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Technetium-99m hexamethylpropylene amine oxime labelled leucocyte scintigraphy in ulcerative colitis and Crohn's disease

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Abstract. Technetium-99m hexamethylpropylene amine oxime labelled leucocyte scintigraphy (LS) was performed on 45 occasions in 30 patients with ulcerative colitis and on 53 occasions in 34 patients with Crohn's disease. Serial images were taken following re-injection of the labelled leucocytes. The segmental extent of the inflammation and the grade of the leucocyte uptake were calculated, and compared with the laboratory results and colonoscopy findings. The sensitivity and specificity of LS proved higher in ulcerative colitis (87% and 93%) than in Crohn's disease (53% and 89% in cases with large intestine involvement, and 82% and 100% in cases with small intestine involvement). The activity of the process determined by LS correlates with the α_2-globulin level ($r=0.47$), fibrinogen level ($r=0.50$), fS iron level ($r=-0.57$), sedimentation ($r=0.44$), leucocyte count ($r=0.38$), platelet count ($r=0.34$) and Best index ($r=0.31$) in ulcerative colitis, but not in Crohn's disease.

Key words: Inflammatory bowel disease – Crohn's disease – Ulcerative colitis – Technetium-99m hexamethylpropylene amine oxime labelled leucocyte scintigraphy

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Introduction

The diagnosis of inflammatory bowel diseases (IBD) is based on endoscopy and histology. In the follow-up of IBD patients, knowledge of the current clinical activity and the extent of the involved bowel segments is necessary. The clinical activity can be determined on the basis of laboratory parameters (acute phase protein level, erythrocyte sedimentation rate, and fS iron level) or the Best index [1], the calculation of which is based mainly on the symptoms. The extent of the inflammatory process can be investigated by means of colonoscopy, X-ray examination and labelled leucocyte scintigraphy (LS) [2]. Technetium-99m hexamethylpropylene amine oxime (^{99m}Tc-HMPAO) is a frequently used agent for in vitro leucocyte labelling [3]. In the assessment of inflammatory activity, the grade of leucocyte accumulation was compared with the laboratory parameters and activity indexes [4] and the histological findings [5].

The aim of this study was to establish the value of ^{99m}Tc-HMPAO LS in ulcerative colitis and Crohn's disease.

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Materials and methods

Forty-five investigations were carried out in 30 patients with ulcerative colitis (12 males, 18 females, aged 17–69 years, mean: 40.4 years; history of disease 0–26 years, mean: 6.3 years), as were 53 investigations in 34 patients with Crohn's disease (11 males, 23 females, aged 20–71 years, mean: 36.9 years; history of disease 0–29 years, mean: 7.2 years). The diagnoses had previously been proven by histology or surgery in all patients. In some cases, repeat studies were performed when the patient's clinical condition had altered. The average interval between studies was 14 months (2–23 months).

In vitro leucocyte labelling was achieved by a routine method [6], with some modifications. 60 ml of venous blood was collected in a syringe containing 6 ml of 3.8% sodium citrate and 12 ml of 6% dextran solution. After spontaneous sedimentation of the erythrocytes, the supernatant was centrifuged at $100 \times g$ for 5 min, and the mixed leucocyte pellet was collected.

^{99m}Tc-HMPAO was formed by adding 1800–2500 MBq ^{99m}Tc in 5 ml to HMPAO (Brain-SPECT, OSSKI, Budapest). The mixed leucocytes were resuspended in 1 ml of ^{99m}Tc-HMPAO (360–500 MBq). After incubation for 10 min with careful shaking at room temperature, the unbound ^{99m}Tc-HMPAO was removed by centrifugation ($100 \times g$, 5 min). The lipophilic ^{99m}Tc-HMPAO complex yield was measured by means of solvent extraction [7]. The viability of the labelled cells was checked with the trypan blue incorporation test.

Images were taken in the anterior view 30 min and 2 and 4 h (Figs. 1, 2) following re-injection of the labelled leucocytes. Only a leucocyte uptake visualized on at least two of the three images was interpreted as pathological. The extent of the inflammatory process indicated by LS was localized in five segments: the small

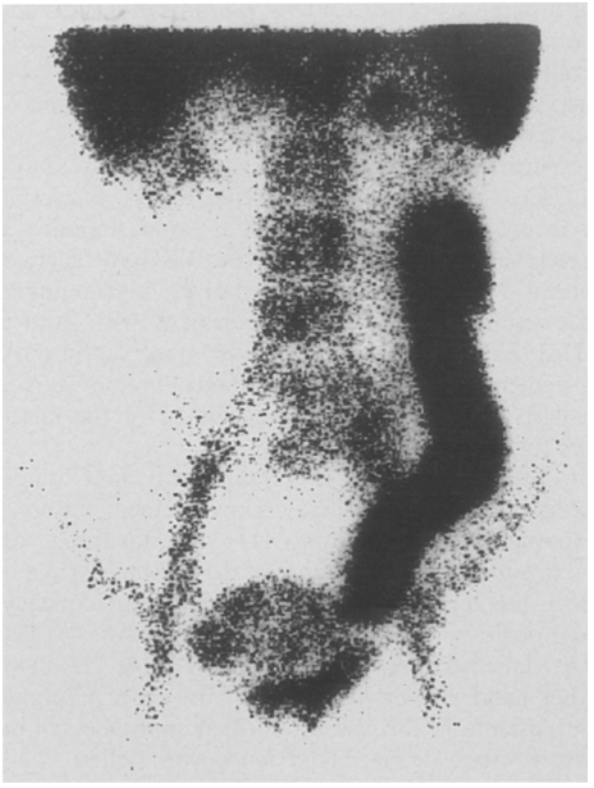

Fig. 1. Four-hour leucocyte scan of a 35-year-old women with ulcerative colitis. Intense leucocyte accumulation in the descending colon and rectosigmoid

intestine, the ascending, transverse and descending colon, and the rectosigmoid. For purposes of comparison, colonoscopy or histology was used in 146 large bowel segments and serial X-ray examinations in small intestine segments. Segments with a histological diagnosis of medium or serious chronic inflammation, abscess, granuloma or ulcer were classified as involved. All the reference investigations and the LS examinations were carried out within 2 weeks.

The uptake of each segment was scored in comparison with the normal bone marrow uptake (0: no uptake; 1: <bone marrow uptake; 2: =bone marrow uptake; 3: >bone marrow uptake). To characterize the grade of the overall inflammatory process, the scintigraphic activity (SA) was calulated by summing the bowel segment scores. The SA values were compared with the laboratory findings (α_2-globulin, fibrinogen, fS iron level, erythrocyte sedimentation rate, and leucocyte and platelet counts) and the Best index.

For a statistical appraisal of the results, the Spearman rank correlation test was used.

Results

Cell labelling

The average lipophilic complex yield of ^{99m}Tc-HMPAO was found to be 93% (range 89%–96%). The mean labelling efficacy was 57% (range 38%–77%). The separation and labelling procedure caused no change in the viability of the leucocytes, which was 98% on average (95%–100%).

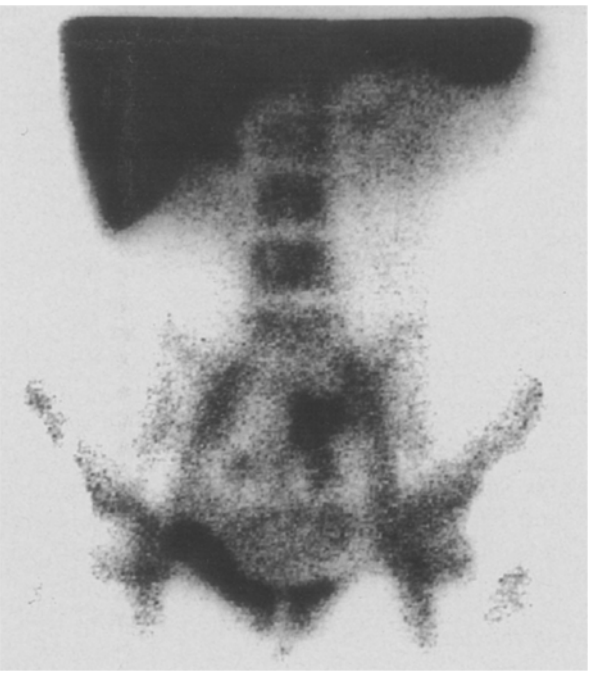

Fig. 2. Four-hour leucocyte scan of a 36-year-old women with Crohn's disease. Circumscribed leucocyte uptake in the abdomen, corresponding to an abscess

Table 1. Comparison of scintigraphic results in determination of segmental extent of inflammation with histological or X-ray findings

	LS findings			
	True-positive	True-negative	False-positive	False-negative
Ulcerative colitis	47	29	2	7
Crohn's disease (large intestine)	17	26	3	15
Corhn's disease (small intestine)	14	5	0	3
Total	78	60	5	25

Extent of the disease

Of the 168 examined segments, 103 were found to be active on colonoscopy, biopsy or X-ray investigation. Scintigraphy proved positive in 78 of the active segments, and false-negative in 25 segments. Of the inactive segments, 60 were normal scintigraphically, and five proved false-positive. The sensitivity, specificity and accuracy in the whole group were 76%, 92% and 82%, respectively. Our results (Table 1) suggested that LS has different values in ulcerative colitis and Crohn's disease. The sensitivity of LS was higher in ulcerative colitis (87%) than in Crohn's disease (53% in the large bowel and 82% in the small intestine). The specificity was

Table 2. Correlation of scintigraphic activity and laboratory and clinical findings (Spearman rank correlation)

	Ulcerative colitis	Crohn's disease
Best index	$r=0.31$ ($P<0.050$, $n=45$)	NS
α_2-Globulin	$r=0.47$ ($P<0.005$, $n=39$)	NS
Fibrinogen	$r=0.50$ ($P<0.002$, $n=43$)	NS
fS iron level	$r=-0.57$ ($P<0.0005$, $n=40$)	NS
Sedimentation	$r=0.44$ ($P<0.005$, $n=44$)	NS
Leucoyte count	$r=0.38$ ($P<0.02$, $n=43$)	NS
Platelet count	$r=0.34$ ($P<0.05$, $n=36$)	NS

NS, Not significant

found to be similar in the two diseases (93% in ulcerative colitis, and 89% and 100% in Crohn's disease, corresponding to the large bowel and the small intestine).

Activity of the disease

The correlations between the SA values and the Best index and the laboratory parameters of the inflammation in ulcerative colitis proved to be significant. However, a significant correlation was not found in Crohn's disease patients, regardless of whether the small intestine or the large bowel was involved (Table 2).

Discussion

In vitro labelled LS is a very useful method for the localization of various inflammatory processes [8]. The majority of the published studies have revealed the great importance of this method in patients with IBD [9]. Indium-111 labelled lipophilic agents were initially used for in vitro leucocyte labelling [10]. Nowadays, despite the advantages of ^{111}In, ^{99m}Tc-labelled HMPAO is most frequently used [3] because of its better radiopharmaceutical characteristics, cost and availability.

The extent and activity of the disease are probably the most important factors that influence the management of IBD. For large bowel diseases, colonoscopy with multiple biopsies is regarded as an accurate method for determination of the extent and activity of the inflammatory process. As for the small intestine, in the absence of routine enteroscopy, serial X-ray examination or enteroclysis is applied to characterize the extent of the disease [11].

Patients with severe disease should generally undergo only limited examinations, and various indices and laboratory parameters have therefore been suggested for determination of the activity of the inflammation [12].

Previous studies demonstrated the usefulness of LS in IBD, but without comparing the findings in Crohn's disease and ulcerative colitis. Separate analysis of the two diseases appears reasonable because of some pathological differences between them: Crohn's disease is described mainly as a lymphocyte infiltration, and ulcerative colitis as a granulocyte predominance within the mucosa. We have calculated the sensitivity and the specificity in each disease.

We performed LS in patients with previously diagnosed IBD. Colonoscopy with multiple biopsy and serial X-ray investigations were used as a "gold standard". The reference investigations and the LS were carried out within 2 weeks. We found a relatively high number of false-negative segments, in agreement with Almer et al. [13]. False-positive findings were observed in only a few segments. We suggest that the intraluminar movement of the labelled leucocytes accounts for the false-positive findings.

Our results revealed a poor sensitivity in Crohn's disease patients with large bowel involvement, in contrast to the ^{111}In LS findings [14]. An advantage of ^{111}In oxine is the high resolution of the late images available with this technique. Because of the high frequency of false-positive findings, late images with ^{99m}Tc-HMPAO-labelled leucocytes are not feasible [15]. On the other hand a disadvantage of ^{111}In LS in Crohn's disease patients is the use of purified granulocytes in contrast to the use of mixed leucocytes. Allen et al. found that ^{99m}Tc-HMPAO is the agent of choice in detecting active IBD, with optimal resolution of the 1- to 3-h images after re-injectin [16].

In cases of small intestine involvement, our results proved better than those of Crama-Bobouth et al. [17]. The difference originated mainly from the different X-ray methods.

Our results indicate a higher sensitivity and specificity of the method in the determination of the extent of the inflammation in ulcerative colitis than in Crohn's disease. The different histological character of the two diseases (mainly lymphocyte infiltration in Crohn's disease, and granulocyte predominance within the mucosa in ulcerative colitis) may account for this.

Comparison of the scintigraphic findings with the laboratory parameters and the Best index revealed marked differences in ulcerative colitis and Crohn's disease. The activity determined by scintigraphy correlated weakly with the laboratory parameters and the Best index in ulcerative colitis. Unlike Schölmerich et al. [4], we found no correlation with the activity index in cases of Crohn's disease.

In conclusion, the accuracy of ^{99m}Tc-HMPAO-labelled LS differs in ulcerative colitis and Crohn's disease. We suggest that the different characters of these diseases account for this.

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