

Reassessment of faecal α -1-antitrypsin excretion for use as screening test for intestinal protein loss

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SUMMARY Faecal α -1-antitrypsin and ^{51}Cr -albumin losses in 42 patients with either gastrointestinal or hepatic disease were compared. The reference range was derived from measurements in 20 controls without gastrointestinal disease.

Alpha-1-antitrypsin excretion was increased in patients with excessive ^{51}Cr -albumin loss, and correlations were found between α -1-antitrypsin clearance and ^{51}Cr -albumin excretion. Because of the considerable overlap of faecal α -1-antitrypsin excretion between controls and patients, sensitivity and specificity of the test were only 58% and 80%, respectively. This poor reliability could not be explained by sampling error or temporal variations in α -1-antitrypsin excretion.

These results show that although faecal α -1-antitrypsin excretion correlates with ^{51}Cr -albumin excretion when whole groups of patients are studied, its poor sensitivity makes it an unreliable measure of enteric protein loss.

Gastrointestinal protein loss is traditionally measured by estimating faecal excretion of ^{51}Cr -albumin.¹ Because this test requires the use of a radioisotope and a minimum five day faecal collection, measurement of α -1-antitrypsin excretion has been proposed as a less troublesome and equally reliable alternative. Alpha-1-antitrypsin is a broad spectrum protease inhibitor that is synthesised in the liver and is resistant to proteolytic degradation within intestinal secretions and faeces²; its intestinal clearance is thought to parallel that of albumin.

Only two groups of workers have attempted to validate α -1-antitrypsin excretion as a measure of enteric protein loss by direct comparison with ^{51}Cr -albumin excretion. Florent *et al*³ found a highly significant correlation between the two methods, but Haeney *et al*⁴ did not. Despite this disagreement α -1-antitrypsin excretion has since been adopted by several investigators as the method of choice in measuring faecal protein loss.⁵⁻⁷

We therefore set out to compare α -1-antitrypsin and ^{51}Cr -albumin excretion in patients with a wide range of gastrointestinal diseases, and we also sought to examine the possibility that the discrepant results of previous studies might be due to differences or errors in the methods used to estimate α -1-antitrypsin excretion.

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Patients and methods

Faecal protein loss was measured in 42 patients with gastrointestinal or hepatic disease by simultaneous determinations of faecal α -1-antitrypsin and ^{51}Cr -albumin excretion: 22 patients had Crohn's disease, five had cirrhosis, four had ulcerative colitis, two each had postgastrectomy malabsorption, immunodeficiency, chronic pancreatic insufficiency, and coeliac disease, and there were single cases of gastrojejunocolic fistula, intestinal lymphangiectasia, and radiation enteritis.

In 35 of these patients α -1-antitrypsin and ^{51}Cr -albumin excretion were compared over five days; in seven patients (four with Crohn's disease; one each with coeliac disease, intestinal lymphangiectasia, and radiation enteritis), of whom five had ^{51}Cr -albumin excretion of >1%, the study period was extended to eight days.

To provide a reference range faecal excretion of α -1-antitrypsin was measured in random stool samples (24 hour collection only) from a group of 20 inpatients without gastrointestinal symptoms or disease. None had conditions or was receiving any drugs likely to cause a protein losing enteropathy.

Approval for this study was given by the ethical committee appointed by the Salford District Health Authority, Salford.

MEASUREMENT OF FAECAL ^{51}Cr -ALBUMIN LOSS

Faecal ^{51}Cr -albumin loss was measured after intravenous injection of 4mBq $^{51}\text{CrCl}_3$ (Amersham International, United Kingdom), stool samples being collected as described above. On the third day a venous blood sample was obtained for measurement of ^{51}Cr -albumin activity and serum α -1-antitrypsin concentration. Radioactivity was measured with a LKB Compugamma gamma counter (LKB Instruments Ltd, South Croydon, Surrey, United Kingdom) and a counting time of 100 seconds.

Faecal ^{51}Cr -albumin excretion was expressed as a percentage of the injected dose excreted during the five or eight day collection period in all 42 patients with gastrointestinal or hepatic disease. In 25 of these faecal ^{51}Cr -albumin excretion was also expressed as a clearance rate (ml/24 hours) derived from the formula, $C = FV/S$, where F is mean faecal radioactivity (cpm/l); V is mean faecal volume (ml/24 hours); and S is serum radioactivity (cpm/l). A faecal ^{51}Cr -albumin loss exceeding 1% of the injected dose is widely accepted to be abnormal and thus indicative of protein losing enteropathy.^{1 8 9}

MEASUREMENT OF FAECAL α -1-ANTITRYPSIN

In 35 patients with gastrointestinal or hepatic disease faeces were collected for five days. From each five day collection, one 5 g sample was removed, frozen at -20°C , and analysed separately. The remainder of the five day collection was homogenised with deionised water in an homogeniser (AJ Seward, London, United Kingdom) for five minutes and a 15 ml aliquot frozen at -20°C . These frozen samples were lyophilised and ground to a fine powder with a pestle and mortar. Lyophylate (250 mg) was dissolved in 5 ml 0.15 sodium chloride by vigorous mixing in a mechanical shaker for 60 minutes. The sample was centrifuged at 1000 g for 10 minutes and the supernatant used for determining the concentration of α -1-antitrypsin.

In a further seven patients with gastrointestinal disease 24 hour faecal collections were made on eight consecutive days. Each 24 hour sample was separately homogenised and then processed as described above.

CONTROLS

Faeces were collected for 24 hours only, and a 5 g sample was removed, frozen, and lyophilised. The remainder of this collection was homogenised, lyophilised, and reconstituted as described above.

MEASUREMENT OF α -1-ANTITRYPSIN CONCENTRATIONS

α -1-antitrypsin was measured by single radial immunodiffusion (LC Partigen, Behringwerke, West Germany). Ring diameters were read at 72 hours and

the concentration calculated from a reference curve prepared from a commercial standard (LC-V, Behringwerke).

Faecal α -1-antitrypsin excretion was expressed as: (i) mg α -1-antitrypsin/g dry weight of lyophilised faeces; (ii) g α -1-antitrypsin/l of faeces; and (iii) as an intestinal clearance derived from the formula $C = FV/S$, where C is α -1-antitrypsin clearance in ml/24 hours; F is faecal α -1-antitrypsin concentration in g/l; V is faecal volume in ml/24 hours; and S is serum α -1-antitrypsin concentration in g/l.

To evaluate the recovery of the assay system α -1-antitrypsin concentration was measured in six 20 g stool samples. These were obtained from a single 24 hour collection from a normal volunteer. To each sample a given volume (0, 50, 100, 200, 300 and 400 μl) of commercial standard α -1-antitrypsin (concentration 1.7 g/l) (Sigma Laboratories, Poole, Dorset, United Kingdom) was added. The concentration of added α -1-antitrypsin, which was unknown to the individual performing the assay, was determined and this result was then compared with that of the calculated concentration.

ANALYSIS OF DATA

Data from 24 hour collections in each of 20 normal subjects, from five day collections in each of 35 patients with gastrointestinal or hepatic disease, and from eight consecutive 24 hour collections in each of seven such patients were available for analysis. α -1-antitrypsin excretion in 5 g samples and in total 24 hour or five day collections were also compared in the 20 normal subjects and in the 35 patients with gastrointestinal or hepatic disease who completed five day collections.

The results were analysed in two ways: firstly, in terms of the overall correlation between various measures of α -1-antitrypsin and ^{51}Cr -albumin excretion, and, secondly, in terms of the sensitivity and specificity of α -1-antitrypsin as a measure of intestinal protein loss, when compared with ^{51}Cr -albumin.

The Shapiro-Wilk test¹⁰ was used to examine the distribution of faecal α -1-antitrypsin excretion within the control population. Excretion of ^{51}Cr -albumin and α -1-antitrypsin were compared by calculation of the correlation coefficient. Group means were compared by one way analysis of variance.

Results

RECOVERY OF THE α -1-ANTITRYPSIN ASSAY

Calculated and measured α -1-antitrypsin concentrations from the "spiked" samples correlated closely over the range of α -1-antitrypsin concentrations tested ($r = 0.956$; $p < 0.001$; slope 0.87), thus showing good recovery of α -1-antitrypsin by the extraction

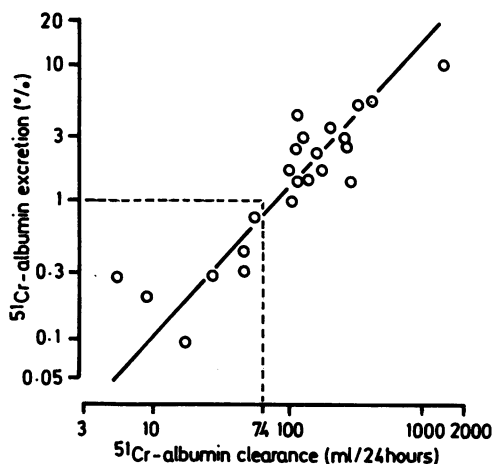


Fig 1 Relation of ^{51}Cr -albumin excretion (as percentage of injected dose) to ^{51}Cr -albumin clearance in 23 patients with gastrointestinal or hepatic disease. Excretion of 1% is equivalent to ^{51}Cr -albumin clearance of 74 ml/24 hours.

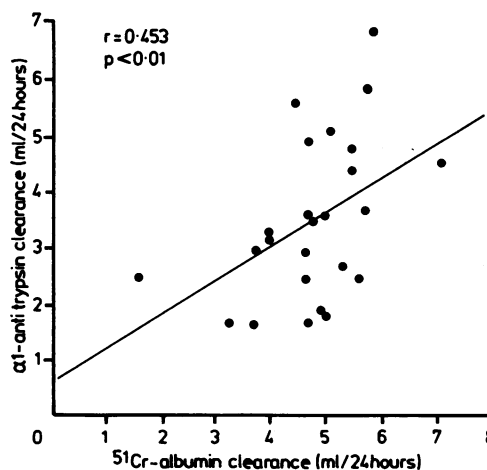


Fig 2 Comparison of clearances of ^{51}Cr -albumin and α -1-antitrypsin in 25 patients with gastrointestinal or hepatic disease in whom clearance studies were performed. Note that data are expressed logarithmically.

procedure used and confirming the precision of the radial immunodiffusion technique.

^{51}CR -ALBUMIN EXCRETION

^{51}Cr -albumin excretion exceeded 1% in 23 of 42 patients with gastrointestinal or hepatic disease: 19 with Crohn's disease; two with coeliac disease; and single cases of ulcerative colitis and gastrojejunal fistula. ^{51}Cr -albumin excretion and ^{51}Cr -albumin clearance showed a close linear correlation (fig 1), an excretion of 1% being equivalent to a ^{51}Cr -albumin clearance of 74 ml/24 hours.

α -1-ANTITRYPSIN EXCRETION

The distribution of α -1-antitrypsin values in control patients was positively skewed; consequently, all data were transformed logarithmically before statistical testing.

A highly significant correlation ($r = 0.94$; $p < 0.001$) was found between faecal α -1-antitrypsin concentrations in 5 g samples and in complete collections (either 24 hour or five day), thus confirming that a 5 g sample provides a representative measure of α -1-antitrypsin excretion. Furthermore, there was a

good correlation between α -1-antitrypsin excretion (mg/g dry faecal weight) and α -1-antitrypsin clearance ($r = 0.84$; $p < 0.001$ for 5 g samples; $r = 0.85$; $p < 0.001$ for both 24 hour and five day collections).

Analysis of results from the seven patients in whom each of eight consecutive 24 hour collections were analysed separately did not show any significant day to day variation in α -1-antitrypsin excretion; in particular, values for days 6, 7, and 8 did not differ significantly from days 1-5.

CORRELATIONS BETWEEN α -1-ANTITRYPSIN AND ^{51}CR -ALBUMIN

Linear regression analysis showed a significant correlation between clearances of α -1-antitrypsin and ^{51}Cr -albumin (fig 2) and between some measures of α -1-antitrypsin excretion and ^{51}Cr -albumin loss (expressed as a percentage loss of radioactivity) (table 1).

One way analysis of variance showed that α -1-antitrypsin excretion was significantly increased in the 23 patients with gastrointestinal or hepatic disease with excessive faecal ^{51}Cr -albumin loss ($> 1\%$) compared with that of controls ($p < 0.001$) and in the

Table 1 Correlation between various measures of α -1-antitrypsin excretion and ^{51}Cr -albumin loss

| Method of sampling and measurement | No of samples tested | Correlation coefficient <i>r</i> | Significance of <i>p</i> value |
|--|----------------------|----------------------------------|--------------------------------|
| α -1-antitrypsin excretion in 5 g samples (measured as mg/g dry weight) | 25 | 0.58 | <0.005 |
| α -1-antitrypsin excretion in 24 hour and 5 day collections (measured as mg/g dry weight) | 38 | 0.38 | <0.05 |
| α -1-antitrypsin excretion in 24 hour collections (measured as g/l faeces) | 34 | -0.14 | >0.05 |

Table 2 Diagnostic specificities and sensitivities of α -1-antitrypsin estimation using different methods of sampling, compared with those used for ^{51}Cr -albumin excretion

| Sampling method | Normal upper limit ($M + 1.645 \text{ SD}$) | Diagnostic specificity (%) | Diagnostic sensitivity (%) |
|-------------------|--|-------------------------------|-------------------------------|
| 24 hour sample | 4.10 mg/g | 62 | 52 |
| 24 hour g/l | 1.26 g/l | 67 | 52 |
| 24 hour clearance | 45.6 ml/day | 80 | 58 |

19 patients with gastrointestinal or hepatic disease with a normal ^{51}Cr -albumin loss ($p < 0.025$). In this second group of patients with "normal" ^{51}Cr -albumin loss there was still a significant excretion of α -1-antitrypsin compared with that of controls (for samples, $p < 0.01$; for complete collection, $p < 0.025$).

SENSITIVITY AND SPECIFICITY OF α -1-ANTITRYPSIN

Based on a normal upper limit of 1% for faecal ^{51}Cr -albumin loss (equivalent to a ^{51}Cr -albumin clearance of 74 ml/24 hour) and our calculated upper limits of the reference range for α -1-antitrypsin excretion

and clearance (calculated as mean $M + 1.645 \text{ SD}$), the diagnostic specificities and sensitivities of each of the α -1-antitrypsin measures studied were calculated (table 2).

Fig 3 shows the overlap between the control subjects and the two categories of patients with gastrointestinal or hepatic disease for each of the measures of α -1-antitrypsin excretion.

Discussion

The major finding of this study was that while significant correlations could be shown between

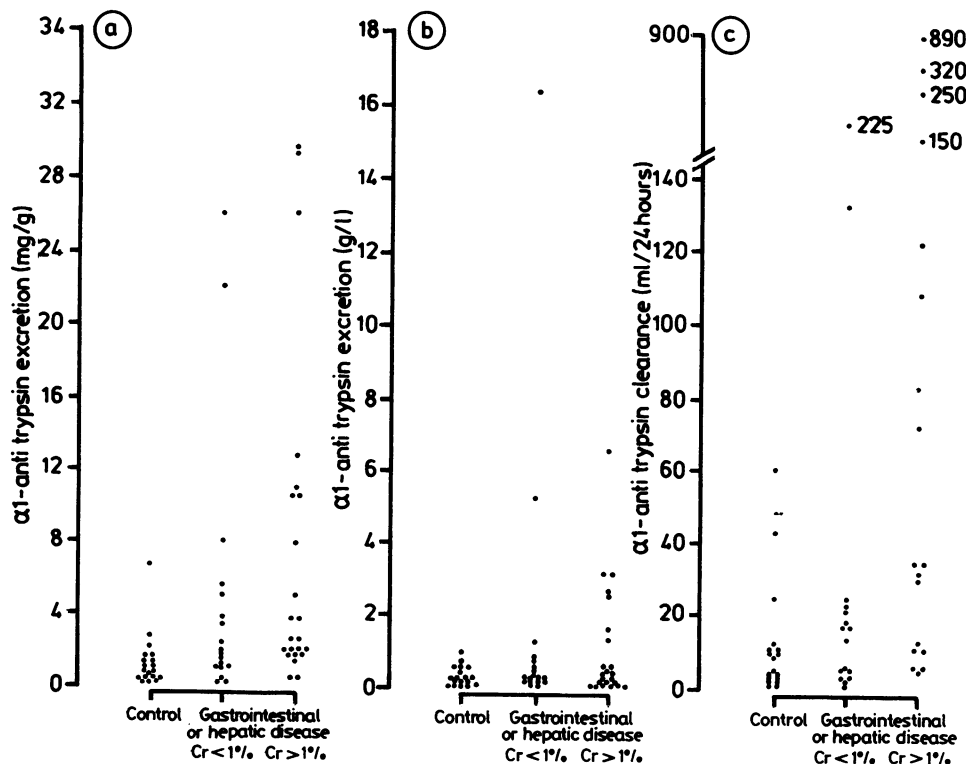


Fig 3 α -1-antitrypsin excretion measured as (a) mg/g dry weight of faeces; (b) g/l of faeces; and (c) clearance (ml/24 hours) in control subjects and in patients with gastrointestinal or hepatic disease with ^{51}Cr -albumin excretion of $< 1\%$, or ^{51}Cr -albumin excretion of $> 1\%$.

Faecal α -1-antitrypsin excretion

α -1-antitrypsin and ^{51}Cr -albumin excretion in the group of 42 patients with gastrointestinal or hepatic disease, the sensitivity and specificity of α -1-antitrypsin as a measure of intestinal protein loss in individual patients was poor. While confirming previous observations that an increase in α -1-antitrypsin excretion is usually associated with an increased loss (>1%) of ^{51}Cr -albumin, this study also showed that an increased excretion of α -1-antitrypsin does not invariably accompany an excessive loss of ^{51}Cr -albumin: conversely, α -1-antitrypsin excretion was normal in 10 of 18 patients with protein losing enteropathy (defined as a ^{51}Cr -albumin loss >1% of the injected dose).

The disappointing sensitivity of α -1-antitrypsin measurement cannot be explained in terms of faecal sampling errors, or the time over which collections were made. Indeed, we have shown that α -1-antitrypsin is homogeneously distributed in faeces, such that a 5 g sample always gives a representative value for α -1-antitrypsin excretion. Moreover, α -1-antitrypsin excretion measured over an extended study period of eight days, with individual analyses of each 24 hour collection, failed to show any significant day to day variation.

It was clearly established that α -1-antitrypsin excretion in an inpatient control population is not normally distributed: data must be expressed as a log normal distribution, a procedure which raises the upper limit of the reference range, thus reducing the sensitivity of the test. It should be noted that previous workers have assumed that their data were "normally" distributed^{3 6 7 11 12}; indeed, recalculation of the data of Haeney *et al*⁴ confirms a log normal distribution for α -1-antitrypsin clearance in their control patients; insufficient information precluded recalculation of distributions from data of other workers.

Alpha-1-antitrypsin should be the ideal marker for enteric protein loss because it is resistant to proteolytic digestion and its clearance is thought to parallel that of albumin. Proof that α -1-antitrypsin is a valid test, however, depends on showing an absolute correlation with enteric protein loss. Direct measurement of gastrointestinal protein loss is precluded both by the impossibility of achieving total recovery of all gastrointestinal secretions and by the intraluminal degradation of protein by digestive enzymes with subsequent absorption of the constituent amino acids.

Traditionally, enteric protein loss has been measured by determining the faecal excretion of a radioactive label following intravenous administration of radiolabelled macromolecules. Of the various radiolabels that have been studied, ^{51}Cr -albumin has proved the most reliable.¹³ ^{51}Cr radioactivity in

stools closely reflects albumin excretion, and the label is neither significantly absorbed from, nor secreted into, the gut¹⁴; this has therefore been used as the "gold standard" in this study. It must be pointed out, however, that the accepted value for the upper limit of the reference range for ^{51}Cr -albumin loss—that is, 1% of the injected dose—is derived from the published reports and is not usually validated in individual laboratories because of the ethical constraints limiting radioisotopic studies in healthy people.^{1 8 9}

Although we agree with Florent *et al*³ that there is a significant correlation between ^{51}Cr -albumin loss and α -1-antitrypsin excretion in selected groups of patients, this relation does not necessarily hold for individual cases. The presence of false negative and false positive results (table 2) affirms that α -1-antitrypsin excretion does not invariably reflect increased enteric protein loss. There may be several reasons for this discrepancy. If the factors affecting the clearance of α -1-antitrypsin and albumin were identical then the slope of the regression line should be 1. In fact, Florent *et al*³ found a slope of only 0.55, while in this study it was 0.61, indicating that for every unit increase in ^{51}Cr -albumin clearance, the increase in α -1-antitrypsin clearance is proportionally less than that predicted. It therefore seems evident that the mechanisms of clearance of these two proteins, or the factors affecting their distribution within the gastrointestinal tract, are not identical.

One possible explanation is that the clearance of these two proteins depends on their rates of biliary excretion. Although albumin and α -1-antitrypsin are both present in bile, albumin clearance is probably independent of bile flow, as in healthy subjects most of the albumin within the intestinal lumen is completely digested and reabsorbed.¹⁵ If bile flow were reduced, however, for example by starvation, the clearance of α -1-antitrypsin should be affected more than that of albumin.

Alternatively, a constant amount of α -1-antitrypsin may be destroyed in the gastrointestinal tract. α -1-antitrypsin is known to be degraded at pH < 3; a falsely high α -1-antitrypsin clearance would therefore be expected in patients with hypochlorhydria or achlorhydria.³ Similarly, α -1-antitrypsin excretion will underestimate protein loss from the stomach, as shown in Menetrier's disease.¹⁶

The situation is further complicated by the recent demonstration that α -1-antitrypsin is excreted in faeces in two forms; as a protease-antiprotease complex and in a form that is relatively unchanged compared with serum α -1-antitrypsin.¹⁷ The proportion of α -1-antitrypsin excreted as a complex showed considerable variation between subjects. Furthermore, formation of the protease-antiprotease complex leads to an artefactual decrease in the apparent

α -1-antitrypsin concentration when either radial immunodiffusion or immunonephelometry are used to measure α -1-antitrypsin.

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