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Quantification of pulmonary uptake of indium-111 labelled granulocytes in inflammatory bowel disease

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Abstract. This study describes a method for quantifying the pulmonary trapping of indium-111 labelled polymorphonuclear (PMN) cells in patients with inflammatory bowel disease (IBD) in comparison to non-inflamed controls. Twenty patients with extensive IBD were studied by ^{111}In-PMN scintigraphy. Gamma-camera images were obtained at 2.5–4 h (early) and 20–25 h (late) after the injection of autologous PMNs labelled in plasma with ^{111}In-tropolonate. Local uptake in the chest, iliac bone marrow, spleen and liver was quantified as the counts per pixel per second per MBq of injected ^{111}In for both early and late scans. Fourteen subjects without inflammatory disease were studied as controls. IBD patients showed significantly greater loss of splenic activity between early and late scans compared with controls (mean ± SD: −35.7% ± 16.6% versus −4.5% ± 6.1%, $P < 0.001$). There was no significant difference between control and IBD groups with respect to liver and bone marrow uptake on both early and late scans. Chest uptake was significantly higher in patients with IBD on both early (6.4 ± 1.6 cps/MBq/pix) and late (5.6 ± 1.5cps/MBq/pix) scans, compared with the controls (4.8 ± 1.3 cps/MBq/pix, $P < 0.005$ and 3.4 ± 1.0 cps/MBq/pix, $P < 0.001$ respectively). The chest uptake in the control group on the late scans demonstrated a significant linear correlation with iliac uptake (y=0.23x + 0.41, r=0.87, n=14). Assuming in controls that there is no parenchymal uptake of ^{111}In, this regression enables an estimate to be made, based on iliac counts, of the count rate from bone marrow in the chest wall. After subtraction of this from the total chest count rate, the true parenchymal uptake was derived, which averaged 2.86 ± 0.91) cps/MBq/pix in the IBD group compared to zero assumed in the control group. The higher lung ^{111}In-PMN uptake on the early scans in IBD compared to controls is suggested to reflect a combination of increased margination, compared to controls, and early migration, whilst the excess ^{111}In-PMN retention on late scans represents extravascular migration only. The bone marrow correction technique for quantification of pulmonary migration of ^{111}In-PMNs should prove useful for the evaluation of PMN kinetics in disease.

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Introduction

Imaging of inflammatory disorders by means of indium-111 labelled neutrophils (polymorphonuclear cells, PMNs) or mixed white blood cells is now widely used as a diagnostic and research technique [1, 2]. Specific patterns of neutrophil kinetics in organs distant from local sites of inflammation, with intravascular delay (margination) or extravascular migration, have been described [3] and are a possible explanation for disorders of pulmonary [4], hepatic and renal function, such as in pancreatitis [5, 6] or systemic sepsis [7]. There is evidence suggesting that abnormal sequestration of PMNs within the pulmonary microvasculature is an important prerequisite for the full expression of permeability abnormalities in inflammation-related acute respiratory distress syndrome [8–10]. Nevertheless, the description of such phenomena remain based upon qualitative criteria, or visually recognised scan patterns [11, 12].

Diffusely increased uptake of ^{111}In-PMNs in the lungs has been described on very early views as a result of cell activation sustained in the course of the labelling procedure [13, 14]. Diffusely increased lung uptake, displaying different kinetics from those resulting from ex vivo cell activation, has been described recently in inflammatory bowel disease (IBD) [15] and systemic vasculitis [16] on the basis of a semiquantitative estimate of the lung to liver count ratio. This may be the result of increased cell adhesiveness and stiffness [17]

promoted by cytokine-induced activation [18], or of pulmonary endothelial abnormalities [19].

The existence of increased lung PMN traffic, and specifically the separate entities of intravascular delay (described in this text as "margination") and extravascular migration, is clearly of great importance in relation to PMN-mediated lung injury. In the current work we have quantified the absolute pulmonary uptake of ^{111}In in patients with IBD in an attempt to test the suggestion that the lungs of such patients show abnormal accumulation of PMNs both on early views, when ^{111}In uptake reflects margination, and on late views, when uptake reflects migration. A second more specific aim of our work was to develop a technique to correct for the chest wall activity in the bone marrow of the ribs which is present in the delayed views, and thereby to derive a signal which exclusively represents pulmonary PMN migration. This approach was based on the relationship between ^{111}In-PMN accumulation in chest and iliac bone marrow observed in patients with no evidence of inflammatory disease (controls), and in whom pulmonary extravascular migration of PMNs is assumed to be absent. With a technique for quantifying migration as well as margination, we would then be in a position to test the hypothesis that migration but not margination is responsible for PMN-induced lung injury [19, 20].

Materials and methods

Patients. Twenty patients with active IBD were studied. All were referred to the nuclear medicine unit for the evaluation of clinically suspected bowel inflammation. In each case the ^{111}In-PMN images were visually judged as positive for IBD on the basis of extensive abnormal granulocyte uptake in bowel. To quantify the disease activity we used a technique based on the rate of loss of splenic ^{111}In activity, which correlates closely with the whole-body loss of granulocytes in IBD and has previously been applied to disease quantification [21–23]. None of the patients had any known lung pathology.

The 14 control subjects were initially referred for white cell scanning for suspected osteomyelitis (n=10), abscess (3) or pericarditis (1). In all these, the ^{111}In-PMN scans were definitely negative for local inflammation and, on the basis of clinical follow-up, were regarded as normal. None of the controls had any known haematological disorders or splenic or hepatic pathology.

The age ranges were similar in both groups: 24–67 years in the IBD group and 26–65 years in controls.

^{111}In labelling of PMNs. Autologous neutrophils were isolated from 100 ml of freshly drawn venous blood anticoagulated by acid citrate dextrose (Formula A, NIH) and labelled with ^{111}In-tropolonate by a standard technique as described previously [24]. Briefly, the PMNs were isolated by separation on a Percoll-plasma gradient. PMNs were not separated from plasma at any stage. The injected dose of ^{111}In-PMN was 7.3–12.5 MBq.

Gamma-camera data acquisition and analysis. Two series of planar scans (anterior and posterior views) were recorded in each patient at 2.5–4 h (early scans) and 20–25 h (late scans) after injection of ^{111}In-labelled PMNs. One of the posterior scans included in the same field of view the lower lobes of the lungs, the thoracic and lumbar spines and iliac bones.

Regions of interest (ROIs) were placed over the lower lobe of the right lung, the thoracic spine, both iliac areas, the right lobe of the liver and the spleen. ROIs over the right lung, the right lobe of the liver and the iliac marrow were of similar areas in order to avoid interference from scattering [25]. All ROIs were redrawn in late scans in the same position and with the same dimensions as in the early scans.

For each ROI the total count and average count per pixel were calculated. All local mean counts per pixel were corrected for ^{111}In physical decay and divided by time of acquisition and by the injected activity for normalisation as cps/MBq/pix (uptake).

For the spleen ROIs the fraction of activity lost between early and late scans (fall in splenic activity, %) was calculated as:

$$\frac{(\text{splenic uptake on late scan}) - (\text{splenic uptake on early scan})}{(\text{splenic uptake on early scan})} \times 100.$$

All studies were performed with an IGE400A or 400T large field of view gamma-camera (General Electric Co.) on-line to dedicated MDS (Micas) computer system for data processing.

Statistics. Comparisons were made between the two patient groups by means of Student's t-test. Parametric linear regression analysis was used for quantification of the relationship between iliac and chest bone marrow uptake of ^{111}In. Changes between early and late scans were evaluated by the paired t-test.

Results

Uptake in spleen

Individual values for splenic uptake are shown in Fig. 1 for both groups. Patients of the control group demonstrated a small decline ($P < 0.05$) in splenic activity from early to late scans(mean ± SD: -4.5% ± 6.1%, range from +4.15% to −11.14%). Patients with IBD showed a greater decline in splenic activity from early to late scans (−35.7% ± 16.6%, range from −15.3% to −73.0%, $P < 0.0005$). The intergroup difference in the

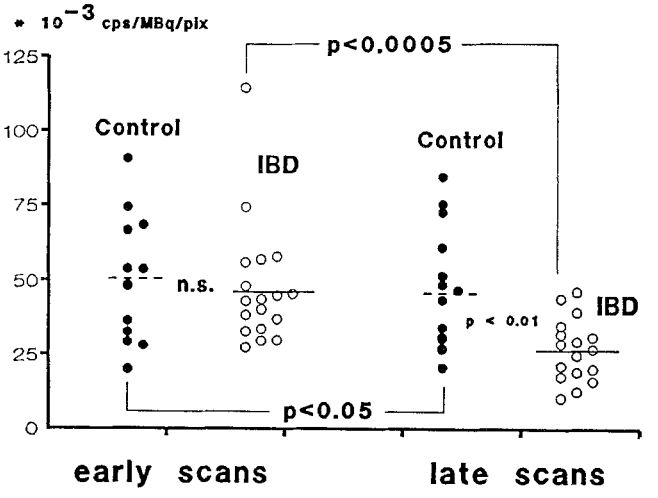

Fig. 1. Splenic ^{111}In-PMN uptake on early and late scans in the IBD group and controls

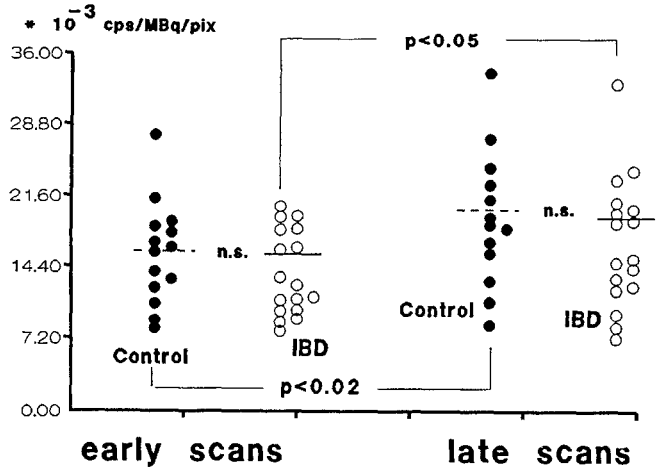

Fig. 2. Liver ^{111}In-PMN uptake on early and late scans in the IBD group and controls

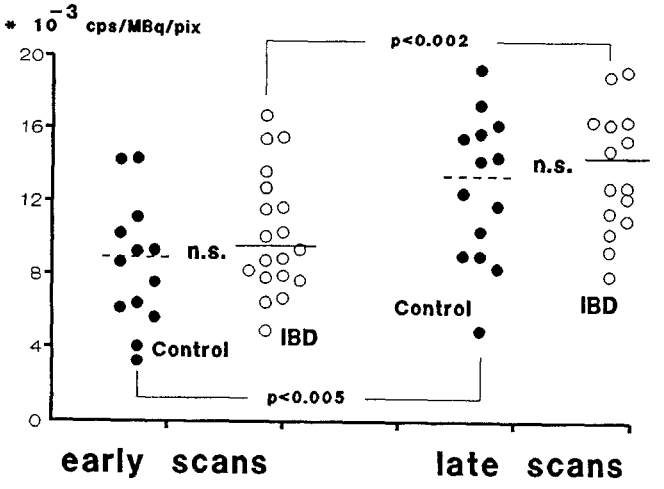

Fig. 3. Iliac bone ^{111}In-PMN uptake on early and late scans in the IBD group and controls

decrease in splenic activity was also highly significant ($P < 0.001$).

Uptake in liver

The kinetics of liver ^{111}In uptake did not differ significantly between the two groups (Fig. 2). There was a progressive increase in liver activity from early to late scans in controls (from 15.7 ± 5.0 to 20.4 ± 7.7 cps/MBq/pix, $P < 0.02$), similar to the IBD group (from 16.3 ± 7.9 to 18.1 ± 7.9 cps/MBq/pix, $P < 0.05$).

Uptake in bone marrow

Uptake of ^{111}In-PMNs in iliac bone was separated on the gamma-camera images as far as possible from inflamed bowel. There was an increase in iliac activity between early and late scans, which was of similar degree in both groups (controls: from 8.7 ± 3.25 to 13.3 ± 4.2 cps/MBq/pix, $P < 0.005$); and in the IBD group from 10.2 ± 3.2 to 13.9 ± 3.2 cps/MBq/pix, $P< 0.002$) (Fig. 3).

Uptake in chest

Thoracic counts were significantly higher in patients with IBD compared with controls on both early ($P < 0.005$) and late ($P < 0.001$) scans (Fig. 4). In the control group there was a decrease in ^{111}In chest uptake between early and late scans from 4.8 ± 1.3 to 3.4 ± 1.0 cps/MBq/pix ($P < 0.001$). A decrease was also seen in the IBD group (from 6.4 ± 1.6 to 5.6 ± 1.5 cps/MBq/pix, $P < 0.02$). The percentage decrease in chest activity between early and late scans was significantly different between the two patient groups: 34%± 4% in controls and 12% ± 3% in the IBD group ($P < 0.001$).

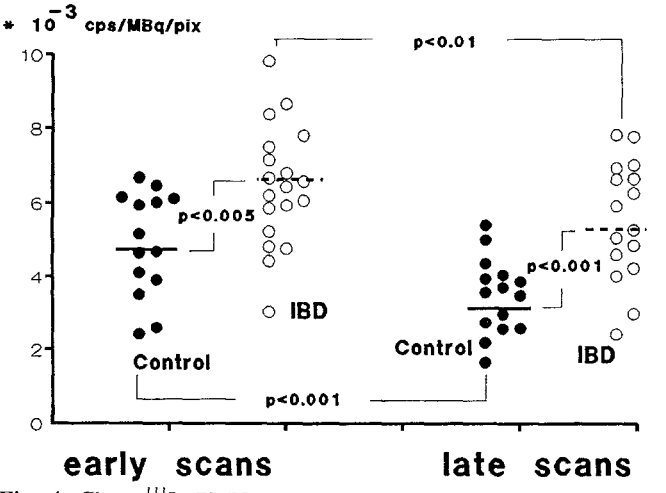

Fig. 4. Chest ^{111}In-PMN uptake on early and late scans in the IBD group and controls

Quantified as the ratio of chest to liver uptake (chest/liver index) [15], chest uptake tended to be higher in the IBD group at 4 h (0.41 ± 0.12) compared with controls (0.33 ± 0.14, $0.1 > P > 0.05$) but was significantly higher on 24-h scans (0.32 ± 0.11 vs 0.18 ± 0.08, $P < 0.001$).

Corrected chest uptake

The chest uptake in the control group on late scans demonstrated a significant linear correlation with iliac marrow uptake (y=0.23x + 0.41, r=0.87, n=14, $P < 0.005$) (Fig. 5), which can be explained as the result of the same local uptake of ^{111}In in different anatomical bone marrow sites. Provided there is no pulmonary accumulation of ^{111}In-PMNs at 24 h in the lungs of controls, this relationship provides a means of quantifying chest bone marrow ^{111}In activity. We used this regression

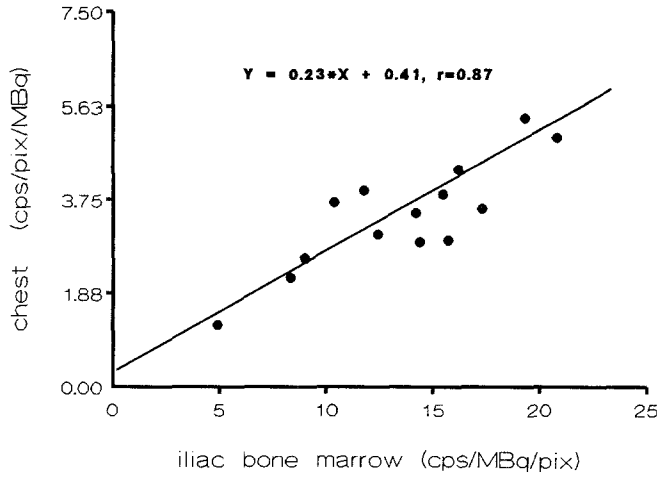

Fig. 5. Chest uptake versus iliac bone uptake of ^{111}In-PMNs on the late scans in the control group

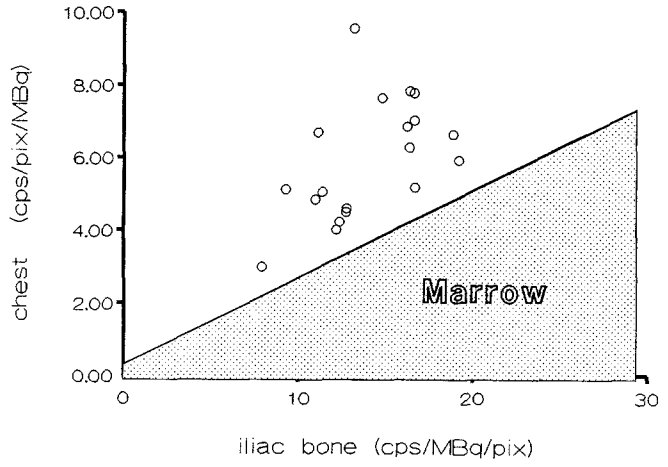

Fig. 6. Chest uptake versus iliac bone uptake of ^{111}In-PMNs on late scans in the IBD group. The "marrow" triangle represents the area of normal rib bone marrow background values

equation to estimate the chest bone marrow signal in the IBD group. By subtraction of the bone marrow signal from total chest uptake, the "pure" pulmonary uptake of ^{111}In-PMN was derived (Fig. 6). The mean value of the background-corrected uptake in the IBD group calculated in this way was 2.86 ± 0.91 cps/MBq/pix, significantly different from 0 ($P < 0.001$). The pulmonary uptake did not correlate significantly with any of the variables studied and particularly not with the fall in splenic activity between early and late scans.

Discussion

The most striking findings in the patient group with active IBD compared to the controls were the high chest retention of ^{111}In-PMNs in early and late scans (Fig. 4) and the low retention in the 24 h splenic scans (Fig. 1). The uptake in liver (Fig. 2) and bone marrow (iliac bone) (Fig. 3) was not significantly different between the two groups. This suggests: (1) that chest and splenic neutrophil kinetics reflect, in different ways, the inflammatory changes in patients with IBD, and (2) that the ^{111}In-PMN count rates in liver or bone marrow might be used as normalizing factors to quantify abnormal neutrophil kinetics in other organs in inflammatory conditions.

Splenic uptake

After margination in the spleen, the ^{111}In-PMNs are eventually recruited for migration in IBD and then lost through bowel, so splenic counts fall between early and late scans, whereas in controls there is a relative balance in the spleen between margination and destruction [21]. In bowel inflammation, faecal loss of ^{111}In-PMNs reflects the inflammatory activity [21–23]. Splenic counts decrease between early and late scans in IBD as a result of faecal excretion of previously pooled cells [26–28]. The degree of spleen loss can be used as an index of IBD activity [21, 22]. Thus, the prominent difference in this index between the control and IBD groups confirms the clinical criteria of IBD activity that were used for the inclusion of patients in this group.

Liver uptake

One might expect margination of inflammatory pre-activated PMNs in hepatic capillaries, as 75% of hepatic perfusion comes from portal blood flow; there is histological evidence to support this view in an animal model following soft tissue trauma or pancreatitis [5, 29]. Nevertheless, in patients with IBD we did not find any hepatic retention (Fig. 2). If there is hepatic retention in IBD, it must be balanced by loss to other organs. Previously Jonker et al. [15] used the chest/liver ratio of counts per pixel on the posterior gamma-camera image and reported a significant increase in this ratio in IBD on early and late scans. But, as shown in Fig. 5, there is a considerable background signal in the chest image from marrow activity in the ribs which will reduce the sensitivity of the chest/liver ratio. In our study, the chest/liver ratio in IBD averaged 135% in the early scan and 188% in the late scan, as compared to controls. This intergroup difference was significant ($P < 0.01$), but it was smaller than the difference between the corrected pulmonary signal of 2.86 on late scans in IBD, compared to zero assumed for controls ($P < 0.001$), as shown graphically in Fig. 6.

Bone marrow uptake

The iliac crest was chosen as the most representative region of the bone marrow. Lumbar and thoracic spine

regions were examined too but the counting geometry was less favourable. The ^{111}In-PMN kinetics in iliac bone marrow were similar for the IBD and control groups (Fig. 3). If an organ pools ^{111}In-granulocytes, then its radioactivity signal should be less in patients with inflammatory disease as compared with controls at 24 h, as in the spleen. We did not find this for bone marrow although the iliac bone marrow signal may have been overestimated in IBD as a result of superimposed radioactivity from diseased bowel. If so, then by applying the regression equation based on control patients to the IBD patients, chest bone marrow would also have been overestimated, thereby underestimating the true degree of neutrophil migration in the lung parenchyma in IBD.

Chest uptake

The chest uptake in the IBD group was significantly higher than in the controls in the early and late scans. The higher uptake in the early scan reflects a combination of increased margination and probably some early migration, but the excess ^{111}In-PMN retention at 24 h should represent extravascular migration [30]. In this paper we are focusing on the late scan and its quantification as a measure of neutrophil migration.

We have already argued (Fig. 5) that the chest uptake in the late scan in the control group represents bone marrow uptake in the ribs. When this "marrow correction" (i.e. subtraction of calculated rib bone marrow background from total chest signal) is applied to the IBD group (Fig. 6), a true pulmonary signal, taken to reflect ^{111}In-PMN migration, is achieved. The "corrected" chest parenchymal signal averaged 2.86 ± 0.91 cps/MBq/pix in IBD patients (control group, by definition, being zero).

The count rate per pixel per MBq of injected ^{111}In recorded from the chest at 24 h, after correction for bone marrow (Fig. 6), is 5–7 times less than the iliac bone marrow count rate and 12–20 times less than the splenic count rate. Thus, pulmonary neutrophil migration will be difficult to recognise visually, separate from the background of the marrow in the chest, emphasising the importance of a quantitative approach.

Mechanisms for pulmonary ^{111}In-PMN retention

Several mechanisms probably interact to cause neutrophils to be retained in the lung. Pre-activation of PMNs in inflamed regions by agents such as endotoxin [30, 31], peptides and prostaglandins [32] promotes an increase in local adhesiveness to endothelium [33] and increased mechanical stiffness of neutrophils [17]. Increased adhesiveness of pulmonary endothelium [34, 35] is possibly also a factor.

In the current study we have no data concerning the mechanisms of PMN migration into the lung parenchyma nor can we with certainty localise the trapped PMNs in IBD patients to the extravascular pulmonary compartment. Although this study does not address the question of the mechanism of pulmonary neutrophil retention, the development of a quantitative technique is important if abnormal PMN kinetics are to be studied.

In conclusion, in this study we have (a) described a regression equation which can be used to correct for chest wall bone marrow activity, thereby deriving a signal for parenchymal activity on 24-h scans; (b) used this more robust quantification approach to the pulmonary ^{111}In signal, conforming the earlier observation of Jonker et al [15] that in IBD there is increased retention of PMNs on both early and late scans; and (c) been unable to show a correlation between retention and the disease activity.

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