OUTCOME AND DETERMINANTS OF NEUTROPENIC ENTEROCOLITIS IN PEDIATRIC CANCER PATIENTS

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Abstract

Background Neutropenic enterocolitis (NEC) is a dreaded complication of chemotherapy. There is scant literature regarding incidence, clinical features, and determinants. The understanding of gut dysbiosis in NEC and pediatric cancer is evolving. Methods Pediatric cancer patients with neutropenia and gastrointestinal symptoms were evaluated for NEC with CECT abdomen. Clinical, imaging, and laboratory features were analysed. Fecal samples were analysed for fecal calprotectin by sandwich ELISA and gut microbiota by conventional culture and compared with healthy controls and children without NEC. Results NEC was diagnosed in 44 children based on clinical and imaging features with incidence of 7.4% (Four had recurrent episodes). Common manifestations included fever(98%), pain abdomen(88%), and diarrhoea(83%). Hypoalbuminemia was observed in 78% patients. Large bowel involvement(94%) with diffuse bowel involvement(63%) and pancolitis(64%) were common. Fecal calprotectin was significantly elevated in NEC group than non-NEC group and healthy controls (median 87, 53, and 42 µg/g respectively). Higher degree of gut dysbiosis was observed in children with NEC with higher isolation of Bacteroides and infrequent isolation of Lactobacilli.. Mortality rate of 23% was observed. Only presence of free fluid predicted higher mortality. Though levels of fecal calprotectin and gut dysbiosis were higher in NEC, they didn't increase mortality. Isolation of Bacteroides and absence of Lactobacilli predicted longer duration of intravenous alimentation. Conclusion NEC caused significant morbidity and mortality in pediatric cancer patients. Gut dysbiosis was significantly higher in NEC group suggesting role in pathogenesis and influencing outcome. This highlights role of targeted interventions towards gut dysbiosis like prebiotics and probiotics.

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Abbreviations

NEC	Neutropenic enterocolitis
CECT	Contrast enhanced computerised tomography
ELISA	Enzyme linked immunosorbent assay
HSCT	Hematopoietic stem cell transplant
IBD	Inflammatory bowel disease
SCFA	Short chain fatty acid
CMV	Cytomegalovirus
PCR	Polymerase chain reaction
RCM	Robertson cooked meat medium
BHIBA	Brain heart infusion blood agar
BBEA	Bacteroides bile esculin agar
CCFA	Cefoxitin cycloserine fructose agar
MRSA	deManRogosa agar
AML	Acute myeloid leukemia
ALL	Acute lymphoblastic leukemia
NHL	Non Hodgkin lymphoma

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Abstract

Background

Neutropenic enterocolitis (NEC) is a dreaded complication of chemotherapy. There is scant literature regarding incidence, clinical features, and determinants. The understanding of gut dysbiosis in NEC and pediatric cancer is evolving.

Methods

Pediatric cancer patients with neutropenia and gastrointestinal symptoms were evaluated for NEC with CECT abdomen. Clinical, imaging, and laboratory features were analysed. Fecal samples were analysed for fecal calprotectin by sandwich ELISA and gut microbiota by conventional culture and compared with healthy controls and children without NEC.

Results

NEC was diagnosed in 44 children based on clinical and imaging features with incidence of 7.4% (Four had recurrent episodes). Common manifestations included fever(98%), pain abdomen(88%), and diarrhoea(83%). Hypoalbuminemia was observed in 78% patients. Large bowel involvement(94%) with diffuse bowel involvement(63%) and pancolitis(64%) were common. Fecal calprotectin was significantly elevated in NEC group than non-NEC group and healthy controls (median 87, 53, and 42 μg/g respectively). Higher degree of gut dysbiosis was observed in children with NEC with higher isolation of*Bacteroides* and infrequent isolation of *Lactobacilli..*

Mortality rate of 23% was observed. Only presence of free fluid predicted higher mortality. Though levels of fecal calprotectin and gut dysbiosis were higher in NEC, they didn't increase mortality. Isolation of *Bacteroides* and absence of *Lactobacilli* predicted longer duration of intravenous alimentation.

Conclusion

NEC caused significant morbidity and mortality in pediatric cancer patients. Gut dysbiosis was significantly higher in NEC group suggesting role in pathogenesis and influencing outcome. This highlights role of targeted interventions towards gut dysbiosis like prebiotics and probiotics.

Introduction

The field of pediatric oncology has witnessed stunning success over the years with remarkable improvement in outcomes of various pediatric cancers.[1] Intensive chemotherapy is a key component of the management of pediatric cancers, which is associated with a host of treatment-related toxicity like bone marrow suppression, and mucositis.[2,3]

Neutropenic enterocolitis (NEC) is a life-threatening gastrointestinal oncological emergency observed in cancer patients during chemotherapy.[4] It has been synonymously termed typhlitis, cecitis and ileo-caecal syndrome. The term typhlitis was derived from the Greek word- *Typhlon* , meaning caecum, indicating the most common region of bowel involved in NEC.[5] It was initially described in children with acute leukemia, however it has been described in adults as well as in other solid malignancies and non-neoplastic conditions like aplastic anemia, post hematopoietic stem cell transplantation (HSCT) and retroviral infection.[6,7]

The true incidence of neutropenic enterocolitis in Indian setup is unknown. Though described as a triad of fever, neutropenia and pain abdomen, all the features may not be seen in the same patient. Hence, ultrasonography and CT abdomen have been used to supplement the diagnosis. Most retrospective analyses

report incidence of NEC from 1.7% to 16.2%.[8-14] However, there is scant literature regarding the incidence of neutropenic enterocolitis in developing nations.

Biomarkers like serum citrulline, faecal and serum calprotectin, intestinal fatty acid binding protein, matrix metalloprotease 2,3 and 9 and interleukins IL-1 and IL-6 have been evaluated as biomarkers for chemotherapy and radiotherapy related mucositis with variable results.[15-18] However, none of them have been evaluated in NEC. Calprotectin, a 36 kDa calcium binding protein and major constituent of neutrophil granules, forms an attractive biomarker for NEC.

Microbiome refers to the summation of all the microorganisms residing in and on the body. It is highly complex in composition and diversity. The understanding of microbiome and role in health and disease is still evolving. Any dysregulation in the interaction, quantity or quality of microbiota is termed as dysbiosis. Qualitative alteration may take the form of loss of alpha diversity (degree of microbial species diversity within a single anatomical site) or beta diversity (degree of microbial diversity in the same site between two individuals or groups).[19]

Gut dysbiosis has been well described in diseases like inflammatory bowel disease (IBD), irritable bowel syndrome and *Clostridiodes difficle* infection. However, its role in pediatric oncology has not been adequately explored. Gut dysbiosis has been proposed to influence various aspects of cancer therapeutics- therapeutic effect, toxicity including mucositis, HSCT, and even long-term effects like obesity and neurocognitive functions.[20-22] Conversely, gut dysbiosis has been noted in relation to cancer chemotherapy, radiotherapy, immunotherapy and antimicrobials- both prophylactic and therapeutic.

Gut dysbiosis has been postulated to play a key role in the pathogenesis of gastrointestinal mucositis. Various mechanisms like modulation of inflammatory and oxidative stress, production of protective short chain fatty acids (SCFA), promotion of goblet cell function and epithelial repair and promotion of local immunity have been proposed.[23] Gut dysbiosis in response to chemotherapy, as noted in preclinical as well as human studies, includes decrease in Shannon index, a marker of alpha diversity, and alteration in the relative abundance of various species. Decrease in the abundance of*Firmicutes* including *Lactobacilli* and increase in*Bacteroides* [24-26] has been noted in few studies, whereas the converse has been observed in others.[27,28]

With the above gaps in knowledge, a prospective observational study was planned to study the incidence, clinical and laboratory features, outcome and its determinants in pediatric patients with NEC. It also aimed at exploring gut dysbiosis in patients with neutropenic enterocolitis and the utility of biomarker- fecal calprotectin.

Methodology

Objectives

The objective of the study was to determine the incidence of NEC in pediatric cancer patients receiving chemotherapy in age group 6 months to 18 years. The other objectives were to assess the clinical and microbiological profile, outcome and its predictors. We also planned to evaluate the presence of gut dysbiosis and its significance and role of fecal calprotectin as a biomarker in NEC.

Study design and subjects

It was a descriptive study, conducted in a tertiary health care centre in India between January 2019 to December 2020. Pediatric cancer patients aged 6 months to 18 years were included in the study. Clearance from institute ethics committee was obtained and informed consent was taken from the parents.

Sample size

The incidence of NEC has varied greatly across the studies.[8-10] There were no studies regarding NEC in India. Hence a pilot study was planned to include the eligible population receiving chemotherapy at the centre during the study period as the study population, which served as the denominator for calculation of incidence.

Definitions

Neutropenic enterocolitis :

There is no standardized definition for NEC with wide variability in the definition across the studies.[8-11] All neutropenic cancer patients with gastrointestinal symptoms (pain abdomen, vomiting, loose stools, blood in stools or abdominal distension) were eligible for evaluation into the study. We planned to classify these patients into three groups i). NEC excluded ii.) probable NEC and iii.) definite NEC. This was done after other causes like pancreatitis, gastritis and ileus due to metabolic derangements like hypokalemia were ruled out and there was persistence of symptoms beyond 48 hours.

1. **NEC excluded:** Patients having any of the gastrointestinal symptoms but no abnormal findings on physical examination (tenderness, rebound tenderness, rigidity) and no radiological abnormality
2. **Probable NEC:** Patients with gastrointestinal symptoms and also having any one/more of abnormal physical findings but no radiological abnormality/ imaging not done
3. **Definite NEC** : Patients having neutropenia any of the gastrointestinal symptoms, with abnormal findings on physical examination and positive radiological criteria.

Both probable & definite NEC were considered as NEC for this study.

Radiological criteria : Patients with suspected NEC satisfying entry criteria underwent CECT abdomen. Findings (one/more) suggestive of NEC served as radiological criteria- bowel wall thickening $>$ 3mm, abnormal bowel dilatation, pneumatosis intestinalis or free intraabdominal gas.

Acute pancreatitis: Any two of three features- characteristic pain abdomen, elevation of amylase $>$ 3 times upper normal limit and compatible imaging finding, based on modified Atlanta classification[29].

Acute gastritis : Characteristic pain abdomen in epigastrium or umbilical region, responding to proton pump inhibitors and no subsequent recurrence of pain.

Screening and evaluation

Children presenting with neutropenia and gastrointestinal symptoms were screened. After excluding patients with acute gastritis, acute pancreatitis and acute appendicitis by appropriate clinical evaluation and investigations, informed consent was taken and were enrolled in the study.

The enrolled patients subsequently underwent CECT abdomen. Based on the physical findings and radiological investigations, they were classified into: Definite and probable NEC and NEC excluded. Patients with definite and probable NEC underwent further evaluation, which included serial blood counts, bacterial and fungal work up for isolation of offending organism, stool work up and blood CMV PCR.

Children diagnosed as definite and probable NEC were treated according to institutional protocol, which included intravenous antibiotics, bowel rest, intravenous fluids, correction of electrolyte imbalances, transfusion support, parenteral nutrition and growth factors. Surgical intervention was considered in case of complications like perforation, uncontrolled haemorrhage and uncontrolled sepsis despite appropriate intravenous antibiotics. They were followed up till discharge. The outcome variables including death or discharge, duration of hospital stay, duration of bowel rest and requirement of vasoactive agents and mechanical ventilation were recorded.

Faecal microbiota and faecal calprotectin were analysed in the enrolled patients and healthy controls. Healthy controls included siblings of children with cancer, and were required to be asymptomatic and not having received any antibiotics in the preceding one month.

Evaluation of gut microbiota

Conventional stool culture methods were used to evaluate the predominant bacterial flora in the stool samples. Fresh stool samples were immediately transported to the laboratory for evaluation of microflora. MacConkey agar and Blood Agar were used for isolation of aerobic bacteria, whereas Robertson Cooked

Meat medium (RCM), brain heart infusion blood agar (BHIBA), Bacteriodes Bile Esculin agar (BBEA), cefoxitin-cycloserine fructose agar (CCFA) and deManRogosa agar (MRSA) were used for isolation of anaerobic bacteria. BHIBA is a general enriched medium that supports the growth a wide range of anaerobic bacteria. CCFA medium is a selective medium for *Clostridiodes difficile* , with the addition of antibiotics-cycloserine and cefoxitin, which inhibit the growth of other aerobic and anaerobic bacteria. To detect spore bearing *Clostridia* spp. the stool samples were subjected to alcohol shock treatment before culturing on CCFA, wherein about O.5 ml. of the sample was first treated with equal amounts of 100% ethanol for 60 min. at 37 ^{0}C (alcohol treatment) prior to plating. BBE medium is used for isolation of *Bacteroides*species, the medium is rendered selective by addition of oxgall and gentamicin, which inhibit growth of other bacteria. MRS medium is a selective medium for *Lactobacillus* species. Further species identification was done using matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS bioMerieux).

Estimation of fecal calprotectin

Faecal calprotectin was estimated by sandwich ELISA technique using commercially available kits. Fresh stool samples were collected and preserved at -80^{o}C and processed in batches. The degree of colour change was measured through change in optical density. The concentration of faecal calprotectin was estimated using standard plots obtained from standards and controls having known concentration of faecal calprotectin.

Results

A total of 590 children with cancer received chemotherapy during the study period. There were 224 children who presented with neutropenia with gastrointestinal symptoms and were screened for neutropenic enterocolitis. Three children had evidence of acute pancreatitis based on biochemical and imaging findings. A diagnosis of acute gastritis made after in 149 children. The rest 72 episodes were evaluated for NEC and enrolled into the study.

They were evaluated for NEC based on the study protocol. Of the 72 episodes evaluated for NEC, 37 had abnormalities on physical examination. CECT abdomen was done in 64 children, out of which 36 had findings in imaging studies suggestive of NEC. Based on the findings in physical examination and imaging studies, diagnosis of NEC was made in 48 children, 36 had definite enterocolitis and 12 had probable NEC. Diagnosis of NEC was excluded in 24 episodes. There were 4 children with repeat episodes of neutropenic enterocolitis contributing to the 48 episodes of neutropenic enterocolitis, with incidence of 7.4%.

Baseline characteristics

There were 25 males and 23 females in the study cohort. Most of them belonged to age group 3-5 years (42%) followed by children aged 10-18 years (25%). Acute myeloid leukemia (AML) was the most common underlying diagnosis among the children with NEC (31%), followed by acute lymphoblastic leukemia (ALL) (29%), non-Hodgkin lymphoma (NHL) (13%) and relapsed ALL (8%). However, the proportion of children developing NEC was the highest in NHL (40%), followed by AML (32%) and relapsed ALL (20%). Fifty percent of children received anthracyclines and etoposide prior to development of NEC. Cytarabine and methotrexate-based chemotherapy was administered prior to 45% and 20% of episodes respectively. Steroids were administered in 35% of the cases. Alkylating agents were administered in 29% of the cases. In 54% episodes, children developed neutropenic enterocolitis following the first cycle of chemotherapy.

Fever was the most common symptom in the study population, present in 98% of the cases. It was followed by pain abdomen (88%) and diarrhoea (83%). Vomiting, abdominal distension and blood in stools were less common, present in 27%, 19% and 15% respectively. Coexisting oral mucositis was observed in 50% of the cases, most of them had grade II-III mucositis (Table 1). Hypoproteinaemia was prominent with median of 5.4 g/dL, 28 of 46 (60%) had serum protein less than 5.5 g/dL. Hypoalbuminemia was also evident, with median serum albumin of 2.9 g/dL, 78% of the children had serum albumin less than 3.5 g/dL.

On etiological evaluation, 17% of the cases showed blood culture positivity. The most common isolate, *Klebsiella pneumoniae* , was isolated in 6 cases. *Staphylococcus aureus* and*Stenotrophomonas maltophila* were isolated in one patient each. Serum procalcitonin, a biomarker associated with bacterial sepsis, was

elevated in 36 of 43 children (84%) (Cut-off 0.5 ng/mL). Blood fungal culture and urine for fungus were negative. However, serum galactomannan was elevated in 34% of children, which may reflect concomitant invasive aspergillosis elsewhere. Blood for CMV PCR was negative in all patients. Stool routine examination showed presence of Giardia and Entamoeba in 3 patients each. However, modified acid-fast staining did not show any atypical organisms like Cryptosporidium in any of the cases.

CECT abdomen was done in 42 of 48 cases. Bowel wall thickening was present in 36 children. Isolated small bowel involvement was seen in 2 children, isolated large bowel involvement was observed in 21 subjects, involvement of both large bowel and small bowel was seen in 13 of 36 patients. Of those with colonic involvement, 16 children had involvement of entire large intestine and 13 children had involvement of right sided colon only. Isolated left colonic involvement was seen in 5 children. Maximum bowel wall thickening ranged from 3.5 mm to 18 mm, with median wall thickening of 6.5 mm. Seven children had thickening of 10 mm or more. Bowel involvement was localised in 36 % and diffuse in 64%. Abnormal bowel wall enhancement and mural stratification were observed in 67% and 61% respectively. Abnormal fat stranding and upstream bowel wall dilatation adjacent to involved segment were seen in 8% and 17% respectively. 26% of children had associated free fluid. One child had evidence of pneumatosis intestinalis, none had evidence of pneumoperitoneum (Table 2).

Faecal calprotectin was significantly elevated in children with NEC as compared to children without NEC and healthy controls with median values of 87 µg/g, 52.5 µg/g and 42 µg/g respectively (p value <0.001) (Table 3).

Gut microbiota

Bacteroides were isolated with a significantly higher frequency in the NEC group (42%) than non- NEC group (14%) and healthy controls (13%), which was statistically significant. There was a nonsignificant trend towards higher isolation of *Clostridium* in children with NEC (37% vs 19%). Interestingly, neither *Clostridiodes difficile*nor *Clostridium septicum,* a well-recognised pathogenic species, were isolated in any sample. The 'healthy bacteria'- *Lactobacillus* species were isolated in 26% of children with NEC as compared to 74% and 80% in non-NEC group and healthy controls respectively, which was statistically significant. The other 'healthy bacteria' *Bifidobacterium* was not isolated in any of the samples. The proportion of other anaerobic bacteria like *Veillonella* and aerobic flora like *E. coli, Klebsiella, Enterococcus* and *Citrobacter* was similar among the groups (Table 4).

Outcome

Eleven children with NEC died, with a mortality rate of 23%. There was also considerable morbidity with prolonged hospitalisation and bowel rest and need for parenteral nutrition. The median duration of hospital stay and bowel rest were 10 days (Range: 7-48 days) and 6 days (Range: 3-18 days) respectively. Vasoactive support was required in 22 of the 48 children (45%), whereas mechanical ventilation was needed in 11 children with median duration of 5 days and 4 days respectively.

The baseline demographic parameters, underlying malignancy and nature of chemotherapy were similar in the NEC and non- NEC group. Only faecal calprotectin and anaerobic flora- *Bacteroides* and*Lactobacillus* showed statistically significant difference among the groups.

Prognostic factors

On univariate analysis, presence of free fluid and elevated serum procalcitonin were associated with mortality. However, only presence of free fluid was found to have independent association with mortality with odds ratio of 24 (95% CI: 2.2-252.5). Though there was higher mortality with higher fecal calprotectin and gut dysbiosis (isolation of*Bacteroides* and absence of *Lactobacilli*), this did not attain statistical significance.

Female gender, lower absolute neutrophil count, longer duration of neutropenia. first cycle of chemotherapy and presence of bowel wall dilatation were independently associated with longer duration of hospital stay. Similarly, lower absolute neutrophil count and isolation of *Lactobacilli* were associated with lesser requirement

of bowel rest. On the contrary, younger age, first cycle of chemotherapy, presence of bowel wall thickening $>$ 10 mm, presence of free fluid and isolation of *Bacteroides* were associated with longer duration of bowel rest.

Diagnostic value of fecal calprotectin

Faecal calprotectin was significantly elevated in patients with NEC. Using the faecal calprotectin of the study cohort, at a cut off value of 75 µg/g, faecal calprotectin as a diagnostic tool yielded a sensitivity of 65% and specificity of 63% with AUC of 0.74 (Figure 1). Increasing the cut off to 100 µg/g increased the specificity to 100%, however it resulted in a loss of sensitivity to 40% only.

Discussion

The incidence of neutropenic enterocolitis in the current study was 7.4%. Fever and pain abdomen were the most common symptoms. However, diarrhoea, haematochezia and mucositis were observed at a higher frequency than in other studies (Table 1).

All the patients had neutropenia, severity and duration of which correlated with various outcome parameters. Significant hypoproteinaemia and hypoalbuminemia observed in 60% and 78% of children respectively can be explained by the loss of serum proteins and albumin through the inflamed gastrointestinal mucosa. Similar findings were noted by User et al[14] and Moran et al[30], where hypoalbuminemia was observed in 50% and 46% respectively.

No definite risk factors for neutropenic enterocolitis could be identified in the current study. *Clostridiodes difficile*infection, found as a risk factor by El-Matary et al[10], was not isolated in any patient. Anthracyclines, etoposide and cytarabine based chemotherapy were administered in half of children with neutropenic enterocolitis; however, this association was not found to be statistically significant. The degree of gut dysbiosis was higher in children with NEC, with significantly higher isolation rates of *Bacteroides* species, nonsignificant higher isolation of*Clostridium* species and lower growth of *Lactobacillus*species. Though this doesn't establish the causality of gut dysbiosis, underlying dysbiosis could predispose a susceptible individual to neutropenic enterocolitis, and a 'healthy flora' may be protective for development of mucositis and neutropenic enterocolitis.

On etiological evaluation, blood culture positivity rate was 17%, majority being contributed by *Klebsiella pneumoniae.* Fungal cultures and CMV PCR were non-contributory. Other series have reported culture positivity rates of 8-25%; majority of isolates being gram negative bacilli.[8,31,32,33] *Stenotrophomonas maltophila* , isolated in one patient, has been rarely implicated in the causation of neutropenic enterocolitis[34].

The current study found only the presence of free fluid to be an independent adverse prognostic factor predicting mortality. However, factors like female gender, younger age, duration and severity of neutropenia and bowel wall dilatation and thickening were associated with other parameters like longer duration of hospital stay and bowel rest. Notably, isolation of *Bacteroides* and lack of*Lactobacillus* were associated with longer requirement of bowel rest, thus suggesting a possible role of gut dysbiosis in pathogenesis of neutropenic enterocolitis. Cartoni et al[35] found higher mortality in patients with bowel wall thickening $>$ 10 mm (60% vrs 4.3%). McCarville et al[8] also found a similar association with bowel wall thickening and duration of neutropenia. User et al[14] found that the presence of hypoalbuminemia, hypokalemia and metabolic acidosis were associated with poor outcome, which was not found in the current study.

Faecal calprotectin was elevated in patients with NEC as compared to patients without NEC and healthy controls. Stringer et al found a similar elevation of faecal calprotectin in patients with chemotherapy related diarrhoea, though the degree of elevation was significantly less.[28] Wedlake et al found a significant rise in faecal calprotectin after pelvic radiotherapy.[17]However, van Vliet et al found faecal calprotectin to be below detectable range in a majority of patients with chemotherapy related mucositis and diarrhoea.[18]

Though prognostic value of fecal calprotectin couldn't be demonstrated, it appears to have diagnostic value. Using a cut off value of 75 µg/g, faecal calprotectin as a diagnostic tool yielded a sensitivity of 65% and

specificity of 63% with AUC of 0.74.

Various studies have uniformly noted a decrease in alpha diversity as measured by Shannon Index as a marker of gut dysbiosis. They also reported decrease in isolation of *Lactobacillus* and*Bifidobacterium* . However, there are conflicting reports on*Bacteroides* and other *Firmicutes* as markers for dysbiosis of faecal microbiota. van Vliet et al, Huang et al and Stringer et al report a decrease in Bacteroides species as a marker of gut dysbiosis, whereas Rajagopala et al[36], Montassier et al[24] and Zwielehner et al[25] reported increase in *Bacteroides* and decrease in *Firmicutes* . This difference in patterns of gut dysbiosis may be explained by racial, geographical and dietary differences and difference in age. In the current study, increase in *Bacteroides* and reduction in*Lactobacilli* were noted in children with NEC. (Table 6)

The relative abundance of each flora did not have significant impact on mortality, this might have been contributed by the small sample size in the non survivors. However, isolation of *Bacteroides* and absence of *Lactobacilli* did have an impact on requirement of prolonged bowel rest; isolation of *Bacteroides* increased the requirement of bowel rest by 2.2 days and presence of *Lactobacilli* tended to decrease the requirement of intravenous alimentation by 2.4 days. This observation supports the hypothesis and mechanism of role of gut microbiota as proposed by van Vliet et al[23]. This gives basis for further studies on gut microbiota in the field of pediatric oncology and role of interventions like prebiotics and probiotics to target gut dysbiosis.

Conflict of interests: None

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Table 1: Comparison of symptomatology of children with neutropenic enterocolitis

S. No	Study	Sample size (n)	Fever	Pain abdomen	Loose stools	Blood in stools	Abdomi
1	Moran et al[30]	42	75	89	64	-	33
2	Altnel et al[31]	10	90	100	50	-	10
3	Fike et al[12]	17	41	88	18	6	-
4	McCarvile et al[8]	91	84	91	72	-	-
5	Mullassery et al[32]	40	88	78	50	5	-
6	Rizzatti et al[9]	231	74	67	45	3	10
7	Sundell et al[33]	12	100	100	92	-	-
8	Index study	48	98	88	83	15	19

Table 2: Findings of CECT abdomen in children with neutropenic enterocolitis, n = 42

S. No	Findings in CECT abdomen	Findings in CECT abdomen	Number (n)
	Bowel wall thickening	Bowel wall thickening	36
	Site of bowel wall thickening	Small bowel	2
		Large bowel	21
		Both	13
	Pattern of colonic involvement	Right sided colitis	13(36.1%)
		Pancolitis	16 (44.4%)
		Left sided colitis	5 (13.8%)
	Maximum bowel wall thickening	Maximum bowel wall thickening	Range: 3.5 to 18 mm, Median: 6.5 mm
	Extent of bowel involvement	Localised	13 (36.1%)
		Diffuse	23 (63.9%)
	Abnormal bowel wall enhancement	Abnormal bowel wall enhancement	24 (66.6%)
	Mural stratification	Mural stratification	22 (61.1%)

S. No	Findings in CECT abdomen	Findings in CECT abdomen	Number (n)
	Abnormal fat stranding	Abnormal fat stranding	35 (83.3%)
	Bowel wall dilatation	Bowel wall dilatation	7 (16.6%)
	Free fluid in abdomen	Free fluid in abdomen	11 (26.1%)
	Pneumatosis intestinalis	Pneumatosis intestinalis	1 (2.3%)
	Pneumoperitoneum	Pneumoperitoneum	0

Table 3: Faecal calprotectin in children with and without neutropenic enterocolitis and healthy controls

S. No	Group	Number	Ρανγε (μγ/γ)	Μεδιαν(μγ/γ)	p value
1	Neutropenic enterocolitis	43	25-380	87	< 0.001
2	Neutropenic enterocolitis excluded	22	24-99	52.5	
3	Healthy controls	30	3-87	42	

Table 4: Faecal microbiota in children with and without neutropenic enterocolitis and healthy controls

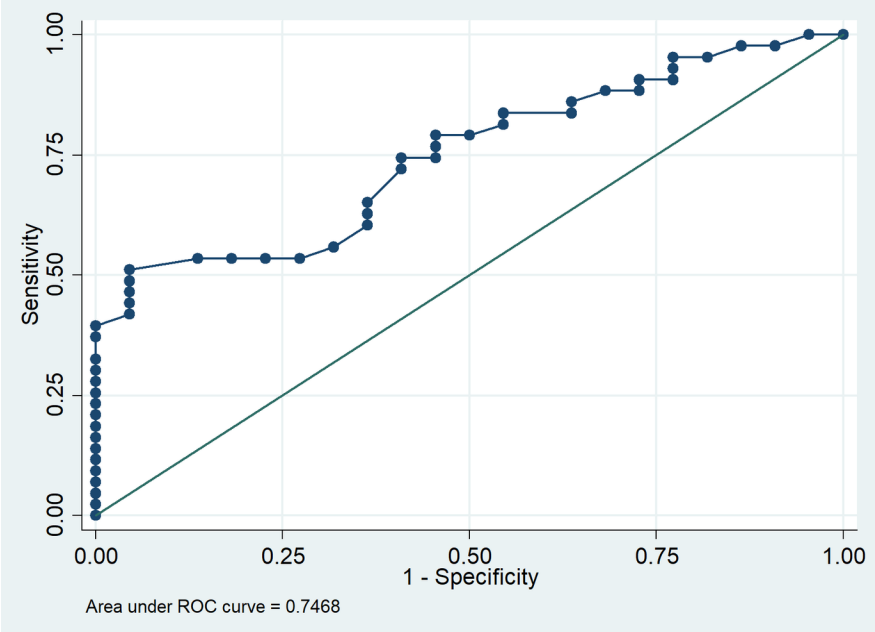
S. No	Microorganism	NEC n = 38	NEC excluded n = 21	Healthy controls n = 30	p value
	Clostridium	14 (36.8)	4 (19.0)	4 (13.3)	0.06
	Bacteroides	16 (42.1)	3 (14.2)	4 (13.3)	0.01
	Veillonella	4 (10.5)	1 (4.7)	1 (3.3)	0.46
	Lactobacilli	5 (26.3)	15 (73.9)	24 (80)	<0.001
	E. coli	30(78.9)	16 (76.1)	26 (86.6)	0.59
	Enterococcus	8 (21.0)	3 (14.2)	6 (20)	0.80
	Klebsiella	4 (10.5)	3 (14.2)	6 (20)	0.54
	Citrobacter	3 (7.8)	2 (9.5)	1 (3.3)	0.63

Table 5: Alteration in gut microbiota with chemotherapy or infections

S. No	Study	Population	Sample size	Technique	Results
1.	Van Vliet et al[27]	Pediatric, AML	9	PCR	Decrease in Shannon index even prior to initiation of chemotherapy Decrease in all genus with chemotherapy *(Clostridium, Bacteroides, Bifidobacterium)* Increase in *Enterococcus* Partial recovery during subsequent cycle of chemotherapy
2.	Huang et al[37]	Pediatric, ALL	36 patients 36 controls	PCR	Lower total bacterial counts, lower *Bifidobacteria, Lactobacilli* and *E. coli* than healthy controls prior to chemotherapy Further fall in bacterial counts, *Bifidobacteria, Lactobacilli* and *E. coli* after high dose methotrexate
3	Stringer et al[28]	Adults	16 patients	PCR	Decrease in *Bacteroides, Lactobacilli* and *Bifidobacterium* Increase in *E. coli* and *Staphylococci*

S. No	Study	Population	Sample size	Technique	Results
4.	Rajagopala et al[36]	Children and young adults	28 patients 23 healthy controls	PCR	Lower Shannon index in patients Lower *Lachnospaerae*, higher *Bacteroides* than healthy controls No change in Shannon index post chemotherapy, but increased subsequently
5.	Montassier et al[24]	Adults with NHL for HSCT	8 patients	PCR	Decrease in Shannon index after starting chemotherapy Increase in *Bacteroides*, decrease in *Fermecutes* and *Bifidobacterium*
6	Zwielehner et al[25]	Adults	17 patients	PCR	Decrease in total bacterial count and diversity after starting chemotherapy Increase in *Bacteroides* and *Clostridium* cluster IV Decrease in *Bifidobacterium* and *Clostridium* cluster IVa
7	Bamole VD et al[38]	Adult, Ca colon	8 patients, 16 controls	Culture and PCR	Higher Bacteroides: Firmicutes ratio in patients Lactobacillus not grown in any patient, 57% in controls Dietary variation observed

S. No	Study	Population	Sample size	Technique	Results
8	Chua LL et al[26]	Pediatric; ALL	7 patients	PCR	Decrease in alpha diversity even prior to initiation of chemotherapy Relative decrease in firmicutes and abundance of Bacteroides prior to therapy The difference diminished after completion of therapy, but didn't normalise
9	Index study	Pediatric	-NEC: 38 -Non-NEC: 21 -Healthy controls: 30	Culture methods	Increase in *Bacteroides* and decrease in *Lactobacilli* in NEC group Trend towards higher isolation of Clostridium in NEC group



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