Investigation of chronic diarrhoea in acquired immunodeficiency syndrome. A prospective study of 155 patients

C Blanshard, N Francis, B G Gazzard

Abstract

Background and Aims—The optimum diagnostic investigation for patients with acquired immunodeficiency syndrome (AIDS) and diarrhoea is not known. Often no pathogen is detected and it is unclear whether this is because pathogens are absent in some patients or the investigations used fail to detect them. The hypothesis that AIDS related diarrhoea is usually due to an infection, which can be identified by a simple diagnostic strategy based on the results of intensive investigation of a cohort of such patients, was investigated.

Methods—155 patients with AIDS and chronic diarrhoea underwent contemporaneous examination of stools, duodenal, jejunal, and rectal biopsy specimens and duodenal aspirate for bacterial, protozoal, and viral pathogens. A decision tree analysis was used to determine the best sequential diagnostic strategy for clinicians.

Results—128 of 155 patients investigated (83%) had at least one potential pathogen. The presenting clinical features could not predict the presence or site of the pathogens. Stool analysis identified the most pathogens (93 of 199, 47%). Rectal biopsy was essential for the diagnosis of cytomegalovirus and adenovirus. Duodenal biopsy was as helpful as jejunal biopsy and detected some treatable pathogens missed by other methods. Electron microscopy, impression smears, and duodenal aspirate yielded little extra information. If gut biopsy was reserved for patients without a stool pathogen, some treatable pathogens would have been missed.

Conclusion—Most patients with AIDS and chronic diarrhoea have at least one gut pathogen, which can be identified by stool analysis and light microscopic examination of duodenal and rectal biopsies. Some pathogens will be missed unless all these investigations are done on all such patients.

(Gut 1996; 39: 824-832)

Keywords: diarrhoea, investigation, human immunodeficiency virus, acquired immunodeficiency syndrome, diagnosis, decision analysis.

A variety of opportunistic and non-opportunistic pathogens may cause diarrhoea in

patients infected with the human immunodeficiency virus (HIV), and some patients have no pathogen identified even after extensive gastroenterological investigations.¹⁻¹⁵ It is unclear whether this is because known pathogens are genuinely absent or because the investigations performed lack sensitivity. Several algorithms have been proposed to investigate HIV related diarrhoea, with the aim of identifying all pathogens or detecting treatable pathogens at least cost. In 1990, Johanson and Sonnenberg¹⁶ wrote an influential paper in which a medical decision analysis suggested that the most cost effective way of alleviating the diarrhoea in a cohort of 1000 theoretical patients was to perform a single stool culture, with specific treatment for those patients with an identifiable bacterial pathogen and symptomatic treatment with diphenoxylate for the rest.

However, this model is no longer applicable. The authors assumed that diarrhoea would resolve in 67% of patients treated with diphenoxylate, regardless of the cause, based on a small uncontrolled study of patients with cryptosporidial diarrhoea.¹⁷ It was also assumed that there was no effective treatment available for CMV colitis, which is no longer true.¹⁸⁻²² Furthermore the prevalence of infections may have changed as microsporidia have become more widely recognised. The accurate identification of the causes of diarrhoea in HIV positive patients allows appropriate treatment and counselling about the prognosis, as well as entry into trials of new potential treatments. However, performing extensive investigations on all patients is expensive, time consuming, and potentially uncomfortable for the patient. It is not known which of the currently available investigations are the most useful or the order in which they should be carried out. We have therefore prospectively studied a large cohort of AIDS patients with chronic diarrhoea to see how many have pathogens and discover if the results of an intensive investigation protocol can be used to construct guidelines for the investigation of diarrhoea in such patients.

Methods

PATIENTS

The aim of the study was to enrol all patients presenting over a two year period with a CD4

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Accepted for publication
22 July 1996

lymphocyte count of less than 200 cells/ μ l and uninvestigated diarrhoea for at least four weeks (defined as the passage of at least three liquid or semi-liquid stools daily on at least five days of each week). Patients were recruited from a hospital HIV Clinic, two hospital Genito-urinary Medicine clinics, and hospital in-patients. All the staff working with HIV seropositive patients in the clinics and on the wards were made aware of the aims and design of the study and encouraged to refer patients. In addition there was an open meeting for patients and their carers at which the investigation and management of diarrhoea was discussed, and a notice about the study appeared in the 'Body Positive' newsletter, which is widely read by HIV infected subjects. Regular verbal and written reminders were circulated.

Completeness of case ascertainment

The completeness of case ascertainment was checked to exclude the possibility of referral bias in the cohort. This was done in three ways.

(1) A record was kept of patients referred who did not attend for appointments or investigation or who did not complete investigations were noted.

(2) A computer search was made to identify those patients not included in the cohort presenting with a known cause of chronic diarrhoea presenting during the study period; those with a diagnosis of 'diarrhoea for more than a month, no cause found', and those with wasting syndrome (weight loss of greater than 10% of normal body weight with diarrhoea or fever and weakness for more than a month).

(3) Pharmacy records were used to identify those patients who had received more than a month's supply of antidiarrhoeal drugs.

For all the above patients not included in the cohort the notes were reviewed to ascertain whether they fitted the inclusion criteria.

INITIAL ASSESSMENT

All patients were assessed by one author (CB) and a standardised clinical history taken. A full physical examination was performed including fundoscopy.

Blood was drawn for the following investigations if they had not been performed during the past two weeks: full blood count, CD4 count, serum urea and electrolytes, liver enzymes, protein, albumin, bilirubin, calcium, phosphate, vitamin B12, folate, red cell folate, blood glucose.

Patients were advised to bring three stool specimens with them by the referring member of staff (one from the previous morning, one from the previous evening, and one from that morning). These were labelled and transported to the laboratory, and containers were issued for a further three specimens to be brought at the next visit. The study was explained to participants and informed consent obtained.

ENDOSCOPIES

Sigmoidoscopy and endoscopy were performed within seven days of initial assessment, whether or not a potential pathogen was identified in the initial three stool specimens.

All of the endoscopies were done by one author (CB), using an Olympus GIFQ20 or GFXQ20 endoscope and intravenous sedation with midazolam in a dose titrated to produce drowsiness. The oesophagus and stomach were examined and biopsy specimens taken of any macroscopic abnormality. The endoscope was then passed into the duodenum and duodenal fluid aspirated into a sterile container after discarding the first 2–3 ml. Eight pinch biopsy specimens were taken from random sites in the distal second part of the duodenum and immediately placed in fixative or used to make impression smears.

Providing the procedure had been well tolerated a Watson capsule was then mounted on the endoscope, which was introduced a second time. The capsule was passed out of sight into the jejunum, air aspirated from the intestine, and the knife mechanism fired. The biopsy specimen was oriented on card and divided into three unequal portions, the larger for light microscopy, a small portion for electron microscopy, and a smear was made with the remainder.

Sigmoidoscopy was performed while the patient remained sedated, using a rigid plastic disposable sigmoidoscope. Biopsy specimens were taken from any areas of macroscopic abnormality or from three random sites.

HISTOPATHOLOGY

Impression smears were made from two duodenal biopsy specimens and part of the jejunal biopsy sample by holding them in fine forceps, allowing to dry for a few seconds, dabbing lightly several times on one end of a clean dry microscope slide, and then drawing the specimen across the slide several times to make a smear one cell thick. Four to five slides were prepared for each biopsy specimen. The smears were air dried and fixed for one to two minutes in absolute methanol, then stained in 10% Giemsa for 35 minutes, and examined under oil immersion ($\times 1000$).

A total of four duodenal biopsy specimens, part of the jejunal biopsy sample, and the rectal biopsy specimens were fixed in 10% formyl saline for at least three hours. The fixed specimens were dehydrated through graded ethanols, cleared, and embedded in paraffin wax. Sections 1–5 μ m thick were cut using a rotary microtome, placed on glycerine albumin coated slides and heated on a hot-plate for 15 minutes. After dewaxing and dehydrating in xylene and methanol the slides were washed in tap water and stained with haematoxylin and eosin, periodic acid Schiff (PAS), PAS-diastase, and Ziehl-Neelsen stains. At least two levels per block were examined.

Immunohistochemistry

Immunoperoxidase staining for CMV was done on all rectal and duodenal biopsy specimens as well as any gastric and oesophageal specimens obtained from areas of abnormality. Sections 3.5 µm thick were cut from two different levels, dewaxed, and treated with trypsin for 12 minutes. A mouse monoclonal antibody (Dako M757) was used as the CMV primary antibody at a dilution of 1 in 30 and incubated for two hours at room temperature in a humidified chamber. After washing, biotinylated rabbit antimouse was applied for 30 minutes at a dilution of 1 in 500. Avidin-biotin complex (1 µl each in 25 µl) was applied for 30 minutes, washed, and developed with diaminobenzidine.²³

Immunohistochemical staining for adenovirus was carried out on the rectal biopsy specimens using adenovirus primary antibody (Serotec No MCA 489) at a dilution of 1 in 1000. Sections were mounted on poly-L-lysine coated slides, subjected to 15 minutes protease digestion, and incubated with primary antibody for one hour. Secondary antibody and avidin-biotin complex were incubated for one hour each and, after washing, developed with diaminobenzidine for five minutes.²⁴

Electron microscopy

Two duodenal biopsy specimens and part of the jejunal biopsy sample were fixed in 3% glutaraldehyde in 0.1 molar cacodylate buffer (pH 7.3) for between six and 12 hours at 4°C. After washing they were cut into 1–2 mm cubes, post-fixed in 1% osmium tetroxide, dehydrated through graded methanols and block stained with uranyl acetate, impregnated with propylene oxide, and embedded in Taab resin. The specimens were oriented villi upwards under the dissecting microscope during embedding. Sections 70 nm thick were stained with uranyl acetate and lead citrate and examined using a JEOL 100 CX microscope.

MICROBIOLOGY

Stool specimens were cultured for faecal pathogens on Salmonella/Shigella agar medium, Campylobacter selective medium, and Selanite F enrichment medium. Concentrated wet preparations were examined by direct microscopy for ova cysts and parasites. Smears were stained with auramine for cryptosporidia: if positive the diagnosis was confirmed by a modified acid-fast method. Ziehl-Neelsen stained smears were examined for acid-fast bacilli and specimens were inoculated onto Lowenstein-Jensen slopes and cultured for mycobacteria. The slopes were examined weekly for eight weeks and if colonies were observed Ziehl-Neelsen staining was used to confirm that they were mycobacteria. Concentrated preparations were prepared and examined for cryptosporidia if six auramine stained smears were negative and diarrhoea persisted.

A tissue culture cytotoxicity assay for *Clostridium difficile* toxin B was performed on one stool sample, using the neutralised, buffered supernatant of a 20% stool suspension inoculated into human foreskin fibroblast cultures. A positive test was the presence of a cytopathic effect at 24–48 hours, neutralised by *Cl sordelli* antitoxin (Wellcome Ltd, Dartford, UK).

Blood cultures for mycobacteria and other pathogens were obtained from all febrile patients using a C14 radiometric assay (Bactec; Becton Dickinson Diagnostic Instrument Systems Ltd, Tucson, Maryland, USA).

VIROLOGY

Stool specimens from all patients were tested for rotavirus antigen using a commercial rotavirus latex agglutination test (Wellcogen, Wellcome, Dartford, UK) and if positive the diagnosis was confirmed by electron microscopy. Those from all patients recruited after November 1991 were examined for viral particles using electron microscopy. A 10% suspension of the stools was made in phosphate buffered saline and cleared by low speed centrifugation. The supernatant was then ultracentrifuged and the pellet stained with 2% phosphotungstic acid (pH 7) and examined with the electron microscope. These patients also had part of their rectal biopsy specimen processed for electron microscopy and examined for viral particles.

OTHER INVESTIGATIONS

Further investigations were done as clinically indicated. Although tests of malabsorption were not part of the investigation protocol many of the cohort had xylose absorption tests and Schilling tests (using the double radio-labelled Dicopac method) as part of the routine investigation of weight loss and these data are included where appropriate.

FOLLOW UP

One patient was lost to follow up immediately after investigation. The remainder were reviewed regularly until the diarrhoea settled or until death or for a minimum of six months (median follow up nine months, range two weeks–30 months). Those whose diarrhoea resolved were returned to the care of their primary physician but advised to reattend if the symptoms recurred.

Further investigation in patients with persisting 'pathogen negative' diarrhoea

All patients who had persistent diarrhoea and negative initial investigations had further investigations as follows:

- (1) Six further stool specimens were sent for stool culture and microscopy as before.
- (2) Three stool specimens were examined by electron microscopy for viral particles and tested for rotavirus using a latex test.

(3) Colonoscopy was carried out following bowel preparation with 'Klean-Prep' (Norgine, Headington, Oxford, UK). Patients were sedated with midazolam and pethidine and the colonoscope was advanced proximally to the caecum. Serial biopsy specimens (three from each of terminal ileum (if possible), ascending colon, transverse colon, descending colon, and rectosigmoid) were obtained for light and electron microscopy.

(4) The upper gastrointestinal endoscopy was repeated with biopsies and duodenal aspiration as before.

(5) A double contrast (per os) barium study of the small bowel was performed.

DATA RECORDING AND ANALYSIS

A case record form was kept for each patient and the data entered into a computer using SPSS Data Entry. Data analysis was done using SPSS (SPSS Inc, Chicago, Illinois).

To determine whether features in the history, examination or basic haematological and biochemical tests could be used to distinguish between patients who did and did not have a pathogen, a number of features in these two groups were compared by univariate analysis, using the Students' *t* test for normally distributed numerical variables such as CD4 count or stool frequency, the Mann-Whitney test for non-normally distributed variables such as serum folate, and the χ^2 test (with Yates's correction if the expected values in any cell were less than 5) for categorical variables such as the presence of abdominal pain. The baseline clinical findings were further analysed in the same way to determine any significant differences between patients with small and large bowel pathogens.

To identify the best sequence of investigations a decision tree analysis was used in

which the initial choice of investigations was analysis of one, three or six stools, followed by duodenal or rectal biopsy specimen or both if no pathogen was found in the stools (Fig 1). The analysis was repeated for 'definite' and treatable pathogens, as defined by consensus view in the current literature. The probabilities at each chance node were calculated directly from the results of the study.

Results

Between 1 August 1990 and 31 July 1992, 2086 patients with HIV infection attended the unit, of whom 887 had an AIDS defining opportunistic infection or malignancy and 1019 had a CD4 lymphocyte count persistently below 200/ μ l. A total of 155 patients meeting the inclusion criteria (15% of those with a CD4 count of less than 200/ μ l) were recruited and completed investigations. Most (91%) were primary (self) referrals with a smaller number of referrals from local general practitioners or other hospitals.

A further 20 patients fitted the inclusion criteria but were not included in the study: five patients did not wish to participate, two failed to attend for investigation, and nine were not referred because they had a diagnosis made by another physician (including six patients with cryptosporidiosis and two with both cryptosporidium and CMV infection). Two patients tolerated endoscopy poorly so that biopsy specimens could not be obtained and two patients were too ill for further investigations or died before investigations were complete.

The characteristics of the cohort are given in Table I and were very similar to that of the clinic population in general, except 151 (97%) were homosexual or bisexual men, compared with 81% of the clinic population. ($\chi^2=24.5$, $p<0.0001$).

Figure 1: Decision tree for the diagnosis of HIV related chronic diarrhoea. The squares are decision nodes and the circles chance nodes. The probability at each chance node was calculated directly from the study data.

TABLE I Characteristics of the cohort of 155 patients investigated

Male:female	151:4
Risk group: Homosexual/bisexual men	150
Heterosexual	4
Blood transfusion	1
Age (y)	36.8 (7.9)
Weight at presentation (kg)	62.9 (9.3)
Weight loss since onset (kg)	6.2 (9.8)
Body mass index	20.2 (2.9)
CD4 cells/ μ l	43.0 (45.6)
Previous AIDS defining opportunistic infection or wasting syndrome:	n=108
Duration of diarrhoea (months)	4.4 (6.4)
Stools/day	6.2 (3.3)
Consistency – liquid:semiformed	115:40
Blood in stools	n=10
Mucus in stools	n=23
Abdominal pain	n=84
Abdominal tenderness	n=22
Nausea	n=58
Vomiting	n=33

Age, weight, weight loss body mass index, duration of diarrhoea and stool frequency are given as mean (SD).

Some 128 patients (83%) had at least one potential cause of diarrhoea identified after completing investigations, including 46 (30%) with more than one (Table II).

FINDINGS AT UPPER GASTROINTESTINAL ENDOSCOPY

Two patients had CMV ulcers in the oesophagus and two had CMV gastritis. Eleven had non-specific gastritis or gastric ulceration and seven had duodenal ulcers or duodenitis. Five patients had Kaposi's sarcoma of the upper gastrointestinal tract and 24 patients had candidal oesophagitis.

Twenty five patients had 'velvety' or 'cobblestoned' duodenal mucosa all of whom had a small bowel pathogen (microsporidia in 22, MAI in two, and cryptosporidia in one).

TABLE II Pathogens and potential pathogens identified in the cohort of 155 patients after completing the investigation protocol (The numbers indicate the number of patients with each pathogen)

Untreatable definite pathogens*	
Cryptosporidium	47
Microsporidium (<i>E. bienersi</i>)	37
Treatable definite pathogens*	
Mycobacterium avium intracellulare	12
Cytomegalovirus	29†
Giardia	18
Salmonella	2
Shigella	1
Campylobacter	7
Toxigenic Clostridium difficile	2
Isospora	1
Microsporidium (<i>Septata intestinalis</i>)	1
Untreatable possible pathogens*	
Adenovirus	10
Rotavirus	2
Coronavirus	3
Small round virus	3
Treatable possible pathogens*	
Blastocystis hominis	6
Spirochaetes	7
Entamoeba histolytica	2
Staphylococcus aureus (in duodenal aspirate)	1
Pseudomonas sp (in duodenal aspirate)	1
Invasive duodenal Candida	1
Non-infectious causes	
Radiation proctitis	1
Crohn's disease	1
Extensive small bowel KS	1
Small bowel lymphoma	2

By consensus view in the literature. †In addition six patients had CMV infection of the oesophagus or stomach only. KS=Kaposi's syndrome.

SIGMOIDOSCOPIC FINDINGS

Evidence of proctitis with contact bleeding or superficial ulceration was found in 17 of the patients, 12 of whom had CMV inclusions on biopsy.

INVESTIGATION RESULTS

Some pathogens were identified by only one investigation, others by more than one. No investigation had 100% sensitivity for the identification of any pathogen (Table III).

Stool analysis

Stool analysis identified more pathogens (93 of 199, 47%) than any other investigation. The diagnostic yield increased as up to six specimens were examined. In seven of 47 cases of cryptosporidiosis the organisms were identified in biopsy specimens when the stools were persistently negative (Fig 2).

Small bowel biopsy

Duodenal biopsy alone identified 87 potential pathogens in 77 patients. Watson capsule jejunal biopsy specimens were successful in 111 patients and gave an identical pathological diagnosis in all but two cases, both of whom had pathogens seen only on duodenal pinch biopsy specimens.

When compared with light microscopy, electron microscopy missed one case of giardia infection, one of microsporidia, one of MAI, three of small bowel cryptosporidiosis, and all but one of the cases of small bowel CMV. No additional pathogens were found at electron microscopy.

Nine small bowel protozoal infections diagnosed by other means were not seen in touch preparations of duodenal biopsy material (five cryptosporidia, one giardia, and three microsporidia), but one case of microsporidiosis and one of giardiasis were found that were not identified by any other diagnostic method.

Duodenal aspirate

Duodenal aspirate had a low overall sensitivity when compared with duodenal biopsy but detected one case of giardiasis not diagnosed by any other method.

Rectal biopsy

Rectal biopsies identified 65 potential causes of diarrhoea in 52 patients. Only 17 of the biopsy specimens were histologically normal, the remainder showing a diffuse infiltrate of chronic inflammatory cells even in the absence of a specific pathogen. Eight biopsy specimens showed *Melanosis coli*. This was always associated with a pathogen (microsporidiosis in six, CMV and adenovirus in one and, cryptosporidiosis in one).

The diagnostic yield of some of these investigations is summarised in Figures 3–5.

TABLE III Diagnostic yield of various investigations

Investigation	Total pathogens identified (%)	Pathogens found	Pathogens missed
One stool	37 (18.6)	<i>cryptosporidium</i> (18) <i>blastocystis</i> (3) <i>entamoeba</i> (2) <i>giardia</i> (3) <i>salmonella</i> (1) <i>campylobacter</i> (2) MAI (4) rotavirus (1) adenovirus (1) coronavirus (1) small round virus (1)	<i>cryptosporidium</i> (29) <i>blastocystis</i> (3) <i>giardia</i> (15) <i>isospira</i> (1) <i>salmonella</i> (1) <i>shigella</i> (1) <i>campylobacter</i> (5) MAI (8) <i>Cl difficile</i> (2) rotavirus (1) adenovirus (9) coronavirus (2) small round virus (2)
Three stools	77 (38.7)	<i>cryptosporidium</i> (32) <i>blastocystis</i> (5) <i>entamoeba</i> (2) <i>giardia</i> (9) <i>salmonella</i> (1) <i>shigella</i> (1) <i>campylobacter</i> (7) MAI (9) <i>Cl difficile</i> (1) rotavirus (2) adenovirus (4) coronavirus (2) small round virus (2)	<i>cryptosporidium</i> (15) <i>blastocystis</i> (1) <i>giardia</i> (9) <i>isospira</i> (1) <i>salmonella</i> (1) MAI (3) <i>Cl difficile</i> (1) adenovirus (6) coronavirus (1) small round virus (1)
Six stools	93 (46.7)	<i>cryptosporidium</i> (39) <i>blastocystis</i> (6) <i>entamoeba</i> (2) <i>giardia</i> (10) <i>salmonella</i> (1) <i>shigella</i> (1) <i>campylobacter</i> (7) MAI (10) <i>Cl difficile</i> (2) rotavirus (2) adenovirus (5) coronavirus (3) small round virus (3)	<i>cryptosporidium</i> (8) <i>giardia</i> (8) <i>isospira</i> (1) <i>salmonella</i> (1) MAI (2) adenovirus (5)
Blood culture	7 (3.5)	MAI (4) <i>salmonella</i> (1) <i>shigella</i> (1) <i>strep viridans</i> (1)	MAI (8) <i>salmonella</i> (1)
Rectal biopsy	65 (32.7)	<i>cryptosporidia</i> (24) CMV (25) adenovirus (9) spirochaetes (7) radiation proctitis (1)	<i>cryptosporidia</i> (23) CMV (4) adenovirus (1)
Duodenal biopsy	87 (43.7)	<i>cryptosporidia</i> (23) <i>microsporidia</i> (37) <i>giardia</i> (11) <i>isospira</i> (1) CMV (8) MAI (6) <i>candidiasis</i> (1)	<i>cryptosporidia</i> (24) <i>microsporidia</i> (1) <i>giardia</i> (7) CMV (21) MAI (6)
Jejunal biopsy (in 111 patients with 142 pathogens)	54 (38)	<i>cryptosporidium</i> (16) <i>microsporidium</i> (27) <i>isospira</i> (1) <i>giardia</i> (3) MAI (3) CMV (4)	<i>cryptosporidium</i> (1) MAI (1)
Touch preparation (in 135 patients with 170 pathogens)	49 (28.8)	<i>microsporidia</i> (33) <i>cryptosporidia</i> (10) <i>giardia</i> (9)	<i>microsporidia</i> (3) <i>cryptosporidium</i> (5) <i>giardia</i> (1)
Duodenal aspirate	12 (6.1)	<i>cryptosporidia</i> (4) <i>giardia</i> (4) <i>isospira</i> (1) MAI (1) <i>pseudomonas</i> (1) <i>staph aureus</i> (1)	<i>cryptosporidia</i> (19) <i>giardia</i> (7) MAI (6)

In 'pathogens found' and 'pathogens missed' the numerals in parentheses refer to the number of cases. *Includes one patient with *S. intestinalis*. For jejunal biopsy, touch preparation, and duodenal aspirate the 'pathogens missed' is by comparison to duodenal biopsy.

COMPARING PATIENTS WITH AND WITHOUT AN IDENTIFIED CAUSE FOR THE DIARRHOEA

Patients who had a cause of diarrhoea identified had a significantly lower mean CD4 cell count, a lower mean weight at presentation, and had lost on average more weight than those without a pathogen (Table IV). The history and examination findings were

not helpful in predicting whether a pathogen might be identified except that abdominal tenderness was significantly commoner in patients with CMV infection than in those with any other diagnosis ($\chi^2=23.9$, $p=0.00001$). Surprisingly, weight loss of more than 5 kg or positive malabsorption tests were not correlated with the presence of a small bowel pathogen (compared with a large bowel pathogen or no pathogen) although high stool volume was (Table V). Nor was patient reported blood and mucus in the stool correlated with the presence of a large bowel pathogen.

Decision tree analysis

A sequential approach, stopping once the first pathogen is identified, results in a number of pathogens, including treatable ones, being missed. (Table VI).

We have repeated the analysis, assuming that examination of six stools would also have 100% sensitivity for the diagnosis of microsporidiosis. This investigation was not routinely available when this study was started but has since been suggested to have an excellent sensitivity and specificity.^{25 26} If rectal biopsy and stool analysis was performed on all patients 82% of all pathogens would be identified (missing some cases of *giardia* MAI and *isospira*, CMV duodenitis, pathogenic bacteria in duodenal aspirates, and the small bowel malignancies). If rectal biopsy was done only in those patients with negative stools, and duodenal biopsy in those with negative stools and rectal biopsy specimens, the diagnostic yield would be 71%.

Discussion

A number of previous publications have suggested algorithms for the investigation of diarrhoea in HIV seropositive patients,^{10 11 13 16 27-31} but no study has attempted to assess the utility of the proposed investigations in clinical practice. We present the results of a large prospective study of chronic diarrhoea in AIDS patients. We chose to study patients with a CD4 count of less than 200/ μ l and diarrhoea for more than a month as our previous studies have shown that most pathogens occur in this patient group.³² The study population contained a greater proportion of homosexual men than the clinic population from which it was derived. This may be because the homosexual population have more advanced disease, reflecting the changing epidemiological pattern of AIDS in the United Kingdom; in addition homosexual men may have a higher incidence of gut pathogens. However we see no reason why the conclusions of the study could not be extrapolated to other Western populations.

The key feature of the investigation protocol was that stool analysis, small bowel and large bowel biopsy were performed on all patients to maximise the chance of identifying all pathogens in multiply infected patients and produce accurate prevalence figures for the

Figure 2: Pie chart illustrating the contribution of stool examination, rectal biopsy, and duodenal biopsy in the diagnosis of cryptosporidiosis. The figures refer to the number of patients diagnosed by each method. 'Other' = bile microscopy and ampullary biopsy.

different infections. The high rate of multiple infections (29%) compared with some series may be due to this approach. The identification of such multiple infections is important not only to explain treatment failures but because the elimination of a second treatable pathogen may result in improvement of the diarrhoea or even disappearance of the untreatable pathogen.³³

This is the first study to give a true indicator of the prevalence of microsporidiosis in unselected AIDS patients with chronic diarrhoea, as most previous series have looked for microsporidia only in patients in whom other pathogens have been excluded.^{34 35}

Although there were significant differences in CD4 counts, stool volumes, the rate of weight loss, and vitamin B12 values between patients with and without a pathogen there was a large overlap and such clinical features could not be used to identify those patients most likely to have a pathogen. Nor could the site of the pathogen be predicted accurately by features in the history and examination. These findings together with the high prevalence of pathogens suggest that all patients should be offered investigation.

Stool analysis is sensitive and non-invasive. The sensitivity increases considerably between one and three samples, particularly for the detection of cryptosporidia, but only slightly for subsequent samples. However in eight patients with cryptosporidiosis, organisms were persistently absent from the stools but present on gut biopsy.

Rectal biopsy is the only reliable way to diagnose CMV and adenovirus proctocolitis and rigid sigmoidoscopy is easily performed in the gastroenterology outpatient clinic. There has been concern that CMV infection may be confined to the right side of the colon in up to 30% of cases²⁰ but we identified CMV on colonoscopy after initially negative rectal biopsies in only two cases. However colonoscopy was performed only on the 35 patients with initially negative investigations.

The most common pathogens primarily infect the small bowel and so biopsy of the duodenum has a high diagnostic yield with some cases of cryptosporidiosis, giardiasis, MAI, CMV being diagnosed only by this method. Jejunal biopsy has no advantage. Characteristic histological changes in the duodenum of patients with MAI are strongly suggestive that the organism may be the cause of the diarrhoea whereas culture of it from the stool may only indicate colonisation. The diagnostic utility of duodenal biopsy will be reduced but not eliminated if the new stool methods for the diagnosis of microsporidia are as sensitive and specific as biopsy.

Although in some series³⁶ duodenal aspirate, obtained via nasoduodenal intubation, has been sensitive in the diagnosis of small bowel pathogens we have not found this to be the case, perhaps due to the lower incidence of small bowel parasites in this series or the different technique used. In expert hands impression smears have a high diagnostic yield for protozoal pathogens particularly microsporidia but we found them difficult to prepare and they added little extra sensitivity to

Figure 3: Histogram summarising the results of stool examination of duodenal biopsy specimens in the diagnosis of various pathogens. The total height of the bars show the number of patients with each pathogen and the heavily shaded portion the number detected by the method. The results of one, three, and six stools are shown separately.

Figure 4: Histogram summarising the results of light microscopy of duodenal biopsy specimens in the diagnosis of various pathogens.

Figure 5: Histogram summarising the results of light microscopy of rectal biopsy specimens in the diagnosis of various pathogens.

TABLE IV Comparison of patients with and without a pathogen

	Pathogen present	No pathogen	p Value
Mean age (y)	37.3	34.7	0.067*
Mean weight (kg)	62.0	65.6	0.038*
Mean total weight loss (kg)	7.3	2.7	0.001*
Mean rate of weight loss (kg/month)	2.9	0.8	>0.001*
BMI (kg/m ²)	20.0	20.7	0.20*
CD4 cells/ μ l	37	62	0.017*
Time since AIDS diagnosed (months)	7.6	10.5	0.15†
Mean stool frequency (per day)	6	6	0.85*
Mean stool volume (ml)	678	428	0.01*
Median serum vitamin B12 (ng/ml)	200	315	0.003†
Mean serum folate (μ g/l)	4.5	3.4	0.71†
Median red cell folate (mg/ml)	250	242	0.79†
Xylose absorption: normal	1	1	0.11‡
reduced	31	1	
Dicopac test: normal	1	2	0.90‡
malabsorption	35	10	
Nausea: present	45	13	0.85‡
absent	74	23	
Vomiting: present	27	6	0.44‡
absent	92	30	
Incontinence: present	40	13	0.78‡
absent	79	23	
Blood in the stool: present	6	2	0.59‡
absent	113	34	
Mucus in the stool: present	17	5	0.95‡
absent	102	31	

* Student *t* test. †Mann-Whitney test. ‡ χ^2 test.

duodenal biopsy. Electron microscopy was less sensitive than light microscopy and is not necessary for routine use.

None of the investigations we examined identified more than 50% of pathogens when used alone. Therefore a combination of investigations is needed to make the correct diagnosis in patients presenting with diarrhoea. In clinical practice a stepwise diagnostic approach is often used, with less invasive investigations first (stool analysis) reserving more invasive tests (gut biopsy) for those without a pathogen in the stools. This is particularly likely when patients are initially seen by primary care physicians without immediate access to sigmoidoscopy and endoscopy. However we have shown that this results in pathogens being missed, whether the aim is to identify all potential pathogens or just those for which a specific treatment is available. Thus we would recommend that all HIV positive people with a CD4 count of less than 200/ μ l should have at least three stools analysed and light microscopy of duodenal and rectal biopsies. There is a high rate of acceptability of such intensive investigations to both patients and referring physicians: only 13% of those meeting the inclusion criteria of this study failed to complete investigations.

This clearly has important workload implications for gastroenterologists and pathologists, but it should be emphasised that this protocol only applies to those HIV positive patients with a CD4 count of less than 200/ μ l and chronic diarrhoea; our previous studies have suggested that less intensive investigation is appropriate for less immunocompromised patients or those with acute diarrhoea.

We thank the following for their valuable contributions to this project: Dr D Shanson and the Microbiology Department of the Westminster Hospital, London, for analysis of the stool specimens and blood cultures. Dr D Ellis and Mr DG Tovey of the Electron microscopy Department of the London School of Hygiene and Tropical Medicine, for electron microscopy. Professor EU Canning and Dr W Hollister of the Parasitology Department, Imperial College, London, for examination of the small bowel impression smears.

TABLE V Comparison of patients with and without a small bowel pathogen

	Small bowel pathogen	
	Present	Absent
Stool volume <500 ml	4	27 p<0.0001
≥500 ml	45	27
<1000 ml	26	46 p<0.001
≥1000 ml	23	8
Xylose absorption test normal	0	2 p=0.11
abnormal	22	10
Dicopac (Schilling) test normal	0	3 p=0.06
abnormal	32	19
Weight loss <5 kg	34	63 p=0.10
≥5 kg	28	30

Small bowel pathogen is defined as identification of an organism in duodenal or jejunal biopsy specimens examined by any method.

TABLE VI Probability of identifying the cause of diarrhoea with different sequences of investigation (decision tree analysis)

	One stool				Three stools				Six stools			
	Nil	RBx	OGD	Both	Nil	RBx	OGD	Both	Nil	RBx	OGD	Both
1st												
2nd												
3rd												
All causes	0.186	0.520	0.548	0.665	0.357	0.649	0.684	0.719	0.417	0.683	0.683	0.724
Treatable conditions	0.173	0.403	0.481	0.500	0.357	0.591	0.602	0.663	0.388	0.612	0.582	0.612
Definite pathogens	0.200	0.538	0.618	0.650	0.388	0.700	0.756	0.763	0.456	0.725	0.763	0.775
Treatable definite pathogens	0.158	0.461	0.513	0.513	0.368	0.605	0.671	0.684	0.395	0.632	0.645	0.645

RBx: rectal biopsy, OGD: oesophagogastrroduodenoscopy with duodenal biopsy for light microscopy and aspiration of duodenal fluid; Both: sigmoidoscopy and rectal biopsy and OGD performed at the same visit. All causes include all the possible causes of diarrhoea in the cohort studied, including those not found on initial investigations. Definite pathogens and treatable pathogens are as defined in Table IV. The treatable conditions also includes the non-infectious causes of diarrhoea given in Table IV.

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