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

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SUMMARY Intestinal protein loss in chronic inflammatory bowel diseases may be easily determined by measurement of alpha-1-antitrypsin (α_1 -AT) stool concentration and α_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 α_1 -AT, faecal excretion and clearance of α_1 -AT did not correlate with ESR, serum-albumin, orosomucoid, and two indices of disease activity. A comparison of α_1 -AT faecal excretion and clearance with the faecal excretion of ¹¹¹In 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 α_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 ⁵¹Cr-albumin. Bernier¹ and Crossley² introduced the faecal excretion of alpha-1-antitrypsin (α_1 -AT) as a reliable and easy method for detecting a protein losing enteropathy. Several authors have shown that α_1 -AT clearance reflected intestinal protein loss just as well as the ⁵¹Cr-albumin method.^{3,4}

Quantification of the faecal excretion of intravenously infused autologous ¹¹¹In-oxin-labelled white blood cells seems to be a highly specific parameter of bowel inflammation.⁷ We and others found that measuring faecal excretion of ¹¹¹In-labelled granulocytes was a reliable parameter to distinguish active from inactive inflammatory bowel disease.^{7,8} Previously published data suggested that enteric protein

loss was related to intestinal inflammation⁹ and the extent of intestinal damage.¹⁰ The aim of this prospective study, therefore, was to compare faecal excretion and clearance of α_1 -AT as parameters of intestinal protein loss with the faecal excretion of ¹¹¹In-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).¹¹ Ulcerative colitis was characterised by typical endoscopic and histological features and the

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characteristic localisation 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 coloileoscopy, routine laboratory parameters, orosomucoid, serum α_1 -AT and clinical indices (CDAI, AI, Truelove index)¹²⁻¹⁴ 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 α_1 -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;¹⁴ 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 α_1 -glycoprotein) was measured by radial immunodiffusion using partigen plates (Behring AG, FRG).

DETERMINATION OF ALPHA₁-ANTITRYPSIN

Serum α_1 -AT concentration was measured by laser nephelometry with a monospecific antiserum against α_1 -AT (Behring AG, FRG) and expressed in mg/dl.

Faecal α_1 -AT was determined by radial immunodiffusion using commercially available partigen plates (LC-partigen, Behring AG, FRG). According to the method of Crossley and Elliott² 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 α_1 -AT concentration. Faecal α_1 -AT was expressed in mg α_1 -AT/g dry stool weight.

Median daily stool weight was determined from the four days stool collection and α_1 -AT clearance was calculated by the formula: $C = F \times W/P$ (C = clearance, F = faecal α_1 -AT [mg/g stool], W = daily stool weight [g] and P = serum α_1 -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.¹⁵ ¹¹¹In-activity of stool samples was measured in a gamma-counter and expressed as per cent of the reinjected dose (4.2 MBq = 250 µCi) after decay correction.

STATISTICAL ANALYSIS

Statistical analysis was undertaken using the arithmetic mean and its standard error of the mean ($\bar{x} \pm \text{SEM}$) and the Spearman's rank correlation coefficient. All correlation coefficients with $p < 0.05$ were considered significant. Using a rank variance analysis according to Kruskal and Wallis significance was considered on a level of $\alpha = 0.05$.

Results

Serum α_1 -AT was significantly higher ($\alpha < 0.001$) in patients with chronic inflammatory bowel diseases than in normal controls or in patients with non-inflammatory bowel diseases (Table, Fig. 1). The patients with active disease had significantly ($\alpha < 0.05$) higher values than those with inactive 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 ($\alpha < 0.001$) 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 α_1 -AT concentrations than those with inactive disease (Table, Fig. 2), but the difference

Table Serum α_1 -AT (mg/dl), faecal α_1 -AT (mg/g), α_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

	Serum α_1 -AT (mg/dl)	Faecal α_1 -AT (mg/g)	α_1 -AT clearance (ml/d)	Faecal excretion (%)
CIBD	377±50.4	1.50±0.17	115.7±22.4	
Active disease	423.8±29.4	1.65±0.33	92±41.9	21.7±4.9
Inactive disease	239.9±13.6	1.43±0.18	117.9±20.8	2.1±0.4
Previous resection	299±28.5	1.48±0.42	231±91.1	
No resection	303±16.9	1.56±0.21	73.3±10.8	
NIBD	238±11	0.60±0.21	49±16	1.5±0.5
Normal controls	241±11	0.58±0.11	39±8.4

Fig. 1 α_1 -AT serum concentration (mg/dl) in normal controls, patients with inactive and active inflammatory bowel diseases and patients with non-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 α_1 -AT and the extent of inflamed bowel. Some patients with acute inflammation over a small segment showed higher faecal α_1 -AT concentration compared with those with a more extensive involvement. Furthermore no correlation of faecal α_1 -AT with ESR, serum albumin, orosomucoid, activity index van Hees, or CDAI could be found.

There was a significant difference ($\alpha < 0.01$) in α_1 -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 α_1 -AT clearance and site or extent of bowel inflammation could be established. There was however, a tendency towards a higher α_1 -AT clearance in patients with previous resections compared with unresected patients (Table, Fig. 4). These findings

Fig. 2 α_1 -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 α_1 -AT serum or stool concentrations were compared (Table, Fig. 4).

The percentage faecal excretion of ^{111}In labelled granulocytes was significantly ($\alpha < 0.001$) 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 α_1 -AT ($r = 0.63$, $p < 0.001$) and activity index van Hees ($r = 0.65$, $p < 0.001$), but no statistically significant correlation with α_1 -AT stool concentration or α_1 -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 α_1 -AT and percentage faecal excretion of ^{111}In labelled granulocytes with disease activity whereas faecal α_1 -AT took an obviously different course in at least two patients (StCh, MB, Fig. 6).

Discussion

In accordance with previously published data¹⁶⁻¹⁸ serum α_1 -AT was significantly raised in patients suffering from chronic inflammatory bowel diseases compared with patients with non-inflammatory

Fig. 3 α_1 -AT clearance (ml/d) in normal controls, patients with inactive and active inflammatory bowel diseases and patients with non-inflammatory bowel diseases.

Fig. 4 α_1 -AT clearance (ml/d) and α_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 α_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*¹⁰ 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 Elliott² overcame the disadvantage using radio-labels and described faecal α_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 α_1 -AT clearance and ^{51}Cr -albumin.^{3,4,6} In chronic inflammatory bowel diseases faecal excretion and clearance of α_1 -AT seem to be objective parameters for the grading of intestinal inflammation.^{5,9,19,20}

In accordance with Karbach,³ Thomas,¹⁹ and Meyers²⁰ our results indicate that faecal α_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*,⁹ however, active disease could not be clearly separated from inactive disease by measuring

α_1 -AT stool concentration (Fig. 2). Furthermore faecal α_1 -AT was obviously lower in our patients with chronic inflammatory bowel diseases compared with those of Thomas¹⁹ and Meyers.²⁰ Considering identical stool preparations and similar α_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 Bernier^{1,3} 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.

Fig. 6 Percentage faecal excretion (%) of ^{111}In -oxin labelled granulocytes, α_1 -AT stool (mg/g) and serum (mg/dl) concentration compared with ESR, orosomucoid, AI and disease activity in four patients with Crohn's disease at different states of their disease.

values. Neither faecal α_1 -AT nor α_1 -AT clearance correlated with ESR, serum albumin, serum α_1 -AT, orosomucoid, clinical indices or sites and extent of inflammation as diagnosed by radiological and endoscopic findings. This is contrary to Florent *et al*⁵ who found a linear relationship between α_1 -AT clearance and CDAI or serum albumin, but in accordance with Karbach³ and Thomas⁹ 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 Karbach³ our patients with prior resections had, independent of their actual disease activity, a higher α_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 α_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.^{7,8} Follow up studies in patients with Crohn's disease under therapy have shown that the percent-

age of faecal excretion rapidly reflects any changes in inflammatory activity and is not masked by medical treatment.⁸ 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 (EI) 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 α_1 -AT, however, showed a parallel trend only in two patients (RK, EI) while it differed noticeably in the other two patients (StCh, MB) (Fig. 6). In one patient (StCh) with the lowest α_1 -AT clearance (4 ml/d) resection of an inflamed stenosis lead to an increase of α_1 -AT clearance up to 164 ml/d despite a fall of disease activity. Similar observations were reported by Karbach³ who found raised α_1 -AT clearance in patients after curative resection.

While the percentage of faecal excretion of ^{111}In labelled granulocytes correlated well with serum α_1 -AT as acute phase reactant there was no correlation with α_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 histomorphological 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.²¹ Furthermore it has to be questioned again whether faecal excretion and clearance of α_1 -AT is really an accurate correlate of protein loss. Contrary to Karbach, Florent and Hill¹⁴ Haeney *et al.*²² failed to confirm a correlation between faecal α_1 -AT and ⁵¹Cr-albumin. On the other hand Saverymattu *et al.*⁷ found in a small number of patients a correlation between faecal ¹¹¹In leucocyte excretion and faecal ⁵¹Cr protein excretion. Further investigations are necessary to elucidate these discrepancies.

We conclude that the percentage of faecal excretion of ¹¹¹In 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 α_1 -AT are easily available. Assessment of inflammatory activity in an individual patient cannot be based upon faecal α_1 -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.

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