Alteration of gut microflora and role of gut dysbiosis in modulation of gastrointestinal toxicity in pediatric cancer patients

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Abstract

Background Chemotherapy related mucosal toxicity is a major hindrance to successful therapy in pediatric cancers. The role of gut dysbiosis in modulation of chemotherapy related gastrointestinal toxicity is poorly understood. Methods Pediatric cancer patients with neutropenia and gastrointestinal symptoms were evaluated for neutropenic enterocolitis (NEC) with CECT abdomen. Clinical features, fecal calprotectin and microbiological data were analysed. Fecal Gut microbiota was evaluated in children with NEC and compared with children where NEC was excluded and healthy controls using conventional culture method. Results Of 590 children receiving chemotherapy during study period, 44 were diagnosed with NEC. Significantly higher frequency of isolation of Bacteroides was observed in children with NEC (42%) as compared to non- NEC group (14%) and healthy controls (13%). Isolation of Lactobacilli was infrequent in NEC group (26%) than non- NEC group (74%) and healthy controls (80%). There was nonsignificant trend towards higher isolation of Clostridium in children with NEC. Clostridiodes difficle or Clostridium septicum were not identified in any group. Isolation of other bacterial flora was similar in the sub groups. No significant association of survival with gut dysbiosis could be established. Isolation of Lactobacilli was associated with reduction in duration of intravenous alimentation by 2.4 days, whereas isolation of Bacteroides prolonged the requirement of bowel rest by 2.2 days. Conclusion Gut dysbiosis was significantly higher in NEC group and associated with higher morbidity suggesting its role in pathogenesis. This highlights role of interventions towards gut dysbiosis like prebiotics and probiotics in pediatric cancer patients.

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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
IBS	Irritable bowel syndrome
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

NEC	Neutropenic enterocolitis
CCFA	Cefoxitin cycloserine fructose agar
MRSA	deManRogosa agar
AML	Acute myeloid leukemia
ALL	Acute lymphoblastic leukemia
NHL	Non Hodgkin lymphoma
GVHD	Graft versus host disease
COG	Children's Oncology Group

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Results

Of 590 children receiving chemotherapy during study period, 44 were diagnosed with NEC. Significantly higher frequency of isolation of Bacteroides was observed in children with NEC (42%) as compared to non- NEC group (14%) and healthy controls (13%). Isolation of Lactobacilli was infrequent in NEC group (26%) than non- NEC group (74%) and healthy controls (80%). There was nonsignificant trend towards higher isolation of *Clostridium* in children with NEC.*Clostridiodes difficle* or *Clostridium septicum* were not identified in any group. Isolation of other bacterial flora was similar in the sub groups.

No significant association of survival with gut dysbiosis could be established. Isolation of *Lactobacilli* was associated with reduction in duration of intravenous alimentation by 2.4 days, whereas isolation of *Bacteroides* prolonged the requirement of bowel rest by 2.2 days.

Conclusion

Gut dysbiosis was significantly higher in NEC group and associated with higher morbidity suggesting its role in pathogenesis. This highlights role of interventions towards gut dysbiosis like prebiotics and probiotics in pediatric cancer patients

Introduction

Neutropenic enterocolitis (NEC) is a life-threatening oncological emergency observed in cancer patients during chemotherapy.[1] 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 this disease.[2] Initially described as a triad of neutropenia, pain abdomen and fever occurring in children with acute leukemia, 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.[3,4]

The determinants of NEC are incompletely understood. Chemotherapy related mucosal damage and neutropenia along with superadded infection have been considered as the pathogenic mechanisms of NEC. The relative avascularity and distensibility of certain segments like caecum contribute to the pathogenesis. However, the role of gut dysbiosis in etiopathogenesis of NEC is incompletely understood.

Human microbiome refers to the summation of all the microorganisms residing in and on the human body. It is highly complex in composition and diversity. The gut microbiota constitutes the largest microbiota in the human body, accounting for about 70% of microbial flora. 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).[5]

Gut dysbiosis has been well described in diseases like inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and *Clostriodes difficile* infection. However, the role of gut dysbiosis 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 and even long-term effects like obesity and neurocognitive functions.[6] Gut dysbiosis has also been linked to development of graft vs host disease and increased non-relapse mortality during HSCT.[7,8] Conversely, gut dysbiosis has been noted in relation to cancer chemotherapy, radiotherapy, immunotherapy and antimicrobials: both prophylactic as well as therapeutic.

Gut dysbiosis has been proposed to play a key role in the pathogenesis of gastrointestinal mucositis. Proposed mechanisms include 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.[9] 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* [10-12] has been noted in few studies, whereas the converse has been observed in others.[13,14]

With the above gaps in knowledge, a prospective observational study was planned to assess the gut dysbiosis in pediatric cancer patients with NEC and the prognostic importance of the same.

Methodology

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. Ethical clearance from the IRB was taken. Informed consent was taken. All the children receiving chemotherapy during the study period formed the denominator of the study

Screening and evaluation

Children presenting with neutropenia and any gastrointestinal symptoms (pain abdomen, vomiting, abdominal distension, diarrhoea, or haematochezia) were evaluated. Conditions like acute gastritis, acute pancreatitis and acute appendicitis were excluded from the study by appropriate clinical evaluation and investigations. After excluding the above patients, patients were enrolled in the study.

The enrolled patients underwent CECT abdomen. Based on the physical findings and findings of CECT abdomen, they were classified into: Definite NEC, Probable NEC and NEC excluded. Patients with definite and probable NEC underwent further laboratory evaluation, which included serial blood counts, bacterial and fungal work up for isolation of offending organism, stool work up, blood CMV PCR and fecal calprotectin. Fecal calprotectin was analysed by sandwich ELISA method using commercially available kits.

Children diagnosed as NEC were admitted and given standard care according to institutional protocol, which included intravenous antimicrobials, bowel rest, intravenous fluids, correction of electrolyte imbalances, transfusion support and parenteral nutrition. Use of growth factors was decided based on unit protocol that was guided by clinical picture, underlying malignancy and phase of chemotherapy. Surgical intervention was considered in case of complications like perforation, uncontrolled haemorrhage and uncontrolled sepsis despite appropriate intravenous antimicrobials. They were followed up till discharge. The outcome variables including death or discharge, duration of hospital stay, duration of bowel rest, requirement of vasoactive agents and need for mechanical ventilation were recorded.

Faecal microflora was analysed in the enrolled patients- those with diagnosis of NEC and NEC excluded, as

well as in healthy controls. The 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.

Definitions

Neutropenic enterocolitis :

There is no standardized definition for neutropenic enterocolitis, with wide variability in the definition across the studies. For purpose of current study, all patients on cancer chemotherapy who were neutropenic and had gastrointestinal symptoms were eligible for evaluation. Patients were classified into three groups i). NEC excluded ii.) probable NEC and iii.) definite NEC. This was done after other causes like pancreatitis, gastritis appendicitis and ileus due to metabolic derangements like hypokalemia were ruled out and there was persistence of symptoms beyond 48 hours.

NEC excluded: Patients having neutropenia along with any of the gastrointestinal symptoms but no abnormal findings on physical examination (tenderness, rebound tenderness, rigidity) and no radiological abnormality.

Probable NEC: Patients having neutropenia and gastrointestinal symptoms with abnormal physical findings, but no radiological abnormality.

Definite NEC : Patients having neutropenia and 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 $>$ 3 mm, abnormal bowel dilatation, pneumatosis intestinalis or free intraabdominal gas.

Statistical analysis

Descriptive statistics were used to analyse the demographic and clinical characteristics of the patients. Mean (SD) and median (IQR) were performed for continuous variables. Comparison between categorical variables was performed using the Pearson Chi-square test or Fisher's exact test and between continuous variables using the t-test or Wilcoxon rank-sum/ Mann-Whitney test. Independent association between variables and outcome was assessed by logistic regression. The level of significance was set at a p-value of $<$0.05. The analyses were performed using Stata software version 20 (STATA, College Station, TX).

Evaluation of gut microflora

Conventional stools 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. Various media were used to isolate and identify both aerobic and anaerobic 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 anaerobic bacteria. MacConkey agar and blood agar are incubated aerobically, the other media used for anaerobic bacteria are incubated under anaerobic conditions. 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 at a concentration less than 80µg/mL, 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).

Results

A total of 590 children with cancer received chemotherapy during the study period of January 2019 to December 2020. There were 224 children who presented with neutropenia with presence of gastrointestinal symptoms and were screened for NEC. Evidence of acute pancreatitis based on biochemical and imaging findings was detected in three children and were excluded. A diagnosis of acute gastritis, made after therapeutic trial of proton pump inhibitors, or rapid resolution of symptoms within 48 hours was observed 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. Based on the physical findings and radiological investigations, they were classified into probable and definite NEC and NEC excluded. NEC was excluded in 24 cases and the diagnosis was considered in 48 episodes, 36 had definite NEC according to the definition and 12 had probable NEC. These 48 episodes were observed in 44 children, with 4 children developing recurrent episodes.

Baseline characteristics

Of the 48 cases with NEC, there were 25 males and 23 females. Most of them belonged to age group 3-5 years (42%) followed by adolescent children (25%). Acute myeloid leukemia (AML) was the most common underlying diagnosis, accounting for 31% of the episodes. It was followed by acute lymphoblastic leukemia (ALL) (29%), non-Hodgkin lymphoma (NHL) (13%) and relapsed ALL (8%). 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 based chemotherapy was administered prior to 45% of episodes. Twenty percent of the children received methotrexate-based chemotherapy. Steroids were administered in 35% of the cases. Alkylating agents were administered in 29% of the cases. In 26 of 48 episodes (54%), children developed NEC following the first cycle of chemotherapy.

Gut microflora

Gut microflora was available in 38 children with NEC, 21 children where NEC was excluded and 30 healthy controls. *Bacteroides* were isolated with a significantly higher frequency in the NEC group than non- NEC group and healthy controls, with isolation rates of 42%, 14% and 3% respectively, which was statistically significant. Similarly, there was a trend towards higher frequency of isolation of *Clostridium* in children with NEC; it could be isolated in 37% of children with NEC and 19% in children without NEC as compared to 13% in healthy controls, however this was not statistically significant. Interestingly, *Clostridiodes difficile* was not isolated in any of the samples. *Clostridium septicum,* a well-recognised pathogenic species, was not grown in any of the groups. The 'healthy bacteria', *Lactobacillus* species were isolated in 26% of children in NEC group as compared to 74% and 80% in non-NEC group and healthy controls respectively ($p < 0.001$). The other 'healthy bacteria' *Bifidobacterium* was not isolated in any of the samples. The proportion of other anaerobic flora like *Veillonella* and aerobic flora like *E. coli, Klebsiella, Enterococcus* and *Citrobacter* was not significantly different in any of the groups. (Table 1)

Prognostic significance of gut microflora

A mortality rate of 23% (n = 11) was observed in the study cohort. Higher isolation rates of *Clostridium* and *Bacteroides* were observed in children who didn't survive the illness; however, this difference was not statistically significant. The isolation of other bacterial flora was similar in the non survivors and survivors. (Table 2)

Presence of gut dysbiosis was found to be associated with prolonged requirement of bowel rest and intravenous alimentation. Isolation of *Lactobacilli* was associated with reduction in duration of intravenous alimentation by 3.8 days by univariate analysis, whereas the isolation of *Bacteroides* prolonged the requirement of bowel rest by 2.3 days. This association retained statistical significance on multivariate analysis, with adjusted

coefficient of -2.4 and 2.2 respectively. No association was noted between gut dysbiosis and other outcome parameters like duration of hospitalization and requirement of vasoactive support and mechanical ventilation.

Faecal calprotectin was found to be significantly elevated in patients where *Enterococcus* was not isolated as compared to those who showed growth of *Enterococcus* (151µg/g vs 64µg/g, p = 0.005). However, isolation of no other bacteria showed any association with faecal calprotectin. (Table 3) There was no significant association between gut microflora with serum procalcitonin or degree of neutropenia.

Discussion

The concept of gut microbiota is highly complex and is incompletely understood. Interaction of the gut microbiome with the intestinal mucosa plays a key role in the maintenance of mucosal integrity. The proposed mechanisms include stimulation of local mucosal immunity, production of antimicrobial substances like bacteriocin that inhibit growth of pathogenic microorganisms and sustenance of the intestinal mucosal cells by production of vitamins and short chain fatty acids.[15-18] Gut dysbiosis may be affected due to several causes like geography, age, diet, antibiotics exposure and toxic exposure. Gut dysbiosis has been studied in several disease states like IBD, IBS, *Clostridiodes difficile* infection and carcinoma colon.[18-21] In fact, few therapeutic approaches target the gut dysbiosis with interventions like fecal microbiota transplant.[22,23]

Several preclinical studies done in murine models have demonstrated the alteration of gut microbiota in response to cancer chemotherapy. Most of them have demonstrated a reduction in alpha diversity and reduction in*Lactobacilli* , while there are conflicting results regarding*Bacteroides* and *Clostridium* .[24-28] Of note, Bultzingslowen et al found increase in gut translocation of gram-negative bacteria to mesenteric lymph nodes after chemotherapy, which was ameliorated with addition of *Lactobacillus plantarum 299v* .[29] There is scant literature regarding role of gut dysbiosis in setting of pediatric cancer, more so in mucositis and neutropenic enterocolitis. Various aspects of therapy of cancer including chemotherapy, radiotherapy, immunotherapy, infections and use of antibiotics have been known to alter the gut microbiota.[30-33]. The presence of gut dysbiosis also has bearing on various aspects of cancer therapeutics- therapeutic effect[34-36], chemotherapy related toxicity including mucositis[37-39] and even long-term effects. In setting of HSCT, it also has been proposed to influence the incidence of bacteraemia[40,41] and development of graft vs host disease (GVHD)[7,8] and non-relapse mortality[42].

In the current study, gut dysbiosis was observed in form of higher isolation of *Bacteroides* and lower isolation of*Lactobacilli* in patients with NEC as compared to healthy controls. A trend towards higher isolation of *Clostridium* was also observed in patients with NEC, however, it was not statistically significant. Various studies have uniformly noted a decrease in alpha diversity, as measured by Shannon index, as a marker of gut dysbiosis.[5] Most of them also reported decrease in isolation of *Lactobacillus* and *Bifidobacterium* as a part of gut dysbiosis. However, there are conflicting reports on*Bacteroides* and other *Firmicutes* as markers for dysbiosis of faecal microbiota. A systematic review on the alteration of gut microbiota in cancer chemotherapy related mucositis found a significant reduction of alpha diversity as measured by Shannon index and reduction in *Lactobacilli, Bifidobacterium, and Clostridium* species (*Firmicutes*), coupled with increase in *Bacteroides*species.[5,49] (Table 4) Van Vliet et al studied the gut dysbiosis in nine pediatric AML patients prior to initiation of chemotherapy and after each cycle of chemotherapy.[37]There was decrease in alpha diversity as measured by Shannon index, even prior to initiation of chemotherapy, and after each cycle of chemotherapy. There was a decrease in number of all genera after chemotherapy except *Enterococcus* . Huang et al found a similar trend of lower bacterial counts in pediatric patients with ALL, which was exacerbated after administration of high dose methotrexate.[43] There were also lower counts of*Bifidobacterium, Lactobacillus* and *Escherichia coli* in the patients as compared to controls. Administration of chemotherapy resulted in further lowering of counts of the above bacteria. Stringer et al studied the gut microbiota in 16 adult patients on various chemotherapy, they were found to have decrease in *Bacteroides, Bifidobacterium* and *Lactobacillus* along with increase in*E. coli* and *Staphylococci* as compared to healthy controls.[44] The above studies observed decrease in*Bacteroides* along with *Bifidobacterium* and*Lactobacillus* as features of gut dysbiosis. Contrastingly, other studies by Rajagopala et al[45], Montassier et al[46], Zwielehner et al[47] and Chua LL et al[48] have observed increase in *Bacteroides*and fall in *Firmicutes* (*Clostridium* and*Lactobacillus)*

as markers of gut dysbiosis. Rajagopala et al found a lower alpha diversity in pediatric cancer patients, lower*Lachnospaerae* and abundance of *Bacteroides* as compared to controls. Chua et al observed gut dysbiosis in pediatric ALL patients in form of fall in Shannon index, increase in *Bacteroides* and decrease in *Firmicutes* . A similar finding was observed by Montassier et al in adult patients undergoing HSCT. Zwielehner et al also found a decrease in alpha diversity and increase in*Bacteroides* and *Clostridium* cluster IV with decrease in*Bifidobacterium* and *Clostridium* cluster IVa. This difference in features of gut dysbiosis may be explained by racial, geographical, and dietary differences and difference in age group.

The relative abundance of each bacteria did not have any significant association with mortality, which may be explained by the small sample size in the non survivors. However, isolation of *Bacteroides* and absence of *Lactobacilli* did have an overwhelming impact on requirement of prolonged intravenous alimentation. 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 points to a strong protective effect of flora like *Lactobacillus* and disruptive effect of *Bacteroides* on the integrity of gastrointestinal tract. This observation supports the hypothesis and mechanism of role of gut microbiota as proposed by van Vliet et al[9].

Various mechanisms have been proposed to explain the role of 'healthy' gut microbiota in modulating the chemotherapy related gastrointestinal toxicity. Gut microbiota and their secretions have been proposed to decrease the inflammation by decreasing NFκB activation and thus downregulating TLR and IL-6, while inducing anti-inflammatory agents like IL-10. Short chain fatty acids (SCFA) like butyrate secreted by the microbiota may also ameliorate the inflammatory and oxidative damage in the setting of infection. Microbiota may decrease the villous atrophy and epithelial cell loss induced by chemotherapy. Certain bacteria like*Lactobacillus rhamnosus GG* and *Lactobacillus acidophilus*upregulate the expression of *MUC- 2* and *MUC- 3* genes, which promotes goblet cell repair and mucin production. They also promote the repair of the mucosa though protective effect of SCFAs. Finally, they contribute to local immunity through secretion of protective molecules like C type lectins, secretory IgA and cathelicidins, which have antibacterial activity and prevent gut translocation of bacteria. Findings of the study strongly support the role of gut dysbiosis in the pathogenesis of neutropenic enterocolitis and protective role of 'healthy flora'.

This observation supports the hypothesis that use of appropriate prebiotics or probiotics may improve the gut dysbiosis in pediatric cancer patients and help improve the treatment related morbidity and mortality. There are only limited studies evaluating role of prebiotics or probiotics in pediatric cancers. (Table 5) Ekart et al observed a reduction in incidence of febrile neutropenia with minimal increase in adverse effects in children who were administered *Lactobacilli*with prophylactic cotrimoxazole as compared with others receiving a cocktail of prophylactic antibiotics.[50] Zheng et al observed a favourable alteration in gut microbiota in patients administered fructooligosachharide, which served as prebiotics.[51] Wade et al found a reduction in incidence of fever and antibiotic exposure along with increase in stool SCFA in children who received probiotics.[52] Another study found a reduction in grade 3-4 diarrhoea and mucositis in adults receiving prebiotics during HSCT, though it did not translate into a benefit in survival.[53] A recent RCT in adults evaluating role of probiotic- *Lactobacillus rhamnosus* for prevention of GVHD was prematurely terminated owing to lack of efficacy. However, the intervention was started only on day 14 of transplant. A pilot study in pediatric patients undergoing HSCT to evaluate safety of probiotic- *Lactobacillus plantarum* did not show any increase in side effects.[55] Children's Oncology Group (COG) ACCL 1663 trial is currently underway to study the efficacy and safety of the probiotics in children undergoing HSCT.

Limitations and strengths of the study

Analysis of gut microbiota by NGS couldn't be done in view of financial constraints, hence analysis of gut microflora was done by conventional culture method. While it showed the growth of the predominant microbiota, bacterial flora present in smaller number were not detected. This also precluded as estimation of Shannon index.

This was the first prospective study on NEC during its commencement. A strict and robust diagnostic

criterion was used for the diagnosis of NEC. Role of the biomarker- faecal calprotectin was evaluated in this setting for the first time. There are no studies which studied the role of gut microbiota in pediatric cancer patients with neutropenic enterocolitis. The presence of gut dysbiosis seen in the study may reflect a pathogenic mechanism of neutropenic enterocolitis. This gives basis for further studies on gut microbiota in the field of pediatric oncology and possible role of interventions like prebiotics and probiotics.

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References

1. Davila ML. Neutropenic enterocolitis. Curr Opin Gastroenterol, 2006;22(1):44-7
2. Nesher L, Rolston KV. Neutropenic enterocolitis, a growing concern in the era of widespread use of aggressive chemotherapy, Clin Infect Dis 2013; 56: 711-717
3. Weinberger M, Hollingsworth H, Feuerstein IM, Young NS, Pizzo PA. Successful surgical management of neutropenic enterocolitis in two patients with severe aplastic anemia. Case reports and review of the literature. Arch Intern Med 1993;153(1):107-113
4. Tinsa F, Necib N, Guesmi M, Bousnina O, Douira W, Bousetta K, et al. Fanconi anemia complicated by neutropenic enterocolitis. Tunis Med 2008;86(11):1011-1013
5. Rotz SJ, Dandoy CE. The microbiome in pediatric oncology. Cancer. 2020;126:3629-3637.
6. Galloway-Peña JR, Smith DP, Sahasrabhojane P, Ajami NJ, Wadsworth WD, Daver NG, et al. The role of the gastrointestinal microbiome in infectious complications during induction chemotherapy for acute myeloid leukemia. Cancer. 2016;122:2186-96.
7. Jenq RR, Taur Y, Devlin SM, Ponce DM, Goldberg JD, Ahr KF, et al. Intestinal Blautia Is Associated with Reduced Death from Graft-versus-Host Disease. Biol Blood Marrow Transplant. 2015 Aug;21(8):1373-83.
8. Köhler N, Zeiser R. Intestinal Microbiota Influence Immune Tolerance Post Allogeneic Hematopoietic Cell Transplantation and Intestinal GVHD. Front Immunol. 2019; 9:3179
9. van Vliet MJ, Harmsen HJ, de Bont ES, Tissing WJ. The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. PLoS Pathog. 2010;6:e1000879.
10. Montassier E, Batard E, Massart S, Gastinne T, Carton T, Caillon J, et al. 16S rRNA gene pyrosequencing reveals shift in patient faecal microbiota during high-dose chemotherapy as conditioning regimen for bone marrow transplantation. Microb Ecol. 2014;67:690-9.
11. Zwielehner J, Lassl C, Hippe B, Pointner A, Switzeny OJ, Remely M, et al. Changes in human faecal microbiota due to chemotherapy analyzed by TaqMan-PCR, 454 sequencing and PCR-DGGE fingerprinting. PLoS One. 2011;6:e28654.
12. Chua LL, Rajasuriar R, Lim YAL, Woo YL, Loke P, Ariffin H. Temporal changes in gut microbiota profile in children with acute lymphoblastic leukemia prior to commencement-, during-, and post-cessation of chemotherapy. BMC Cancer. 2020: 24;20:151.
13. van Vliet MJ, Tissing WJ, Dun CA, Meessen NE, Kamps WA, de Bont ES, et al. Chemotherapy treatment in pediatric patients with acute myeloid leukemia receiving antimicrobial prophylaxis leads to a relative increase of colonization with potentially pathogenic bacteria in the gut. Clin Infect Dis. 2009;49:262-70.
14. Stringer AM, Al-Dasooqi N, Bowen JM, Tan TH, Radzuan M, Logan RM, et al. Biomarkers of chemotherapy-induced diarrhoea: a clinical study of intestinal microbiome alterations, inflammation and circulating matrix metalloproteinases. Support Care Cancer. 2013;21:1843-52.
15. Sánchez B, Delgado S, Blanco-Míguez A, Lourenço A, Gueimonde M, Margolles A. Probiotics, gut microbiota, and their influence on host health and disease. Mol Nutr Food Res. 2017 Jan; , 1600240
16. Mazmanian SK, Liu CH, Tzaianabos AO, Kasper DL et al. An immunomodulatory module of symbiotic bacteria directs maturation of the host immune system. Cell. 2005; 122:107–118
17. Salzman NH, Underwood MA, Bevins CL. Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa. Semin Immunol. 2007; 19:70–83.

18. Bamola VD, Ghosh A, Kapardar RK, Lal B, Cheema S, Sarma P, et al. Gut microbial diversity in health and disease: experience of healthy Indian subjects, and colon carcinoma and inflammatory bowel disease patients. Microb Ecol Health Dis. 2017 May 19;28(1):1322447.
19. Scanlan PD, Shanahan F, Clune Y, Collins JK, O'Sullivan GC, O'Riordan M, et al. Culture-independent analysis of the gut microbiota in colorectal cancer and polyposis. Environ Microbiol. 2008; 10:789–798.
20. Moore WE, Moore LH. Intestinal floras of populations that have a high risk of colon cancer. Appl Environ Microbiol. 1995; 61:3202–3207.
21. Hope ME, Hold GL, Kain R, El-Omar E.M, et al. Sporadic colorectal cancer-role of the commensal microbiota. FEMS Microbiol Lett. 2005; 244:1–7
22. Cammarota G, Ianiro G, Gasbarrini A. Faecal microbiota transplantation for the treatment of Clostridium difficile infection: a systematic review. J Clin Gastroenterol. 2014 Sep;48(8):693-702.
23. Qazi T, Amaratunga T, Barnes EL, Fischer M, Kassam Z, Allegretti JR. The risk of inflammatory bowel disease flares after faecal microbiota transplantation: Systematic review and meta-analysis. Gut Microbes. 2017 Nov 2;8(6):574-588.
24. Stringer AM, Gibson RJ, Logan RM, Bowen JM, Yeoh AS, Hamilton J, et al. Gastrointestinal microflora and mucins may play a critical role in the development of 5-Fluorouracil-induced gastrointestinal mucositis. Exp Biol Med (Maywood). 2009 Apr;234(4):430-41.
25. Stringer AM, Gibson RJ, Logan RM, Bowen JM, Yeoh AS, Keefe DM. Faecal microflora and beta-glucuronidase expression are altered in an irinotecan-induced diarrhea model in rats. Cancer Biol Ther. 2008 Dec;7(12):1919-25.
26. Lin XB, Dieleman LA, Ketabi A, Bibova I, Sawyer MB, Xue H, et al. Irinotecan (CPT-11) chemotherapy alters intestinal microbiota in tumour bearing rats. PLoS One. 2012;7(7):e39764.
27. Fijlstra M, Ferdous M, Koning AM, Rings EH, Harmsen HJ, Tissing WJ. Substantial decreases in the number and diversity of microbiota during chemotherapy-induced gastrointestinal mucositis in a rat model. Support Care Cancer. 2015 Jun;23(6):1513-22.
28. Johnson, L.B., Riaz A.A., Adawi D, Wittgren L, Bach S, Thornberg C, et al. Radiation enteropathy and leucocyte-endothelial cell reactions in a refined small bowel model. BMC Surg 4**,** 10 (2004).
29. Von Bültzingslöwen I, Adlerberth I, Wold AE, Dahlén G, Jontell M. Oral and intestinal microflora in 5-fluorouracil treated rats, translocation to cervical and mesenteric lymph nodes and effects of probiotic bacteria. Oral Microbiol Immunol. 2003 Oct;18(5):278-84.
30. Shreiner AB, Kao JY, Young VBThe gut microbiome in health and in disease, Curr Opin Gastroenterol 2015, 31:69–75.
31. Rajagopala SV, Yooseph S, Harkins DM, Moncera KJ, Zabokrtsky KB, Torralba MG et al. Gastrointestinal microbial populations can distinguish pediatric and adolescent Acute Lymphoblastic Leukemia (ALL) at the time of disease diagnosis. BMC Genomics. 2016 Aug 15;17(1):635.
32. Galloway-Peña, J.R., Smith, D.P., Sahasrabhojane, P, Wadsworth W.D, Fellman B.M, Ajami N.J, et al. Characterization of oral and gut microbiome temporal variability in hospitalized cancer patients. Genome Med, 2017:9, 21.
33. Rattanathammethee T, Tuitemwong P, Thiennimitr P, Sarichai P, Na Pombejra S, Piriyakhuntorn P, et al. Gut microbiota profiles of treatment-naïve adult acute myeloid leukemia patients with neutropenic fever during intensive chemotherapy. PLoS One. 2020 Oct 28;15(10): e0236460.
34. Alexander JL, Wilson ID, Teare J, Marchesi JR, Nicholson JK, Kinross JM. Gut microbiota modulation of chemotherapy efficacy and toxicity. Nat Rev Gastroenterol Hepatol. 2017; 14:356-365.
35. Geller LT, Barzily-Rokni M, Danino T, Jonas OH, Shental N, Nejman D, et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science. 2017; 357:1156-1160.
36. Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science. 2013; 342:967-970.
37. van Vliet MJ, Tissing WJ, Dun CA, Meessen NE, Kamps WA, de Bont ES, et al. Chemotherapy

treatment in pediatric patients with acute myeloid leukemia receiving antimicrobial prophylaxis leads to a relative increase of colonization with potentially pathogenic bacteria in the gut. Clin Infect Dis. 2009 Jul 15;49(2):262-70.
38. Hakim H, Dallas R, Wolf J, Tang L, Schultz-Cherry S, Darling V, et al. Gut Microbiome Composition Predicts Infection Risk During Chemotherapy in Children with Acute Lymphoblastic Leukemia. Clin Infect Dis. 2018 Aug 1;67(4):541-548.
39. Galloway-Peña JR, Smith DP, Sahasrabhojane P, Ajami NJ, Wadsworth WD, Daver NG, et al. The role of the gastrointestinal microbiome in infectious complications during induction chemotherapy for acute myeloid leukemia. Cancer. 2016 Jul 15;122(14):2186-96.
40. Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, et al. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clin Infect Dis. 2012 Oct;55(7):905-14.
41. Montassier E, Al-Ghalith GA, Ward T, Corvec S, Gastinne T, Potel G, et al. Pretreatment gut microbiome predicts chemotherapy-related bloodstream infection. Genome Med. 2016 Apr 28;8(1):49.
42. Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, et al. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. Blood. 2014 Aug 14;124(7):1174-82.
43. Huang Y, Yang W, Liu H, Duan J, Zhang Y, Liu M, et al. Effect of high-dose methotrexate chemotherapy on intestinal Bifidobacteria, Lactobacillus and Escherichia coli in children with acute lymphoblastic leukemia. Exp Biol Med (Maywood). 2012 Mar;237(3):305-11.
44. Stringer AM, Al-Dasooqi N, Bowen JM, Tan TH, Radzuan M, Logan RM, et al. Biomarkers of chemotherapy-induced diarrhoea: a clinical study of intestinal microbiome alterations, inflammation and circulating matrix metalloproteinases. Support Care Cancer. 2013 Jul;21(7):1843-52.
45. Rajagopala SV, Yooseph S, Harkins DM, Moncera KJ, Zabokrtsky KB, Torralba MG, et al. Gastrointestinal microbial populations can distinguish pediatric and adolescent Acute Lymphoblastic Leukemia (ALL) at the time of disease diagnosis. BMC Genomics. 2016 Aug 15;17(1):635.
46. Montassier E, Batard E, Massart S, Gastinne T, Carton T, Caillon J, et al. 16S rRNA gene pyrosequencing reveals shift in patient faecal microbiota during high-dose chemotherapy as conditioning regimen for bone marrow transplantation. Microb Ecol. 2014 Apr;67(3):690-9.
47. Zwielehner J, Lassl C, Hippe B, Pointner A, Switzeny OJ, Remely M, et al. Changes in human faecal microbiota due to chemotherapy analyzed by TaqMan-PCR, 454 sequencing and PCR-DGGE fingerprinting. PLoS One. 2011;6(12):e28654.
48. Chua LL, Rajasuriar R, Lim YAL, Woo YL, Loke P, Ariffin H. Temporal changes in gut microbiota profile in children with acute lymphoblastic leukemia prior to commencement-, during-, and post-cessation of chemotherapy. BMC Cancer. 2020: 24;20(1):151.
49. Touchefeu Y, Montassier E, Nieman K, Gastinne T, Potel G, Bruley des Varannes S, et al. Systematic review: the role of the gut microbiota in chemotherapy- or radiation-induced gastrointestinal mucositis - current evidence and potential clinical applications. Aliment Pharmacol Ther. 2014 Sep;40(5):409-21.
50. Ekert H, Jurk IH, Waters KD, Tiedemann K. Prophylactic co-trimoxazole and lactobacilli preparation in neutropenic patients. Med Pediatr Oncol. 1980;8(1):47-51.
51. Shan Zheng, Philippe Steenhout, Dong Kuiran, Wang Qihong, Wang Weiping, Corinne Hager, et al. Nutritional support of pediatric patients with cancer consuming an enteral formula with fructooligosaccharides, Nutrition Research, 26;4, 2006:154-62
52. Wada M, Nagata S, Saito M, Shimizu T, Yamashiro Y, Matsuki T, et al. Effects of the enteral administration of Bifidobacterium breve on patients undergoing chemotherapy for pediatric malignancies. Support Care Cancer. 2010 Jun;18(6):751-9.
53. Iyama S, Sato T, Tatsumi H, Hashimoto A, Tatekoshi A, Kamihara Y, et al. Efficacy of Enteral Supplementation Enriched with Glutamine, Fiber, and Oligosaccharide on Mucosal Injury following Hematopoietic Stem Cell Transplantation. Case Rep Oncol. 2014 Oct 22;7(3):692-9.
54. Gorshein E, Wei C, Ambrosy S, Budney S, Vivas J, Shenkerman A, et al. Lactobacillus rhamnosus GG probiotic enteric regimen does not appreciably alter the gut microbiome or provide protection against

GVHD after allogeneic hematopoietic stem cell transplantation. Clin Transplant. 2017 May;31(5).
55. Ladas EJ, Bhatia M, Chen L, Sandler E, Petrovic A, Berman DM, et al. The safety and feasibility of probiotics in children and adolescents undergoing hematopoietic cell transplantation. Bone Marrow Transplant. 2016 Feb;51(2):262-6.

TABLE 1: Faecal microflora 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 2: Association of faecal microflora with survival in patients with neutropenic enterocolitis

S. No	Faecal microbiota	Survivors, n = 29 n (%)	Non survivors, n = 9 n (%)	p value
1	Clostridium	10 (34.5)	4 (44.4)	0.43
2	Bacteroides	12 (41.3)	5 (55.5)	0.35
3	Veillonella	3 (10.3)	1 (11.1)	0.67
4	Lactobacilli	4 (13.8)	1 (11.1)	0.66
5	E coli	24 (82.7)	7 (77.7)	0.53
6	Enterococcus	7 (24.1)	2 (22.2)	0.64
7	Klebsiella	4 (13.8)	0 (0)	0.32
8	Citrobacter	2 (6.9)	1 (11.1)	0.56

TABLE 3: Association of specific genera in faecal microflora with faecal calprotectin, n = 38

S. No	Faecal microbiota	Φαεcαλ cαλπροτεcτιν ιν cπεcιφις γενερα (μγ/γ)	Φαεcαλ cαλπροτεcτιν ιν
1	Clostridium	137 (25-359)	126 (46-380)
2	Bacteroides	150 (25-359)	115 (29-380)
3	Veillonella	143 (78-278)	129 (25-380)
4	Lactobacilli	81 (36-126)	138 (25-380)
5	E coli	142 (25-380)	79 (49-126)
6	Enterococcus	64 (25-126)	151 (29-380)
7	Klebsiella	165 (79-278)	127 (25-380)
8	Citrobacter	138 (29-286)	130 (25-380)

TABLE 4: Alteration in gut microbiota with chemotherapy or infections

S. No	Study	Population	Sample size	Technique	Results
1.	Van Vliet et al[37]	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[43]	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[44]	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[45]	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[46]	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[47]	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 al1[8]	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[48]	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
8	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

TABLE 5: Studies targeting gut dysbiosis in cancer patients

S. No	Study	Sample size	Intervention	Result
1.	Ekert et al[50], Australia, 1980	68 children with cancer	Arm I- Lactobacilli + Cotrimoxazole Arm II- Framycetin + Nystatin + Metronidazole	Arm I showed lower incidence of febrile neutropenia Minimal side effects, well tolerated
2.	Zheng et al[51], China, 2006	67 pediatric cancer patients	Intervention (32): Prebiotic- Fructooligosachharide Control: 35	Higher lactobacilli count in intervention group than control Trend towards higher Bifidobacterium in intervention group (Not significant)

S. No	Study	Sample size	Intervention	Result
3.	Wada et al[52], Japan, 2010	42 pediatric cancer patients	Intervention (19): Probiotic-*Bifidobacterium breve* strain Yakult Control: 23	Lower incidence of fever and antibiotic exposure in intervention group Lower count of enterococci in intervention group Higher SCFA in intervention group
4.	Iyama et al[53], Japan 2014	44 adult cancer patients undergoing HSCT	Intervention (22): Prebiotic combination-Glutamine, fiber and oligosachharide Control: 22	Significantly lower incidence and duration of grade 3-4 diarrhoea and mucositis Decreased weight loss and requirement of parenteral nutrition Trend towards lower gut bacterial translocation in intervention group No difference in OS, GVHD rates, though some survival benefit at day 100
5	Gorshein et al[54], USA, 2017	31 adult cancer patients undergoing HSCT	Intervention (20): Probiotic Lactobacillus rhamnosus GG started post engraftment Control: 10	No reduction in TRM, rates of acute or chronic GVHD No difference in microbiome as compared to controls Trial terminated early due to lack of efficacy
6	Ladas et al[55], USA, 2016	30 children undergoing HSCT, pilot study	Probiotic *Lactobacillus plantarum* started from day -8 of transplant	Feasible Safe, no increase in toxicity

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