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Alpha$_1$-antitrypsin, a reliable endogenous marker for intestinal protein loss and its application in patients with Crohn's disease

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SUMMARY Intestinal protein loss is generally determined by radio-labelled macromolecules. Alpha$_1$-antitrypsin has been proposed as an endogenous marker for protein losing enteropathy, but different opinions exist about its reliability. In 25 patients with Crohn's disease faecal protein loss was studied with intestinal alpha$_1$-antitrypsin (α_1AT) clearance. Simultaneously, in 10 patients α_1AT clearance was compared with faecal ^{51}Cr clearance after intravenous ^{51}Cr-albumin injection. There was a linear relation ($p<0{\cdot}05$) between α_1AT clearance and ^{51}Cr clearance in these cases. In all patients α_1AT clearance was raised above control values. α_1AT clearance, however, did not correlate with the activity index of Crohn's disease.[1] This index does not contain direct critieria of intestinal inflammation, does not take into account localisation or extent of inflammation, and includes complications such as extraintestinal manifestations, fistuli, stenoses not necessarily related to actual mucosal involvement. It is concluded that α_1AT is a reliable marker for intestinal protein loss and that the intestinal changes of Crohn's disease generally lead to an increased protein exudation into the gut.

Gastrointestinal loss of plasma proteins can be detected by a variety of labelled macromolecules: ^{59}Fe-labelled dextran[2] and ^{131}I-PVP[3] are not split by digestive enzymes; the radioactive isotopes ^{51}Cr-albumin,[4] ^{67}Cu-ceruloplasmin[5] or ^{95}Nb-albumin[6] are poorly absorbed by the alimentary tract. These methods are expensive and the patients are exposed to radioactivity and therefore cannot be used routinely as screening-tests or to monitor the course of illness. Crossley and Elliot[7] showed that α_1AT, a protease inhibitor synthesized by the liver and present in serum at a concentration of 2–5 g/l, is not degraded by digestive enzymes nor reabsorbed by the intestine. It has been shown to be a reliable endogenous marker of intestinal protein loss by Bernier and colleagues.[8] These authors reported that both faecal α_1AT concentration and α_1AT clearance were significantly higher in patients with suspected exudative enteropathy than in controls. Their results were not confirmed by Haeney *et al*,[9] who compared α_1AT concentration and α_1AT clearance with the conventional ^{51}Cr-albumin method. According to Keaney and Kelleher[10] the contradictory results could be caused by a difference in methods and by comparison of different parameters.

We examined the validity of α_1AT as a reliable indicator of intestinal protein loss in patients with Crohn's disease, comparing α_1AT clearance with the clearance of ^{51}Cr-albumin and studied problems of methodology.

Finally, we tried to correlate intestinal α_1AT clearance with site, extent, and activity of intestinal inflammation or resection in Crohn's disease.

Methods

PATIENTS

Alpha$_1$-antitrypsin clearance was determined in 10 healthy controls and in 25 patients with Crohn's disease (mean age 31·6±2·5, range 18–63 years; 17 women, eight men). The site and extent of inflammation was documented radiologically and/or by endoscopy and histology (Table). Ten patients had undergone intestinal resection, five of them suffered from anastomosal relapse. Twelve patients had complications: intestinal stenosis occurred in six

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Table *Morphological, clinical, and laboratory data of patients*

Patient (no.)	*Resection*	*Involvement*	*Complications*	*CDAI*	*α_1AT clearance (ml/d)*	*ESR (mm/1 h)*	*Serum albumin (g%)*	*HCT (%)*	*Treatment*
1. MD	Ileoascendost	0	0	27	20·7	15	3·6	41	0
2. RC	Ileoascendost	0	Anastomosal stenosis	354	46·2	4	4·2	42	0
3. GM	Ileoascendost	0	0	115	40·6	20	3·4	31	0
4. HE	Ileotransver-sostomy	0	0	35	44·7	8	4·8	38	0
5. BU	Subtotal colectomy	0	0	29	39·5	10	4·1	40	0
6. EH	Ileoascandost	AR	0	118	203·6	—	3·5	42	SASP, ST
7. WE	Ileotrans-versostomy	AR	Anastomosal stenosis	158	307·8	3	4·5	46	SASP, ST
8.GP	Ileotrans-versostomy	AR	Anal fistula	92	34·5	38	4·0	37	METRO, ST
9. FM	Ileotrans-versostomy	AR	0	136	105·9	13	3·7	39	SASP, ST
10. KP	Ileosigmoid-ostomy	AR	Anal fistula	431	40·6	22	3·2	44	METRO, ST
11. SH	0	tC	0	50	54·5	20	4·3	37	SASP
12. KH	0	TI (10 cm)	Stenosis (TI) anal fistula	227	23·0	9	4·8	43	SASP, ST
13. WG	0	TI (20 cm), tC	EIM	151	53·3	26	3·2	41	SASP, ST
14. HM	0	TI (20 cm)	0	64	105·7	14	4·3	38	SASP, ST
15. JM	0	TI (30 cm)	Stenosis (TI)	58	57·2	10	3·6	38	SASP
16. MW	0	TI (25 cm)	Stenosis (TI)	88	59·1	26	3·9	40	SASP, ST
17. SB	0	TI (30 cm), CA	Enteral fistula	124	25·3	42	3·7	38	SASP, ST
18. SU	0	TI (20 cm), CA	0	244	103·4	13	3·7	39	SASP, ST
19. RM	0	TI (10 cm) CT (20 cm)	Stenosis (TI)	242	26·7	26	3·8	35	SASP, ST
20. FG	0	TI (5 cm), tC	Enteral fistula EIM	485	80·9	18	3·7	41	SASP, ST
21. RP	0	TI (30 cm), tC	Enterovaginal fistula	261	123·0	115	2·7	33	SASP, ST
22. WL	0	TI (30 cm), tC	0	125	377·7	13	3·9	39	SASP, ST
23. DH	0	TI (30 cm), tC	0	182	168·6	66	3·1	41	SASP, ST
24. ZK	0	tC	0	48	42·1	24	4·4	38	SASP
25. ZI	0	tC	0	185	67·1	65	2·5	22	SASP, ST

Abbreviations: TI=terminal ileum, tC=total colon, CA=ascending colon, TC=transverse colon, CD=descending colon, AR=relapse at anastomosis, EIM=extraintestinal manifestation, SASP=salazosulphapyridine, ST=methylprednisolone, METRO=metronidazole.

patients (one of them also having anal fistula), extraintestinal manifestations (arthritis and erythema nodosum) in two patients (one also having anal fistula), perianal complications only in two patients and internal fistulae occurred in two patients.

In all patients the CDAI[1] was established. In the Table data on ESR, serum albumin concentrations, haematocrit and therapy are also given.

In 10 patients α_1AT clearance was compared with faecal excretion of intravenously injected ^{51}Cr-albumin and with the intestinal ^{51}Cr clearance.

DETERMINATION OF ALPHA$_1$-ANTITRYPSIN

Alpha$_1$-antitrypsin of serum and faeces was determined by radial immunodiffusion. The diameter of the precipitation ring of the plates containing a monospecific antigen against α_1AT (Behringwerke) was measured after 72 hours and compared with a reference curve established from the diameters of rings of a standard solution. Faeces and sera were kept at −20°C until used. The volume of the three day stool samples was calculated by weight and specific gravity. Specific gravity was determined by weighing a volume of 5 ml of the homogenised stool. An aliquot of 2 g of the homogenised stool was diluted 1:4 with normal saline or for comparison with aqua dest, and again homogenised for 10–60 minutes. After centrifugation at 1500–3000 *g* for 10–30 minutes 5 μl of the supernatant was applied to the plates. The sera obtained at the beginning of stool sampling were diluted 1:5 in normal saline and 5 μl of the samples were applied to the plates. In five samples the

determinations were repeated after storage of faeces and sera for eight months at −20°C. Faecal α_1AT loss was calculated from faecal α_1AT concentration and stool volume. α_1AT clearance was calculated by the formula

$$\alpha_1\text{AT clearance} = \frac{\text{faecal volume} \times \alpha_1\text{AT faecal concentration}}{\alpha_1\text{AT serum concentration}}$$

^{51}Cr-ALBUMIN METHOD

The radioactivity of the three day stool samples (Behringwerke) was measured, after intravenous injection of 10 μCi ^{51}Cr-albumin, with a gamma counter. The ^{51}Cr-serum concentration was calculated from the administered ^{51}Cr-dose and from the total serum volume calculated by conventional formulas.[11] The formula for computing the ^{51}Cr clearance corresponded to that for α_1AT.

Results

There was a good correlation between α_1AT clearance and ^{51}Cr excretion (r=0·84, α=5%) or ^{51}Cr clearance (r=0·87, α=5%). The relation between α_1AT and ^{51}Cr clearance as expressed by a linear equation was y=0·73+4·42 (Fig. 1). The different methods of treating the faeces with normal saline or aqua dest, the duration of homogenisation, and the rpm of centrifugation, had no influence on the results. After storage of the stool and the serum at −20°C for eight months the recovery rate of α_1AT was more than 90%.

In our experiments the faecal loss of α_1AT in patients with Crohn's disease (average 308 mg/d, range 22–785 mg/d) was higher than in controls (average 54 mg/d, range 0–136 mg/d) (Fig. 2). When the clearance of α_1AT was calculated there was a better separation (Fig. 2) of patients with Crohn's disease (average 93 ml/d, range 19–367 ml/d) from controls (5 ml/d on an average, range 0–22 ml/d) and with only one exception α_1AT clearance was raised above control values in all patients. As shown in Fig. 3 there was no difference between patients with involvement of the small intestine only, and the colon only. The clearance of α_1AT in patients with skip lesions of the large bowel was similar to those with total colonic inflammation and the same findings applied to the inflammation of the ileum. Three patients with inflammation of both colon and ileum had higher values than those who had only the ileum or the colon involved. On the other hand three patients with relapse of Crohn's disease over a small segment adjacent to the anastomosis had higher values than others with total colonic and/or ileal involvement. Even after curative resection

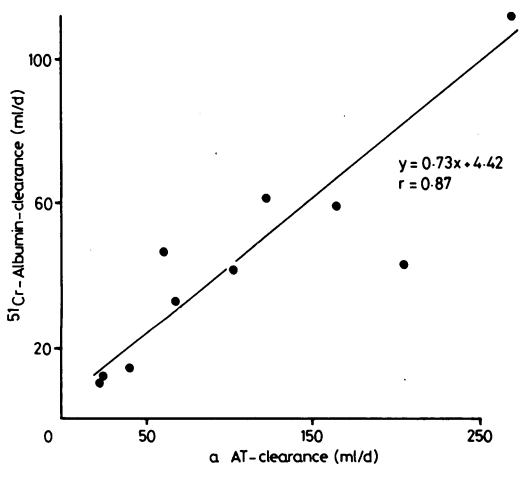

Fig. 1 *Relation between α_1AT clearance and ^{51}Cr-albumin clearance.*

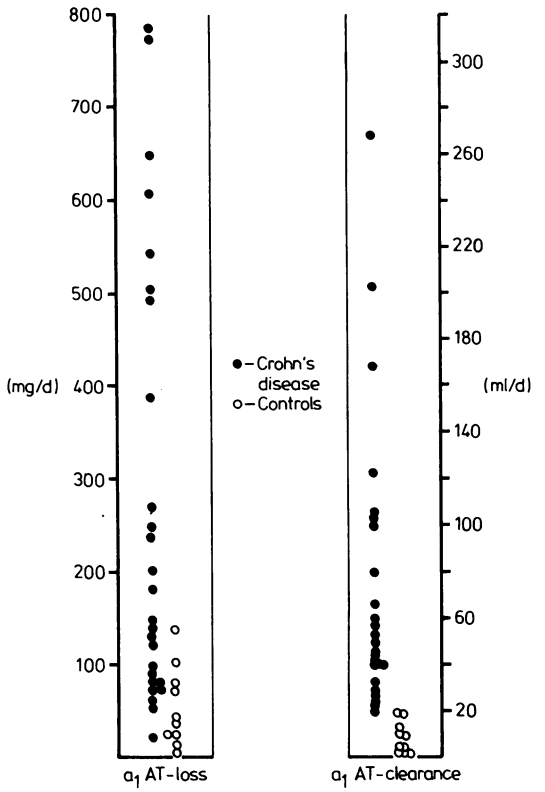

Fig. 2 *Faecal α_1AT loss and intestinal α_1AT clearance in patients with Crohn's disease and controls.*

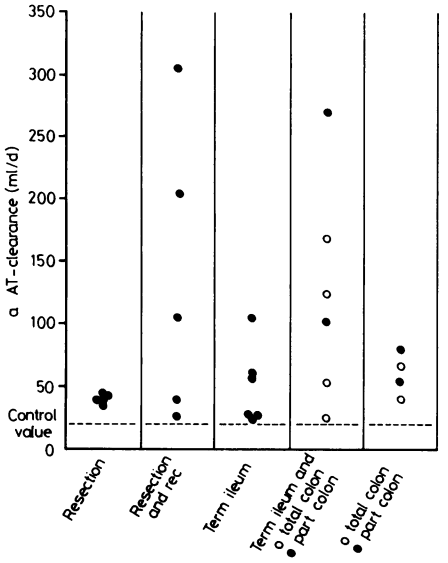

Fig. 3 *Site and extent of Crohn's disease and α_1AT clearance (rec=recurrence, term ileum=terminal ileum, part colon=partial colon).*

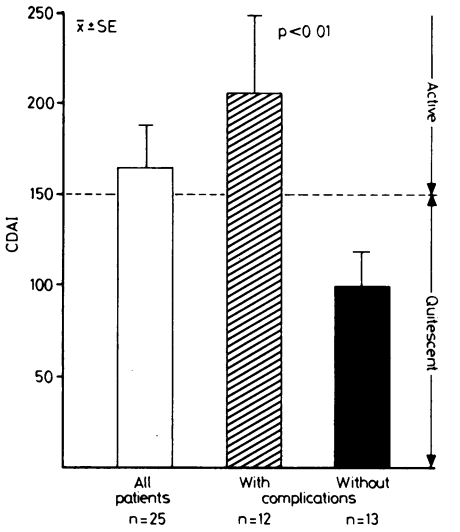

Fig. 5 *Activity index (CDAI) in patients with Crohn's disease and under consideration of complications such as intestinal stenosis, anal fistula, and extraintestinal manifestations.*

faecal α_1AT was raised. The increase of faecal α_1AT in the resected patients was about twice above control values. There was no correlation between activity index and α_1AT clearance (Fig. 4). Patients with inflammation in remission (activity index <150) had values of α_1AT clearance similar to patients with a CDAI higher than 400.

In patients with complications such as fibrous strictures, anorectal fistulation, or extraintestinal manifestations, the mean CDAI (206±43) was significantly higher ($p<0{\cdot}02$) than in patients without complications (99±19) (Fig. 5).

The mean α_1AT clearance in patients without complications (104·9±26·5 ml/d) was raised above

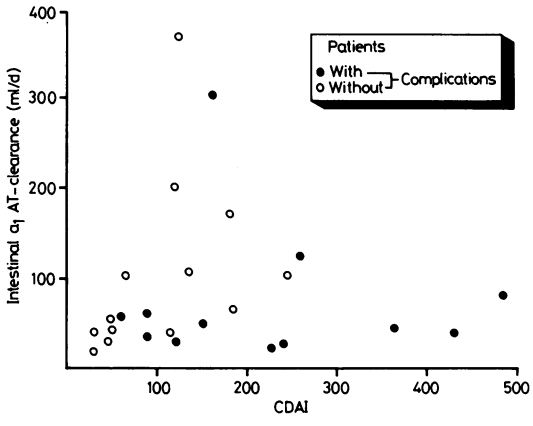

Fig. 4 *Activity index (CDAI) in patients with Crohn's disease in comparison to intestinal α_1AT clearance.*

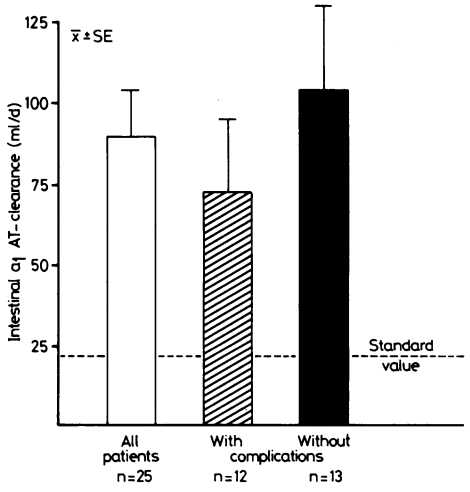

Fig. 6 *Intestinal α_1AT clearance in patients with Crohn's disease and under consideration of complications.*

the values of patients with complications (73·1±22·8 ml/d) (Fig. 6).

Discussion

Similar to the results of Florent and coworkers[12] we found a linear relationship of the intestinal protein loss measured by ^{51}Cr or by α_1AT clearance. The slope of the curve comparing α_1AT and ^{51}Cr-albumin clearance (Fig. 1) should be unity provided both proteins follow the same mechanism of transmucosal exudation, whereas a value of 0·73 was calculated from our results. There is some destruction of α_1AT in gastric juice of pH below 3,[13] but from the results of Crossley and Elliot[7] it is unlikely that α_1AT is degraded in the small or large bowel to any significant degree nor is ^{51}Cr absorbed to any appreciable extent.[14] Moreover, the molecular weights of α_1AT[15] and albumin are comparable and no significant difference exists in exudative enteropathy in the loss of small or large proteins. Höfer *et al*[16] found that chrominated albumin is present in a monomer fraction and in an aggragated ^{51}Cr-albumin fraction. The metabolic behaviour of the aggregate and monomer fraction is different. The aggregate fraction is stored in the liver in a greater amount than the monomer fraction followed by a smaller intestinal exudation, which could possibly explain the difference between α_1AT and ^{51}Cr clearance.

Differences in preparing stool such as dilution, homogenisation, or centrifugation, had no influence on the determination of α_1AT. Therefore the contradictory results of Bernier *et al*[8] and Haeney *et al*[9] cannot be explained in that way.

Our results confirm those of Bernier *et al*[8] in that no overlap of α_1AT clearance was seen in patients and controls, and all patients had raised α_1AT clearance levels (Fig. 2). If all patients with Crohn's disease lost serum proteins into the intestinal lumen one should expect a quantitative correlation of intestinal protein loss or serum albumin concentrations and extent and/or severity of disease.[13 17] This was not so in our cases. Even patients with apparent curative resection had raised values while others with a high activity index or those with extensive involvement had relatively minor protein losses (Figs. 3, 4). An explanation for this paradox may be that normal macroscopic appearance of the mucosa does not exclude histological involvement with changes characteristic of Crohn's disease.[3 18 19] Lymph drainage may be impaired and protein exudation increased if the mesenteric and lymph nodes are involved, features which cannot be diagnosed by radiology. On the other hand quiescent disease may still show impressive radiological changes.

Florent *et al*[13] found an exceptionally good correlation between the intestinal α_1AT clearance and the clinical activity assessed by the CDAI. From our results it is not possible to ascertain any coherence between CDAI and intestinal protein loss. The important objection to the CDAI is, that it is largely determined by subjective variables that may not generally be reliable indicators of the intestinal inflammatory activity but often result from complications or residual effects of the disease. It is obvious that considerable subjective complaints like fibrous strictures, anorectal fistulation, extra-intestinal manifestations, or conglomerate tumour may not necessarily be related to the severity of intestinal inflammation.

Accordingly, in patients with complications the mean CDAI was higher than in patients without complications (Fig. 5). In contrast the mean α_1AT clearance in patients without complications was raised above the values of patients with complications (Fig. 6). From this comparison it is obvious that in patients suffering from complications the CDAI reflects a much higher clinical activity as it is evident from the intestinal protein loss indicating severity of the local intestinal inflammation.[14]

Discrepancies between the quantity of intestinal protein loss and the activity of Crohn's disease estimated by the Best index may be explained by the absence of mucosal parameters as determining factors for this index. The same reason may apply for the six of 14 patients of Florent *et al*[13] who had raised α_1AT clearances in spite of a normal Best index and developed a relapse on follow up.

Selection of patients may be another cause explaining differences of the studies. Twenty-eight out of the 30 patients of Florent *et al*[13] with Crohn's disease had marked hypoalbuminemia (mean serum albumin concentration 2·73±0·53 g%). Only nine out of 26 of our patients had albumin concentrations below 3·6 g% (mean value 3·80±0·57 g%).[17 20] Furthermore, these authors studied patients without previous intestinal surgery whereas 10 of our patients had undergone intestinal resection and complications were not documented.

Protein loss *via* the intestinal wall seems to be one of the most accurate parameters to measure the inflammatory activity in the intestine.[14] So the determination of α_1AT by radioimmunodiffusion may be a convenient and reproducible objective method to assess severeness of intestinal inflammation.

Further investigations will show if the α_1AT clearance is to be of use in following the course of illness or the efficacy of therapy in patients with Crohn's disease.

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