Faecal $\alpha_1$ antitrypsin as a marker of gastrointestinal disease in HIV antibody positive individuals

D Sharpstone, A Rowbottom, M Nelson, B Gazzard

Abstract
Hypoalbuminaemia and diarrhoea are common complications of HIV infection and substantial causes of morbidity, but the specific intestinal pathologies that cause enteric protein loss have not been clearly defined. Two hundred and twenty stool samples from patients with a variety of HIV related conditions were analysed for faecal $\alpha_1$ antitrypsin. Patients with intestinal Kaposi’s sarcoma had a significantly raised faecal $\alpha_1$ antitrypsin value and hypoalbuminaemia. A faecal $\alpha_1$ antitrypsin value of greater than 0·3 mg/g wet stool has a sensitivity of 94% and a specificity of 76% for the diagnosis of intestinal Kaposi’s sarcoma in HIV positive individuals.

Patients with cytomegalovirus and bacterial enteritis had raised faecal $\alpha_1$ antitrypsin values but levels were normal for all other intestinal pathologies compared with pathogen negative stool. The combination of faecal $\alpha_1$ antitrypsin concentration greater than 0·2 mg/g, a negative stool culture for enteric bacteria, and the absence of palatal Kaposi’s sarcoma has a sensitivity of 55% and specificity of 88% for the diagnosis of enteric cytomegalovirus infection.

(Gut 1996; 38: 206–210)

Keywords: faecal $\alpha_1$ antitrypsin, Kaposi’s sarcoma, cytomegalovirus.

Hypoalbuminaemia is a substantial cause of morbidity and an independent marker of mortality in subjects with AIDS. A low serum albumin may be caused by decreased protein intake,2 malabsorption,3 decreased synthesis,4 increased turnover,5 and enteric losses.6 $\alpha_1$ Antitrypsin concentrations increased in stool samples of patients with diarrhea, and this may result in stool loss of 1·0 mg/g protein.7 Increased malabsorption7 and increased protein intake8 contribute to diarrhoea. Decreased faecal $\alpha_1$ antitrypsin concentrations were associated with diarrhoea, which suggests that diarrhoea may play a role in the development of hypoalbuminaemia.

Patients and methods
All patients with diarrhoea and a CD4 lymphocyte subset count of less than 200 cells/mm3 attending the HIV outpatient clinics at the Chelsea and Westminster Hospital were requested to provide six routine stool samples that were cultured for faecal pathogens on appropriate media. Smears were acid-fast stained with auramine-phenol for cryptosporidia and acid-fast bacilli; if positive the diagnosis was confirmed by a modified Ziehl-Neelsen stain. Specimens were inoculated onto Löwenstein-Jensen slopes and cultured for mycobacteria. A standard formol-ether concentration method was used to look for ova and parasites by direct microscopy. Concentrated preparations were also examined for cryptosporidia if six standard preparations were negative and diarrhoea persisted. Three stools were also examined for microsporidia; spores were suspended in a calcofluor stain and Weber’s strong trichrome method. A cytotoxicity assay for Clostridium difficile toxin was carried out on one stool sample from all patients. If stool microscopy and culture were negative and the diarrhoea persisted for more than two weeks, oesophagogastroduodenoscopy with distal duodenal biopsies and sigmoidoscopy with biopsy were performed. Impression smears were made from one duodenal biopsy, air dried, and fixed for one or two minutes and then stained with Giemsa and examined under oil immersion ($\times$1000). All histological biopsy specimens were fixed in 10% formal saline and paraffin sections 2–5 $\mu$m thick were stained with haematoxylin and eosin, periodic acid Schiff (PAS), PAS-diastase, and Ziehl-Neelsen
stains. At least two levels per block were examined. Immunoperoxidase staining for cytomegalovirus (CMV) and adenovirus was performed on all rectal and duodenal biopsy specimens.

Between 1/9/93 and 1/12/94, 193 patients were referred to the specialist HIV-diarrhoea clinic at the Chelsea and Westminster Hospital and 159 provided a stool sample for measurement of faecal α1 AT and a serum sample for albumin before taking specific treatment. Endoscopy was performed within one month of the stool sample. Sixteen subjects with endoscopically diagnosed intestinal KS and a control group of 45 asymptomatic HIV positive individuals with peripheral CD4 counts of less than 200 cells/mm³ and no known enteric pathogens also provided stool specimens for faecal α1 AT and microbiology. Controls and subjects were matched with regard to CD4 count (±20 cells/mm³). Intestinal KS was diagnosed by typical endoscopic appearances and histological confirmation on biopsy specimens. Weight loss was defined as a loss of greater than 5% of pre-morbid weight.

MEASUREMENT OF FAECAL α1 AT

Stool samples were promptly frozen after collection and stored at −20°C for less than one month. All analyses were performed on coded samples so that the investigator was unaware of their origin. The stool was thawed at room temperature and 200 mg were weighed into a universal tube and emulsified in 2 ml of 0-9% (w/v) sodium chloride. This solution was then mixed on a rotamixer for 60 minutes at room temperature. The homogenised stool samples were then centrifuged at 10 000 g for 10 minutes at room temperature. Finally, the supernatant was removed and treated with β-propiolactone to inactivate the HIV. Samples were then filtered using a disposable 5 μm pore size filter to remove cellular debris. A rate nephelometer was used to quantify faecal α1 AT (Beckman Ltd, UK). The assay was standardised against the supraregional protein calibrant SPS-01. The interassay coefficient of variation (CV%) for faecal α1 AT was 12.1, and the intra-assay CV% was 7.6. Faecal α1 AT assays on duplicate samples from 18 patients and replicate determinations on 31 specimens showed no significant variance (p>0.05). The mean differences of the paired observations were 0-07 and 0-05 mg/g wet stool respectively. All stool samples were tested for occult blood using Okokit II (Hughes and Hughes Ltd, Somerset).

STASTICS

Results were analysed using the Mann-Whitney U test. A p value of less than 0.05 was taken as significant. Pearson’s coefficient was used to assess correlation between faecal α1 AT, weight loss, and serum albumin. Student’s t test was used to analyse duplicate and replicate samples. Mean (SD) values are given. Sensitivity, specificity, and positive predictive value (PPV) were calculated by standard methods. The receiver operating characteristic curves were plotted using sensitivity against one minus specificity.

Results

A total of 159 HIV positive subjects and diarrhoea, 16 with intestinal KS, and 45 controls provided a specimen for faecal α1 AT (Fig 1, Table I). Patients with bacterial enteritis comprised four with Salmonella spp, four with Campylobacter spp and three with Clostridium difficile. The “other” group comprised seven patients with dual infections, seven with Mycobacterium avium complex, six with Giardia lamblia, six with Entamoeba histolytica, three with enteric viruses, one with a small bowel lymphoma, one with Isospora belli, and one with a rectal carcinoma.

Faecal α1 AT was significantly greater in subjects with enteric CMV compared with all groups other than bacterial enteritis or intestinal KS. Enteric CMV comprised four patients with oesophageal, one with gastric, and six with colonic disease. The mean faecal α1 AT value in patients with colonic CMV (0.6 (0.5) mg/g) was higher than in patients with upper gastrointestinal CMV (0.4 (0.3) mg/g), although the difference was not statistically significant. One patient with CMV colitis and one with CMV gastritis had stool positive for occult blood (faecal α1 AT of 1 mg/g and 0.7 mg/g, respectively).

Faecal α1 AT was significantly raised in people with intestinal KS compared with all other patient groups. The mean faecal α1 AT
value in subjects with both upper and lower gastrointestinal KS was significantly greater than in patients with KS isolated to either the stomach or duodenum (Table II). The mean faecal α1 AT was significantly higher in stool positive for faecal occult blood than in negative stool. There was no significant difference in the faecal α1 AT values in patients receiving systemic chemotherapy compared with untreated subjects.

There was no correlation between weight loss or serum albumin and faecal α1 AT, although the mean serum albumin in the intestinal KS group (23·5 (7), range 12–36 g/dl) was significantly less than the mean serum albumin (32 (4·6), range 10–44 g/dl) of all study subjects.

Serum albumin or faecal α1 AT were not correlated with abnormalities of other liver function tests (seen in 58%) or positive serology for hepatitis B or C (3%) or antiretroviral drugs (zidovudine, zalcitabine, or didcoxysenadine) (38%).

Table II  Faecal α1 antitrypsin (Fa1 AT) and intestinal Kaposi's sarcoma (KS)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (SD) Fa1 AT (range) mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated gastric KS (3)</td>
<td>0·6 (0·2) (0·5–0·8)</td>
</tr>
<tr>
<td>Isolated duodenal KS (3)</td>
<td>0·7 (0·2) (0·2–1·3)</td>
</tr>
<tr>
<td>Upper gastrointestinal KS (6)</td>
<td>1·2 (1) (0·2–3·6)</td>
</tr>
<tr>
<td>Upper and lower gastrointestinal KS (4)</td>
<td>2·3 (2·5) (0·5–6·0)</td>
</tr>
<tr>
<td>Positive FOB (4)</td>
<td>3·2 (1·0) (1·5–6·0)</td>
</tr>
<tr>
<td>Negative FOB (12)</td>
<td>1·0 (0·7) (0·2–2·6)</td>
</tr>
<tr>
<td>Systemic chemotherapy (12)</td>
<td>1·6 (1·7) (0·2–6·0)</td>
</tr>
<tr>
<td>No systemic chemotherapy (4)</td>
<td>1·2 (0·7) (0·5–2·1)</td>
</tr>
</tbody>
</table>

FOB = faecal occult blood

Discussion

The main aim of this study was to see whether enteral protein loss was a common feature of HIV disease, was associated with diarrhoea, or, as shown, was seen only in individuals with specific gut pathologies.

Mild villus blunting and an increase in small bowel permeability are features of HIV infection at all stages of disease as assessed by CD4 count and these abnormalities become more pronounced in enteric protozoal infection. As protein losing enteropathy is more related to the integrity of the vascular endothelium within the gut than permeability, it is not surprising that faecal α1 AT is not high in asymptomatic HIV infection, and pathogen negative or protozoal diarrhoea. This concept is supported by the raised faecal α1 AT value found in bacterial diarrhoea, CMV and intestinal KS.

Bacterial diarrhoea is associated with high faecal α1 AT values in people who are not immunosuppressed, presumably because of vascular disturbances associated with cytokine release. CMV has a predilection for vascular endothelial cells and an ischaemic colitis is well described in CMV colitis in HIV positive patients. The neoplastic cell line of KS remains debated but it is clearly a tumour with a major vascular component. Although the raised faecal α1 AT seen in our patients might be due to a generalised abnormality of gastrointestinal vascular integrity associated with KS, this seems unlikely as the faecal α1 AT values were related to the tumour burden.

The second aim of this study was to see if a raised faecal α1 AT value predicts specific pathologies within the gastrointestinal tract of HIV positive patients. Although stool analysis readily diagnoses bacterial infection, both intestinal KS and CMV can present diagnostic problems and may require extensive investigation. Visceral KS is frequently asymptomatic but is associated with a worse prognosis. Some groups have shown the value of palatal KS in the diagnosis of intestinal KS but others have not confirmed this.

Although in our study palatal KS is probably a better qualitative measure of intestinal KS than faecal α1 AT, with a higher PPV and specificity but a lower sensitivity, it is not a quantitative measure. This may be important as higher faecal α1 AT values were found in individuals with a larger tumour load, which is likely to carry a worse prognosis.

Figure 2: A receiver operating characteristic curve for faecal α1 antitrypsin in the diagnosis of intestinal Kaposi's sarcoma.
Although the present study was prospective and the results obtained were statistically significant, the value of faecal $\alpha_1$ AT in the evaluation of enteric protein loss and the diagnosis of KS of the gut can be criticised. The sensitivity, specificity, and PPV apply only in an HIV-positive population. If the HIV status of an individual is unknown, the likelihood of KS or CMV of the intestine is much lower and the sensitivity, specificity, and PPV would be different. Faecal $\alpha_1$ AT values in intestinal KS may be reduced by systemic chemotherapy as has been described with abdominal radiotherapy and may reflect different KS measured in a cohort associated with systemic impairment and with serum concentrations associated with systemic disease might have an impact on the faecal values and were not measured in this study. Previous careful balance studies using 24 hour stool collection indicate that serum values do affect the assessment of enteric protein loss, and a study of HIV-positive patients with cryptosporidiosis has shown that the faecal $\alpha_1$ AT value may be mildly raised if such studies are performed. The complexity and difficulty of performing these studies, however, probably outweigh the slight improvement in accuracy of the results obtained.

Further studies are needed to assess fully the PPV of raised faecal $\alpha_1$ AT values in HIV-positive individuals. A larger study of individuals with skin KS, both with and without diarrhea and before and after chemotherapy, would be useful to assess the clinical relevance of faecal $\alpha_1$ AT in these patients.

CMV of the gut requires endoscopy and biopsy, and in perhaps a third of individuals with isolated right sided colitis, total colonoscopy is required. It is possible that a raised faecal $\alpha_1$ AT in individuals with diarrhea without palpable KS or bacterial enteritis might be a useful guide to performing such investigations. Confirmation of the utility of faecal $\alpha_1$ AT again requires prospective studies.

Hypoalbuminaemia carries a poor prognosis in HIV positive patients and its causes are multifactorial. Protein losing enteropathy may also play a part in individuals with enteric KS where hypoalbuminaemia seems to be particularly common in the terminal phase. This has previously been described in case reports, and our results suggest this might be a more general phenomenon. The multifactorial nature of hypoalbuminaemia in these patients makes it unsurprising that faecal $\alpha_1$ AT values did not correlate with serum albumin, although this does not negate the impact of enteric protein loss in late stage HIV disease in some individuals.


