[1] at 5 rads per mCi. The mean dose used in this study was 180 microcuries.

CLINICAL GRADING SYSTEM FOR PATIENTS WITH INFLAMMATORY BOWEL DISEASE

Mild

This grade included those with mild symptoms, bowel frequency once or twice a day; no recent weight loss; and minimal abnormality or normal appearance at sigmoidoscopy in the patients with proctitis.

Moderate

In this grade were those with intermittent abdominal pain, bowel frequency three to five times a day; weight loss less than 3 kg; sigmoidoscopy showing either a granular or reddened mucosa in patients with proctitis.

Severe

This grade included those with continuous abdominal pain; bowel frequency more than five times per day; weight loss greater than 3 kg.

SCANNING TECHNIQUES

Gamma camera (GEC 400T) scans over the abdomen to include liver, spleen, and pelvis were performed at approximately three to five hours (early scans) and 18–26 hours (late scans). A medium energy parallel hole collimator and a dual energy analyser centred on the two photo-peaks of ^{111}In (173 and 247 Kev) were used. Counts were collected for up to 10 minutes.

INTERPRETATION OF SCANS

Scans were assessed by an experienced nuclear medicine physician who had no knowledge of the clinical state of the patients but was aware of the extent of previous surgery. Scans were designated positive if activity outside the normal distribution were present. Positive scans were further classified as showing small and large intestinal localisation according to the distribution of abnormal activity on the scan. In those cases where bowel loops were clearly visible, the extent of disease on scan was compared with the extent of disease assessed by radiology.

FAECAL COLLECTION

After administration of the labelled cells a four day faecal collection was made in daily aliquots. The total ^{111}In content of each daily aliquot was counted on an ARMAC counter.

In eight cases the fraction of particulate bound radioactivity was determined by thoroughly mixing approximately 0·5 g faeces in 10 ml 0·15M NaCl, and comparing the radioactivity in 1 ml of the mixture to the supernatant obtained after 10 minutes centrifugation at 2000 *g*.

STATISTICS

Correlations were calculated using Spearman's rank correlation.

Results

ABDOMINAL SCANS

Examples of scans obtained from control patients and patients with inflammatory bowel disease are shown in Figs 1–6. After injection of labelled cells all patients showed the expected distribution of radioactivity in spleen, liver, and bone marrow. The control patients with the irritable bowel syndrome and organic non-inflammatory bowel disorders showed no other intra-abdominal localisation (Fig. 1). Figure 2 illustrates an early scan on a patient with moderately active Crohn's colitis. Figures 3 and 4 show the barium follow-through and early white cell scan on a patient in whom disease was severely active with small bowel recurrence of Crohn's disease proximal to an ileotransverse anastomosis.

Later scans (18–26 hours) showed activity in the bowel lumen at a site distal to that visualised on initial scans (Figs 4 and 5). This distal movement of activity from the initial site of localisation was found in all patients with the exception of a patient with an abscess and enterocutaneous fistula (Fig. 6) where activity remained at the same site on later scan. In view of this transit of labelled cells, early scans were used to assess disease localisation and extent.

Early scans (three to five hours) were positive in

Table 1 *Clinical data*

Patient	*Disease*	*Distribution*	*Severity*	*CDAI*	*Bowel frequency*	*WBC* $\times 10^9/l$	*ALB g/l*	*ESR mm in 1st h*	*CRP*	*Treatment*
1 RR (1)	Crohn's	Small bowel	Severe	335	5	6·3	40	25	32	Azathioprine
2 RR (2)	Crohn's	Small bowel	Severe	360	7	7·4	41	10	25	Azathioprine, prednisolone
3 VN (1)	Crohn's	Small bowel	Severe	422	10	9·4	35	25	112	—
4 MY	Crohn's	Small and large bowel	Severe	534	16	8·8	33	80	83	—
5 PA	Crohn's	Small bowel	Severe	414	5	5·8	31	49	67	—
6 HC	Crohn's	Large bowel	Severe	401	12	14·0	37	110	121	Prednisolone, salazopyrin
7 LB	Crohn's	Large bowel, abscess	Severe	224	2	12·2	39	31	69	Prednisolone, salazopyrin
8 VN (2)	Crohn's	Small bowel	Moderate	216	3	11·6	33	25	28	—
9 MB (1)	Crohn's	Large bowel	Moderate	295	3	6·1	23	50	32	—
10 MW (1)	Crohn's	Small bowel	Moderate	179	4	8·7	38	27	20	—
11 CP	Crohn's	Small and large bowel	Moderate	239	5	9·0	37	34	38	Salazopyrin
12 HH	Crohn's	Small and large bowel	Moderate	236	7	10·6	38	90	82	—
13 HW	Crohn's	Small bowel	Moderate	213	4	7·4	34	20	13	—
14 AH	Crohn's	Large bowel	Moderate	176	3	11·3	38	22	31	Prednisolone, proxicromil
15 MG	Crohn's	Small bowel	Moderate	—	3	10·2	39	70	36	—
16 PF	Crohn's	Small bowel	Mild	171	2	7·3	46	4	2	—
17 MB (2)	Crohn's	Large bowel	Mild	159	1	6·8	32	30	0	Salazopyrin
18 MW (2)	Crohn's	Small bowel	Mild	149	2	7·1	38	22	14	—
19 IT	Crohn's	Small bowel	Mild	100	2	8·3	40	15	0	—
20 MW	Crohn's	Small bowel	Mild	136	3	10·3	47	6	0	—
21 IJ	Crohn's	Small bowel	Mild	117	2	6·7	38	10	3	—
22 DB	Crohn's	Small bowel	Mild	94	1	5·2	41	5	1	—
23 MP	UC	Large bowel	Severe	—	5	9·1	31	48	—	—
24 SP	UC	Large bowel	Severe	—	5	11·3	39	103	29	Salazopyrin
25 DM	UC	Large bowel	Severe	—	8	13·0	33	72	36	Salazopyrin
26 DK	UC	Large bowel	Severe	—	12	9·3	41	22	12	—
27 PS	Radiation enteritis	Small bowel	Severe	—	6	4·8	28	108	144	—
28 AS	Graft *vs* host disease	Large bowel	Severe	—	10	6·2	23	60	—	Cyclosporin A
29 MN	UC	Large bowel	Moderate	—	4	12·2	36	9	2	Prednisolone
30 SS	UC	Large bowel	Moderate	—	5	15·9	36	45	19	—
31 JC	Radiation enteritis	Small bowel	Moderate	—	1	7·3	44	27	—	Prednisolone
32 ML	Radiation enteritis	Small bowel	Moderate	—	3	4·1	30	80	—	—
33 MN	Vasculitis	Small bowel	Moderate	—	1	20·0	30	20	—	—
34 TC	Ischaemic colitis	Large bowel	Moderate	—	1	14·8	28	100	46	Prednisolone, azathioprine
35 ER	UC	Large bowel	Mild	—	1	7·3	41	6	1	—
36 CH	UC	Large bowel	Mild	—	1	6·4	42	12	0	—
37 RK	Intestinal TB	Small bowel	Mild	—	1	7·7	37	22	1	—
38–48	IBS	—	—	—	3·5 (±1·0)	6·9 (±1·3)	45 (±2·0)	5·6 (±2·4)	—	—
49–62	Non-IBD		—	—	1·7 (±1·3)	10·1 (±6·0)	37·8 (±2)	43 (±26)	—	—

Values for inflammatory bowel disease and non-inflammatory bowel disease represent mean ± 1 SD. UC=ulcerative colitis.

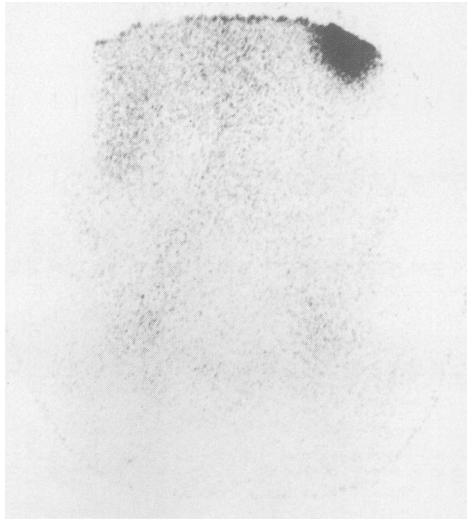

Fig. 1 *Abdominal ^{111}In leucocyte scan in a patient without inflammatory bowel disease showing normal distribution of activity in spleen, liver, and bone marrow.*

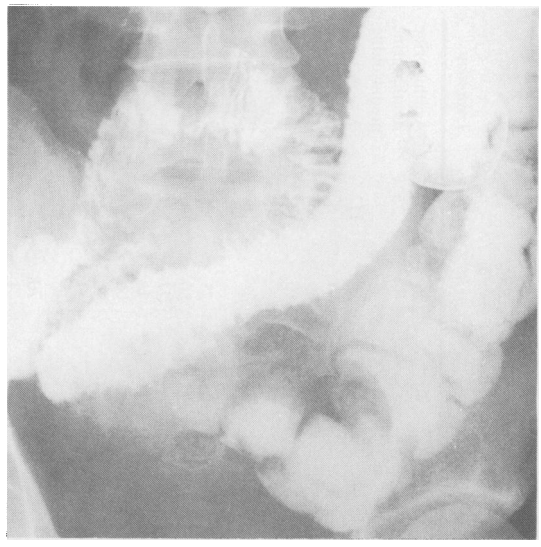

Fig. 3 *Barium follow-through in a patient with a recurrence of Crohn's disease proximal to an ileotransverse anastomosis.*

all patients with moderate or severe inflammatory bowel disease. Table 2 summarises the relation between disease activity, scan positivity, and agreement between scan and radiology with regard to disease localisation and extent. Small bowel disease alone or large bowel disease alone were correctly localised on scan by their respective central or peripheral distribution of activity. In the three patients with both small and large bowel disease, irrespective of disease activity only one or other site (two small bowel, one large bowel) was localised. In scans where bowel loops were clearly outlined (four

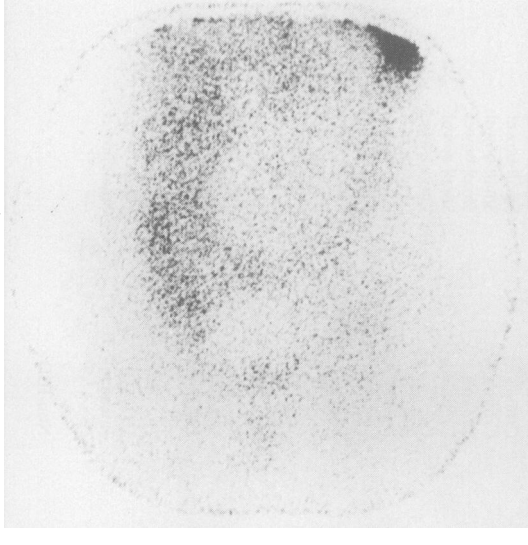

Fig. 2 *Early abdominal scan from a patient with Crohn's colitis.*

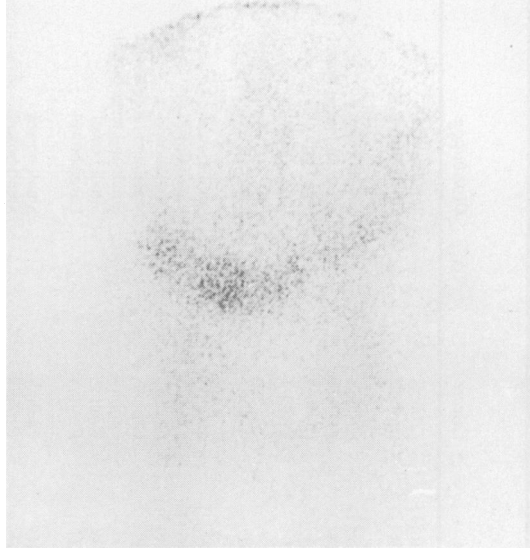

Fig. 4 *Early abdominal scan from patient in Fig. 3.*

Fig. 5 *Late abdominal scan from patient in Fig. 3.*

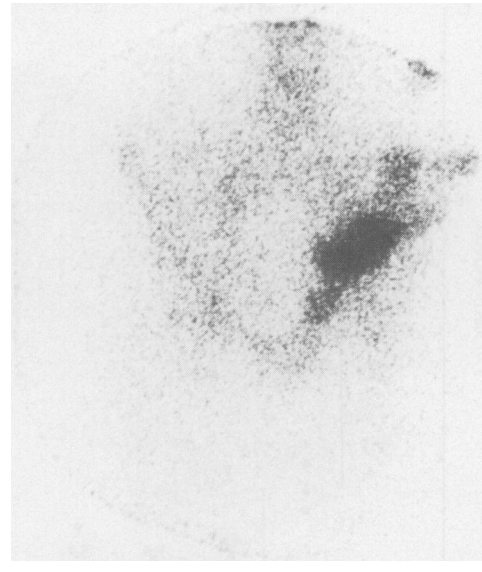

Fig. 6 *Early abdominal scan in a patient with Crohn's enterocutaneous fistula and abscess.*

small bowel and 10 large bowel) there was good agreement with disease extent assessed by standard radiology.

FAECAL EXCRETION

Four day faecal white cell excretion of ^{111}In was low in patients with irritable bowel syndrome (0·1–1·0% dose, men 0·46±SD 0·3) and organic non-inflammatory bowel disease (0·1–1·6, mean 0·5±SD 0·4). In the patients with inflammatory bowel disease, white cell excretion was raised compared with control groups and increased progressively with disease severity (mild 0·2–3·0, mean 1·2±SD 0·98; moderate 0·5–11·0, mean 5·2±SD 2·8; or severe 3·6–13·1, mean 8·8±SD 2·1) (Fig. 7). Seventy-five per cent (±SD 12·3) of ^{111}In activity was found to reside in the particulate fraction of faeces. There was good correlation between faecal white cell excretion and serum C-reactive protein ($r=0·74$, $p<0·001$), an objective laboratory parameter of disease activity.[12] In patients with Crohn's disease an excellent correlation between white cell excretion and CDAI ($r=0·78$, $p<0·001$) was found.

Discussion

This study demonstrates that ^{111}In labelled leucocytes can be used to obtain gamma camera images of inflamed bowel in a wide variety of inflammatory bowel diseases. False positive scans were not observed in the irritable bowel syndrome or bowel malignancy. Determination of faecal excretion of ^{111}In provided an objective assessment of disease severity and correlated with both clinical and

Table 2 *Scan result and comparison with radiology and colonoscopy*

Disease category	*Scan result*		*Disease localisation*			*Disease extent*		
	+ve	*−ve*	*Small bowel*	*Small and large bowel**	*Large bowel*	*Small bowel*	*Small and large bowel**	*Large bowel*
Severe	11	0	5/5	0/1	5/5	3/5	0/1	5/5
Moderate	14	0	7/7	0/2	5/5	1/7	0/2	3/5
Mild	0	10	—	—	—	—	—	—
Total	25	10	12/12	0/3	10/10	4/12	0/3	8/10

* Either small or large bowel localised, but not both.

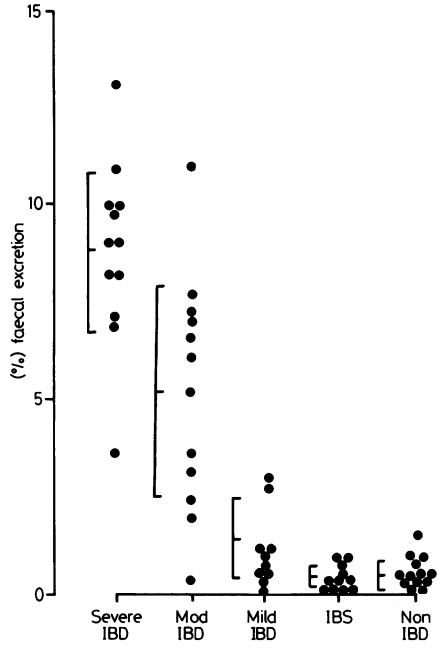

Fig. 7 *Faecal ^{111}In excretion expressed as percentage of injected dose in inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and non-inflammatory bowel disease (non-IBD). Bars represent mean ± SD.*

biochemical indices of activity.

^{111}In leucocyte scanning is now established in routine use in many medical centres. Its advantages are that it is not invasive, not uncomfortable for the patient, and no bowel preparation is needed; it is thus safe in the acutely sick patient where conventional methods of radiological imaging may be hazardous. Inflamed bowel or a complicating abscess can be localised rapidly within three to five hours of injecting the labelled cells.

Although by gamma scanning the localisation of disease to small or large bowel was usually clear cut, the correlation between the extent of disease on scan and extent of radiological abnormality was different in small and large bowel disease. In colonic disease the agreement with extent was excellent, while in small bowel involvement the image was frequently not clear enough for accurate assessment of the length of involved bowel. The poor quality of the small bowel image is due to a combination of factors which include background blood pool and bone marrow activity, and bowel movement during the scanning time. This latter movement appears to be the most important factor, as in the four studies performed in patients with previous surgery (where the bowel was thought to be relatively fixed) small bowel loops could be clearly visualised.

The current technique of ^{111}In leucocyte scanning is probably superior to the alternative techniques of imaging inflamed bowel using Gallium-67 citrate.[13–15] Gallium-67 is normally excreted into the bowel leading to false positive bowel images in normal subjects.[16–17] Furthermore, Gallium-67 scanning has been shown to have of limited value in localising inflamed bowel in Crohn's disease.[14 18]

The rapid excretion of ^{111}In into the bowel allows accurate quantification of faecal excretion. ^{111}In binds avidly to subcellular constituents,[19] so faecal activity represents ^{111}In within leucocytes or attached to stable cell constituents and this is supported by demonstrating most of the activity in the particulate fraction. The faecal excretion expressed as a percentage of the injected dose probably underestimates the leucocyte influx into the bowel, as the original cell population using the current technique is not a pure neutrophil preparation. Results with pure neutrophil leucocytes show higher percentage faecal excretions (unpublished observations).

Faecal excretion of ^{111}In increased progressively with disease activity either assessed on a clinical grading system or biochemically. Furthermore, in the subgroup of Crohn's disease patients, there was a strong correlation between activity assessed by the Crohn's Disease Activity Index and faecal excretion. All patients with mild Crohn's disease, however, had negative scans. The magnitude of the faecal excretion was useful diagnostically, as no patient with the irritable bowel syndrome was found to have a faecal excretion of ^{111}In greater than 1%.

^{111}In leucocyte scanning provides a novel approach to the problem of imaging and assessment of inflammatory bowel disease. Quantitative faecal excretion of ^{111}In provides an objective assessment of disease activity which should prove useful in evaluating treatment regimes. Future studies with pure leucocyte populations may provide valuable information about white cell kinetics in inflammatory bowel disease.

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