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European Journal of Cancer 40 (2004) 571–578

European Journal of Cancer

www.ejconline.com

Severity of enterocolitis is predicted by IL-8 in paediatric oncology patients

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Received 8 May 2003; received in revised form 15 September 2003; accepted 31 October 2003

Abstract

Enterocolitis in oncology patients remains an important complication, but there is a lack of insight into its likely severity from microbial, pathological and inflammatory aspects. Paediatric oncology patients admitted with neutropenic fever, who developed abdominal pain and diarrhoea, were monitored by the takers of rectal biopsies, cultures, and inflammatory marker measurements. Twenty-five patients were included (mean age 7.1 years). 8 patients (32%) needed intensive care treatment, 3 (12%) patients died. Gram-positive bacteraemia was diagnosed in 4 patients (16%). Most patients had negative blood and stool cultures. Predictors of a severe clinical course of the enterocolitis were an increased serum interleukin-8 (IL-8) (>1000 pg/ml) level and an increased serum C-reactive protein level (CRP) (>150 mg/l) level, both measured on the first day of clinical illness. Relative risks (RR) for admission to an Intensive Care Unit (ICU) were 11.3 (95% Confidence Interval (CI) 1.6–77.9) for elevated IL-8 levels and 6.4 (95% (CI) 0.92–45.1) for increased CRP levels. Rectal biopsies and pathology could not predict outcome ($P=0.22$). IL-8 analysis at the onset of enterocolitis symptoms can identify high-risk patients, which might be used clinically to design future intervention trials.

Keywords: Neutropenic enterocolitis; *Cl. difficile*; IL-8; Prognostic markers

1. Introduction

Neutropenic enterocolitis represents a complex spectrum of inflammatory processes of the colon seen in immunocompromised hosts after intensive chemotherapy for malignancies. It ranges from pseudo-membranous colitis caused by *Clostridium difficile* to typhlitis [1–3]. The clinical picture ranges from mild infection to severe transmural colitis with a high mortality-rate (50–100%) [4]. Neutropenic enterocolitis was initially defined as a clinical–pathological entity through retrospective review of autopsy findings in leukaemic patients first described by Cooke in 1933 [5]. The main causative organisms are Gram-negative bacilli, mainly *Pseudomonas aeruginosa* and *Escherichia coli*, followed by *Clostridium difficile*, *Clostridium septicum* and fungal pathogens [6]. The pathogenesis of this disorder is thought to be due to a multifactorial disruption of the mucosal barrier, in which the bacterial flora, neutropenia and cytotoxic therapy play a role. The invasive infection leads to ischaemia followed by necrosis of the various layers of the bowel wall. Although the process may have a pre-dilection for the terminal ileum and caecum, any segment of the bowel can be involved [7,8].

To date, prognostic inflammatory markers in neutropenic enterocolitis have not been defined, however, many studies have evaluated prognostic inflammatory markers in patients with fever in neutropenia irrespective of any symptoms of an impending enterocolitis [9–11]. Low serum interleukin-8 (IL-8) levels at the onset of fever can define a low-risk subgroup of neutropenic patients who can safely be treated with antibiotic monotherapy instead of combination therapy [12]. In similar terms, Bont and colleagues could define a low-risk group at the start of fever by the use of IL-8 and

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doi:10.1016/j.ejca.2003.10.021

IL-6 as plasma parameters: the IL-8 and IL-6 plasma concentrations were significantly increased in patients with chemotherapy-related neutropenia and fever due to bacteraemia (mainly Gram-negative bacteraemia) compared with fever of non-bacterial origin [10,13].

In neutropenic enterocolitis, both toxic and ischaemic bowel injury may play an important role. The response of the inflammatory cascade to pathogens attacking the gastrointestinal mucosa involves mainly the cytokines IL-6, IL-8, IL-10 and tumour necrosis factor-α (TNF-α) [14]. The role of these cytokines may be important in the pathophysiology of the inflammatory responses in neutropenic enterocolitis in children [11,15].

Therefore, a prospective single unit study was started to identify the incidence of enterocolitis in a paediatric oncology unit, to gain insight into the pathogenetic mechanisms, and to identify clinical and inflammatory prognostic markers.

2. Patients and methods

2.1. Patient selection

Entry criteria were neutropenic paediatric oncology patients who had abdominal pain, diarrhoea and fever for more than 24 h. Neutropenia was defined as <500/μl absolute neutrophils. Diarrhoea was defined as having at least grade 2 diarrhoea (4–6 times in 24 h correlating to the common toxicity criteria (CTC)). Fever was defined as a temperature >38.5 °C. All types of malignancies were included.

The study was performed in a single paediatric oncology unit. Approval from the medical ethics committee was obtained. Informed consent was obtained from the parents and from the child if >12 years of age.

2.2. Patient analyses

On the first day of the study the following procedures were done: (1) history and clinical check list, (2) physical examination (3) collection of stool cultures, (4) rectal biopsy, (5) blood investigations and (6) measurement of serum levels of inflammatory markers.

On days 3 and 7, the above investigations were repeated. The rectal biopsy was only repeated if the findings on day 1 were abnormal. The next course of chemotherapy was started when all of the abnormal findings had normalised.

2.2.1. Procedure (1): history and clinical check list

The food intake of the week before the onset of symptoms was recorded, as well as any medication taken before the onset of symptoms. Clinical signs and symptoms of the illness (abdominal pain, nausea, diarrhoea, blood loss and pattern of fever) were recorded. Definitions used: Fever normalised within 48 h was classified as 'short duration', fever which continued >48 h was classified as 'long duration' and if fever spiked above 38.5 °C every 24 h, and the temperature normalised in between this was classified as 'spiking'. Abdominal pain was classified as 'cramps', 'stool-related', or 'continuous', and the frequency of vomiting was recorded. Stools were recorded as 'bloody stools' or as 'watery frequent'.

2.2.2. Procedure (2): physical examination

State of consciousness, respiratory rate, hypotension, abdominal distension, bowel sounds, mucositis and any other clinically important abnormalities were reported. Mucositis was recorded as present when there were oral lesions in the mouth. The clinicians scoring the history and physical examination were blinded for the inflammatory marker results and the results of the biopsy findings.

2.2.3. Procedure (3): stool cultures

Stool cultures were performed for bacteria, viruses, parasites and fungi, using routine microbiological procedures. Faeces was tested for Clostridial cytotoxin. Detection of *C. difficile* was performed as follows. Faeces was tested by enzyme immunoassay (Premier™ Toxins A&B, Meridian Bioscience, OH, USA) to detect *C. difficile* toxins A and B. For cultures, faeces was pretreated by mixing with ethanol 96% during 1 h. *C. difficile*-like colonies were identified using standard microbiological procedures.

2.2.4. Procedure (4): rectal biopsy

Rectal mucosal biopsies were taken by an experienced paediatric gastroenterologist. This was performed by a flexible adult sigmoidoscope which was introduced no more than 15 cm, no anaesthetics were needed; in smaller children conscious sedation was given 30 min before the procedure. The procedure was only done when the platelets were above 50×10^9/l. If the platelets were $<50 \times 10^9$/l a platelet transfusion was given 30 min prior to the procedure. Insight was gained into the macroscopic and the microscopic aspects of the rectal mucosa. Microscopically four categories were distinguished in order of increasing severity: 'no changes', 'infiltrate only', 'pseudomembranes' and 'ulcerative changes with fibrin exudate'. The diagnosis of *C. difficile*-related disease was made if two out of three of the following findings were included: pseudomembranes on biopsy, toxin-positivity in the stools and/or a positive culture for *C. difficile*, either in the blood or stools.

2.2.5. Procedure (5): blood investigations

Full blood count, C-reactive protein (CRP), liver-enzymes, creatinine, viral serology and blood cultures were performed, according to standard laboratory procedures.

2.2.6. Procedure (6): inflammatory markers

CRP was measured in blood. This assay was performed as described by Wolbink and colleagues in Ref. [16] using anti-CRP mAb KH61 (2 μg/ml) as the coating mAb, and biotinylated anti-CRP mAb 5G4 to detect the bound CRP. IL-6, IL-8, IL-10 and TNFα concentrations were analysed using commercially available enzyme-linked immunosorbent assays (ELISAs) with detection limits of 3.0, 15.0, 15 and 5.0 pg/ml, respectively (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), Amsterdam, The Netherlands).

3. Statistics

To compare the Intensive Care Unit (ICU) group versus the non-ICU group, the Mann–Whitney U test was used on the continuous data and the χ-square test for categorical data; if the expected number of patients was below 5 in one of the cells we performed a Fisher's exact test. To examine the prognostic value of elevated inflammatory markers we used both cut-offs mentioned in the literature (for IL-8 above 1000 pg/ml [10] CRP above 150 mg/l [11]) and optimal cut-offs from the data were calculated using the Youden index (sensitivity + specificity − 1) as the criterion. To estimate the predictive value of increased inflammatory parameters, we calculated relative risks (RR) with 95% Confidence Intervals (CI). All reported *P*-values are two-sided.

4. Results

4.1. Patients' characteristics

Over a 3-year period (November 1998–January 2002), 452 new patients with oncological disorders were admitted to the unit. Of these, 25 patients fulfilled the entry criteria of the study and were included, 15 were male and 10 were female. The diagnoses at presentation showed 11 haematological disorders (acute lymphoblastic leukaemia (ALL)/ANLL), four B-cell lymphoma's, nine solid tumours and one haemophagocytic syndrome. The mean age at diagnosis was 7.1 years (range 1.0–17.1 years). Enterocolitis presented in most cases within the first 3 months after diagnosis and the start of chemotherapy.

The clinical symptoms are summarised in Table 1. Most patients had signs of mucositis (92%), 40% of patients had less than 48 h of fever and 60% had fever >48 h. The stool pattern was recorded as 'watery and frequent' in 60% of patients and 'bloody diarrhoea' was recorded in 28% of patients. The abdominal pain was classified as 'cramps' in 72% of patients, and 24% of patients had continuous pain.

Of the 25 patients, 8 were admitted to the ICU due to cardiovascular symptoms for which inotropic support was indicated. Of these patients, 5 did not have a proven bacteraemia. 3 died following the enterocolitis episode, even though these patients received adequate antibiotic treatment and extensive inotropic support. The first

Table 1
Patient characteristics

	N (%)
Gender	
Male	15 (60)
Female	10 (40)
Diagnosis	
Haematological	11 (44)
Lymphoma	4 (16)
Solid	9 (36)
Haemophagocytic syndrome	1 (4)
CVC	
PAC	12 (48)
Broviac	10 (40)
No CVC	3 (12)
Clinical parameters	
Fever	
<48 h	10 (40)
Long and spiking	6 (24)
Long and continuous	9 (36)
Diarrhoea	
Watery and frequent	15 (60)
Bloody	7 (28)
Other	3 (12)
Abdominal pain	
Cramps	18 (72)
Stool related	1 (4)
Continuous pain	6 (24)
Mucositis	
Yes	23 (92)
No	2 (8)
Vomiting	
<4×/24 h	11 (44)
>4×/24 h	2 (8)
No vomiting	12 (48)
Pathology results	
No changes	12 (48)
Infiltrate only	9 (36)
Pseudomembranes	3 (12)
Ulcerative changes	1 (4)
Outcome	
ICU admission	8 (32)
Mortality	3 (12)

CVC, central venous catheter; PAC, port-a-cath internal CVC device; Broviac, external CVC device. Pathology results are ranked according to severity. In the outcome row, the Intensive Care Unit (ICU) admission is described, and mortality due to enterocolitis.

patient with a haemophagocytic syndrome had pseudomembranous colitis due to *C. difficile*, she underwent surgical resection of an ischaemic part of the bowel, but her condition deteriorated and she died. The 2 other patients suffered from acute myeloid leukaemia (AML), and had positive blood cultures at the time of colitis (*Candida tropicalis* and *Staphylococcus aureus*). They both deteriorated rapidly and died, despite appropriate antibiotic treatment and inotropic support. In all patients, cardiomyopathy was excluded by echocardiography.

4.2. Etiology

Microbiological results are shown in Table 2. A variety of organisms were cultured, but most stool and blood cultures remained negative (76 and 68%, respectively). 4 patients had a Gram-positive bacteraemia but none of these patients had a positive stool culture. One patient had an adenovirus in the faeces, the blood culture remained negative, 2 patients had protozoa in the faeces, 1 of these patients also having a Gram-negative bacteraemia (*Klebsiella* spp). Only 2 patients had *C. difficile* in the stool culture and 1 of these patients had a positive blood culture with *C. difficile* (the same strain was found in the blood culture as in the stool culture). In 3 patients, *C. difficile* toxin stool tests were positive.

Histological examination of the rectal biopsies resulted in 'no changes' in 12 patients (48%), 'infiltrate only' without pseudomembranes in 9 patients (36%), 'pseudomembranes' in 3 patients (12%) and 1 patient had 'ulcerative changes' with fibrin exudates (4%).

Overall, in 4 patients (16%), two out of three parameters for *C. difficile*-positive colitis were found to be positive.

Table 2
Micobiological results

		Organism	$N=25$
Stool culture[a]	Bacterial		
	Gram-positive	*Clostridium difficile*	2
	Gram-negative	*Klebsiella* spp.	1
	Parasitic	*Cryptosporidium parvum*	1
		Ascaris lumbricoides	1
	Viral	*Adenovirus*	1
	Negative	–	19
Blood-culture[a]	Bacterial		
	Gram-positive	*Strep. sanguis*	2
		Staph. aureus	1
		Coagulase-neg. Staphylococcus	1
		Clostridium difficile	1
	Gram-negative	*Klebsiella* spp.	1
		Escherichia coli	1
	Fungal	*Candida tropicalis*	1
	Negative		17

[a] Stool and blood culture results obtained during the same study period.

To explain the low rate of *C. difficile* positive-colitis, we analysed the use of i.v. antibiotics prior to study inclusion. 19 patients (76%) had received i.v. antibiotics prior to the rectal biopsy (mean of 5.5 days), these antibiotics were administered because of fever in neutropenia of which 13 patients (52%) had been pretreated with vancomycin (Fig. 1). Whereas none of these patients pretreated with vancomycin was *C. difficile*-positive, 5 patients of this group showed abnormal rectal biopsies. The pathological changes, the negative stool cultures and the relative small number of positive blood cultures suggest a multifactorial aetiology of neutropenic enterocolitis.

4.3. Prognostic factors

To identify possible factors predictive of the clinical course, we used admittance to the ICU with need for inotropic support during the enterocolitis episode as our primary endpoint.

4.3.1. Clinical parameters

Patients included in the ICU group were 3 patients with ANLL, 3 patients with Burkitt's lymphoma, 1 patient with high-risk leukaemia, and 1 patient with a haemophagocytic syndrome. In the non-ICU group, there were three patients with ANLL, 1 Burkitt's lymphoma, 4 patients with leukaemia and 9 solid tumours. There was no difference in the chemotherapies used in the two groups. The chemotherapies used in both groups included high dose cytarabine, daunorubicin, etoposide, prednisone and methotrexate. Abdominal pain, stool pattern, amount of vomiting and mucositis between the ICU and the non-ICU group were compared and no significant difference between the two groups was found. However, the pattern of fever did show differences between the groups if fever was grouped into two categories, (fever <48 h after onset of

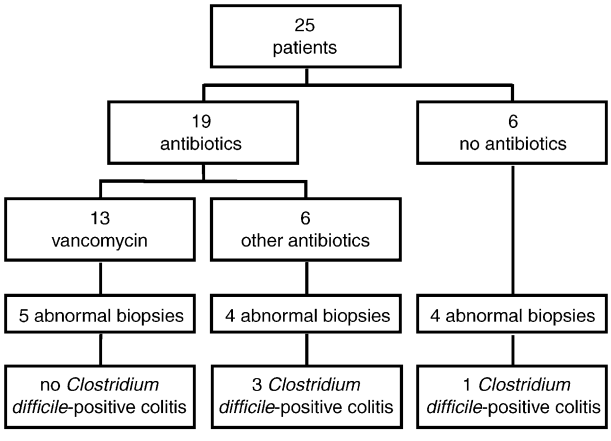

Fig. 1. Intravenous (i.v.) use of antibiotics at the start of the study and biopsy results. The chart shows the number of patients pretreated with antibiotics, the rectal biopsy and *Clostridium* test results.

the enterocolitis, and fever > 48 h after onset colitis) In the ICU group, 5 out of 8 patients (63%) had fever > 48 h compared with 4 out of 17 patients (24%) in the non-ICU group; this was borderline significant ($P = 0.056$). In the original classification, using the three categories, no significant difference was found between the groups (Table 3).

4.3.2. Microbiological results, rectal biopsy and other laboratory results

Neither the stool nor the blood cultures showed any significantly different patterns in the ICU group compared with the non-ICU group. The white cell count, platelet count, liver functions, renal function and histopathological results were also not significantly different in the two groups (Table 3).

4.3.3. Inflammatory markers

Of the evaluated inflammatory serum markers, only CRP and IL-8 showed a significant difference between the two groups (Figs. 2 and 3). The median CRP levels in the ICU and the non-ICU group were 239 mg/l (interquartile range (IQR) 193–292 mg/l) versus 88.2 mg/l (IQR 119–187 mg/l), respectively ($P = 0.023$). However, there was overlap between the two groups (Fig. 2). At a cut-off level of 150 mg/l ([11], Receiver

Table 3
Clinical risk factors for admittance to ICU

	ICU	Non-ICU	P-value
Number of patients (25)	8 (32%)	17 (68%)	
Clinical findings			
Abdominal pain			
Cramps	4 (50%)	14 (82%)	
Stool-related	1 (13%)	0	$P = 0.15$
Continuous	3 (38%)	3 (18%)	
Diarrhoea[a]			
Watery frequent	4 (50%)	12 (71%)	
Bloody	4 (50%)	3 (18%)	$P = 0.34$
Other	0	2 (12%)	
Fever			
Short and continuous	0	10 (59%)	
Long and spiking	3 (38%)	3 (18%)	
Long and continuous	5 (63%)	4 (24%)	$P = 0.15$
Mucositis			
Yes	8 (100%)	15 (88%)	
No	0	2 (12%)	$P = 0.34$
Blood culture			
Gram-positive	2[b]	3	
Gram-negative	1[b]	1	
Fungal	1		
Cl toxin-positive	2	1	
Rectal biopsy			
Infiltrate only	2 (25%)	7 (41%)	
Pseudomembranes	2 (25%)	1 (6%)	$P = 0.22$
Ulcerative changes	1 (13%)	0	
No changes	3 (38%)	9 (53%)	
Laboratory findings median (IQR)[c]			
WBC	0.25×10^9/l (0.1–0.52)	0.30×10^9/l (0.2–0.5)	$P = 0.39$
Platelet count	39×10^9/l (22–48)	32×10^9/l (14–83)	$P = 0.79$
SGOT	4.5 U/l (6–44)	17 U/l (11–25)	$P = 0.59$
creatinine	26 mg/l (16–35)	24 mg/l (18–37)	$P = 0.97$
Inflammatory parameters median (IQR)[c]			
CRP (day 1)	239 mg/l (239–292)	118 mg/l (85–188)	
(day 3)	160 mg/l (82–212)	75 mg/l (27–146)	
(day 7)	85 mg/l (10–219)	24 mg/l (6.3–72)	
IL-8 (day 1)	2882 pg/ml (1228–4536)	146 pg/ml (66.7–213.5)	
(day 3)	393 pg/ml (157–1708)	41 pg/ml (30.5–113)	
(day 7)	221 pg/ml (11.6–430)	31 pg.ml (15–33)	

WBC, white blood cells; IL-8, interleukin-8; CRP, C-reactive protein; SGOT, aspartate aminotransferase.

[a] *C. Difficile*-positive enterocolitis was present in 1 ICU patient and 3 non-ICU patients.

[b] One patient in ICU had a positive blood culture for both *C. difficile* and *E. coli*.

[c] IQR = interquartile range (P25–P75).

Operating Curve (ROC) cut-off 190 mg/ml), 7 out of 8 ICU patients had a high CRP compared with 6 of 17 patients in the non-ICU group. Patients with a CRP > 150 mg/l had a 6.4 times higher chance of developing a severe enterocolitis compared with patients with a CRP < 150 mg/l, (95% CI 0.92–45.1). Using the ROC cut-off level of this cohort of patients (190 mg/l), the chance of ICU admission was 10.5× higher (95% CI 1.5–72.8) compared with patients with a CRP < 190 mg/l.

The IL-6 levels in both groups were extremely low (the cut-off value of IL-6 at 250 pg/ml was not reached [11]). The mean in the ICU group was 108 pg/ml and in the non-ICU group 32 pg/ml. In non-neutropenic patients with sepsis, the values of IL-6 are at least 1000–10 000-fold higher. Because of these extremely low values of IL-6, statistical analysis on this parameter were not possible.

IL-8 levels measured on day 1 correlated most significantly with outcome (Fig. 3). On day 1, the median value in the ICU group was 2882 pg/ml (IQR 1228–4536 pg/ml) and the median in the non-ICU group was 146 pg/ml (IQR 66.7–213 pg/ml) (Fig. 3; $P = 0.001$). Two data points are missing; one in each group. IL-8 was not measured at that time-point. Using a cut-off value of 1000 pg/ml([10], ROC cut-off 980 pg/ml) 6 out of 7 patients were found to have a high IL-8 in the ICU group compared with 2 out of 16 patients in the non-ICU group (Table 4). The risk of ICU admittance was 11.3 times ((95% CI 1.6-77.9) higher in the elevated IL-8 group than in the non-ICU group. All 3 patients who died had a IL-8 level of > 1000 pg/ml.

The longitudinal data showed a decrease in the levels of IL-8 and CRP, but not a normalisation within 48 h (see Table 3). All other inflammatory marker levels were not significantly different between the 2 groups. Values of IL-10 were extremely low (< 15 pg/ml) and did not show any correlation with outcome. It was noted that there was no rise of TNF-α levels in any patient at any time-point (< 5 pg/ml).

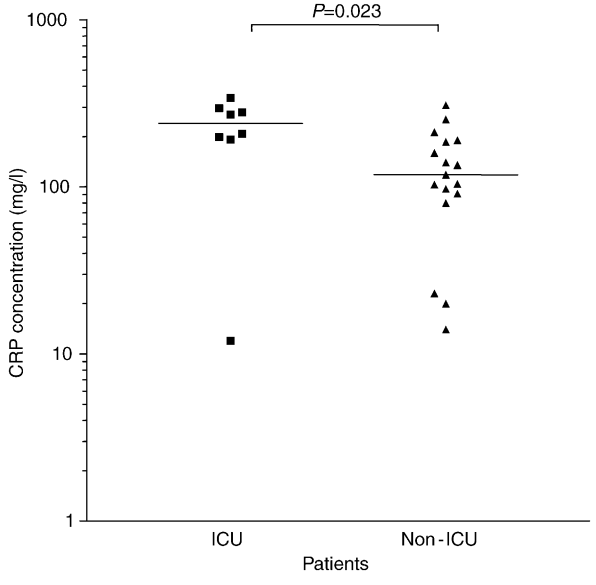

Fig. 2. Scatter plot of C-reactive protein (CRP) values on day 1 in Intensive Care Unit (ICU) and non-ICU patients. Horizontal line indicates the median value.

5. Discussion

The annual incidence of enterocolitis in our single paediatric oncology unit is estimated at 4–6% of newly-diagnosed patients over this 3-year period. In review articles, the annual incidence ranges from 12 to 46% [17–19]. Of the included patients only 16% had a *C.difficile* positive colitis. the carriage rate of *C. difficile* in

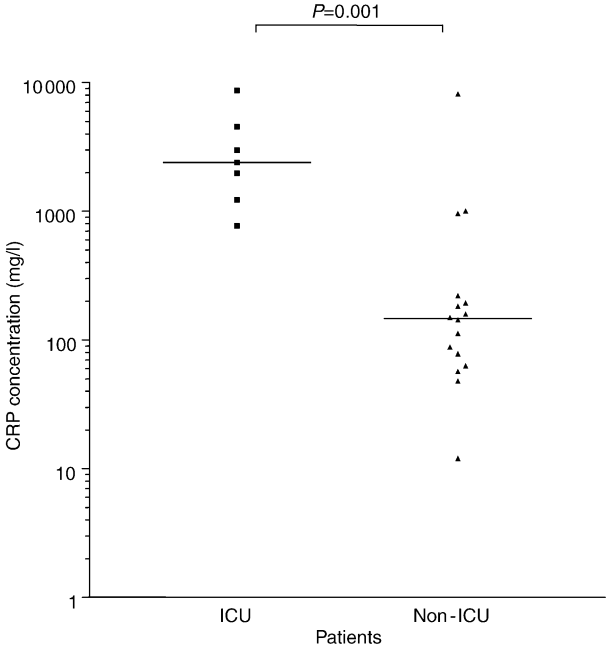

Fig. 3. Interleukin-8 (IL-8) results comparing the ICU and the non-ICU group. A scatter plot is presented. The horizontal line indicates the median value.

Table 4
IL-8 as prognostic tool in neutropenic enterocolitis

	IL-8 > 1000 pg/ml	IL-8 < 1000 pg/ml	
ICU	6[a]	1	7
Non-ICU	2	14	16
	8	15	23

Relative Risk (RR) 11.3 (95% Confidence Interval (CI) 1.6–77.9). There are two missing values: (i) If the IL-8 value missing in the ICU group was < 1000 pg/ml and the IL-8 value in the non-ICU group was > 1000 pg/ml, then the RR is 5.3. (ii) If the IL-8 value missing in the ICU group is > 1000 pg/ml and the IL-8 value in the non-ICU group is < 1000 pg/ml, then the RR 12.8.

[a] All 3 patients who died were found in this group.

oncology patients is 15–30%. 70% of these patients will show signs of clinical illness [20,21]. The low percentage of *Clostridium-positive* colitis in our series can be explained by the fact that 19 out of 25 patients (76%) were pretreated with antibiotics, of which 13 patients (52%) had received vancomycin prior to rectoscopy (Fig. 1). However, in this group, 5 patients (38%) still showed pathological changes by rectoscopy, which indicates that these patients may develop colitis in spite of adequate antibiotic treatment. This illustrates that a variety of factors are involved in causing and modulating mucosal barrier injury, including the chemotherapy given, the involvement of pro-inflammatory and other cytokines, such as endotoxins across the mucosal barrier, translocation of the resident microflora and their products, and the exposure to antimicrobial agents [8].

In the older literature reports, severe enterocolitis was mainly seen in haematological malignancies [22,23], but with the general intensification of chemotherapy it has been more common to find these enterocolitis entities in both solid and haematological malignancies [24]. These findings were confirmed in this study: 44% of the patients had a haematological malignancy and 52% had a solid tumour.

Microbiological studies revealed bacteraemia in 24% of our patients: of these cultures, 57% showed growth of Gram-positive organisms, 29% Gram-negative organisms, and 14% fungal organisms. Katz and colleagues [23] reported bacteraemia in 84% of patients and fungaemia in 16%. One possible explanation for the lower incidence of bactaeremia in our unit is that all patients were started on selective decontamination of the intestinal tract shortly after chemotherapy. Another explanation might be that with special techniques to detect cell-wall deficient strains more episodes of bactaeremia (approximately 5%) might be detected [25].

The main aim of the study was to identify patients likely to develop a severe enterocolitis. The clinical symptoms diarrhoea, abdominal pain and mucositis showed no significant difference between the ICU and the non-ICU groups. This is also described in the literature [7]. The pattern of fever had a low prognostic value in the sense that the ICU group had more 'long and continuous fever' than the non-ICU group.

The rectal biopsy findings did not contribute to treatment decisions, it was shown that the findings of pseudomembranes on rectal biopsy correlated fully with *C. difficile* toxin positivity or culture positivity. None of the patients had any complications after the procedure or found the procedure painful. Because the yield was so low, we would not recommend to perform this procedure in future trials, although it might be worth looking at inflammatory parameters in the rectal dialysate instead. Routine laboratory investigations could not distinguish the severe cases from the less severe cases. Sloas and colleagues [26] who (retrospectively) investigated 24 confirmed cases of typhlitis also reported that routine laboratory investigations were not informative. Imaging was not included in the study. Most of the patients had an abdominal ultrasound performed on day one and in most of these ultrasounds widened bowel loops were found and thickened bowel walls. However, this was not scored accurately or prospectively validated, therefore this was not included as an outcome measure. This should be considered in future trials as bowel wall thickening is becoming a diagnostic tool [27,28].

The inflammatory parameters in our study showed that CRP and IL-8 were of prognostic value. The extent and course of serum concentrations of IL-8 and IL-6 in the patients with colitis symptoms were reported to be significantly different from those seen in septic conditions in non-neutropenic children [29]. In non-neutropenic patients, concentrations of IL-8 are at least 10–100-fold higher, and the IL-6 levels are at least 100–1000-fold higher [29]. IL-8 used at a cut-off point of 1000 pg/ml was a strong prognostic factor. Although decreasing over time, the IL-8 levels in our patient cohort did not normalise within 24–48 h after administering intensive care treatment and support, as is usually observed in children with non-neutropenic sepsis. Although the chemotactic activity towards neutrophils is the most important function of IL-8, we know that in these patients neutrophils are missing and also absent in the extravascular tissues. Taken together, these data indicate that IL-8 in neutropenic patients is less likely derived from enterocytes and myeloid cells, but from tissue cells such as endothelial cells, and fibroblasts [15,30]. The chronic low flow and hypoxia-prone situation is not impossible as a contributing factor for endothelial cell-induced IL-8 generation. This might be mediated by hypoxia-sensitive AP-1 and nuclear factor – kβ (NF-kB)-like binding sites in the *IL-8* promoter site. In such patients, a role for IL-8 as an angiogenesis-regulating factor may be more important. Strikingly, the IL-6 values were found to be low. The role of IL-6 remains unclear, although IL-6 is a multifunctional cytokine regulating B and T cell function and the acute phase response [31], neither prolonged fever nor CRP levels correlated with serum IL-6.

With this possible pathophysiological mechanism in mind, we hypothesise that the increase in IL-8 seen in our patients reflects the extent of damage of the intestinal wall, and could therefore predict the severity of the disease. For the daily clinical practice, we would strongly advise to initiate early aggressive supportive care in neutropenic patients with abdominal cramps and diarrhoea in whom IL-8 levels are elevated, of course, the measurement of such inflammatory parameters may very much depend on the assay used and will require further standardisation. With regard to the supportive care, inotropic support on the first day like dopamine at a

dose of 5 μg/kg/min to increase the gastrointestinal blood-flow should be given. Future intervention trials should concentrate on the use of granulocyte-transfusions, enterocyte-healing factors such as epidermal growth factor (EGF) and anti-inflammatory factors.

Acknowledgements

We are grateful to our paediatric gastroenterology department for performing the rectoscopies and mucosal biopsies, to our pathology department doing the microscopy, and to our clinical and epidemiological biostatistics department for their statistical advice. Financial support was received from Stichting Kindergeneeskundig Kankeronderzoek (SKK) a research foundation financing paediatric oncological research (Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands).

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