European Journal of
Nuclear
Medicine

Original article

Technetium-99m hexamethyl propylene amine oxine leucocytes in the assessment of disease activity in inflammatory bowel disease

Eila Lantto[1], Kari Järvi[2], Ilkka Krekelä[3], Tuomo Lantto[4], Matti Taavitsainen[5], Heikki Vedenkangas[3], and Martti Vorne[4]

Departments of [1] Radiology, [2] Pathology, [3] Internal Medicine and [4] Nuclear Medicine, Päijät-Häme Central Hospital, Keskussairaalankatu 7, SF-15850 Lahti, and [5] Department of Diagnostic Radiology, Helsinki University Central Hospital, Helsinki, Finland

Received 16 May 1991 and in revised form 6 August 1991

Abstract. The inflammatory activity in 108 bowel segments of 40 patients with suspected or known inflammatory bowel disease was assessed macroscopically by endoscopy, histology and technetium-99m hexamethyl propylene amine oxine (^{99m}Tc-HMPAO) leucocytes using a numerical grading system (scores 0–3). A 4-h series of scintigrams showed a significant correlation with both histological and macroscopical assessment of disease activity (rho = 0.850, $P < 0.001$ and rho = 0.773, $P < 0.001$, respectively). Sensitivity, specificity and accuracy of scintigraphy in detecting active inflammatory segments were 85%, 92% and 89%, respectively. A normal scintigram did not completely exclude mild inflammatory activity, especially in the rectosigmoid area. ^{99m}Tc-HMPAO leucocytes offer an accurate and non-invasive alternative for the assessment of disease activity in ulcerative colitis and Crohn's disease.

Key words: Inflammatory bowel disease – Crohn's disease – Ulcerative colitis – technetium-99m hexamethyl propylene amine oxine (^{99m}Tc-HMPAO) leucocytes – Inflammatory activity

Eur J Nucl Med (1992) 19:14–18

Introduction

Several methods have been used for the evaluation of inflammatory activity and extent in ulcerative colitis and Crohn's disease, including clinical indices, laboratory tests, radiology, endoscopy both with and without mucosal biopsies and radiolabelled autologous leucocytes (Camilleri and Proano 1989). A recent innovation is scanning with technetium-99m hexamethyl propylene amine oxine (HMPAO) leucocytes. Results of previous studies have shown a significant correlation between scanning with ^{99m}Tc-HMPAO leucocytes and radiology, endoscopy or histology in the assessment of disease extent (Schuemichen and Schoelmerich 1987; Schölmerich et al. 1988). In the assessment of inflammatory activity ^{99m}Tc-HMPAO leucocytes have been compared with clinical indices (Crohn's disease activity index, CDAI, and van Hees index) or laboratory measurements (erythrocyte sedimentation rate, ESR, and serum C-reactive protein, CRP), and only a weak correlation has been found between the results of the leucocyte scan and other activity indices (Schuemichen and Schoelmerich 1987; Schölmerich et al. 1988). However, ^{99m}Tc-HMPAO leucocytes have not been compared in the assessment of inflammatory activity with colonoscopy and histological assessment of mucosal biopsy specimens, which are regarded as the golden standard.

The aim of the present study was to evaluate the worth of the ^{99m}Tc-HMPAO leucocyte scan in the assessment of disease activity in inflammatory bowel disease when histology is used as a reference method.

Offprint requests to: E. Lantto

Material and methods

A total of 40 consecutive patients with suspected or known inflammatory bowel disease (32 men, 8 women, aged 19–75 years) were prospectively studied by endoscopy with mucosal biopsy specimens and by ^{99m}Tc-HMPAO leucocytes. In 29 patients ulcerative colitis and in 5 patients Crohn's disease were diagnosed 0–35 years before the study by clinical, endoscopical and histological criteria. In 6 patients inflammatory bowel disease was excluded. Some 29 patients were receiving sulphasalazine, steroids and/or 5-aminosalicylic acid as treatment at the time of the study. The study was accepted by the Hospital Ethics Committee.

Mixed leucocytes were isolated and labelled as described previously (Vorne et al. 1989). A 40-ml sample of venous blood was taken up in a 60-ml plastic syringe containing 10 ml acid citrate dextrose and 10 ml 6% hydroxyethyl starch. After sedimentation

© Springer-Verlag 1992

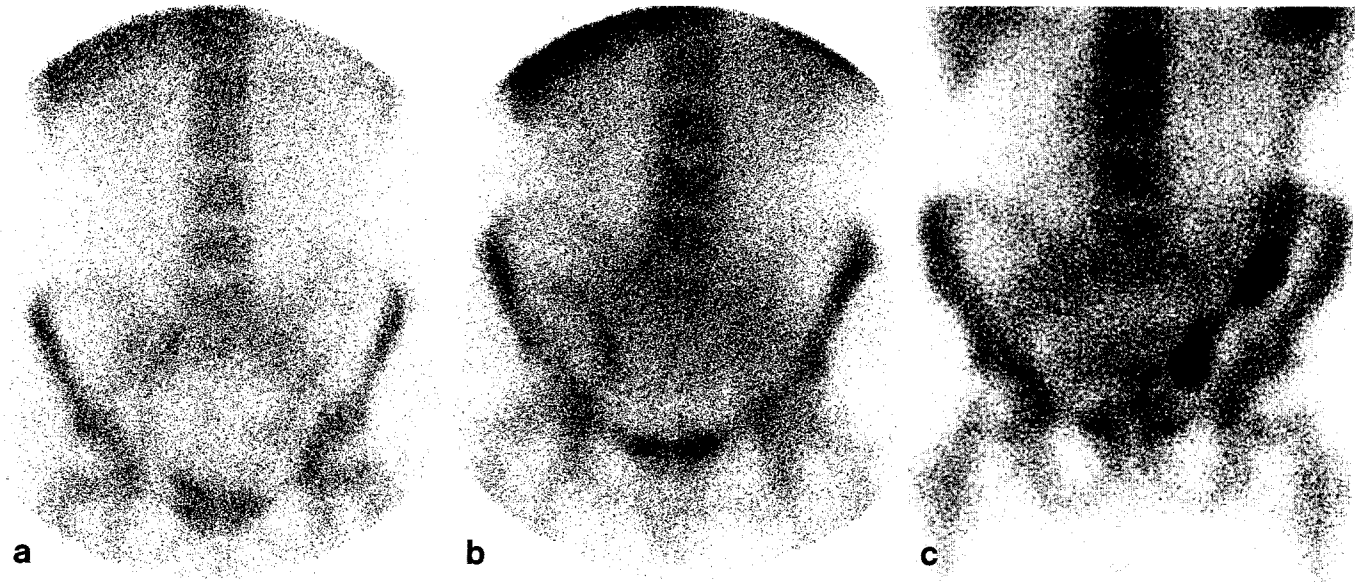

Fig. 1a–c. The 4-h scans of three patients imaged with technetium-99m hexamethyl propylene amine oxine (^{99m}Tc-HMPAO) leucocytes. Mild inflammatory activity (grade 1) is seen in the descending and rectosigmoid segments of a patient with ulcerative colitis (**a**), moderate activity (grade 2) in the terminal ileum of a patient with Crohn's disease (**b**) and intense activity (grade 3) in the descending and rectosigmoid segments of a patient with ulcerative colitis (**c**)

for 1 h at room temperature, the supernatant was centrifuged in sterile tubes at $100 \times g$ for 5 min. The platelet-rich supernatant was isolated and centrifuged at $2000 \times g$ for 5 min to obtain cell-free plasma. Leucocytes were suspended into 1 ml of cell-free plasma. ^{99m}Tc-HMPAO was formed by adding 600 MBq ^{99m}Tc in 6 ml isotonic saline to a vial containing HMPAO (Ceretec, Amersham Int.). Next, 5 ml (500 MBq) of ^{99m}Tc-HMPAO complex was added to the leucocyte suspension, which was left for 10 min at room temperature. The suspension was centrifuged at $100 \times g$ for 5 min, resuspended in 5 ml of cell-free plasma and reinjected intravenously. The average cell labelling efficiency was 41% (range 20%–86%), and the mean dose injected was 185 MBq (range 100–300 MBq).

Images were obtained at 2 min, 0.5, 2 and 4 h after the injection of labelled leucocytes with a large field-of-view gamma-camera in anterior abdominal projection. Posterior abdominal, right and left anterior oblique and pelvic outlet views were often used to localize the tracer uptake. Scintigraphy was performed within 3 weeks (mean 6 days) of the endoscopy.

The scintigrams were independently evaluated by two nuclear medicine physicians without knowledge of the clinical history or endoscopic and histological findings. The bowel was divided into five segments (rectosigmoid, descending, transverse and ascending colon and terminal ileum), and the inflammatory activity for each segment was graded in the whole series of images by comparing the abnormal uptake in the bowel with vertebral bone marrow and liver (grade 0 = no abnormal activity; grade 1 = abnormal activity with an intensity less than vertebral bone marrow; grade 2 = more than bone marrow, less than liver; grade 3 = more or equal to liver; Fig. 1). The gradings of 2-h and 4-h images were taken for the final statistical analysis of the inflammatory activity of bowel segments. Most readings were equal, and those with some discrepancy were re-evaluated together, and a consensus of the readings was made.

A total of 18 colonoscopies and 22 sigmoidoscopies with mucosal biopsies were performed by two endoscopists. Altogether, 108 bowel segments were studied, and only these segments were taken to the final statistical analysis. The inflammatory activity in the same regions was assessed macroscopically at endoscopy by a standard grading system (grade 0 = normal appearance; grade 1 = loss of vessel pattern and oedema; grade 2 = contact haemorrhage; grade 3 = ulceration or surface mucopus).

Multiple mucosal biopsy specimens (at least 2–4) were taken from all segments studied. The specimens were blindly examined by the same pathologist, and the inflammatory activity was scored by using a grading system (grade 0 = no inflammatory activity; grade 1 = slight inflammatory infiltrate which includes polymorphonuclear leucocytes; grade 2 = moderate inflammatory activity, polymorphonuclear leucocytes infiltrate epithelium; grade 3 = marked inflammatory activity, epithelial destruction and/or crypt abscesses).

ESR and CRP were used as laboratory measurements in the assessment of inflammatory activity.

Spearman's rank correlation was used for statistical analysis of results.

Results

Inflammatory activity determined by ^{99m}Tc-HMPAO leucocytes showed a significant correlation with both histological and endoscopic assessment of disease activity (rho = 0.850, $n = 108$, $P < 0.001$, and rho = 0.773, $n = 108$, $P < 0.001$, respectively). Also histological and macroscopical assessment of disease activity at endoscopy showed a significant correlation (rho = 0.865, $n = 108$, $P < 0.001$). Results of scintigraphy correlated with CRP (rho = 0.400, $n = 108$, $P < 0.001$), but no correlation was found between scintigraphy and ESR (rho = 0.143, $n = 108$, NS).

Active inflammatory bowel disease was seen histologically in 55 of 108 segments studied. The corresponding

Table 1. Corresponding grades of inflammatory activities assessed by labelled leucocyte scanning and histology

		Leucocyte scan				
	Grade	0	1	2	3	Total
Histology	0	49	3	1	0	53
	1	5	9	6	4	24
	2	3	3	8	5	19
	3	0	0	1	11	12
Total		57	15	16	20	108

Table 2. Corresponding grades of inflammatory activities assessed by labelled leucocyte scanning and macroscopically at endoscopy

		Leucocyte scan				
	Grade	0	1	2	3	Total
Macroscopy	0	48	4	4	1	57
	1	6	6	0	5	17
	2	0	4	7	0	11
	3	3	1	5	14	23
Total		57	15	16	20	108

Table 3. Relationship between the inflammatory activity determined by histological grading and imaging time at which the leucocyte scan became positive

Imaging time	Inflammatory activity by histological grading				
	0	1	2	3	Total
2 min	2	10	11	10	33
0.5 h	1	1	2	2	6
2 h	0	5	2	0	7
4 h	1	3	1	0	5
False negative	–	5	3	0	8
Total	4	24	19	12	59

activity scores by scanning and histology and by scanning and endoscopy are presented in Tables 1 and 2. The relationship between histological grades of inflammatory activity and the imaging time at which the scintigrams became positive are seen in Table 3.

False-positive tracer activity was seen in 4 segments from 3 patients. In 1 patient the leucocyte scan showed grade 1 activity and endoscopy grade 2 activity, but histological biopsy specimens revealed no inflammatory activity. One patient evidenced grade 1 inflammatory activity in two segments and 1 patient grade 2 inflammatory activity in one segment as assessed by scintigraphy, but both endoscopy and histology results were negative. In 2 segments the false-positive activity was seen after 2 min, in 1 segment after 0.5 h and in 1 segment at 4 h. All three patients had at least 2 affected bowel segments with true-positive scintigrams, so the extent of inflammatory active disease was overestimated by the leucocyte scan in these cases. False-negative scintigrams were seen in 8 segments with grade 1 or 2 activity as assessed by histology; 6 of them were in the rectosigmoid region.

The sensitivity, specificity and accuracy of the 4-h series of scintigrams in detecting inflammatory active segments were 85%, 92% and 89%, respectively. The positive predictive value of the leucocyte scan was 90% and negative predictive value 86%.

When the inflammatory activity was assessed by the same grading system in the 2-h series of images, sensitivity, specificity and accuracy were 77%, 91% and 85%, respectively. Inflammatory activity determined on 2-h images also showed a significant correlation with both the histological and endoscopic assessment of disease activity (rho = 0.847, $n = 108$, $P < 0.001$, and rho = 0.772, $n = 108$, $P < 0.001$, respectively).

Discussion

The assessment of disease activity and extent in ulcerative colitis and Crohn's disease is important for the rational choice of therapy and in the evaluation of the effect of drug therapy. Colonoscopy with multiple biopsies is regarded as the most accurate method for this assessment (Gomes et al. 1986; Saverymuttu et al. 1986). However, it cannot be performed in every patient because of discomfort or if the patient is suffering a severe attack or displays features suggestive of toxic megacolon (Camilleri and Proano 1989).

Radiolabelled autologous leucocytes offer a non-invasive alternative for the assessment of disease extent and activity. Recently, autologous leucocytes labelled with ^{99m}Tc-HMPAO were used for imaging intestinal inflammation with promising results (Schölmerich et al. 1988; Allan et al. 1990; Li et al. 1990). The present study revealed a significant correlation between labelled leucocyte scanning and histology or endoscopy in the assessment of disease activity. Scintigraphy could accurately detect segments with inflammatory activity as judged by histological assessment of mucosal biopsy specimens. All patients with marked inflammation (grade 3) on histological study had true-positive scintigrams. However, a normal scintigram did not completely exclude mild inflammatory activity, especially in the rectosigmoid segment in which uptake could be obscured by urinary bladder activity. This disadvantage could be avoided by emptying the bladder before imaging and by pelvic outlet views. In some patients with histologically active inflammatory bowel disease, the leucocyte scan overestimated the disease extent by showing more inflammatory active segments than histology did. This may be due to intestinal shifting of tracer activity from

the proximal segments of inflamed bowel if it appears on later images (4 h). In 3 segments with false-positive scintigrams, the activity became visible not later than 0.5 h after reinjection of labelled cells.

An excellent correlation between ^{99m}Tc-HMPAO leucocytes and barium enema or endoscopy was found by Schölmerich et al. (1988) in the assessment of disease extent in 29 patients with Crohn's disease. Activity as determined by leucocyte scan was, however, only weakly correlated with CDAI and van Hees index. These clinical indices are widely used but rely heavily on subjective factors, such as general well-being, and are cumbersome, since patients must maintain a diary of symptoms (Camilleri and Proano 1989).

Laboratory measurements such as ESR and CRP are useful in confirming the clinical assessment of disease activity and in monitoring the response to therapy (Camilleri and Proano 1989). Changes in these parameters are, however, not specific for bowel inflammation. In the present study activity determined on the leucocyte scan showed a correlation with CRP, but no correlation was found with ESR. Our result is somewhat contradictory to previous reports of Schuemichen and Schoelmerich (1987) and Schölmerich et al. (1988), who found a weak correlation with ESR but no correlation with CRP.

Previously, autologous leucocytes labelled with indium-111 (^{111}In-WBC) were used in the assessment of inflammatory bowel disease extent and activity (Saverymuttu et al. 1982, 1986; Fotherby et al. 1986). A comparison of ^{111}In-WBC scanning with colonoscopy and histology results revealed excellent correlations for the extent and activity of inflammatory bowel disease (Saverymuttu et al. 1986). ^{99m}Tc has many advantages over ^{111}In, including lower radiation dose, better image quality and greater convenience in performance. Because the results of the present and previous studies are comparable with those of ^{111}In-WBC scanning (Schuemichen and Schoelmerich 1987; Schölmerich et al. 1988; Allan et al. 1990; Li et al. 1990) ^{99m}Tc-HMPAO leucocyte scanning should be regarded as a preferable scintigraphic method in the assessment of inflammatory bowel disease. Severity graded by ^{111}In-WBC also showed a close correlation with fecal ^{111}In-granulocyte excretion (Saverymuttu et al. 1986). Because of the short half-life of ^{99m}Tc and the non-specific intestinal excretion of ^{99m}Tc-HMPAO, this method unlike one using ^{111}In-WBC cannot be employed in the assessment of fecal excretion of labelled leucocytes as an index of inflammatory activity. However, stool collection for 96 h in the assessment of inflammatory activity may be inconvenient.

The exact identification of bowel segments affected may be difficult with labelled leucocytes, which is the main disadvantage when compared with radiological techniques, but the severity and extent of inflammatory bowel disease are often underestimated with radiological techniques (Gabrielson et al. 1979).

Intestinal excretion of the tracer has been regarded as a major disadvantage of ^{99m}Tc-HMPAO leucocytes in imaging abdominal inflammations (Mountford et al. 1990). However, non-specific bowel accumulation is also seen with ^{111}In-WBC (Syrjälä et al. 1987). This non-specific bowel accumulation with ^{99m}Tc-HMPAO leucocytes appears 4 h after re-injection, mainly in the caecum and ascending colon (Becker et al. 1988b). In the present study we used sequential imaging between 2 min and 4 h after re-injection to avoid misinterpretations in the evaluation of scintigrams and achieved a specificity of 92%. In most patients scintigrams became positive by not later than 0.5 h, but if later images (2 h and/or 4 h) had not been used, some patients with mild or moderate inflammatory activity could have had false-negative results. By sequential imaging, shifting intraluminal intestinal activity can be distinguished from true inflammatory activity in the intestinal wall. Images after 4 h are not useful because of the non-specific bowel activity (Becker et al. 1988a).

In conclusion, ^{99m}Tc-HMPAO leucocyte scanning can accurately assess the disease activity in ulcerative colitis and Crohn's disease. It can be used as a safe and non-invasive alternative for the assessment of the severity of inflammatory bowel disease. However, a normal scintigram does not exclude mild colonic inflammation.

References

Allan RA, Sladen GE, Bassingham S, Lazarus C, Clarke SEM, Fogelman I (1990) Comparison of simultaneous ^{99}Tc^{m} HMPAO- and ^{111}In-labelled white cell scans in assessment of inflammatory bowel disease. Nucl Med Commun 12:890

Becker W, Fischbach W, Weppler M, Mosl B, Jacoby G, Börner W (1988a) Radiolabelled granulocytes in inflammatory bowel disease: diagnostic possibilities and clinical indications. Nucl Med Commun 9:693–701

Becker W, Schoman E, Fischbach W, Börner W, Gruner KR (1988b) Comparison of ^{99}Tc^{m}-HMPAO and ^{111}In-oxine labelled granulocytes in man: first clinical results. Nucl Med Commun 9:435–447

Camilleri M, Proano M (1989) Advances in the assessment of disease activity in inflammatory bowel disease. Mayo Clin Proc 64:800–807

Fotherby KJ, Wraight EP, Garforth H, Hunter JO (1986) Indium-111 leucocyte scintigraphy in the investigation and management of inflammatory bowel disease. Postgrad Med J 62:457–462

Gabrielson N, Granqvist S, Sundelin P, Thorgeirsson T (1979) Extent of inflammatory lesions in ulcerative colitis assessed by radiology, colonoscopy, and endoscopic biopsies. Gastrointest Radiol 4:395–400

Gomes P, duBoulas C, Smith CL, Holdstock G (1986) Relationship between disease activity indices and colonoscopic findings in patients with colonic inflammatory bowel disease. Gut 27:92–95

Li DJ, Middleton SJ, Wright EP (1990) ^{99}Tc^{m}-HMPAO-labelled leukocyte scintigraphy in inflammatory bowel disease. Nucl Med Commun 12:889

Mountford PJ, Kettle AG, O'Doherty MJ, Coakey AJ (1990) Comparison of technetium-99m-HMPAO leukocytes with indium-

111-oxine leukocytes for localizing intraabdominal sepsis. J Nucl Med 31:311–315

Saverymuttu SH, Peters AM, Hodgson HJ, Chadwick VS, Lavender JP (1982) Indium-111 autologous leucocyte scanning: comparison with radiology for imaging the colon in inflammatory bowel disease. Br Med J 285:255–257

Saverymuttu SH, Camilleri M, Rees H, Lavender JP, Hodgson HJF, Chadwick VS (1986) Indium 111-granulocyte scanning in the assessment of disease extent and disease activity in inflammatory bowel disease. A comparison with colonoscopy, histology, and fecal indium 111-granulocyte excretion. Gastroenterology 90:1121–1128

Schölmerich J, Schmidt E, Schumichen C, Billman P, Schmidt H, Gerok W (1988) Scintigraphic assessment of bowel involvement and disease activity in Crohn's disease using technetium 99m-hexamethyl propylene amine oxine as leukocyte label. Gastroenterology 95:1287–1293

Schuemichen C, Schoelmerich J (1987) Specificity of Tc-99m HM-PAO labelled leukocyte uptake in the large bowel as an indicator of inflammatory bowel disease. Nuclearmedizin 24:370–374

Syrjälä MT, Liewendahl K, Valtonen V, Gripenberg J (1987) Intestinal accumulation of ^{111}In-granulocytes in patients studied because of occult infection. Eur J Nucl Med 13:121–124

Vorne M, Soini I, Lantto T, Paakkinen S (1989) Technetium-99m-HMPAO-labeled leukocytes in detection of inflammatory lesions: comparison with gallium-67 citrate. J Nucl Med 30:1332–1336