SIMPOSIO

CANCRO, MALATTIE CRONICHE INTESTINALI ED INFEZIONI NELL'OSPITE IMMUNOCOMPROMESSO

CANCER, CHRONIC BOWEL DISEASES AND INFECTIONS IN THE IMMUNOCOMPROMISED HOST

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EPIDEMIOLOGY OF AN OUTBREAK OF CLOSTRIDIUM DIFFICILE-ASSOCIATED-DIARRHOEA. APPLICATION OF A TYPING SYSTEM

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Summary. - A prospective epidemiological study of an outbreak of C.difficile associated diarrhoea in an oncology ward is described. A typing scheme for C.difficile based on polyacrylamide gel electrophoresis of ^{35}S-methionine labelled proteins was applied. The results demonstrated nosocomial acquisition of C.difficile and that cross infection occurred among patients with a single epidemic strain: - type X.

Riassunto (Studio su un episodio epidemico di diarrea associata al Clostridium difficile. Applicazione di un sistema di tipizzazione). - Viene descritto uno studio prospettico epidemiologico su un focolaio di diarree associate al C.difficile in una corsia oncologica. E' stato utilizzato uno schema di tipizzazione basato sull'elettroforesi in gel di poliacrilamide di proteine marcate con ^{35}S-metionina. I risultati hanno dimostrato che l'acquisizione di C.difficile e le infezioni crociate avvengono con un solo ceppo epidemico: il tipo X.

Introduction

Antibiotic-associated diarrhoea and colitis (AAD and AAC) are part of the spectrum of disease caused by the anaerobic gram positive rod, Clostridium difficile. It is currently estimated that between 15-20% of all AAD is found in conjunction with an isolate of the organism (1). Most cases of pseudomembranous colitis (PMC) are currently believed to be caused by toxigenic strains of C.difficile (2).

The simple presence of the organism in a faecal specimen does not necessarily implicate it as a pathogen. It seems quite clear that other conditions must pertain in the bowel in order to allow the organism to express its pathological potential. Antibiotics and antineoplastic chemotherapy are amongst the best documented precipitating factors, but iscahemia (3) and inflammatory bowel disease (4, 5) may, perhaps, also predispose to infection with the organism. An understanding of the epidemiology of C.difficile has been hampered until recently by the lack of a comprehensive typing scheme for the organism. Several different approaches to the problem have now been considered, including phage and bacteriocin typing (6), immunoblotting (7), serotyping (8) and analysis of the radiolabelled protein profile produced by the organism using SDS-PAGE (9). We have developed the latter scheme into a simple, easily expandable system and have described to date 9 standard strains of the organism (A-E, W-Z). Using this typing method, we have prospectively studied a large outbreak of Clostridium difficile-associated-diarrhoea (CDAD) on male and female wards housing both immunocompromised and general medical patients.

Materials and Methods

Over a six-month period (May 1983-October 1983) the patients on the mixed male and female oncology/medical wards were prospectively screened for the presence of Clostridium difficile in faecal samples. The patients were housed on a 25 bed open ward, with a central partition separating the oncology and general medical patients. Toilet and bath facilities were shared, while the nursing staff shared the care of both groups of patients. Medical staff tended to look after only one group of patients.

An on-admission faecal specimen, taken within 48 hours of each patient's arrival on the ward, was cultured on selective media (CCFA) under anaerobic conditions for 48 hours. The organisms were identified by their macroscopic morphology, appearance on gram stain, smell and the ability to fluoresce under ultraviolet light. Confirmation of their identify was achieved by gas liquid chromatography in which the characteristic iso-caproic peak was seen. Additional samples were collected weekly for the duration of admission or more frequently if diarrhoea occurred, at which time examination for other enteric pathogens was also undertaken.

Isolates of the organism were stored in Robertson's Cooked Meat Medium (RCMM) until required for typing. Organisms were typed according to the previously described method involving the incorporation of 35S-methionine into bacterial proteins. Briefly, 2-3 colonies from a 48 hour anaerobic culture on blood agar of each strain for typing were inoculated into 100 μC of 35S-methionine. After a 2 hour incubation under anaerobic conditions at 37°C, an equal volume of double strength electrophoresis buffer was added and the tubes were boiled for 2 minutes. Electrophoresis on 12.5% polyacrylamide gels was performed, followed by autoradiography.

Results

Table 1 shows the relevant screening data. 991 faecal specimens were examined, from 249 patients. One hundred and thirtysix were oncology patients,

Table 1. - Screening data on oncology/medical wards for Clostridium difficile (1.5.83 - 31.10.83)

		Male	Female
Number of patients screened	249	136	113
Oncology patients screened	135	76	59
Medical patients screened	113	60	53
Total specimens examined	991		
Number of patients positive for Clostridium difficile	62 (25%)		
Number of oncology patients positive for Clostridium difficile	49 (36.8%)		
Associated medical patients positive for Clostridium difficile	13 (11.5%)		

and 113 were general medical patients. Sixty-two patients (25%) in all had a positive faecal culture for the organism. Forty-nine of the 136 oncology patients (37%) were found to have C.difficile on faecal culture while 13 of the 113 (11.5%) medical patients carried the organism.

The typing results (Table 2) demonstrate that of the 49 oncology patients found to carry the organism, 35 carried the epidemic strain, Type X, whilst the remaining 14 patients carried a range of other strains (A, D, E, W, Y). Three of the general medical patients had X strain, the rest again carrying a variety of other strains.

Patients were also evaluated for acquisition of the organism during their stay in hospital. Of the 49 oncology patients who were found to have the organism, 12 were unevaluable since they were on the ward prior to the start of the screen. All of these patients carried the epidemic strain. Nine patients were found to have the organism on the first specimen that was examined (within 48 hours of admission to the ward) and two of these were X strain. Twenty-eight patients acquired the organism after being on the ward for 48 hours or longer and had had at least one faecal sample negative for the organism. Twenty-two of these patients acquired the epidemic strain, Type X, whilst the remaining six patients acquired other strains.

Discussion

The application of our typing scheme has helped to unravel some of the previously unanswered questions regarding cross-infection and hospital acquisition of C.difficile. The results presented in this paper demonstrate for the first time that C.difficile is nosocomially acquired particularly among the immunocompromised susceptible patients. This study also demonstrated clearly that cross-infection occurs in hospital wards and that it could become a serious clinical problem.

Previous reports of clusters of cases (10) and outbreaks of PMC and AAC were suggestive of nosocomial spread of C.difficile, particularly when the organism was isolated from patients and their hospital environment. However, the epidemiology of this organism had not been fully clarified in the absence of a typing scheme. Several workers have recently initiated various schemes to investigate hospital outbreaks (6-9).

Although a number of different types of C.difficile were isolated on the oncology ward, there was one strain, type X, which predominated. Thirty-five out of the 49 oncology patients had type X. Of the 29 oncology patients who had negative cultures within 48 hours of admission, 22 of those went on to acquire type X. It appears therefore that type X is more virulent and spreads more easily among patients that the other types which were isolated (A, D, W, Y).

The source of the outbreak is unclear, but it is possible that the 12 unevaluable patients who already had type X when the screening started, could have provided the pool for type X.

Measures to control the spread of the epidemic strain in the oncology ward

Table 2. - Types of Clostridium difficile isolated

Types of Clostridium difficile	A	B	C	D	E	W	X	Y	Z
Oncology patients	1	0	0	1	5	1	35	6	0
Associated medical patients*	1	3	0	1	3	0	3	1	0

* one isolate died before typing could be performed.

were instituted by separating and isolating patients with C.difficile from those who were not infected. These procedures were successful in limiting the incidence of new cases (Heard et al., 1986, J. Infect. Dis.).

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FLORA BATTERICA INTESTINALE E TUMORI DEL COLON: ESCREZIONE E DEGRADAZIONE INTESTINALI DEGLI STEROIDI NEUTRI IN SOGGETTI A RISCHIO PER CANCRO DEL COLON

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Riassunto. - La flora batterica intestinale è in grado di metabolizzare gli steroli neutri e acidi in prodotti secondari. Con il nostro studio abbiamo voluto valutare l'escrezione giornaliera degli steroli neutri e la degradazione intestinale del colesterolo a prodotti secondari in soggetti a rischio di cancro colorettale suddivisi in 4 gruppi più 1 di controllo. Campioni di feci venivano raccolti per 3-6 giorni ed i singoli steroli analizzati mediante gas-cromatografia. L'escrezione totale degli steroli neutri non è risultata significativamente diversa tra controlli e i gruppi studiati. In tutti i controlli il colesterolo era ampiamente convertito nei suoi metaboliti, mentre negli altri gruppi alcuni soggetti convertivano poco il colesterolo: questo è dovuto forse ad alterazioni della flora batterica intestinale.

Summary (Intestinal bacterial flora and colon cancer: excretion and degradation of neutral steroids). - The intestinal microflora is able to metabolize the neutral steroids and acids into secondary products. We studied the daily excretion of neutral steroids and the intestinal degradation of cholesterol in 4 groups of high risk subjects for colon-rectal cancer and 1 control group. The total excretion of neutral sterols was not significatively different between the studied groups and controls. The cholesterol was highly degradated in the control group, while in the other 4 groups some individuals were able to metabolize it scarcely: this was probably due to alterations of the intestinal microflora.

Introduzione

Il cancro del grosso intestino è molto più frequente nei paesi ad elevato sviluppo tecnologico, che in quelli meno industrializzati, dove la popolazione consuma, in media, meno carne e grassi animali e più fibre vegetali (1-3). Infatti, studi epidemiologici hanno mostrato una relazione fra consumo di carne e grassi animali e incidenza di cancro colorettale (4).

Nel 1971 Hill e Coll. riferirono che le popolazioni occidentali eliminavano con le feci elevate concentrazioni di acidi biliari e steroli neutri rispetto alle popolazioni africane e dell'estremo oriente (5). In seguito lo stesso gruppo riportò che pazienti con cancro colorettale eliminavano con le feci maggiori quantità di acidi biliari rispetto ai controlli (6). Gli autori ipotizzarono che il cancro colorettale fosse in qualche modo correlato alle alte concentrazioni di prodotti secondari legati all'attività batterica intestinale, sia sugli steroli acidi, sia sui neutri (6, 7).

In seguito sono stati riportati risultati contrastanti su questo argomento, probabilmente a causa del numero relativamente piccolo di soggetti studiati e della frequente mancanza di controllo metabolico.

Gli scopi del nostro studio sono stati quelli di caratterizzare l'escrezione e la degradazione del colesterolo in prodotti secondari in soggetti senza patologie gravi, mantenuti sotto controllo metabolico, e di paragonare questi dati con quelli ottenuti da pazienti con polipi e cancro del grosso intestino.

Materiali e Metodi

Abbiamo studiato i seguenti gruppi: 38 individui senza gravi malattie (gruppo di controllo); 22 pazienti con cancro colorettale (prima dell'intervento); 15 membri di 2 famiglie con alta prevalenza di polipi multipli del colon (fra 3 e 10 polipi) e cancro del colon; 12 soggetti con polipi solitari senza familiarità; 16 membri 4 dei quali affetti e 12 loro parenti di primo grado di 6 famiglie con poliposi familiare del colon, malattia ereditaria trasmessa di generazione in generazione in modo autosomico dominante (8).

I soggetti studiati erano tenuti a dieta standard, contenente quantità note di calorie e colesterolo (400 mg) al giorno. Ai soggetti venivano somministrate dosi giornaliere di β sitosterolo radioattivo e di Cr_2O_3 come "markers" di recupero e di flusso fecale. Tutti i campioni di feci venivano raccolti per almeno 3 giorni ed ognuno di essi analizzato separatamente. I singoli steroli erano isolati mediante TLC e GLC, come precedentemente descritto (9,10), usando 5 α colestano come standard interno. In tal modo il colesterolo ed i suoi principali metaboliti intestinali - coprostanolo e coprostanone - venivano dosati ed analizzati separatamente.

Risultati

L'escrezione media totale giornaliera di steroli neutri nei 5 gruppi studiati è mostrata nella Tab. 1. Abbiamo osservato un'ampia variazione dei valori all'interno di ciascun gruppo con un range compreso fra 200 e 700 mg/die. In ciascuno dei gruppi studiati la differenza dai controlli era statisticamente significativa.

In tutti i gruppi studiati il coprostanolo era lo sterolo più abbondante, compreso fra 50 e 98% del totale: conseguentemente la somma dei 2 metaboliti intestinali, coprostanolo e coprostanone, rappresentava la quota maggiore di steroli neutri escreti. Il "pattern" di degradazione mostrava solo modeste fluttuazioni durante i 3 giorni di raccolta.

All'interno di ognuno dei 3 gruppi di pazienti con polipi e del gruppo di pazienti con cancro colorettale si potevano identificare 2 distinte popolazioni, una con un tipo di degradazione simile a quello dei controlli (Alti Convertitori conversione alta o quasi completa del colesterolo nei prodotti secondari), l'altra (Bassi Convertitori) con una conversione del colesterolo scarsa o assente.

Tabella 1. - Escrezione degli steroli neutri totali nei 5 gruppi studiati (media $\pm$ ES)

N	mg/die	range
Controlli (38)	412 $\pm$ 31	354 - 688
Cancro colorettale	386 $\pm$ 76	364 - 663
Polipi solitari	516 $\pm$ 84	230 - 636
Polipi multipli	505 $\pm$ 52	328 - 679
Poliposi familiare	402 $\pm$ 65	368 - 546

Lo stato di basso convertitore è stato osservato in 6 pazienti su 22 con cancro colorettale, in 5 su 16 membri delle famiglie con poliposi familiare, in 4 soggetti su 12 con polipi solitari, e in 2 su 15 membri di famiglie con polipi multipli. La differenza coi controlli raggiungeva la significatività statistica ($p < 0,01$) solo nel gruppo della poliposi familiare.

Discussione

I nostri risultati indicano che non esiste una differenza significativa nell'escrezione degli steroli neutri totali fra controlli e pazienti a rischio di cancro colorettale. Tuttavia, in una minoranza dei pazienti, il colesterolo è scarsamente degradato in coprostanolo e coprostanone. Livelli di steroli neutri fecali superiori ai controlli sono stati osservati in diversi gruppi di pazienti ad aumentato rischio di cancro colorettale, come nel caso della poliposi familiare, della sindrome di Gardner e della colite ulcerosa (11). In questi studi, tuttavia, per lo più condotti senza controllo metabolico, il parametro studiato era la concentrazione di steroli fecali (mg/g di feci secco) e non la escrezione giornaliera.

Sebbene la maggior parte dei pazienti con cancro colorettale e polipi sembri convertire il colesterolo nei suoi metaboliti, in una minoranza di essi il colesterolo non veniva praticamente degradato. Risultati simili erano già stati riportati in precedenza in pazienti con poliposi familiare, cancro del colon familiare e nei loro parenti di primo grado (8-13). Il nostro studio estende queste osservazioni ad altri gruppi di pazienti con neoplasie del grosso intestino senza una chiara familiarità.

In conclusione, ci sembra che le informazioni disponibili siano ancora insufficienti per chiarire se gli steroli neutri fecali siano correlati al cancro colorettale, e se la definizione della escrezione e della degradazione degli steroli neutri possa essere utile nell'identificazione di individui a rischio.

In particolare occorrono ulteriori studi longitudinali per accertare se lo stato di basso convertitore aumenti la suscettibilità al cancro colorettale e possa quindi permettere la sorveglianza nel tempo di soggetti a rischio.

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FAECAL CARRIAGE OF CLOSTRIDIUM DIFFICILE IN CYSTIC FIBROSIS PATIENTS

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Summary. - Faecal samples from 37 patients with cystic fibrosis (CF) and 40 control patients have been examined for the presence of Clostridium difficile. Overall the isolation rate of this organism from control subjects was similar to that described in other published works. However, there was a high isolation rate (overall, 29.7%) from patients with CF. Many of the isolates could be shown to produce cytotoxin in vitro, and cytotoxin was detected in some of the stool samples. Despite this, C.difficile appeared to be carried asymptomatically by the patients in this study.

Riassunto (Presenza di C.difficile nelle feci di pazienti affetti da fibrosi cistica). - Sono stati esaminati per la presenza di C.difficile campioni fecali di 37 pazienti con fibrosi cistica (CF) e 40 pazienti controllo. Mentre la percentuale di isolamento nei controlli è risultata simile a quella riportata in altri lavori, quella relativa ai pazienti con CF è risultata più elevata (29,7%). Molti dei ceppi isolati erano tossigenici in vitro e la citotossina è stata rilevata anche in alcuni campioni fecali, ma ciò nonostante il C.difficile sembra colonizzare questi pazienti senza produrre malattia.

Introduction

Cystic fibrosis is the most common lethal genetic syndrome among white children and is the cause of much of the chronic progressive pulmonary disease encountered in children.

It is an autosomal recessive disorder characterised by high sodium chloride content in sweat, pancreatic insufficiency (and consequent malabsorption) and hypertrophy of the mucus secreting glands. This latter symptom makes the lungs very susceptible to infection and pulmonary disease accounts for more than 95% of deaths of patients with cystic fibrosis (CF). Because of this, these patients receive almost continual antimicrobial prophylaxis. They would thus appear to be prime candidated for gastrointestinal colonisation with C.difficile, although symptoms of antibiotic associated enteric disease are rare. Wu et al. (1) noted a 50% carriage rate of C.difficile, but most of the isolates did not produce cytotoxin.

The present study was undertaken for 3 reasons. Firstly, to establish the normal carriage rate of C.difficile in the stools of patients of the Brompton Hospital Group, London, (Brompton Hospital and London Chest Hospital), secondly to establish the carriage rate of this organism in CF patients and thirdly to find the incidence of toxigenic strains of C.difficile in these patient groups.

Materials and Methods

Patients. - There were 40 control patients (35 London Chest Hospital, 5 Brompton Hospital) with a range in age of 56 years (range 27-78 years). Twelve control patients were receiving antibiotics. The control patients were suffering from respiratory tract other than CF. This included emphysema, asthma, chronic obstructive airway disease and carcinoma.

The 37 CF patients (8 London Chest Hospital, 29 Brompton Hospital) had a mean age of 19 years (range 6-32 years) and all but two were receiving antibiotics. Most of these patients were also being treated for pancreatic insufficiency which was well controlled.

None of the patients (controls or CF) had diarrhoea at the time of the study.

Faecal samples. - About 10 g stool sample were sent to the laboratory directly or by post. They were stored untreated at -20°C for up to one month.

Bacteriological investigations. - Approximately 0.1 g were seeded onto a prereduced selective medium (CCA) containing D-cycloserine (500 mg/L) and Cefowitin (20 mg/L) (2). Seeded plates were incubated for 48 hours at 37°C in anaerobic jars. Anaerobiosis was achieved with the use of the 'Gas-kit' system and palladium catalyst (Oxoid Ltd., Basingstoke, England).

A similar (0.1 g) sample was inoculated into Robertson's Cooked Meat Medium RCM) and incubated aerobically at 37°C for 48 hours and then subcultured onto CCA. After incubation the primary CCA plates were examined for typical C.difficile colonies. A semi quantitative estimate of the amount of growth was scored: + = growth in the 'pool' area only, ++ = growth in pool and primary streaks, +++ = growth in pool and in primary and secondary streaks. When C.difficile was recovered only from the RCM that faecal sample was scored ± for the organism.

Identification of C.difficile isolates. - Initial identification of isolates was by characteristic colonial morphology, smell (paracreosol), typical Gram reaction and the ability of the colonies to show characteristic fluorescence under 360 nm ultra-violet light. Identification was confirmed by conventional techniques, including gas-liquid chromatography.

Tests for cytotoxin activity. - Isolates were tested for their ability to produce cytotoxin acting against vero cells as described by Larson and Price (3). The neutralizing agent was C.sordellii antitoxin (Wellcome Research Laboratories, Kent, England). One in ten dilutions of stools that were positive for C.difficile were also tested for the presence of cytotoxin.

Results

Isolation of C.difficile from control patients. - C.difficile was isolated from two of the twelve control patients who had received antibiotics - an isolation rate of 16.6% (Table 1). These patients had been treated with cephalosporins (Table 2).

Table 1. - Isolation of C.difficile (CD) from control patients

Treatment	No. tested	No. CD positive	% Isolation rate
Antibiotics	12	2	16.6
No antibiotics	28	1	3.5
Total	40	3	7.5

Table 2. - Control patients carrying C.difficile (CD)

Age	Sex	CD isolated	Cytotoxin in culture	Cytotoxin in stool	Diagnosis	Antibiotic
68	F	+++	+	+	Ca lung	- (Steroid)
31	F	+	+	-	Bronchiectasis	Ceftazadime
64	M	+	-	-	Ca lung	Cefuroxime

The organism was isolated from only one of the twenty-eight control patients who had no antibiotic treatment (3.5%) (Table 1). In total, therefore, 7.5% of the control patients were found to be carrying C.difficile in their stools. Two of these isolates produced cytotoxin in vitro. Cytotoxin activity could also be detected in the stools of one of the control patients carrying C.difficile (Table 2).

Isolation of C.difficile from patients with cystic fibrosis. - Overall, eleven of the CF patients (29.7%) carried C.difficile in their stools (Table 3). There was a marked difference in the isolation rate of the organism from patients at the London Chest Hospital (50%) compared with those at the Brompton Hospital (24%). However, two of the Brompton patients had not received antibiotics and a further seven had received local, nebulized antibiotics. C.difficile was not isolated from them. If these patients are discounted from the total, the isolation rate for this hospital rises to 35%.

All of the CF patients from whom C.difficile was isolated had been treated systemically with a β-lactam antibiotic (Table 4). In addition, many had received an aminoglycoside.

Six isolates from CF patients could be shown to be capable of producing cytotoxin in vitro (Table 4). Cytotoxin was also detected in the stools of three patients.

Discussion

Bearing in mind the small number of patients investigated in the present study, the overall carriage rates of C.difficile in the stools of the control group of patients are similar to those described elsewhere. A carriage rate of 2 - 3% has been noted amongst normal healthy adults (4-6) and 21% amongst adults receiving antibiotics who had no diarrhoea or pseudomembranous colitis (6).

Table 3. - Isolation of C.difficile (CD) from cystic fibrosis patients

Hospital	No. tested	No. receiving antibiotics	No. CD positive	% Isolation rate
LCH	8	8	4	50
BH	29	27*	7	24 (35)
Total	37	35	11	29.7

* 7 patients received only nebulized antibiotics.

Table 4. - Cystic fibrosis patients carrying C.difficile (CD)

Age	Sex	CD isolated	Cytotoxin in culture	Cytotoxin in stool	Systemic antibiotics
18	M	++	+	+	Aminoglycoside + β-lactam
16	M	+	-	-	"
19	M	+++	+	+	"
23	M	++	-	-	"
15	F	+++	+	-	"
21	F	+	+	-	"
16	M	+	-	+	"
26	M	+	+	-	"
21	F	+++	-	-	β-lactam
19	M	+/-	-	-	"
17	M	+	+	-	"

Published data concerning carriage rates of C.difficile amongst patients with CF are rare. However, a carriage rate of 50% has been suggested (1). Our own study has also shown raised carriage rates amongst these patients, but with marked differences between patients from the two participating hospitals. There appeared to be a 50% carriage rate amongst patients at the London Chest Hospital (LCH), whilst the organism was recovered from only 24% of the Brompton Hospital (BH) patients. However, most of the CF patients came from BH and had the study been extended to rectify this imbalance, the differences may well have been less marked. It is unlikely that the organism remained undetected in some of the samples through storage loss. Willey and Bartlett (7) found that C.difficile could be readily recovered from faeces despite freezing of the specimen prior to culture. A more reasonable explanation for our observation concerns local antibiotic usage. Nebulized antibiotic treatment (used by seven patients at BH) may well disturb faecal ecology to a lesser extent than systemic antibiotic administration.

It is interesting to note that in the present study, β-lactam antibiotics had been used in all culture positive patients (both control and CF). Bartlett (8) and George et al. (9) have observed an association between exposures to penicillins and cephalosporins and enteric disease due to C.difficile.

Many of the isolates of C.difficile in the present study were capable of producing cytotoxin, although we encountered no gastrointestinal disease. The value of cytotoxin as a marker of pathogenicity in these patients merits reassessment and further studies must investigate the ability of the isolates to produce enterotoxin together with in vivo pathogenicity tests.

Finally, it must be remembered that whilst C.difficile in CF patients may be carried asymptomatically, the strains may be pathogenic if transferred to other patients. It has been suggested that patients with demonstrable C.difficile in their stools represent a cross-infection risk (10). We note, however, that at the hospitals included in the present study, cross-infection with C.difficile does not appear to be a problem in practice.

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SOME ASPECTS OF COLONIC MICROBIAL ACTIVITY IN IRRITABLE BOWEL SYNDROME ASSOCIATED WITH FOOD INTOLERANCE

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Summary. - Bacteriological studies on stools from patients three months post-hysteroctomy showed that increased score of Irritable Bowel Syndrome (IBS) symptoms was associated with significantly increased levels of aerobic bacteria, whereas in 'naturally arising' IBS patients levels of aerobes were similar to those in control subjects. There was a greater diversity of aerobic species and decreased recovery of Gram positive aerobic bacteria in both groups of patients. No significant differences were observed in moisture, pH or volatile fatty acid content of faeces of patients and controls.

Riassunto (Alcuni aspetti della attività microbica del colon nella sindrome del colon irritabile associata con intolleranza alimentare). - Studi batteriologici sulle feci di pazienti operati da tre mesi di isterectomia mostrano che un aumento di sintomi della sindrome dell'intestino irritabile (IBS) è associato ad un aumento significativo di batteri aerobi, mentre in pazienti con IBS sorto non a seguito di isterectomia le quantità di batteri aerobi sono simili a quelle presenti in soggetti controllo. In entrambi i gruppi di pazienti c'è una maggiore diversità di specie aerobie ed una diminuita frequenza di isolamento di batteri aerobi Gram positivi. Non sono state osservate differenze significative nella quantità di acqua nel pH e negli acidi grassi volatili contenuti nelle feci di pazienti e controlli.

Introduction

Food intolerance is a major factor of Irritable Bowel Syndrome (IBS) and the association between food and symptoms has been tested (1). Several observations point to the significance of the bacterial flora in this phenomenon. In many patients symptoms start after a gut infection or date from courses of antibiotics or from abdominal or pelvic surgery (2). Food challenge in these patients does not produce symptoms of pain, flautance and sometimes diarrhoea until 16-72h after challenge and there is little, if any, evidence of any immunological abnormality (1).

In some patients pilot studies have indicated higher aerobic bacterial counts in faeces of IBS patients than in controls. A more diverse range of Gram negative aerobic bacteria was isolated from patients (3).

In this study levels of aerobic bacteria, moisture content, pH and volatile fatty acids (VFA) were determined on 103 stool samples from 101 subjects. The subjects were a group of patients from a double blind, controlled, prospective hysterectomy study which showed that treatment courses of antibiotics increased the vulnerability of patients to IBS (4), and 'naturally arising' IBS sufferers presenting at clinic, prior to exclusion diet therapy, together with

a control group.

Materials and Methods

Freshly passed stools were collected at home to minimise stress and transported under cool conditions to the laboratory for analysis within 5 hrs. Aerobic bacteria were enumerated using CLED (Oxoid) agar, moisture content was measured by drying samples (c.5g) to constant weight at 100°C and VFA were estimated by headspace analysis. Aerobic bacteria were characterised using the API 20E identification system (API Laboratory Products) or the methods of Cowan (5). Data from samples which were delayed in transit, or where patients were subsequently found to have clinically defined diseases, was discarded. IBS symptom scores in hysterectomy patients were assessed using questionnaires preoperatively and 3 and 18 months post-operatively.

Results

The viable aerobic bacterial counts in faeces were similar for both IBS and control categories (Table 1). Analysis of the data relating to the 'naturally arising' IBS group also showed that counts were not significantly different from the control group.

Table 1. - Faecal properties in food related IBS

	N	Log_{10} aerobic bacteria/g wet weight		Moisture content (%)		pH		N	Total VFA moles/g wet weight	
		Mean	SE	Mean	SE	Mean	SE		Mean	SE
All categories										
IBS	56	7.62	0.14	75.0	1.0	7.10	0.08	38	118	6
Control	35	7.47	0.16	72.2	1.0	7.10	0.08	34	112	5
'Naturally arising'										
IBS	38	7.65	0.14	74.4	1.2	7.09	0.10	26	125	7
Control	16	7.42	0.17	73.8	1.5	7.01	0.14	16	115	8
3-months post-hysterectomy										
Placebo only	5	7.04[a]	0.32							
Prophylaxis	10	6.80[a]	0.47							
Antibiotic treatment	18	7.82[a]	0.16							

[a] Coefficient of contrast p=0.018
(Placebo only and Prophylaxis vs. Antibiotic Treatment)
N = No. of samples tested

Assessment of the symptoms related to IBS in gynaecology patients using questionnaires showed that there was a significantly greater increase in

symptom score in patients who had received post-operative treatment courses of antibiotics than those receiving placebo or Metronidazole prophylaxis ($p=<0.04$) (4), and furthermore that stools from these patients showed higher viable counts of aerobes (Table 1). There was a significant correlation between 3-months post-operative symptom score and aerobic bacterial count irrespective of drug treatment (Fig. 1).

There were no significant differences in moisture content, pH and VFA levels (Table 1) or proportions of individual acids (data not shown) for all categories tested.

Escherichia and Streptococcus were the common genera in the stools. Other genera isolated were Staphylococcus, Proteus, Klebsiella, Enterobacter and Micrococcus. Genera other than Escherichia and Streptococcus were isolated from 17 out of 38 patients from the 'naturally arising' IBS group and 4 out of 16 controls, and from 5 out of 33 hysterectomy patients of whom 4 had a high symptom score. There was a decrease in the % Gram positive aerobic bacteria in the stools in the patient groups (Table 2), although the decrease was not significant.

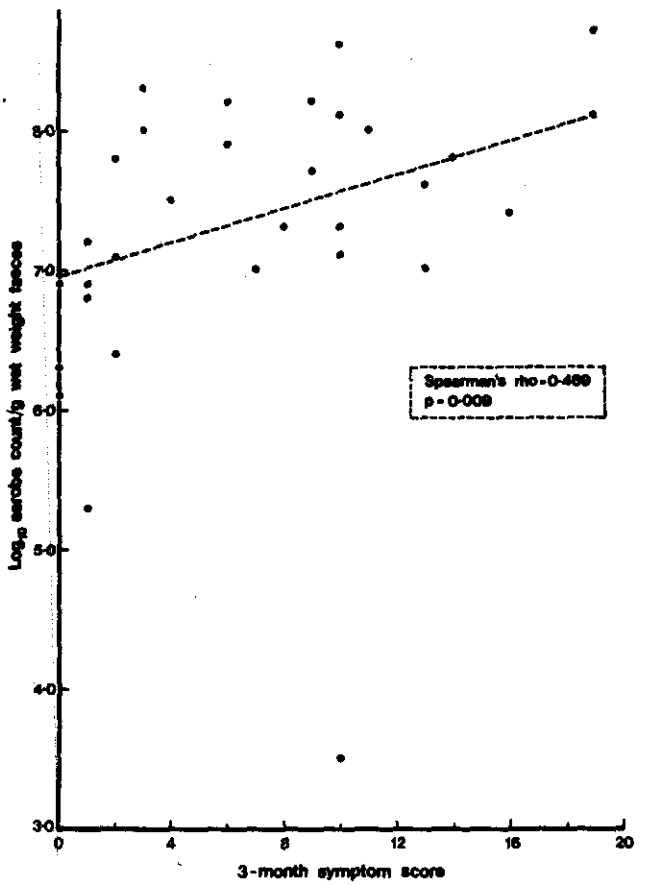

Figure 1. - Correlation between 3-month post-operative symptom score and aerobic stool count.

Table 2. - Gram positive aerobic bacteria present in faeces

	Treatment	N	Mean %	S.E.	P
All categories					
	IBS	55	30.2	5.0	0.61
	CON	34	34.5	6.7	
'Naturally arising'	IBS	42	36.5	6.1	0.42
	CON	19	45.3	9.1	
3-month post-hysterectomy symptom score vs. species					
	Placebo + Prophylaxis	15	35.7	10.0	0.32
	Antibiotic treatment	16	22.0	8.5	

Discussion

In this study we have shown that hysterectomy patients who exhibit IBS symptoms post-surgery excrete higher concentrations of aerobic bacteria in faeces.

In healthy individuals transient increases in numbers of aerobes and the appearance of Klebsiella, Enterobacter and Serratia are known to occur following treatment with Metronidazole (6). In the hysterectomy patients from the study reported here stool samples were taken several weeks after antibiotic treatment suggesting either that antibiotic treatment per se was not significant or that in patients suffering from IBS symptoms the changes in flora resulting from antibiotic treatment were less easily reversed. The trend towards decreased numbers of Gram positive aerobes and the appearance of organisms other than E.coli and streptococci in both groups was not statistically significant, but it is possible that within the limited number of individuals tested more than one microbiologically distinct group existed. These groups are different to those observed by Balsari et al. (7) who showed that with a group of IBS patients whose altered intestinal mobility was described as being 'essentially caused by psycho-functional phenomena' faecal coliforms were significantly reduced. These authors also reported the appearance of Pseudomonas and Enterobacter although it is not clear whether these organisms were associated with hospitalisation. The changes in the flora in the limited number of out-patients studied here supports the idea that in some IBS patients the state of the aerobic gut flora may be an indicator of the disease state, although further work would be required to elucidate the significance of these changes.

There was no significant difference between patients and controls in faecal miosture, pH or VFA content. Many patients with IBS show symptoms of diarrhoea but the difference in moisture content between formed and unformed stools can be as low as 1-2% (C.E. Bayliss: unpublished data).

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THE MICROBIOLOGY OF ILEAL RESERVOIRS FOLLOWING RESTORATIVE PROCTOCOLECTOMY

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Summary. - The bacteriology of ileal reservoirs in 66 patients is reported. The mean total count was 7.2 $\log_{10}$cfu/g consisting of almost equal numbers of aerobes and anaerobes. A correlation was found between the counts of E.coli and the degree of chronic inflammation in the reservoir. The possible significance of this is discussed.

Riassunto (Studio microbiologico del serbatoio ileale in seguito ad intervento di proctocolectomia). - Viene riportata la flora batterica presente in 66 pazienti con serbatoio ileale. La media della conta batterica era 7,2 $\log_{10}$cfu/g con un uguale numero di aerobi ed anaerobi. E' stata riscontrata una correlazione tra conte di E.coli e grado di infiammazione cronica nel serbatoio ileale: ne viene discusso il possibile significato.

Introduction

Patients with diffuse colonic diseases such as ulcerative colitis and familial adenomatosis coli are successfully treated by proctocolectomy but at the cost of a permanent ileostomy. As an alternative, colectomy and ileo-rectal anastomosis to restore intestinal continuity has been widely used. However, this has the disadvantage that not all the diseased tissue is removed, as the rectum is preserved. Proctocolectomy maintaining the normal route of defaecation by ileo-anal anastomosis avoids this disadvantage but function in these patients, in particular frequency of defaecation, is often unsatisfactory.

The operation of restorative proctocolectomy with ileo-anal reservoir (1) may overcome these problems. It appears to eradicate the disease but at the same time restore gastrointestinal continuity and continence. The elimination of all the diseased tissue means that the risk to the patient of developing carcinoma is also negligible. However, the operation leaves the patient with a pouch of small bowel in which faeces may remain for a considerable of time. This could lead to a stagnant loop syndrome as a result of bacterial overgrowth in the reservoir due to stasis (2). Several studies of the operation have now been published but they have been concerned primarily with variations in operative technique and physiological studies of continence and evacuation (3). We previously reported a pathological and physiological assessment of 14 patients who had this operation performed (1).

A more extensive pathophysiological assessment of 66 patients has now been

completed. In this paper we describe a possible correlation between the microbiological findings and chronic inflammation of the pouch.

Materials and Methods

A specimen of pouch mucosa was obtained by sigmoidoscopy. Chronic inflammation was assessed microscopically using a numerical scale on the basis of the degree of villous atrophy and the presence of a chronic inflammatory cell infiltrate consisting mainly of lymphocytes and plasma cells in the lamina propria. Each parameter had a maximum score of 3 and the total maximum score was therefore 6.

A second sigmoidoscopy was carried out to obtain a specimen for bacteriology. The specimen was placed in faecal transport broth (4) and frozen at -70°C until processed. Samples were thawed by placing in an anaerobic chamber, and homogenised by the addition of glass beads and rotamixing for three minutes. They were then serially diluted in the supernatant from pre-steamed Robertson's Cooked Meat. For anaerobic incubation samples (100µl) were spread on the surface of the following media which had been poured in the chamber: Brucella blood agar, neomycin (100µg/ml) - blood agar and Kanamycin (50µg/ml)- Vancomycin (5µg/ml) - lysed blood agar were prepared by previously described methods (5); Rogosa agar (Difco) with a final pH of 5.4 as a selective medium for lactobacilli. The dilution tubes were then removed from the chamber and 100ul samples were spread on plates of the following media for aerobic cultivation: Blood agar, MacConkey agar. Anaerobic culture plates were incubated in the chamber for up to 7 days at 35°C and the aerobic plates were incubated in the chamber for 2 to 3 days at 35°C in an atmosphere of air and 5% CO_2. Different colony types were counted and subcultured onto fresh media for identification. Counts were expressed as the logarithm of the number of colony-forming units per gram ($\log_{10}$cfu/g). The minimum number of bacteria that could be detected was about 3.0 $\log_{10}$cfu/g.

A possible correlation between the bacterial counts and the degree of inflammation was assessed using the contingency coefficient, C (6).

Results

All the specimens apart from one gave some bacterial growth (98%). The mean total bacterial count was 7.2 $\log_{10}$cfu/g, consisting of almost equal numbers of aerobes (7.0 $\log_{10}$cfu/g) and anaerobes (7.1 $\log_{10}$cfu/g). Nearly all the specimens grew aerobes (91%) but only 59% grew anaerobes.

There was some degree of chronic inflammation in nearly all the reservoirs.

Because there was no biochemical, haematological or physiological explanation for the inflammation, a possible correlation with the bacterial findings was considered. It was found that there was a significant correlation between the counts of Enterobacteriaceae, (consisting mainly of E.coli) and the degree of chronic inflammation ($p<0.001$). Specimens with a count greater then 6.0 $\log_{10}$cfu/g always had a high degree of chronic inflammation (Fig 1). In some cases there was a high degree of inflammation but only a low count of Enterobacteriaceae.

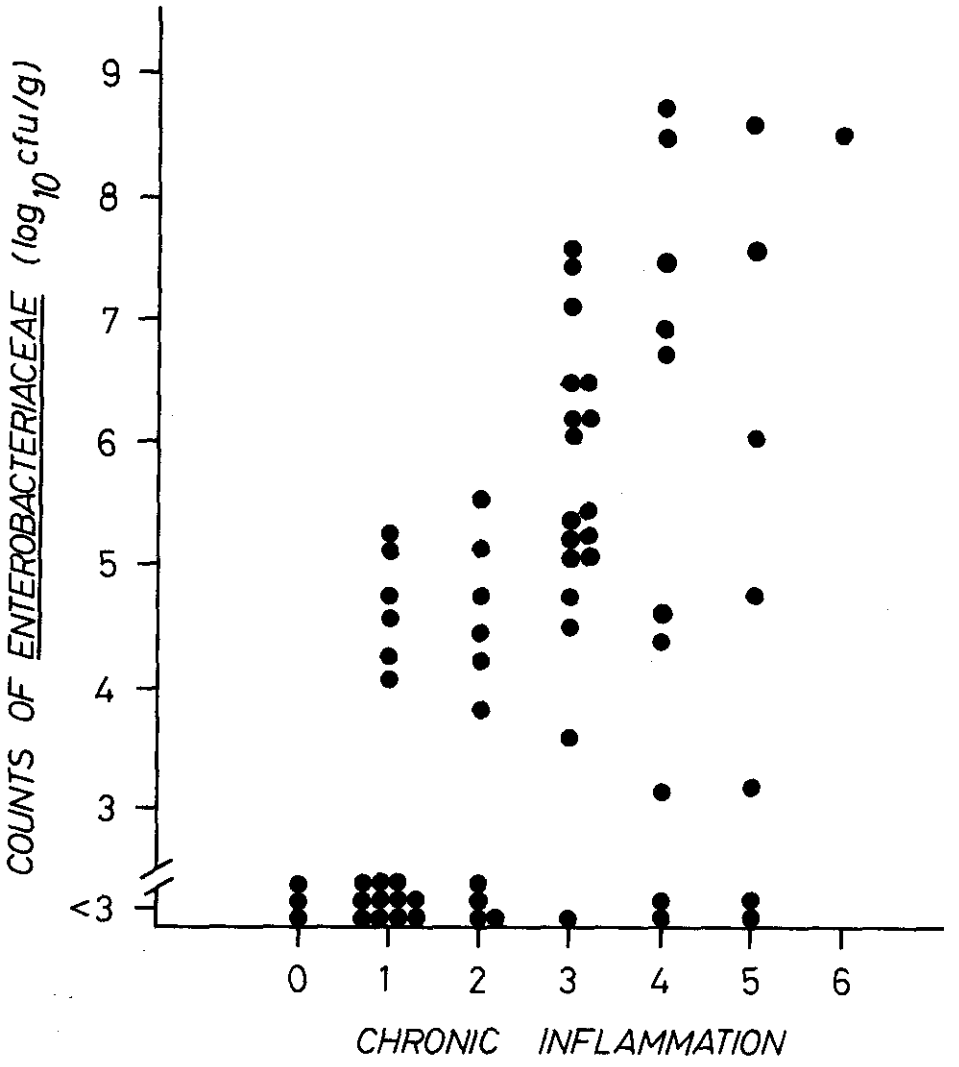

Fig. 1. - Correlation between counts of Enterobacteriaceae and degree of chronic inflammation.

Discussion

If E.coli is responsible for the inflammation then this could be caused by several different mechanisms, e.g. production of toxins or other metabolic products or it could be that the actual stains of E.coli are different in some way, for example that they are invasive. The fact that high counts of E.coli were invariably associated with inflammation makes the first suggestion the more likely. Finally, as inflammation makes the first suggestion the more likely. Finally, as inflammation is also occasionally present in cases where the bacterial counts were low, there may be other causes of inflammation.

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LE COMPLICANZE INFETTIVE NEI PAZIENTI CON EMOPATIE MALIGNE

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Riassunto. - Vennero studiate la frequenza, l'etiologia ed il tipo di infezioni nei pazienti emopatici ricoverati negli anni 1982-84 presso la Cattedra di Ematologia di Roma. 356 di 631 pazienti (56,4%) sottoposti a terapia citostatica svilupparono 504 episodi febbrili di provata o presunta infezione. Di questi, ben 102 casi furono superinfezioni. Viene sottolineata l'emergenza dei funghi e del C.difficile tra gli agenti di superinfezione.

Summary (Infections in patients with haematologic malignancies). - Frequence, etiology and microbiological as well as clinical documentation of infections in patients with hematological malignancies hospitalized at the Department of Hematology of the University of Rome were studied over a span of three years (1982-84). 356 out of 631 patients (56,4%) under cytotoxic therapy underwent 502 febrile episodes of proved or presumed infections. Out of these, 102 febrile episodes were superinfections. The authors underline the emergence of fungi and Clostridium difficile as etiologic agents of superinfections.

Introduzione

La terapia antimicrobica empirica precoce con combinazioni antibiotiche presumibilmente sinergiche nei confronti dei più comuni agenti etiologici ha consentito una notevole riduzione della mortalità per complicanze infettive nei pazienti affetti da emopatie maligne (1). In particolare un grande contributo alla migliorata prognosi dei pazienti granulocitopenici febbrili è stato dato dalle cefalosporine della terza generazione e dalle ureidopenicilline, particolarmente attive nelle infezioni dovute ai bacilli Gram-negativi, che sono stati i più frequenti agenti etiologici degli ultimi anni (2). Tuttavia nuovi patogeni e cocchi Gram-positivi, la maggior parte dei quali resistenti ai trattamenti sopra menzionati, stanno emergendo (3). Lo scopo di questo studio retrospettivo è stato quello di valutare il tipo e la etiologia delle infezioni nei pazienti emopatici ricoverati negli anni 1982, 83, 84 presso la Cattedra di Ematologia della Università di Roma.

Materiali e Metodi

Dal gennaio 1982 al dicembre 1984, 831 pazienti emopatici vennero ricoverati presso la Cattedra di Ematologia. Di questi 631 ricevettero chemioterapia citostatica per l'emopatia maligna di base. Al momento della febbre (T=38,5°C) i pazienti furono subito valutati clinicamente e microbiologicamente e ricevettero entro 2-4 ore combinazioni antibiotiche nelle modalità già descritte (4).

Gli episodi febbrili iniziali furono classificati in accordo con De Jongh et al. (5) come "infezioni microbiologicamente documentate" (sito ed etiologia di infezione identificati), "infezioni clinicamente documentate" (sito di infezione identificato, non l'etiologia), "infezioni possibili" (segni e sintomi equivoci di infezione), "infezioni dubbie" (retrospettivamente, la febbre fu ritenuta di origine non infettiva). Allo stesso modo abbiamo distinto le superinfezioni, vale a dire ogni infezione nello stesso sito iniziale causata da una etiologia diversa da quella iniziale che si sia sviluppata durante la terapia antibiotica o entro sette giorni dalla sua sospensione, o qualsiasi infezione sviluppatasi in tale lasso di tempo in altre sedi. L'analisi statistica dei dati è stata eseguita attraverso la determinazione del chi-quadro.

Risultati

356 pazienti dei 631 emopatici trattati con chemioterapia svilupparono 504 episodi febbrili di probabile o provata infezione. Come mostra la Tabella 1 circa un quinto di essi furono superinfezioni.

Setticemie (170 casi) e polmoniti (65 casi) furono le principali cause di morbilità, rappresentando insieme il 36,8% degli episodi febbrili iniziali e il 72,5% delle superinfezioni. 170/504 episodi febbrili furono associati a setticemia, nella maggior parte dei casi (119/170) il cui focolaio di origine non è stato identificato. Come si vede nella Tabella 2, 172 patogeni responsabili di infezione furono isolati inizialmante in 160 infezioni microbiologicamente documentate.

Tabella 1. - Documentazione dell'infezione negli episodi febbrili iniziali e nelle superinfezioni

	Episodi febbrili iniziali (402 casi)		Superinfezioni (102 casi)	
	n	(%)	n	(%)
Infezioni documentate microbiologicamente:	160$	(39,8)	92§	(90,1)
con batteriemia	119	(29,6)	51	(50,0)
senza batteriemia	47	(11,2)	45	(44,1)
Infezioni documentate clinicamente:	66	(16,4)	10	(9,9)
Infezioni possibili:	139	(34,5)	-	
Infezioni dubbie:	37	(9,3)	-	

$ Sei casi di infezione doppia
§ Quattro casi di infezione doppia

Tabella 2. - Agenti etiologici delle infezioni isolati prima e dopo l'inizio della terapia antibiotica

	prima	dopo
Gram negativi	89(51,7%)	37(36,6%)
Gram positivi	67(38,9%)	24(23,7%)
Anaerobi	3(1,7%)	15(14,8%)
Funghi	13(7,5%)	25(24,7%)

Bacilli Gram-negativi furono osservati nel 51,7% dei casi e microrganismi Gram-positivi nel 38,9% dei casi, mentre funghi furono isolati nel 7,5% e microrganismi anaerobi nell'1,7% dei casi. Fra tutti, E.coli fu la principale causa di infezione iniziale (36/172), seguito da Staph.aureus (32/172) e da Pseudomonas aeruginosa (30/172). In aggiunta a questi agenti etiologici iniziali, le colture ottenute nei pazienti che svilupparono superinfezioni rivelarono altri 101 microrganismi. Come appare in Tabella 2, gli agenti etiologici delle superinfezioni differirono da quelli delle infezioni iniziali. La frequenza dei microrganismi Gram-negativi e Gram-positivi diminuì al 36,6% ed al 23,7% dei casi rispettivamente, mentre funghi ed anaerobi furono isolati, rispettivamente, nel 24,7% e nel 14,8% dei casi. E.coli e Staph.aureus furono isolati soltanto nel 3% e nel 5% dei casi rispettivamente, mentre gli agenti etiologici prevalenti delle superinfezioni furono Ps.aeruginosa (19%) fra i bacilli Gram negativi, C.difficile (11%) fra gli anaerobi, Staph.epidermidis e Strept.faecalis (8%) fra i microrganismi Gram-positivi e Aspergillus sp. (7%) fra i funghi.

Come si vede in Tabella 3, su 262 pazienti che svilupparono una grave neutropenia (100 Neutrofili/mm cubico) durante il periodo di ospedalizzazione, 239 (91%) furono affetti da uno o più episodi febbrili da provata o presunta infezione, mentre 66 (25,1%) svilupparono una superinfezione. I pazienti meno granulocitopenici ebbero un significante minor numero di episodi di infezione e superinfezione.

Discussione

Il nostro studio retrospettivo fa trarre alcune conclusioni. Le infezioni rappresentano ancora oggi una complicanza comune e grave nei pazienti emopatici. L'etiologia più frequente è ancora rappresentata, a dispetto dell'aumento di incidenza delle forme sostenute dai cocchi Gram-positivi, dai bacilli Gram-negativi. Ciò testimonia della importanza dell'intestino quale porta d'ingresso dell'infezione. La terapia antibiotica empirica iniziata precocemente consente di migliorare in modo significativo la prognosi di tali complicanze (1). Tale miglioramento, soprattutto nei pazienti più gravemente neutropenici, può essere inficiato dalla insorgenza di superinfezioni che riconoscono etiologie spesso diverse da quelle dell'infezione iniziale. Il dato più importante in tali pazienti è costituito dall'alta incidenza delle micosi sistemiche sia durante che dopo il trattamento antibiotico.

Questi risultati sembrano confermare precedenti osservazioni (2) secondo le quali l'efficacia dei regimi antibiotici nei pazienti emopatici granulocitopenici dovrebbe essere valutata non solo nelle fasi iniziali del trattamento antimicrobico, ma anche successivamente, se il paziente rimane persistentemente neutropenico ed a rischio di superinfezione.

Tabella 3. - Relazione tra granulociti polimorfonucleati circolanti, episodi febbrili (infezioni provate o presunte) e superinfezioni

PMN/mmcc	N.pazienti	Pazienti con uno o più episodi febbrili (%)		Pazienti con superinfezioni (%)
100	262	91,2	A	25,1
100-499	127	51,2	B	7,8
500-999	41	17,1	C	0
1000	201	22,4	D	0,9
Totale	631	56,4		12,3

A vs B, A vs B+C+D, B vs C, B vs C+D = $p<0,0005$

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INFECTION PREVENTION DURING INDUCTION OF REMISSION IN CHILDREN WITH NEWLY DIAGNOSED ACUTE LEUKEMIA

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Summary. - During a 4 year's period, 96 children were treated for a first remission induction of their newly diagnosed acute leukemia. 65 children (group A) were nursed in a single room and received no prophylactic antibiotics. The other 31 children (group B) were nursed in a partial protective isolation and received selective antimicrobial modulation (cotrimoxazol and/or nalidixic acid and/or polymyxin together with amphotericin or nystatin or ketoconazol). In group A, 26.9% of the children had a severe infection (sepsis/meningitis/pneumonia) and 11% a minor infection (urinary tract infection). In group B, 9.7% of the children had a severe infection and none a minor infection. Selective antimicrobial modulation together with partial protective isolation, gives a significant reduction of infections, during the first induction treatment of children with acute leukemia.

Riassunto (Modulazione antimicrobica selettiva in bambini con leucemia acuta recentemente diagnosticata). - 96 bambini affetti da leucemia acuta di recente insorgenza sono stati trattati, in un periodo di 4 anni, per una prima induzione di remissione. 65 (gruppo A) sono stati ricoverati in camere singole e non hanno ricevuto profilassi con antibiotici. 31 (gruppo B) sono stati isolati solo parzialmente ed hanno ricevuto profilassi antimicrobica selettiva con cotrimoxazolo e/o acido nalidixico e/o polimixina insieme ad amfotericina o nystatin o ketoconazolo. Nel gruppo A il 26,9% dei bambini ha sviluppato infezioni gravi (sepsi/meningiti/polmoniti) e l'11% infezioni minori (infezioni urinarie). Nel gruppo B, il 9,7% dei bambini ha sviluppato infezioni gravi e nessuno infezioni minori. Si conclude che durante il trattamento di induzione nei bambini affetti da leucemia acuta, la profilassi antimicrobica selettiva ed un parziale isolamento portano ad una riduzione significativa delle infezioni.

Introduction

Infection is a major cause of morbidity and mortality in children with leukemia (1, 2). The most important predisposing factors to these infections are severe neutropenia and cytostatic induced mucosal barrier lesions (3, 4). Many of these infections are caused by micro-organisms which are already present in the resident endogenous microflora at the time of admission or by micro-organisms acquired during hospitalization. Prevention of infection in patients with acute leukemia has been studied by limiting the acquisition of new organisms (protective isolation) (5) and/or the suppression of the endogenous potentially pathogenic micro-organisms (decontamination or antimicrobial modulation) (6, 7). To achieve selective antimicrobial modulation (SAM) patients receive a combination of antibiotics with activity against aerobes and

fungi (the potentially pathogenic micro- organisms) but not against anaerobes thus preserving the colonization resistance of the digestive tract (8). We studied in children the efficacy of selective antimicrobial modulation and protective isolation during the first induction of remission in acute leukemia.

Patients and Methods

Patients. - All patients aged 1 to 15 years with newly diagnosed acute leukemia in 2 university children's hospitals were studied during their first induction of remission. Table 1 shows the composition of the patient groups studied with respect to classification of leukemia. The criteria used for high risk ALL were an initial leukocytosis $\geq 50 \times 10^9/l$ and/or an initial leukemic organ infiltration and/or a broadened mediastinum on the chest X-ray.

Treatment for leukemia. - All patients were treated according to the same protocols of the Dutch Childhood Leukemia Study Group. Briefly, all patients received a remission induction regimen of vincristine, prednison and asparaginase (regimen VA). The patients in group VB also received four gifts of rubidomycin.

Patient nursing. - All the patients in hospital A were nursed in single rooms. The patients in hospital B were also nursed in single rooms, but during neutropenic episodes (PMN count $< 500/mm^3$) the patients were nursed in a more protective environment (single rooms, aseptic handling by nurses) and received food with a low bacterial level.

Selective antimicrobial modulation. - All the patients in hospital A received nystatin prophylaxis during hospitalization. The patients in hospital B received a SAM regimen. The skin was daily washed with povidone-iodine soap. The SAM regimen of the gastro-intestinal tract consisted of an antimycotic agent (nystatin or amphothericin B or ketoconazol) and a combination of polymyxin B and cotrimoxazol, all given orally. The first ten patients received monotherapy (either polymyxin or cotrimoxazol) but microbiologic surveillance showed the emergence of resistance of some Enterobacteriaceae in the stool. After combined prophylaxis (polymyxin and cotrimoxazol) no resistant Enterobacteriaceae were cultured any more.

Infections. - The infections were defined as minor infections (superficial infections of the skin and mucosa) or major infections (infections with invasion of deep tissues of organs: pneumonia, meningitis, arthritis, and septicemia). Only the microbiological proven infections are taken into consideration.

Microbiology. - A microbiological inventory of the patients was carried out on admission and furtheron twice a week. Samples of stool and urine and swabs from the nose and skin were cultured on selective and non-selective media.

During periods of fever (axillary temperature > 38°C) blood samples were cultured, both aerobically and anaerobically.

Results

At admission 14 of the 96 patients had a proven infection. In addition 24 of the remaining children had fever without a recognizable localized infection.

Table 2 shows the days of neutropenia in both groups. In hospital A (the control group) 53 out of 65 patients (82%) experienced one or more episodes of

Table 1. - Some characteristics of the patients with newly diagnosed acute leukemia

Hospital A		Hospital B	
ALL 52 children		ALL 29 children	
high risk	17	high risk	8
non high risk	35	non high risk	21
VA	16	VA	9
VB	19	VB	12
ANLL 13 children		ANLL 2 children	

ALL: acute lymphoblastic leukemia; ANLL: acute non-lymphoblastic leukemia. VA and VB are two different treatment regimens

moderate neutropenia (< 500/mm^3) and 36 out of 65 (55%) got through one or more periodes of severe neutropenia (< 100/mm^3). In hospital B (the SAM group) 27 out of 31 children (87%) encountered a periode of moderate neutropenia and 20 patients (65%) periode(s) of severe neutropenia.

Table 3 shows the infections acquired after hospitalization in the neutro-

Table 2. - Neutropenic days in the two patient groups

	Hospital A	Hospital B
< 100 granulocytes/mm^3	379 (n = 36)	226 (n = 20)
mean	10	11
< 500 granulocytes/mm^3	1512 (n = 53)	561 (n = 27)
mean	30	21

Table 3. - Infections acquired after hospitalization in the neutropenic patients (PMN < 500/mm^3)

Hospital A (n=53; total 1512 days) (control group)		Hospital B (n=27; total 561 days) (SAM group)	
Severe infections		Severe infections	
(17 patients / 20 episodes)		(3 patients / 3 episodes)	
sepsis	18	sepsis	2
meningitis	2	bronchopneumonia	1
pneumonia	2		
Minor infections		Minor infections	
(7 patients / 7 episodes)		none	
urinary tract	6		
skin	1		

penic patients. The major pathogens that were responsible for the infections in the control group were Enterobacteriaceae, Pseudomonas aeruginosa and Staphylococcus aureus. Our results show that the incidence of infection was significantly reduced in the SAM group, although the SAM regimen was not totally successful. One patient had a sepsis with Escherichia coli, one patient with Staphylococcus aureus and the third patient had a bronchopeumonia with Haemophilus influenzae. In addition 23 children in the control group received systemic antibiotics because of a probable but not microbiologically proven infection, against only one child in the SAM group. No fungal infections were seen in both patient groups. In the control group 2 patients died as the result of a bacterial infection whereas no deaths attributable to this cause occurred in the SAM group.

Discussion

The data obtained in this study show the effectiveness of selective antimicrobial modulation and protective isolation as a means for infection prevention in neutropenic children during the first induction of remission. Almost all children tolerated the SAM regimen rather well. Poor compliance was seen in the patients with infection but this might be a sign of infection and not an independent variable as suggested by others (6). The effectiveness of SAM can be shown by the lower infection rate, i.e. the percentage of patients with an infection (9). In the control group the infection rate during moderate neutropenia was 45% against 11% in the SAM group. The number of infections per 1000 days at risk was 17.8 in the control group against 5.3 in the SAM group for the same moderately neutropenic patients. The differences are even more striking in the severely neutropenic group. In the control group there was one sepsis every 34 days against 1 every 226 days in the SAM group.

In conclusion, selective antimicrobial modulation and protective isolation show a significant reduction of bacterial infections in children with neutropenia during their first induction of remission in acute leukemia.

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LA COLITE DA CLOSTRIDIUM DIFFICILE IN PAZIENTI EMOPATICI

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Riassunto. - Sono stati studiati 821 pazienti affetti da neoplasie ematologiche sottoposti a terapia antibiotica e antiblastica: di questi, 155 hanno sviluppato durante il ricovero sintomi addominali (diarrea, dolori e/o distensione).

Il C.difficile è stato isolato dalle feci di 28 dei 155 pazienti con sintomi addominali (18%), mentre la citotossina è stata ricercata in 18 pazienti con C.difficile ed è stata dimostrata in 9. Il 96% dei pazienti con C.difficile aveva ricevuto in precedenza farmaci antiblastici e antibiotici.

Il C.difficile è stato isolato anche da pazienti che non presentavano diarrea ma solo dolori e/o distensione addominale (32%). Nei pazienti con C.difficile i sintomi addominali non si sono associati a sepsi polimicrobiche e 6 pazienti hanno presentato ittero.

Summary (Clostridium difficile colitis in patients with leukemia). - 821 patients with haematological malignancies, receiving antibiotic and cytotoxic therapy, have been studied. 155 developed abdominal disturbances (diarrhea, pains and/or distension) while in hospital.

C.difficile was isolated from faeces of 28 out of 155 patients with abdominal symptoms (18%); cytotoxin was found in the stools of 9 patients out of 18 tested.

96% of C.difficile positive patients had previously received antibiotics and cytotoxic drugs.

C.difficile was isolated also from patients who presented with abdominal pains and distension but no diarrhea (32%). C.difficile colitis was not associated with polymicrobic septicemia; only six patients developed jaundice.

Introduzione

Tra i pazienti immunocompromessi, quelli affetti da neoplasie ematologiche e sottoposti a terapie antiblastiche e antibiotiche rappresentano un importante gruppo di pazienti, suscettibile alle infezioni opportunistiche (1). Queste condizioni favoriscono, in alcuni casi, l'insorgenza della colite da C.difficile (2, 3).

Con il nostro studio ci siamo prefissi: di valutare l'incidenza della colite da C.difficile in pazienti affetti da neoplasie ematologiche, che presentano sintomi addominali quali diarrea, dolore e/o distensione, di dimostrare se la terapia antibiotica, antiblastica ed alcune caratteristiche cliniche quali il tipo di emopatia e lo stato di neutropenia rappresentano dei fattori di rischio per l'insorgenza della colite da C.difficile; di valutare la relazione tra colite da C.difficile e caratteristiche cliniche come la comparsa

di ittero o l'insorgenza di sepsi polimicrobiche, la cui presenza da alcuni Autori è stata associata, nei pazienti affetti da neoplasie ematologiche, all'infezione da C.difficile (2).

Materiali e Metodi

Pazienti studiati: tutti i pazienti ammessi alla Clinica Ematologica dell'Università di Roma nel periodo aprile 1982 - maggio 1985 che sviluppavano diarrea, dolori e/o distensione addominale.

Colture: i campioni di feci sono stati esaminati per C.difficile su terreno selettivo CCFA (4).

L'identificazione è stata eseguita secondo le tecniche descritte in precedenza (5).

La ricerca dei comuni enteropatogeni (Salmonella, Shigella, Campylobacter, Yersinia, Protozoi) è stata eseguita con colture convenzionali (6). Saggio di citotossicità: i supernatanti dei campioni fecali positivi per C.difficile sono stati saggiati per la presenza di citotossina secondo il metodo descritto in precedenza (5).

Risultati

Nel periodo tra aprile 1982 e maggio 1985 sono stati ricoverati nella Clinica Ematologica dell'Università di Roma, 821 pazienti. Di questi, 155 pazienti (18,8%) hanno presentato durante il ricovero sintomi addominali quali: diarrea, dolori e/o distensione; per tutti questi è stato eseguito un esame colturale per C.difficile, risultato positivo in 28 (18%). Gli 821 pazienti sono stati studiati in base ad alcune caratteristiche cliniche quali: diagnosi ematologica, terapia antibiotica e antiblastica e neutropenia con polimorfonucleati (PMN) < $1000/mm^3$ e PMN < $100/mm^3$ e comparati, riguardo alle stesse caratteristiche, con gli altri gruppi (vedi Tabella 1).

Tabella 1. - Studio dell'incidenza di sintomi addominali e della malattia da Clostridium difficile in un reparto di emopatici (1982-85)

CARATTERISTICHE CLINICHE	PAZIENTI IN STUDIO (N=821)			PAZIENTI CON SINTOMI ADDOMINALI (diarrea-distensione-dolore) (N=155)			PAZIENTI CON SINTOMI ADDOMINALI E CLOSTRIDIUM DIFFICILE (N=28)	
	n	(%)		n	(%)		n	(%)
DIAGNOSI								
LANL	254	(31)		62	(40)		12	(43)
LLA	208	(25,5)		46	(29,5)		7	(25)
LLC	12	(1,5)		4	(2,5)		1	(3,5)
LMC	36	(4,5)		11	(7)		3	(11)
LINFOMA DI HODGKIN	28	(3,5)		3	(2)		1	(3,5)
LINFOMA NON HODGKIN	90	(11)		15	(10)		2	(7)
ALTRI	191	(23)		14	(9)		2	(7)
TERAPIA								
ANTIBIOTICA	356	(43,5)	p <0,001	101	(65)	p <0,001	27	(96)
ANTIBLASTICA	631	(77)	p <0,001	154	(99,5)		27	(96)
NEUTROPENIA								
PMN $\leq 1000/mm^3$	430	(52)	p <0,001	112	(72)		22	(75)
PMN $\leq 100/mm^3$	262	(32)		41	(27)	p <0,01	13	(46)

Nei 155 pazienti con sintomi addominali, non è stata osservata, rispetto ai pazienti in studio senza sintomi addominali, nessuna differenza significativa rispetto alla diagnosi ematologica.

Si possono notare, invece, differenze statisticamente significative ($p < 0,001$) per quanto riguarda le terapie antibiotiche ed antiblastiche somministrate prima della comparsa dei sintomi addominali e lo stato di neutropenia con PMN < $1000/mm^3$.

Nel gruppo di 28 pazienti con sintomi addominali e C.difficile, abbiamo osservato, rispetto ai pazienti con solo sintomi addominali ma senza C.difficile, una differenza statisticamente significativa per la terapia antibiotica precedentemente somministrata e per la neutropenia con PMN < $100/mm^3$.

Nella Fig. 1 si può osservare la distribuzione dei 155 pazienti sintomatici e dei 28 pazienti con coltura positiva per C.difficile per il periodo aprile 1982 - maggio 1985. Malgrado la distribuzione nel tempo sia abbastanza uniforme, c'è una leggera tendenza dei pazienti con C.difficile a concentrarsi in alcuni periodi; questo fa pensare ad una circolazione dei ceppi di C.difficile tra i pazienti leucemici del reparto.

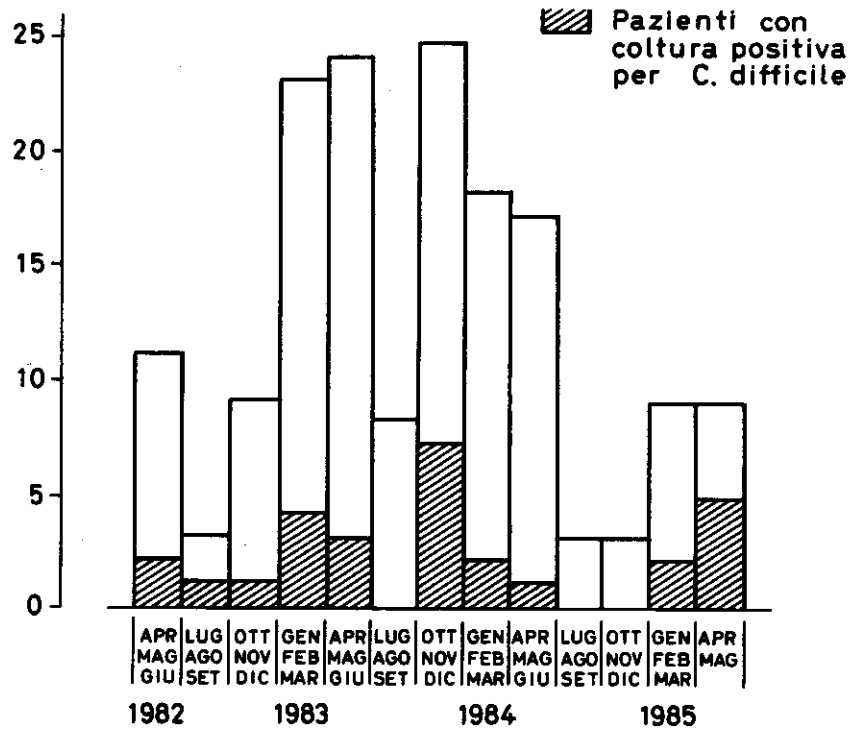

Figura 1. - Distribuzione dei 155 pazienti sintomatici e dei 28 pazienti con coltura positiva per Clostridium difficile per il periodo aprile 1982 - maggio 1985.

I 28 pazienti (13 maschi e 15 femmine) con C.difficile avevano età compresa tra 4 e 64 anni, con mediana di 43.

Gli antibiotici sono stati somministrati al 96% dei pazienti sotto forma di associazioni.

La più utilizzata (68%) è stata quella tra Cefalosporine di terza generazione (cefotaxine e ceftazidime) ed aminoglicosidi (tobramicina, gentamicina, amikacina).

Nel 21,5% dei pazienti è stata utilizzata l'associazione tra aminoglicosidi e penicilline semisintetiche (piperacillina), mentre in un solo paziente non sono stati somministrati antibiotici.

Tra i farmaci antiblastici l'associazione più utilizzata è stata la daunurubicina + citosina arabinoside (32%). Sono state utilizzate altre associazioni antiblastiche nel 43%, mentre in un solo paziente non sono stati somministrati antiblastici. Nei pazienti con colite da C.difficile i sintomi che sono stati rilevati con maggior frequenza sono la diarrea associata a distensione e a dolori addominali (50%), mentre dolori e distensione addominali senza diarrea sono risultati presenti nel 32% dei pazienti.

Nei 28 pazienti con C.difficile abbiamo osservato 7 sepsi (25%) comparse durante episodi di neutropenia. Una sola sepsi è stata polimicrobica, mentre tutte le altre sono state sostenute da un solo microrganismo. Il C.difficile non è stato mai isolato dal sangue.

L'ittero con bilirubina > 2mg% è stato osservato in 6 pazienti (21%). In un paziente era presente anche ascite ed uno solo è giunto a morte entro 7 giorni dalla comparsa dei sintomi di colite.

All'esame autoptico sono stati riscontrati colite pseudomembranosa in un paziente affetto da leucemia linfatica acuta, megacolon tossico in un paziente con leucemia mieloide cronica ed in uno con linfoma di Hodgkin.

La ricerca della citotossina nelle feci è stata effettuata in 19 pazienti con risultato positivo in 10. In vitro tutti i ceppi isolati producevano citotossina. La gravità dei sintomi addominali, soprattutto la diarrea, non si sono correlati con la presenza di citotossina nelle feci.

In 9 pazienti, per la gravità della colite, è stata somministrata vancomicina per os (500 mg ogni 6 ore) per almeno 7 giorni con pronta remissione della sintomatologia addominale e senza ricadute.

Discussione

La valutazione dell'incidenza della colite da C. difficile in pazienti affetti da neoplasie ematologiche con sintomi addominali (diarrea, dolore e/o distensione), presenta delle difficoltà dovute anche alla capacità dei farmaci antiblastici nell'indurre alterazioni intestinali (7). Tuttavia l'isolamento, nel 18% dei pazienti leucemici con sintomi addominali, del Clostridium difficile e della citotossina deve far riflettere sull'importante ruolo svolto da questo microrganismo nel causare quadri clinici che vanno dalla diarrea fino alla colite pseudomembranosa in questo gruppo di pazienti.

La terapia antibiotica somministrata in precedenza e la comparsa di neutropenia con PMN < $100/mm^3$, rappresentano in questi pazienti fattori di rischio per lo sviluppo di colite da C. difficile. Diversamente la diagnosi ematologica, la terapia antiblastica e la neutropenia con PMN tra 1000 e $100/mm^3$, non sono dei fattori di rischio.

Secondo alcuni Autori, la colite da C. difficile nei pazienti leucemici può presentarsi oltre che con i sintomi addominali anche con altre caratteristiche cliniche poco usuali, come sepsi polimicrobiche ed ittero, la "malattia" da Clostridium difficile (2). Nei nostri pazienti non abbiamo rilevato nessuna sepsi polimicrobica, mentre ittero è stato osservato in 6 pazienti. In proposito non è facile approfondire la patogenesi dell'ittero in pazienti leucemici in terapia con farmaci citotossici.

Per concludere, ci sembra opportuno sottolineare l'importanza di riconoscere e trattare la colite e/o malattia da C. difficile in pazienti con neoplasie ematologiche al fine di evitare complicanze anche mortali.

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