Faecal alpha-1-antitrypsin and excretion of \textsuperscript{111}Indium granulocytes in assessment of disease activity in chronic inflammatory bowel diseases

W FISCHBACH, W BECKER, J MOESSNER, W KOCH, AND C REINERS

From the Medizinische Poliklinik and Department of Nuclear Medicine, University of Würzburg, FR Germany

SUMMARY Intestinal protein loss in chronic inflammatory bowel diseases may be easily determined by measurement of alpha-1-antitrypsin (\(\alpha_1\)-AT) stool concentration and \(\alpha_1\)-AT clearance. Both parameters were significantly raised in 36 and 34 patients respectively with chronic inflammatory bowel diseases, compared with eight patients with non-inflammatory bowel diseases, or 19 healthy volunteers. There was wide range of overlap between active and inactive inflammatory disease. Contrary to serum \(\alpha_1\)-AT, faecal excretion and clearance of \(\alpha_1\)-AT did not correlate with ESR, serum-albumin, orosomucoid, and two indices of disease activity. A comparison of \(\alpha_1\)-AT faecal excretion and clearance with the faecal excretion of \textsuperscript{111}In labelled granulocytes in 27 patients with chronic inflammatory bowel diseases, showed no correlation between the intestinal protein loss and this highly specific marker of intestinal inflammation. Enteric protein loss expressed by faecal excretion and clearance of \(\alpha_1\)-AT does not depend on mucosal inflammation only, but may be influenced by other factors.

Intestinal protein loss seems to be common feature in chronic inflammatory bowel diseases such as Crohn's disease or ulcerative colitis. For many years protein loss into the gastrointestinal tract could be only determined by using radiolabelled serum proteins like \textsuperscript{51}Cr-albumin. Bernier and Crossley introduced the faecal excretion of alpha-1-antitrypsin (\(\alpha_1\)-AT) as a reliable and easy method for detecting a protein losing enteropathy. Several authors have shown that \(\alpha_1\)-AT clearance reflected intestinal protein loss just as well as the \textsuperscript{51}Cr-albumin method.\textsuperscript{1-6}

Quantification of the faecal excretion of intravenously infused autologous \textsuperscript{111}In-oxin-labelled white blood cells seems to be a highly specific parameter of bowel inflammation.\textsuperscript{7} We and others found that measuring faecal excretion of \textsuperscript{111}In-labelled granulocytes was a reliable parameter to distinguish active from inactive inflammatory bowel disease.\textsuperscript{7,8} Previously published data suggested that enteric protein loss was related to intestinal inflammation\textsuperscript{9} and the extent of intestinal damage.\textsuperscript{10} The aim of this prospective study, therefore, was to compare faecal excretion and clearance of \(\alpha_1\)-AT as parameters of intestinal protein loss with the faecal excretion of \textsuperscript{111}In labelled granulocytes as parameter of bowel inflammation in patients with chronic inflammatory bowel diseases. Additionally both methods were compared with clinical indices, serum laboratory findings, and morphological parameters.

Methods

Patients

Forty three patients with chronic inflammatory bowel diseases (33.3±15, range 13–78 years, 21 women, 22 men) were investigated. Thirty one patients had Crohn's disease, eight ulcerative colitis and four unspecific colitis. The diagnosis of Crohn's disease was based on the criteria published by the European Cooperative Crohn's disease Study Group (ECCDS).\textsuperscript{11} Ulcerative colitis was characterised by typical endoscopical and histological features and the
characteristic localization pattern. Unspecific colitis was defined as colitis lacking any of the above mentioned criteria for Crohn’s disease or ulcerative colitis. Furthermore, infectious causes have been excluded. As well as small bowel radiography examination and colonoscopy, routine laboratory parameters, orosomucoid, serum α1-AT and clinical indices (CDAI, AI, Truelove index) were evaluated in all patients. Alpha-1-antitrypsin stool concentration could be measured in 36 patients. Because of unreliable stool collection in two cases only 34 patients were available for clearance determination. In 27 patients the percentage of faecal excretion of 111In-labelled autologous granulocytes could be studied simultaneously. All stool and blood collections were done on the same day. Faecal excretion of 111In and α1-AT were measured in identical stool samples. All other investigations such as radiographic and endoscopy were carried out within eight days.

Patients with chronic inflammatory bowel diseases were classified as ‘active’ if the following criteria were fulfilled: for all cases of chronic inflammatory bowel diseases histologically diagnosed acute bowel inflammation; additionally for Crohn’s disease CDAI>150 and/or activity index van Hees>100; for ulcerative colitis ‘severe’ or ‘moderately severe’ according to the Truelove index; and for unspecific colitis clinical signs and laboratory parameters indicating acute inflammation.

In addition four patients with Crohn’s disease could be studied twice at different states of their disease.

Twenty one healthy volunteers (24±2.7, range 21–33 years, 10 women, 11 men) and eight patients with non-inflammatory bowel diseases (irritable bowel syndrome n=7, inactive Whipple’s disease n=1, 43±4±17-1, range 21–68 years, two women, six men) served as controls. All of them completed a reliable stool collection. 111In-faecal-excretion was only available in patients with non-inflammatory bowel diseases and because of radiation safety regulations not in volunteers.

SERUM LABORATORY PARAMETERS
Erythrocyte sedimentation rate was measured in the usual way, serum albumin was determined by automated analysis, using the bromcresol-green method. Orosomucoid (human acid α1-glycoprotein) was measured by radial immunodiffusion using partigen plates (Behring AG, FRG).

DETERMINATION OF ALPHA1-ANTITRYPSIN
Serum α1-AT concentration was measured by laser nephelometry with a monospecific antiserum against α1-AT (Behring AG, FRG) and expressed in mg/dl.

Faecal α1-AT was determined by radial immunodiffusion using commercially available partigen plates (LC-partigen, Behring AG, FRG). According to the method of Crossley and Elliott an aliquot of lyophilised stool (250 mg) was extracted with 5 ml 0-9% saline by gently mixing over 30 minutes at 22°C. After centrifugation (12,000 g for 15 minutes at 4°C) 5 μl of the supernatant were placed into the wells of partigen plates. The diameter of the precipitating rings was measured after 72 hours. As control served a reference curve with serum of known α1-AT concentration. Faecal α1-AT was expressed in mg α1-AT/g dry stool weight.

Median daily stool weight was determined from the four days stool collection and α1-AT clearance was calculated by the formula: C=F×W/P (C= clearance, F=faecal α1-AT [mg/g stool], W=daily stool weight [g] and P=serum α1-AT [mg/dl]).

PER CENT EXCRETION OF 111IN-OXIN
Isolation of white blood cells and labelling of granulocyte preparations with 111In-oxin was carried out as previously described. 111In-oxin was incubated at a level of ~10 Ci (μCi) and could be measured after 72 hours. Faecal α1-AT was measured in mg α1-AT/g dry stool weight.

The patients were divided into two subgroups: patients with active and inactive bowel disease (Table, Fig. 1). Alpha-1-antitrypsin serum concentrations correlated with ESR (r=0.67, p<0.001), serum albumin (r=0.47, p<0.001), orosomucoid (r=0.49, p<0.001) and activity index van Hees (r=0.74, p<0.001), but not with CDAI.

Alpha-1-antitrypsin stool concentration was significantly (α<0.001) raised in patients with chronic inflammatory bowel diseases compared with normal controls or patients with non-inflammatory bowel diseases (Table, Fig. 2). Patients with active disease showed higher α1-AT concentrations than those with inactive disease (Table, Fig. 2), but the difference...
Table  Serum $\alpha_1$-AT (mg/dl), faecal $\alpha_1$-AT (mg/g), $\alpha_1$-AT clearance (ml/d) and percentage faecal excretion (%) of $^{111}$In labelled granulocytes in patients with chronic inflammatory bowel diseases (CIBD), non-inflammatory bowel diseases (NIBD) and in normal controls

<table>
<thead>
<tr>
<th></th>
<th>Serum $\alpha_1$-AT (mg/dl)</th>
<th>Faecal $\alpha_1$-AT (mg/g)</th>
<th>$\alpha_1$-AT clearance (ml/d)</th>
<th>Faecal excretion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIBD</td>
<td>377±50-4</td>
<td>1-50±0-17</td>
<td>115-7±22-4</td>
<td>21-7±4-9</td>
</tr>
<tr>
<td>Active disease</td>
<td>423-8±29-4</td>
<td>1-65±0-33</td>
<td>92±41-9</td>
<td>2-1±0-4</td>
</tr>
<tr>
<td>Inactive disease</td>
<td>239-9±13-6</td>
<td>1-43±0-18</td>
<td>117-9±20-8</td>
<td></td>
</tr>
<tr>
<td>Previous resection</td>
<td>299±28-5</td>
<td>1-48±0-42</td>
<td>231±91-1</td>
<td></td>
</tr>
<tr>
<td>No resection</td>
<td>303±16-9</td>
<td>1-56±0-21</td>
<td>73-3±10-8</td>
<td></td>
</tr>
<tr>
<td>NIBD</td>
<td>238±11</td>
<td>0-80±0-21</td>
<td>49±16</td>
<td>1-5±0-5</td>
</tr>
<tr>
<td>Normal controls</td>
<td>241±11</td>
<td>0-58±0-11</td>
<td>39±8-4</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1 $\alpha_1$-AT serum concentration (mg/dl) in normal controls, patients with inactive and active inflammatory bowel diseases and patients with non-inflammatory bowel diseases.
Faecal alpha-1-antitrypsin in chronic inflammatory bowel diseases

was not statistically significant. Alpha-1-antitrypsin stool concentrations were higher in patients with a combined involvement of small and large bowel (1.7±0.4 ng/mg, n=13) compared with those who had only the ileum (1.4±0.4 ng/mg, n=8) or the colon (1.4±0.2 ng/mg, n=15) involved. There was however, no clear relationship between faecal α1-AT and the extent of inflamed bowel. Some patients with acute inflammation over a small segment showed higher faecal α1-AT concentration compared with those with a more extensive involvement. Furthermore no correlation of faecal α1-AT with ESR, serum albumin, orosomucoid, activity index van Hees, or CDAI could be found.

There was a significant difference (α<0.01) in α1-AT clearance between patients with chronic inflammatory bowel diseases and normal controls or patients with non-inflammatory bowel diseases (Table, Fig. 3). Patients with active disease did not differ from those with inactive disease (Table, Fig. 3). Alpha-1-antitrypsin clearance did not correlate with ESR, serum albumin, orosomucoid, activity index van Hees, or CDAI. No relationship between α1-AT clearance and site or extent of bowel inflammation could be established. There was however, a tendency towards a higher α1-AT clearance in patients with previous resections compared with unresected patients (Table, Fig. 4). These findings

Fig. 2  α1-AT stool concentration (mg/g) in normal controls, patients with inactive and active inflammatory bowel diseases and patients with non-inflammatory bowel diseases.
were independent of the actual inflammatory activity. These two groups did not differ from each other if their α₁-AT serum or stool concentrations were compared (Table, Fig. 4).

The percentage faecal excretion of ¹¹¹In labelled granulocytes was significantly (α<0.001) raised in patients with active inflammatory bowel disease compared with patients with inactive disease or non-inflammatory bowel diseases (Table, Fig. 5). All but one ‘active’ patient excreted more than 4% (Fig. 5). There was a good correlation between the percentage faecal excretion and ESR (r=0.62, p<0.001), serum albumin (r=−0.44, p<0.001), orosomucoid (r=0.51, p<0.001), serum α₁-AT (r=0.63, p<0.001) and activity index van Hees (r=0.65, p<0.001), but no statistically significant correlation with α₁-AT stool concentration or α₁-AT clearance.

Serial studies were possible in 4 patients with Crohn’s disease (Fig. 6). Two of them (RK, StCh) showed a decrease of disease activity, one patient (EI) was stable and another one (MB) developed intestinal fistulae (Fig. 6). The individual studies showed a parallel decrease or increase of serum α₁-AT and percentage faecal excretion of ¹¹¹In labelled granulocytes with disease activity whereas faecal α₁-AT took an obviously different course in at least two patients (StCh, MB, Fig. 6).

**Discussion**

In accordance with previously published data, serum α₁-AT was significantly raised in patients suffering from chronic inflammatory bowel diseases compared with patients with non-inflammatory bowel diseases.
Faecal alpha-1-antitrypsin in chronic inflammatory bowel diseases

Fig. 4 $\alpha_1$-AT clearance (ml/d) and $\alpha_1$-AT stool concentration (mg/g) in patients with CIBD with or without previous resections.

bowel diseases or normal controls (Table, Fig. 1). Active and inactive inflammatory disease were clearly separated. Serum $\alpha_1$-AT indicated the systemic inflammatory activity similarly to clinical indices or other laboratory parameters and run parallel to the course of disease (Fig. 6).

Beeken et al. found that intestinal protein loss reflects well the extent of inflamed bowel segments. Their results were based on intestinal protein clearance utilising $^{51}$Cr-albumin or $^{51}$Cr-chloride. Crossley and Elliott7 overcame the disadvantage using radio-labels and described faecal $\alpha_1$-AT as a reliable test for protein losing enteropathies, suitable for clinical routine because of its simplicity. Several authors have shown that there is a good correlation between $\alpha_1$-AT clearance and $^{51}$Cr-albumin. In chronic inflammatory bowel diseases faecal excretion and clearance of $\alpha_1$-AT seem to be objective parameters for the grading of intestinal inflammation.8-13

In accordance with Karbach, Thomas,14 and Meyers15 our results indicate that faecal $\alpha_1$-AT is significantly raised in chronic inflammatory bowel diseases compared with normal controls or non-inflammatory bowel diseases (Fig. 2). Contrary to Thomas et al.,1 however, active disease could not be clearly separated from inactive disease by measuring $\alpha_1$-AT stool concentration (Fig. 2). Furthermore faecal $\alpha_1$-AT was obviously lower in our patients with chronic inflammatory bowel diseases compared with those of Thomas16 and Meyers.17 Considering identical stool preparations and similar $\alpha_1$-AT stool concentrations in the control subjects we believe that selection of patients rather than a technical problem may explain this discrepancy. Our percentage of patients with high disease activity was relatively low as our group mainly consisted of outpatients.

Alpha-1-antitrypsin clearance was significantly higher in our patients with chronic inflammatory bowel diseases but contrary to Karbach and Bernier18 an unequivocal overlap with our controls was evident (Fig. 3). Alpha-1-antitrypsin clearance of our patients was quite similar to their patient data whereas our normal controls showed distinctly higher

Fig. 5 Percentage faecal excretion (%) of $^{111}$In labelled granulocytes in inactive and active inflammatory bowel disease.
values. Neither faecal $\alpha_1$-AT nor $\alpha_1$-AT clearance correlated with ESR, serum albumin, serum $\alpha_1$-AT, orosomucoid, clinical indices or sites and extent of inflammation as diagnosed by radiological and endoscopical findings. This is contrary to Florent et al. 7 who found a linear relationship between $\alpha_1$-AT clearance and CDAI or serum albumin, but in accordance with Karbach 8 and Thomas 9 who also failed to prove a correlation between intestinal protein loss and extent or localisation of inflammation or severity of disease.

As with the patients of Karbach 8 our patients with prior resections had, independent of their actual disease activity, a higher $\alpha_1$-AT clearance than patients without surgical treatment in their history (Fig. 4). This may be due to copious diarrhoea after extensive previous resections in some patients. We therefore found a tendency towards a higher clearance if large bowel segments had been resected. Measurement of faecal $\alpha_1$-AT alone was not distinctive between resected and unresected patients with chronic inflammatory bowel diseases (Fig. 4).

The percentage of faecal excretion of $^{111}$In labelled granulocytes has been shown to be highly specific for intestinal inflammation, independent of subjective complaints, extraintestinal manifestations of chronic inflammatory bowel diseases or other coexisting diseases. 10 Follow up studies in patients with Crohn's disease under therapy have shown that the percentage of faecal excretion rapidly reflects any changes in inflammatory activity and is not masked by medical treatment. 6 With one exception we found a clear differentiation of inactive and active patients (Fig. 5). Compared with common clinical parameters there was a good correlation to ESR, serum albumin, orosomucoid and activity index van Hees.

The follow up study of four patients with Crohn's disease - two with decreasing disease activity (RK, StCh), one with stable disease (EJ) and one developing intestinal fistulae (MB) - demonstrates that the percentage of faecal excretion of $^{111}$In labelled granulocytes run parallel to disease activity in all cases (Fig. 6). Faecal $\alpha_1$-AT, however, showed a parallel trend only in two patients (RK, EJ) while it differed noticeably in the other two patients (StCh, MB) (Fig. 6). In one patient (StCh) with the lowest $\alpha_1$-AT clearance (4 ml/d) resection of an inflamed stenosis lead to an increase of $\alpha_1$-AT clearance up to 164 ml/d despite a fall of disease activity. Similar observations were reported by Karbach 8 who found raised $\alpha_1$-AT clearance in patients after curative resection.

While the percentage of faecal excretion of $^{111}$In labelled granulocytes correlated well with serum $\alpha_1$-AT as acute phase reactant there was no correlation with $\alpha_1$-AT stool concentration and clearance. Considering these as parameters of intestinal protein loss our results indicate that protein loss from the gut does
not always reflect the actual individual degree of intestinal inflammation. Our own histomorpho-
logical studies seem to confirm this. We found a significant increase of the percentage excretion with the intensity of the granulocyte infiltrate in biopsy specimens, whereas the intestinal protein loss seems to parallel lymphoplasmocellular infiltrates. Furthermore it has to be questioned again whether faecal excretion and clearance of α1-AT is really an accurate correlate of protein loss. Contrary to Karbach, Florent and Hill\textsuperscript{6} Haenay \textit{et al}\textsuperscript{2} failed to confirm a correlation between faecal α1-AT and 51Cr-albumin. On the other hand Saverymuttu \textit{et al}\textsuperscript{7} found in a small number of patients a correlation between faecal \textsuperscript{111}In leucocyte excretion and faecal \textsuperscript{51}Cr protein excretion. Further investigations are necessary to elucidate these discrepancies.

We conclude that the percentage of faecal excretion of \textsuperscript{111}In labelled granulocytes is a highly specific marker of the intestinal inflammation. Changes of individual disease activity are correctly recognised by this objective parameter. Faecal excretion and clearance of α1-AT are easily available. Assessment of inflammatory activity in an individual patient cannot be based upon faecal α1-AT stool concentration and clearance alone. Enteric protein loss does not only depend on the mucosal inflammation but may be also influenced by prior resections, complications like stenosis, extent and localisation of disease or other factors such as bacterial overgrowth, secondary intestinal lymphangiectasia, and unknown parameters.

References


Faecal alpha-1-antitrypsin and excretion of 111indium granulocytes in assessment of disease activity in chronic inflammatory bowel diseases.

W Fischbach, W Becker, J Mössner, W Koch and C Reiners

Gut 1987 28: 386-393
doi: 10.1136/gut.28.4.386

Updated information and services can be found at:
http://gut.bmj.com/content/28/4/386

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/