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NUTRITIONAL MODULATION AND SUPPORT FOR PEDIATRIC PATIENTS WITH CANCER AND UNDERGOING HEMATOPOIETIC CELL TRANSPLANTATION

Presentata da: *Dott. Francesco Venturelli*

Coordinatore Dottorato

Prof.ssa Manuela Ferracin

Supervisore

Prof. Riccardo Masetti

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1. ABSTRACT

Nutritional status has been demonstrated as a crucial aspect in the treatment of pediatric oncology patients, with measurable impact of morbidity and mortality in children with acute leukemias. Thus, great effort should be made to optimize nutritional evaluation and support. In fact, in pediatric patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT), nutritional support (e.g. enteral nutrition) can be effectively utilized to reduce the incidence of bloodstream infections (BSI). Furthermore, nutritional interventions can be utilized to modulate the gut microbiome (GM) configuration, which has implications for severe HCT-related complications such as acute graft-vs-host disease (GvHD). Other interventional strategies including fecal microbiota transplantation (FMT), can be utilized to modify the GM configurations.

In this thesis, we summarize the results of two nationwide survey among the Associazione Italiana di Ematologia ed Oncologia Pediatrica (AIOEP) network aimed to assess the current practices for nutritional evaluation and care, as well as food-handling practices and foodborne infections among pediatric patients undergoing chemotherapy and allo-HCT.

Moreover, we compared nutritional and HCT-related outcomes (such as acute GvHD and BSI) in a cohort of pediatric patients undergoing allogeneic HCT between patients receiving nutritional support with Parenteral Nutrition (PN) and Enteral Nutrition (EN).

Finally, we evaluate the feasibility and safety of FMT in a cohort of children with steroid-refractory gut GvHD and MDR colonization, observing an exceptional safety profile and clinical response.

2. AIM OF THE PROJECT

This thesis aims to provide a comprehensive overview of the research focused on the nutritional support of pediatric patients with cancer in the pediatric hematological oncology department of Sant'Orsola-Malpighi Hospital (Bologna).

Nutritional support is emerging as a crucial aspect, yet often overlooked, in the treatment of pediatric oncology patients. Despite the increased focus on nutritional evaluation and support, there is currently a lack of a systematic approach to this issue. Thus, we aimed to understand the key knowledge gaps to assemble a set of recommendations regarding nutritional evaluation and support of the pediatric patient with cancer, to share with other centers among the AIEOP (Associazione Italiana di Ematologia ed Oncologia Pediatrica) network.

Furthermore, recent evidence suggests that gut microbiome (GM) dysbiosis may also play an important role in pediatric oncology patients undergoing chemotherapy or allogeneic hematopoietic cell transplantation (allo-HCT), influencing the risk of developing complications (such as infections and acute graft-versus-host disease) and survival outcomes. The most recent literature evidence suggests that modulation of the gut microbiota through various tools (such as enteral nutrition and fecal microbiota transplantation) may play a role in the prevention or treatment of such complications.

Moreover, through the analysis of human tissues (such as plasma, saliva, urine, and feces), it is possible to characterize the modifications of the gut microbiome in pediatric patients undergoing chemotherapy following specific therapies (e.g. allo-HCT, fecal microbiota transplantation). We present a brief metagenomic characterization of the gut microbiome of one patient who underwent the Fecal Microbiota Transplantation procedure for MDR colonization.

3. BACKGROUND

a. Nutrition in pediatric cancer

i. Role of nutrition in pediatric acute leukemias

Childhood acute leukemia is characterized by the proliferation of immature, cancerous white blood cells and it is a leading cause of childhood cancer mortality[1]. Factors such as specific genetic defects or response to chemotherapy are well-established prognostic factors and are utilized for the risk stratification and therapeutic management of acute leukemias.

Interestingly, also nutritional status has been linked to pediatric acute myeloid leukemia survival. Specifically, Lange et. al reported that overweight (defined as BMI ≥ 95% of the cohort) and underweight (defined as BMI ≤10% of the cohort) children and adolescents with AML are less likely to survive than patients with BMI in the 11th through 94th percentiles (**Figure 1**) due to early treatment-related mortality, and treatment-related mortality is mostly from infections[2].

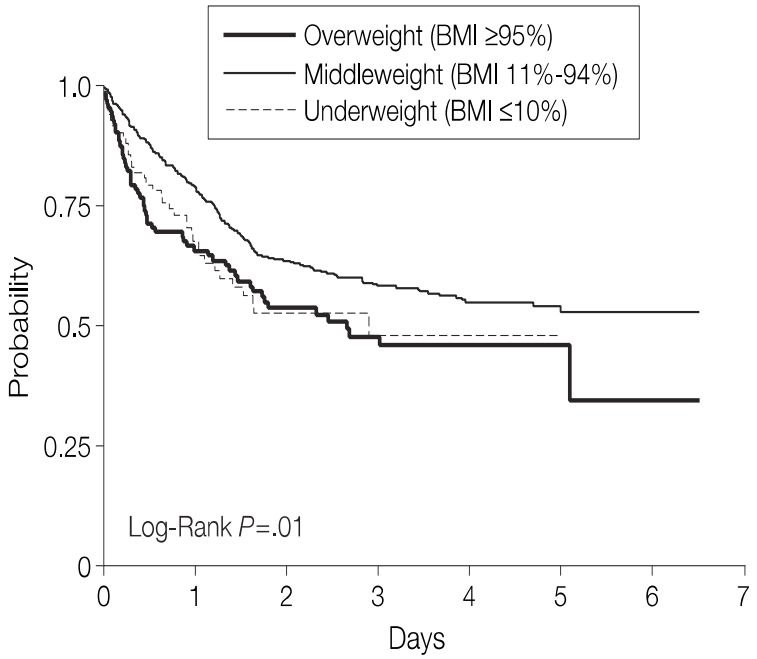

Figure 1: OS in overweight and underweight children with acute myeloid leukemia (Lange et. Al JAMA. 2005)

A following report from a wide cohort of the Children Oncology Group highlighted similar results also for pediatric patients undergoing treatment for acute lymphoblastic leukemia, with children underweight or obese at diagnosis experiencing significantly lower EFS[3] (**Figure 2**).

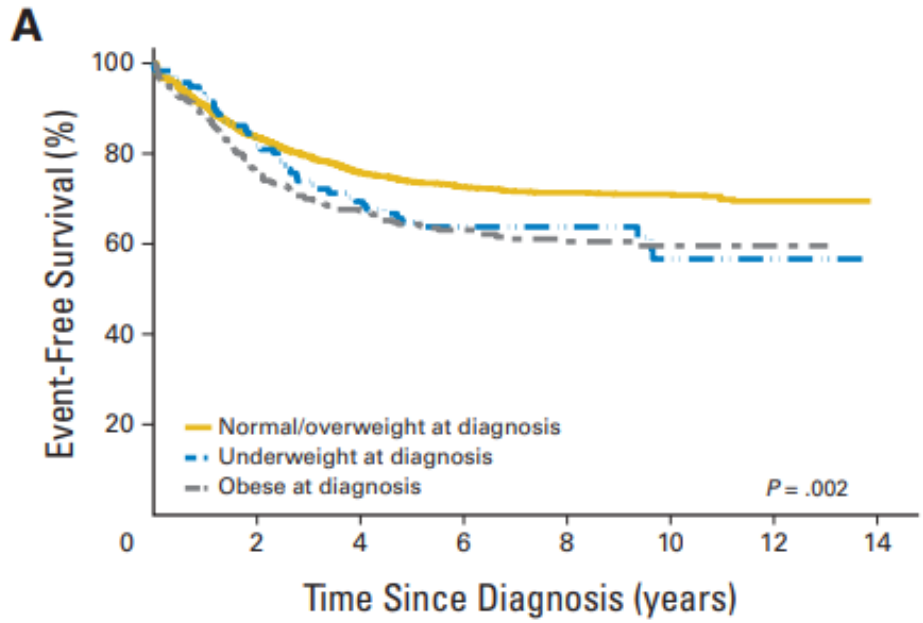

Figure 2: EFS for pediatric patients treated for ALL according to weight and diagnosis (Orgel E et al, JCO 2013)

Triarico et al. also reported a significant reduction of OS and EFS for children treated for various types of cancer experiencing a weight loss ≥ 10% in the first 3 months of chemotherapy[4].

Moreover, children differ from their adult counterparts, because they are at a developmental stage where nutritional imbalances can significantly impact proper growth. Indeed, malnutrition, defined by WHO as both undernutrition and overnutrition, is very common in children with cancer, occurring in up to 70% and between 25% and 75%, respectively[5].

In this context, a proper nutritional evaluation and support are essential from diagnosis, during treatment, and even beyond for long-term survival.

Nevertheless, shared guidelines and recommendations are lacking[6,7]. Recent efforts from single pediatric cancer societies have been made to address this issue[8], but the implementation of shared guidelines in clinical practice remains difficult.

ii. Nutritional assessment and care

The more recent Italian consensus states that assessment of nutritional status using a standardized method should be performed on all patients at diagnosis and repeated periodically throughout the course of treatment, as well as at follow-up. Patients receiving periods of intensive treatment or at high risk of malnutrition require a more strict follow-up schedule[8].

Nutritional status should be assessed using a standardized and cost-effective method, as recommended by the Nutrition Working Group (NWG) of the International Society of Pediatric Oncology (SIOP), Committee on Pediatric Oncology in Developing Countries (PODC)[7]. Specifically, it should consist of:

- Anthropometric measures: the minimal assessment should include body mass index (BMI) and mid-upper arm circumference (MUAC). Triceps Skinfold Thickness (TSFT) and Bioelectrical Impedance Analysis (BIA) can be considered to better characterize body composition.
- Clinical evaluation
- Dietary intake
- Biochemistry exams

One of the main issues is the lack of simple, cost-effective, and validated serum biomarkers to monitor the nutritional status. Serum albumin, prealbumin, and transferrin can add information about a patient's protein status. Serum calcium, magnesium, and vitamin D can add information about bone status. Also, iron studies, vitamin levels, and specific trace elements (such as zinc and vitamins B12, B1, A, D, and E) can be of help. However, it should always be considered that these parameters, due to the tumor itself or treatments, can be altered. More specific laboratory exams, such as retinol-binding protein or transferrin receptor dosage, can be used in severely malnourished children to assess malnutrition over time, but they are hardly available in all centers. Runco et al. analyzed for the first time in children serum concentrations of growth differentiation factor 15 (GDF15), a non-specific marker used in adults to monitor oxidative stress, inflammation, and cachexia. GDF15 levels were higher at diagnosis and during treatment compared to healthy subjects but no association with anthropometric measures or quality of life assessments was found[9]. Also, the caregiver's ability to support their children's nutritional needs should not be neglected, as highlighted recently by LaLonde and colleagues[10].

The consensus focuses also on what professional figures should perform each evaluation (e.g. dietician, clinical nutritionist) and what tools could be useful as screening (such as validated composite scores). Among these, the Screening Tool for Risk of Nutritional Status and Growth (Strong Kids) is suggested, because it is more balanced and it considers many aspects of the disease, such as the clinical status, and contributing factors, especially related to undernutrition.

iii. Nutritional status and allo-HCT outcomes

Allogeneic hematopoietic cell transplantation represents the only curative option to date for several aggressive childhood cancer types. Patients undergoing HCT are at high nutritional risk both in the pre-transplant and the post-transplant phase. The conditioning regimen causes various side effects including nausea, vomiting, mucositis with diarrhea, and odynophagia. Furthermore, the potential onset of gastrointestinal graft-versus-host disease (GVHD) in the post-transplant phase (including the medications used as its treatment) and possible infections must be considered. Acute gut GvHD causes profuse diarrhea, dysphagia, and damage to the intestinal wall, resulting in fluid loss, malabsorption, and weight loss. Among the therapies used, corticosteroids induce bone and muscle catabolism as well as hypertriglyceridemia, hyperglycemia, hypercholesterolemia, and sodium and fluid retention[11]. Cyclosporine also has gastrointestinal side effects that contribute to the onset of malnutrition. Psychological factors also strongly impact the food intake of transplant patients. Furthermore, the use of opioids for pain management in HCT patients leads to side effects including constipation, the most common complication, nausea, vomiting, and reduced appetite[12]. Following HSCT, there is increased catabolism due not only to conditioning regimens but also to the onset of fever, sepsis, GvHD, and organ failure. The tendency towards catabolism, combined with reduced food intake and fluid redistribution in the body, underlies the hypoproteinemia often found after HCT. A decrease in BMI and body weight or an excess of body weight can be observed in the peri-transplant period; both fall within the definition of malnutrition[13]. Among the types of transplantation, allo-HCT is the one at greatest risk of malnutrition.

The impact of nutritional status on allo-HCT-related outcomes is well established. Aplenic et al demonstrated that children with BMI >30 exhibited higher transplant-related mortality (TRM) compared to normal-weight patients, despite a lower disease relapse rate[14]. Another study of pediatric and young adult patients undergoing allogeneic umbilical cord blood (UCB) transplantation revealed that a low body mass index was associated with an increased risk of acute graft-versus-host disease (aGVHD)[15]. Also, pre-transplant hypoalbuminemia (defined as serum albumin < 3.1 g/dL) required more intensive care interventions, such as mechanical ventilation, non-invasive ventilation, and vasoactive agents. Moreover, hypoalbuminemia was associated with increased 6-month mortality[16].

iv. Nutritional support strategies for pediatric patients undergoing allo-HCT

Nutritional support, in the form of enteral or parenteral nutrition, should be initiated when oral intake fails to meet at least 60% of the patient's basal energy requirements (as determined by calorimetry) for three consecutive days[17].

Parenteral nutrition (PN) support through central venous catheter has been considered the gold standard historically[18]. PN was introduced in the 1960s as a support therapy for patients suffering from intestinal insufficiency and was later adopted as one of the main nutritional supports for the oncologic patient[19].

However, PN is associated with several adverse effects, including drug interactions, bone demineralization with risk of fractures, cholelithiasis, hepatopathy, growth retardation in case of inadequate nutritional support, hyperglycemia, hypertriglyceridemia, electrolyte disturbances, and refeeding syndrome. Also, increased levels of pro-inflammatory cytokines, such as IFN-γ and TNF-α have been detected in the intestine of patients receiving PN. Additionally, PN also reduces intestinal transit, leading to a loss of intestinal barrier function and mucosal atrophy in the gastrointestinal tract. Histopathological findings include loss of junctional integrity, villous atrophy, reduced proliferation, and increased apoptosis of intestinal epithelial cells[20]. Furthermore, PN is associated with an increased risk of infection due to hyperglycemia, and the impairment of the intestinal barrier allows for the potential translocation of pathogens from the lumen into the circulation, resulting in sepsis[21].

Due to these adverse effects, both the European Society for Clinical Nutrition and Metabolism (ESPEN) and the American Society for Parenteral and Enteral Nutrition (ASPEN) guidelines recommend enteral nutrition (EN) as the first-line nutritional support option for pediatric patients undergoing hematopoietic stem cell transplantation[22,23].

Thus, enteral nutrition (EN) is considered the optimal nutritional support option in oncology; however, if it is contraindicated or not tolerated, parenteral nutrition (PN) can be used.

b. The human gut microbiome (GM)

i. GM and homeostasis

The term "microbiota" refers to the diverse community of microorganisms, including bacteria, yeasts, and viruses, that inhabit various human body sites such as the gut, skin, lungs, and oral cavity[24]. Notably, the human microbiota, often dubbed as "the hidden organ" contributes over 150 times more genetic information than the entire human genome[25]. While the terms "microbiota" and "microbiome" are often used as synonyms, there are some differences. "Microbiota" describes the living microorganisms found in a defined environment (e.g. Oral, gut microbiota) while "Microbiome" refers to the collection of genomes from all the microorganisms in the environment, including not only the community of the microorganisms, but also the microbial structural elements, metabolites, and the environmental conditions[26].

Trillions of microorganisms reside both internally and on the surface of the human body, where they perform numerous functions contributing to the maintenance of organismal homeostasis under physiological conditions[27]. The gastrointestinal tract hosts the densest microbial niche, mainly composed of anaerobic species, and the capabilities of this ecosystem to create functional connections with other physiological systems means that a shift in its composition might lead to disease involving not only the gut but also other distant organs[28]. To date, 2172 distinct species have been identified within the GM, with 94% belonging to four primary phyla: Actinobacteria, Bacteroides, Proteobacteria, and Firmicutes[29] (**Figure 3**).

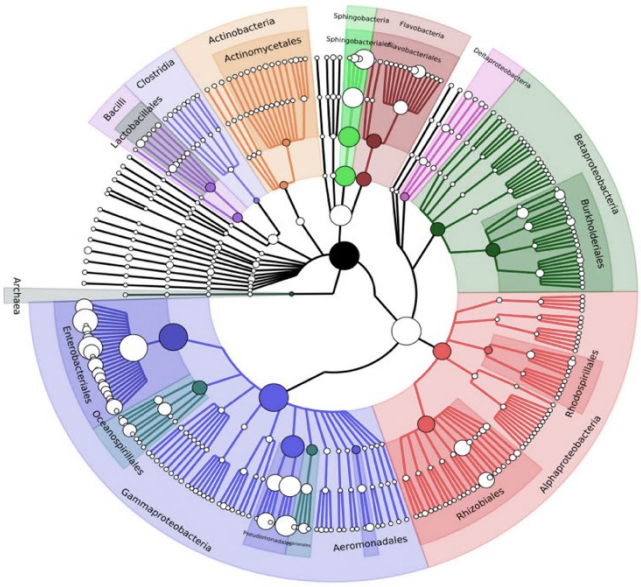

Figure 3: phylogenetic tree of gut microbiota (Joynson R, et al. Front Microbiol. 2017 Nov 8;8:2181.)

The first strategy to investigate GM was based on culture methods. However, only a small fraction of the species comprising the microbiota could be identified through cultures. Currently, the advent of Next-Generation Sequencing (NGS) enabled the exploration of all the diverse bacterial species within the GM. The analysis of the GM starts with fecal samples; the DNA contained therein is first amplified via PCR and then sequenced. NGS is capable of analyzing all DNA fragments present within the sample, allowing us to not only identify which species are part of the GM but also their relative abundance[30]. Specifically, the analyzed DNA region contains the gene for 16S ribosomal RNA (16S rRNA). Analysis of the 16S rRNA gene has facilitated a comprehensive understanding of the GM composition at a reduced cost[31]. An emerging field in the study of the MI, and specifically its interactions with the immune system, is Metabolomics. Metabolomics encompasses a suite of technologies capable of studying metabolites present in various bodily fluids. This investigative method allows us to visualize which metabolites produced by the bacteria comprising the GM are in circulation [32].

A symbiotic relationship exists between the host and the microbiota, providing mutual benefits. The microbiota exerts numerous beneficial effects on the host organism, including maintaining intestinal epithelial integrity and protecting against colonization by pathogenic bacteria. Moreover, GM is capable of influencing both adaptive and innate immunity through the mediation of various molecules produced by resident bacteria, playing a pivotal immunomodulatory role, not only locally but also systemically. Some bacteria belonging to the GM can produce various metabolites from the digestion of proteins and complex carbohydrates, which have effects on immune system cells. Among the metabolites produced by GM, short-chain fatty acids (SCFAs) are of the greatest importance. These are saturated fatty acids with an aliphatic chain composed of fewer than six carbon atoms; acetate, propionate, and butyrate are the most abundant. Once released into the intestinal lumen, butyrate is absorbed by intestinal epithelial cells, acetate is released into the circulation, and propionate is taken up by the liver. Butyrate plays a protective role for intestinal epithelial cells[33,34]. Once in circulation, SCFAs are taken up by innate and adaptive immune cells in tissues distant from the site of origin[35] influencing also other system behavior (**Figure 4**). Through different pathways, short-chain fatty acids can influence not only gene expression but also the differentiation, proliferation, migration, and apoptosis of immune cells. Among the actions performed by the GM is the switch of T lymphocytes from a Th1, Th2, and Th17 phenotype to a regulatory phenotype[36].

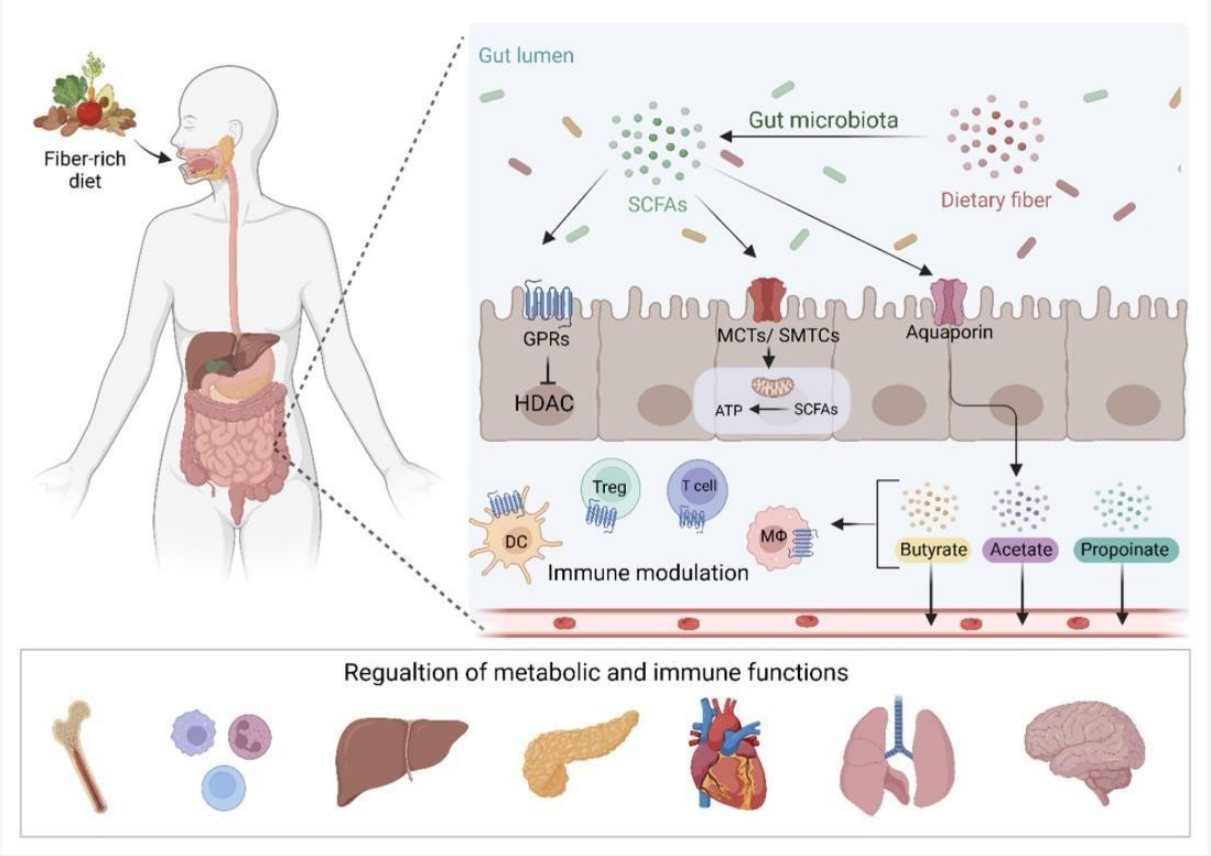

Figure 4: Short-chain fatty acids (SCFA) cross the intestinal mucosa and regulate intestinal immunity or pass into the circulation to modulate metabolic and immune functions in various distant organs (GH Al-Qadami et al. Microorganisms. 2022).

ii. Pediatric trajectories of gut microbiome modifications

GM evolves in symbiosis with the individual's growth, shifting configurations from the neonatal age 37. Perturbations of its configuration in early life are linked to the onset of conditions in the adult 38. Various environmental and host-related factors influence the establishment of microbial communities in infants, beginning from the presence of bacteria in the placenta, umbilical cord, and amniotic fluid[37], and continuing during birth. Children delivered vaginally have initial contact with the maternal vaginal and fecal microbiota, resulting in an abundance of bacteria of the *Lactobacillus* and *Prevotella* genera[38]. Children delivered through a cesarean C-section are colonized mainly due to the interaction with maternal skin microbiota[39]. Different feeding modalities also account for different neonatal GM configurations, with breastfed children characterized by a GM rich in Bifidobacteria, which is linked to the correct development of immunological functions[40]. The solid diet enlarges the GM

alpha diversity, replacing Proteobacteria and Actinobacteria with mainly Firmicutes and Bacteroides[41].

Recent longitudinal GM studies revealed that the development of the gut microbiome during early childhood consists of four different stages (acquisition, developmental, transitional, and stable) that are characterized by typical changes in GM diversity and composition[42]. During the first 3 years of life, the GM exhibits increasing richness within individual samples (α-diversity)[43] and decreasing compositional differences compared with adults (β-diversity), followed by gross stabilization (**Figure 5**).

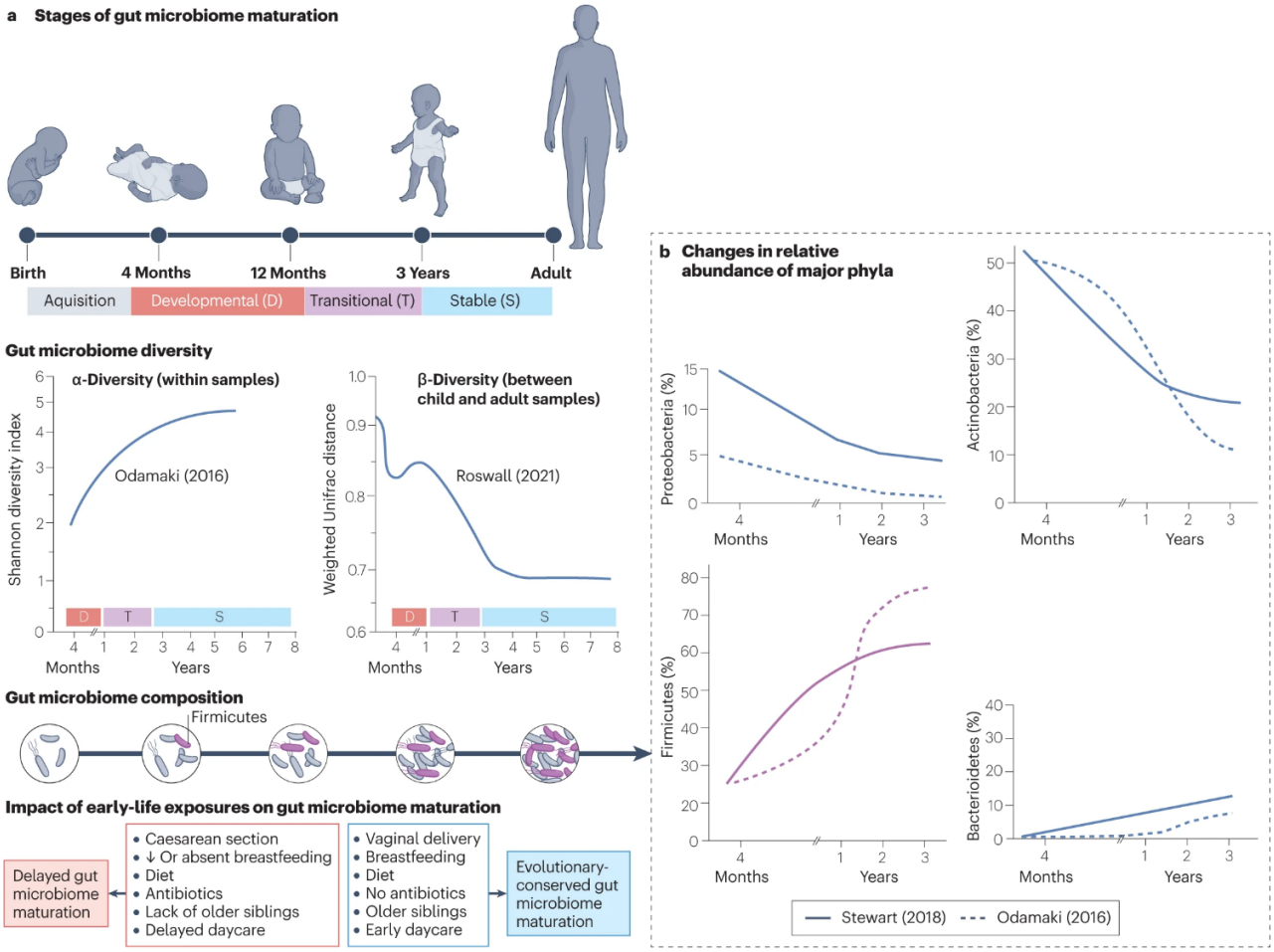

Figure 5: GM trajectories and compositions during life (I. Peppas et al, Nat. Review Cancer 2023)

Despite the dynamic response to dietary changes, antibiotic use, and lifestyle habits, the GM composition remains relatively stable until old age. From the age of 65 years old, an important reduction of α-diversity in which bacteria belonging to the Firmicutes phylum decrease in favor of Bacteroidetes is observed[44].

c. Gut microbiome and allogeneic stem cell transplantation

i. GM modifications during pediatric allo-HSCT

Patients undergoing allo-HSCT are subjected to high doses of chemotherapy, radiation, and antibiotics within a short time frame, which leads to mucosal barrier disruption and microbiota dysbiosis, characterized by reduced diversity, loss of commensals, and expansions of potentially pathogenic bacteria[45–48]. The degree of GM injury and the establishment of specific GM signature are associated with major adverse outcomes. Reduced alpha diversity at engraftment is independently associated with increased mortality after transplantation[49]. Overgrowth of Enterococcus is a risk factor for the development of aGvHD and for increased GvHD-related mortality and all-cause mortality[50], while Blautia is considered a protective factor from lethal GvHD[51]. Intestinal domination, which occurs when a single bacterial taxon comprises 30% or more of the entire GM, is associated with the occurrence of bloodstream infections (BSI)[52].

Immune reconstitution, hepatic sinusoidal obstruction syndrome, febrile neutropenia, pulmonary complications, and relapse of the primary disease have also been associated with intestinal microbiota composition after and/or before transplantation in single or multicenter studies[53,54].

Moreover, children's GM during allo-HCT has different characteristics compared to the adult GM (**Figure 6**).

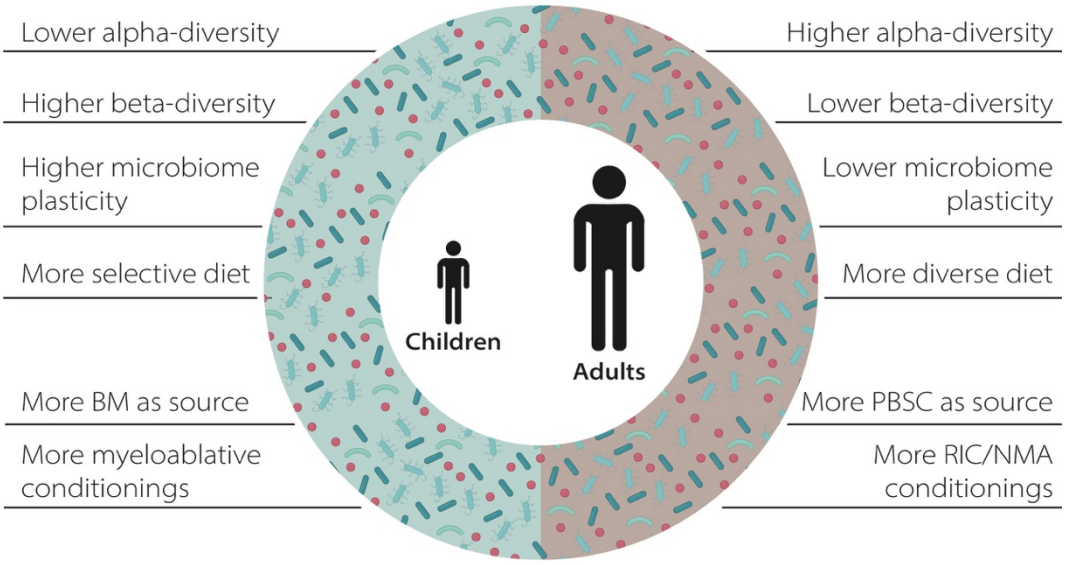

Figure 6: Differences between the pediatric and adult microbiome and allo-HCT.

ii. Pediatric acute graft vs host disease (GvHD)

Although currently one of the only curative treatments for these patients, allo-HCT also comes with significant risks. In pediatric patients, the main causes of morbidity and mortality after allo-HCT are infection and graft versus host disease (GvHD)[55]. The incidence of acute GvHD (aGvHD) in children is approximately 50% of any grade and 20% of grade II-IV, with certain variability based on the characteristics of allo-HCT[56]. About half of patients with grade II-IV aGvHD do not respond to first-line steroids, posing a significant challenge for clinicians[57].
The pathogenesis of GvHD is a complex interplay of factors, including genetic disparities in human leukocyte antigen (HLA) proteins, and the inflammatory milieu induced by conditioning regimens[58]. While HLA disparities can harvest the graft versus leukemia effect[59], it can also increase the risk of GvHD[60]. Conditioning therapies damage tissues, releasing inflammatory cytokines such as TNF-α, IL-1, and IL-6[58]. Additionally, the gut microbiome, a diverse community of microorganisms, plays a crucial role in GvHD pathogenesis.

Conditioning regimens can disrupt the gut microbiome, leading to the release of bacterial DNA and RNA, known as pathogen-associated molecular patterns (PAMPs). PAMPs activate immune cells, promoting the presentation of host antigens to donor T cells. Activated T cells, particularly Th1 and Th17 subtypes, target host tissues, triggering a cascade of inflammatory events involving the release of cytokines like IFN-γ and TNF-α[58]. The balance between regulatory and effector T cells, influenced by the microbiome and other factors, determines the severity of GvHD.

iii. Role of GM in pediatric acute GvHD

Recent research has highlighted the intricate relationship between the gut microbiome and GvHD. The microbiome can modulate tissue injury, inflammatory signaling, and the balance of immune cells, ultimately impacting the development and severity of GvHD. The microbial components known as PAMPSs are recognized by pattern recognition receptors (PRRs) on immune cells, leading to the release of pro-inflammatory cytokines such as IL-1β and type 1 interferons associated with the severity of GvHD[61]. Bacterial metabolites also regulate immune responses with short-chain fatty acids (SCFAs), protein metabolites, and secondary bile acids being central to gut-host communication. SCFAs, more abundant in pediatric microbiomes, support intestinal health and reduce inflammation through mechanisms such as epithelial

growth promotion and the suppression of inflammatory cytokine production. Protein metabolites like indoles, which act through the aryl hydrocarbon receptor (AhR), modulate T cell responses in the gut, in a ligand-dependent manner[62]. Lower AhR ligand production has been correlated with acute GvHD development in adults[63]. Arginine, another bacterial metabolite, may have a role in promoting inflammatory macrophage signaling depending on the inflammatory environment[64], with high ratios of arginine-producing bacteria at transplant predictive of GvHD[65]. Inosine, produced by common pediatric gut bacteria, can promote inflammatory T cell differentiation and may increase GvHD risk when the gut barrier is compromised[66].

iv. Nutritional modulation of GM

Nutritional status and GM have a bidirectional relationship. Disturbances in the microbiome affect the risk for undernutrition and obesity through the alteration of bacterial metabolite production, and malnutrition alters GM function and composition[67–69]. Specific taxonomic and functional markers are associated with increased BMI and glucose metabolism deterioration, such as enrichment in Bacteroides enterotype 2 and impairment of biotin metabolism[67,70], but further studies are needed to better decipher the complex relationship between obesity and GM. In the HSCT setting, the impact of obesity on the GM and GvHD pathogenesis was assessed both in mouse models and humans. In the mouse model, mice with diet-induced obesity had an increased incidence of severe, rapid-onset gut aGvHD with high lethality. Gut aGvHD in obese mice was mediated by donor CD4+ T cells and occurred even with a minor MHC incompatibility. Obese mice presented also increased epithelial cell apoptosis, gut permeability, endotoxin translocation across the gut, and radiation-induced gastrointestinal damage after conditioning. GM analysis pre-transplant, both in humans and mice, revealed reduced GM diversity and decreased Clostridiaceae abundance in obese patients, particularly with a lower abundance of genus *Clostridium*. In the mouse model only, the relative abundance of *Enterococcus* and *Akkermansia muciniphila* significantly increased in obese after transplantation. Therefore, obesity-associated GM alterations may render the patient more prone to gut epithelial damage, inflammation, and gut aGvHD. Interestingly, prophylactic antibiotic treatment in obese mice improved gut GvHD histological severity and mortality, as well as reduced endotoxin translocation across the intestinal epithelium and inflammatory cytokine production, but did not protect against the development of cGvHD of the skin, highlighting the possibility of modulating obesity-associated dysbiosis[71].

Indeed, body mass composition itself could impact HSCT outcome, and GM composition could also influence the complex metabolic pathways regulating body composition. Muscle mass is an independent predictor of survival after HSCT, with sarcopenia associated with worse disease-free and overall survival[72,73]. The GM, and particularly its metabolites, play an important role in muscle metabolism, affecting skeletal muscle mass and function[74]. Administration of soy-whey blended protein for two months in HSCT recipients who failed to improve muscle function within half a year resulted in significantly improved muscle area and muscle strength. However, in a small number of patients, muscle status did not improve. GM Alpha diversity significantly increased in responders to treatment, whereas it did not in non-responders. Moreover, the abundance of most of the butyrate-producing taxa decreased significantly in non-responders. This important SCFA is known to regulate skeletal muscle energy expenditure and metabolism[75]. *Ruminococcus* and *Veillonella* abundance correlated positively with muscle status, whereas *Streptococcus* correlated negatively[76]. *Ruminococcus* species can metabolize monosaccharides and degrade mucin, producing acetic acid and formic acid[77]. *Veillonella* enhances muscle function by converting lactic acid produced by muscles to propionic acid[78]. Amino acid biosynthesis and pentose phosphate pathways were also higher in the GM of responders. These findings underline the important role of the GM on muscle metabolism after HSCT.

Oral intake in the early post-transplantation period is severely impaired due to the side effects of the conditioning regimen, mainly vomiting, and mucositis. Parenteral nutrition (PN) has been historically used as a supportive measure to avoid the deterioration of nutritional status in HSCT recipients[79–81]. However, PN is associated with several metabolic, hepatic, and intestinal complications. In recent years, enteral nutrition (EN) has been increasingly used in the HSCT setting considering the feasibility and clinical benefits of this approach[82,83] and it is currently recommended by international guidelines as first-line nutritional support in transplant recipients if oral intake is insufficient[23].

The clinical positive effects of EN for nutritional support during the neutropenic phase after HSCT have been shown in several studies. In our recent meta-analysis, the use of EN was associated with lower rates of aGVHD, aGVHD grade III-IV, and gastrointestinal aGVHD compared to PN[84]. Some studies also observed reduced infective complications, such as BSI[85]. One possible explanation for these findings is the different effects of the two nutritional strategies on GM composition, as already demonstrated in different clinical and preclinical settings. On one side, PN reduced intestinal transit with subsequent GM dysbiosis and mucosal

atrophy. It also determines a proinflammatory state with resulting loss of epithelial barrier function that leads to microbial translocation and bacteremia. On the other side, EN allows the maintenance of gut transit and seems to improve mucosa integrity. EN exerts a trophic effect on enterocytes, either directly providing nutrients in the gut lumen, or indirectly via the production of SCFA from the GM[86,87] Moreover, EN seems to be essential to maintain a healthy gut mucosal and high GM diversity, as shown in preclinical and clinical models[86–90] (Figure 1). Data on humans are limited, but seems to demonstrate that PN reduces GM diversity and is associated with an increased relative abundance of *Proteobacteria* (particularly *Enterococcaceae*), that proliferate in a nutrient-deprived environment. In contrast, a reduction in *Firmicutes* is observed, which seem to dominate when a valid nutrient supply is guaranteed[90,91]. The effect of PN on the GM of HSCT recipients was investigated in two large observational studies. The first one, on 80 HSCT recipients, groups with different GM diversity showed no differences in terms of PN administration, with similar percentages of patients receiving PN during the pre-engraftment period in the two groups [92]. Differently, in a second study investigating the relationship between GM and GvHD on 115 patients, patients receiving PN for a shorter period (<10 days) showed a higher abundance of genus *Blautia* compared to those receiving PN for a longer duration. Notably, the inverse association of PN and *Blautia* was maintained even for patients who did not receive anaerobe-active antibiotics[93] *Blautia* is an anaerobic commensal producer of SCFA that has been associated with reduced GvHD-related mortality[93]. To date, two studies specifically investigated the impact of EN on GM composition compared to PN in HSCT setting. In a cohort of 23 adult HSCT recipients randomly allocated to receive EN or PN, a shotgun metagenomic sequencing analysis of stool samples 30 days post-transplant, revealed no difference in terms of microbial diversity in the two groups. However, significant differences in GM composition were reported in patients receiving predominantly EN compared to patients receiving predominantly PN. Particularly, the former presented a higher abundance of taxa associated with increased SCFA production, including *R. bromii*, *R. inulinivorans*, *A. hadrus,* and several *F. praunitzii* species. In the PN group, a greater abundance of *Enterococcus* and *Proteobacteria*, such as *Klebsiella*, was noted (**Figure 7**).

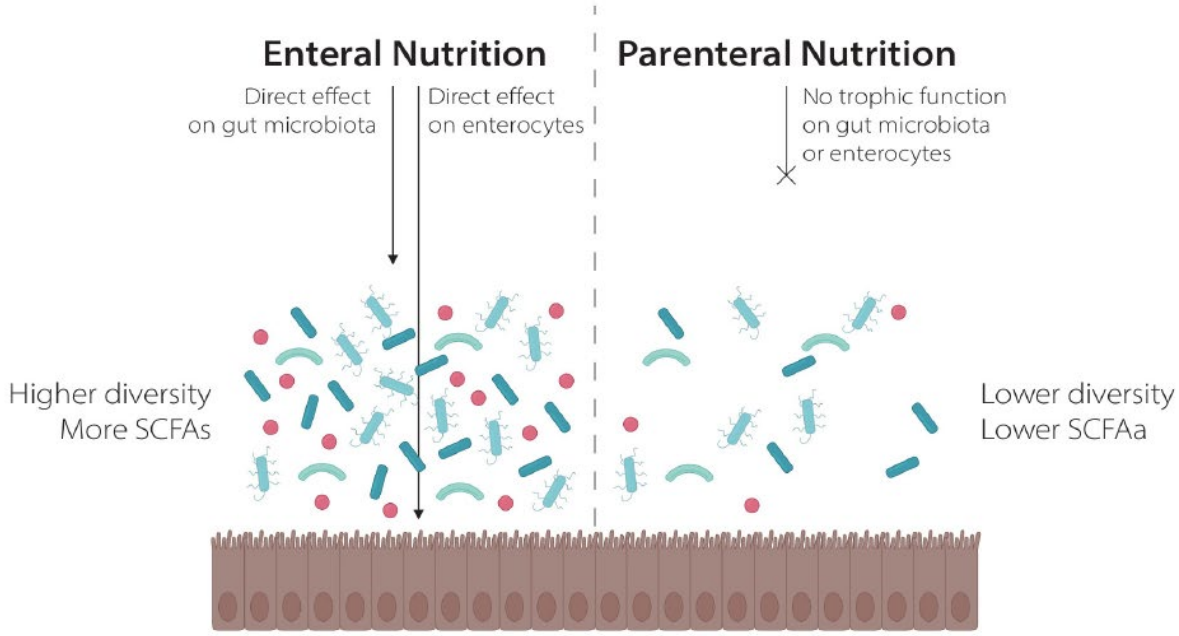

Figure 7: The effect of Enteral and Parenteral Nutrition on the gut ecosystem.

Furthermore, patients who maintained higher levels of oral intake during the phase of nutritional support, whether parenteral, enteral, or a combination of both, present significantly different GM composition with higher microbial diversity and relative abundance of SCFA-producing taxa, including *F. prausnitzii_C*, *R. inulinivorans* and *Blautia.* A greater abundance of potential pathogens, such as *Klebsiella_A*, *Staphylococcus,* and *Enterococcus* was observed instead in patients who maintained a longer duration of minimal oral intake. Unfortunately, the small sample size and the absence of pre-transplant sampling limit the reliability of these findings[94]. We reported a positive effect of EN on GM composition after HSCT in pediatric HSCT recipients. We assessed GM composition at the baseline, close after transplantation, and during the immunological recovery following the HSCT. Patients receiving EN showed lower GM injury and an almost complete recovery of the diversity after HSCT. On the other side, patients receiving PN presented a microbial shift toward a dysbiotic profile and never achieved a return to pre-transplant microbial status. Some genera, including *Faecalibacterum, Dorea*, *Blautia, Bacteroides*, *Parabacteroides,* and *Oscillospira*, were relatively more abundant in EN patients after HSCT, confirming the higher presence of SCFA-producing bacteria in EN-treated patients. As expected, the fecal levels of SCFAs were restored to baseline values only in the EN group[95].

Several other nutritional compounds, such as prebiotics and postbiotics are currently under investigation in preclinical or clinical models, such as Lactoferrin, Inulin, and

Exopolysaccharide, and could be supplemented together with oral or enteral feeding (**Figure 8**).

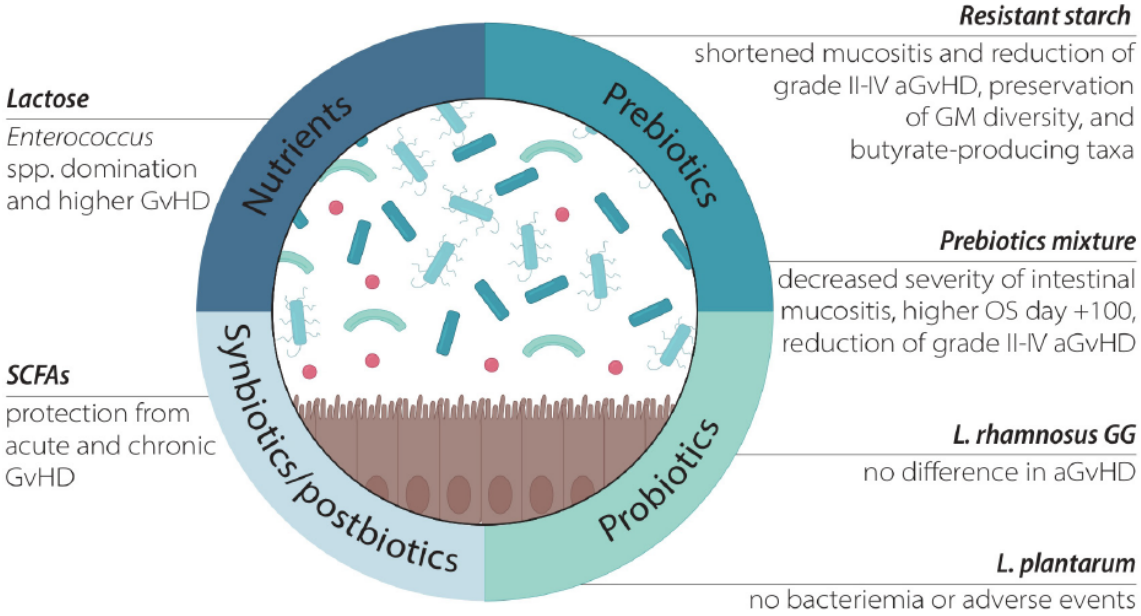

Figure 8: the effects of various nutritional compounds targeting GM.

v. Fecal Microbiota Transplantation

One promising approach to modulate GM is fecal microbiota transplantation (FMT), which consists of the infusion of fecal matter from a healthy donor into the gastrointestinal tract of the recipient[96]. In adult allo-HCT patients, FMT significantly changed the GM, particularly improving GM diversity, reducing overgrowth of pathogens, and promoting the recovery of commensals species[97,98]. In children, an increase in diversity and a variable expansion of potentially beneficial species was observed[99–101]. To date, only 16 pediatric allo-HCT patients have been documented in the literature as receiving FMT: 11 for the treatment of steroid-refractory GvHD and 5 for multi-drug resistant (MDR) bacteria decolonization. FMT prior to transplant in the 5 patients for MDR decolonization resulted in 80% testing negative for MDR within one week, with complete decolonization only achieved in one case [99–103]. In the eleven pediatric patients (age range 5-17) that received FMT for treatment of steroid-refractory GvHD, eight achieved complete remission and three partial remission[99–103]. FMT is generally well tolerated, with few serious adverse events and mainly minor adverse events reported, mostly nausea and abdominal pain[99–104]. In the transplant setting, a major concern is the risk of infectious complications, since live bacteria, viruses, and fungi are administered to an immune-compromised host with impaired gut permeability. Thus, FMT is typically administered before

allo-HCT or after neutrophil engraftment to ensure the presence of neutrophils in the event of bacterial translocation[105]. While rare, FMT-derived bacteremia has been observed in adult allo-HCT studies[106]. Larger studies are needed to fully comprehend the potential of FMT for the treatment of pediatric GvHD.

This evidence underlines the pivotal role that nutritional support has been recognized in recent years for pediatric patients undergoing cancer treatment and hematopoietic stem cell transplantation. Given the lack of shared guidelines in this field, we aim to assess current practices of nutritional assessment and care as well as food safety practices among AIEOP centers. We also explore the role of enteral nutrition and gut microbiota modulation with fecal microbiota transplantation in pediatric patients undergoing allogeneic hematopoietic stem cell transplantation for hematological malignancies.

4. METHODS

a. Current practices for nutritional evaluation and care during the treatment of pediatric oncology patients

i. Aim

The study aimed to assess the current practices for nutritional assessment and care of pediatric patients with cancer and undergoing HCT, to describe potentially addressable knowledge or clinical gaps in routine clinical care. Thus, we conducted a nationwide survey among physicians within the AIEOP network, composed of 49 centers in the country, of which 21 performed HCT.

ii.Data collection

A 25-item web-based questionnaire was circulated to all AIEOP centers as of January 9, 2023. The primary outcome was the assessment of the current practice regarding nutritional evaluation and support in pediatric patients with cancer undergoing chemotherapy or radiotherapy in Italian centers, and the secondary included the nutritional care of patients undergoing allogeneic HCT. Data were analyzed by descriptive statistics.

b. Current Practices for Food Safety and Infection Prevention: a Study by the Infectious Diseases Working Group of AIEOP

i. Aim

The study aimed to assess current recommendations and food-handling practices for pediatric patients with cancer and undergoing HCT and describe in detail all recorded cases of foodborne infections, highlighting potential addressable clinical gaps, ultimately creating a set of shared, nationwide recommendations. Thus, we conducted a second nationwide survey among physicians within the AIEOP network.

ii. Data collection

A web-based questionnaire concerning dietary guidelines, food handling, preparation, and the role of diet was circulated to all AIEOP centers. Cases of foodborne illnesses were collected using a specific eCRF during the period 2014-2024.

c. Impact of Enteral Nutrition (EN) versus Parenteral Nutrition (PN) on nutritional and allo-HCT outcomes

i. Aim

The study aimed to confront the impact of EN versus PN on nutritional and allo-HCT-related outcome.

ii. Study population and design

All pediatric and young adult patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) for both malignant and non-malignant diseases at the Pediatric Hemato-Oncology Transplant Unit of the IRCCS Azienda Ospedaliero-Universitaria di Bologna were prospectively enrolled in this study. Patients included in the cohort underwent transplantation between January 1, 2016, and September 13, 2023. Enteral nutrition (EN) was introduced as the first-line nutritional support for patients undergoing HSCT in 2018. Consequently, patients enrolled between January 2016 and December 2017 received parenteral nutrition (PN) exclusively.

Inclusion criteria for the study were 1) Informed consent, 2) Age up to 21 years, and 3) Allogeneic HSCT for both malignant and non-malignant diseases. There were no exclusion criteria for the study population.

Parenteral nutrition (PN) was used as first-line therapy for all patients transplanted between January 2016 and December 2017. PN was administered via a dual-lumen central venous catheter (CVC), and glucose calories accounted for 70% of non-protein calories, with the remaining 30% derived from a soybean oil emulsion. This lipid component was excluded in cases of febrile neutropenia.

Starting in January 2018, enteral nutrition (EN) via nasogastric tube (NGT) was adopted as the first-line nutritional support for patients undergoing HSCT. Initiation of EN required a prescription from a pediatric gastroenterologist specifying the type of enteral support and the dosing schedule. The exact dose was determined using the Schofield formula, based on the patient's basal metabolic rate[107]. Patients who were unable to consume the prescribed EN dose received glucose solutions to meet their caloric needs. NGT placement was performed by trained nursing staff between days -2 and +2, during sedation by pediatric anesthesiologists. EN was administered continuously for a specific number of hours, starting with low doses and gradually increasing based on individual patient tolerance. If the prescribed dose was not

achieved, the necessary caloric intake was provided through glucose solutions. From January 1, 2018, to October 2021, the EN formula contained synthetic amino acids instead of whole proteins. Starting in November 2021, EN formulas enriched with TGF-β2, an anti-inflammatory cytokine, were used, based on the results of a study conducted in adult patients who had undergone allogeneic HSCT, which demonstrated a reduction in aGVHD, pneumonitis, and severe malnutrition. Nutrition was discontinued, and the NGT was removed when the patient resumed oral intake and was able to consume at least 60% of their daily caloric needs.

Patients who refused enteral nutrition were started on PN as first-line therapy. Patients who showed poor tolerance to EN from the beginning were also switched to PN until they could resume oral feeding.

All patients in the study were offered high-calorie, high-protein oral nutritional supplements throughout the peri-transplant period.

iii. Data analysis

Several nutritional parameters were assessed to evaluate the nutritional status of transplant patients and identify malnutrition.

The following anthropometric parameters were analyzed:

- Weight: Daily weight measurements were recorded by nursing staff. Weights were recorded on the day of admission, day +0 post-transplant, and on days +7, +14, +21, +28, and +35 post-transplant, as well as on the day of discharge. The percentage weight change from admission to discharge was calculated, and a weight loss of at least 10% of the patient's body weight was defined as severe malnutrition according to the American Society for Parenteral and Enteral Nutrition and the Academy of Nutrition and Dietetics Consensus Statement for patients aged 2 to 20 years (229).
- Body mass index (BMI): BMI was calculated using the formula: BMI = weight (kg) / height² (m^2) (230). BMI was calculated at admission, on days +0, +7, +14, +21, +28, and +35, and at discharge.
- Z-score: The z-score was derived from the BMI. This score is used to classify the weight of children and adolescents. It represents the number of standard deviations by which a patient's BMI differs from the mean BMI of a reference population of the same age and sex and follows a non-normal distribution. The z-score can identify malnutrition in pediatric patients, however, like BMI, it cannot determine the percentage of body fat.

The following biochemical parameters indicative of nutritional status were also assessed:

- serum albumin levels were measured daily, as albumin is a primary biochemical marker of nutritional status. Normal serum albumin levels range between 35 g/L and 50 g/L, with lower values correlating with malnutrition (182, 183). Albumin levels were measured at admission, on days +0, +7, +14, +21, +28, and +35, and at discharge.

The type and duration of nutritional support were evaluated, including the use of either enteral nutrition (EN), parenteral nutrition (PN), or both. Patients were categorized based on whether they received exclusive EN for more than 7 days. Additionally, patients receiving EN were further categorized into those who consumed more than 50% of the prescribed dose and those who consumed less. The maximum prescribed and actual EN doses were determined from therapy sheets and gastroenterology consultations. In cases of discrepancies between the prescribed and actual EN sources, caloric equivalents were used to calculate the percentage of EN consumed. Oral intake resumption was also evaluated.

iv. Statistical analysis

Continuous variables were compared using the Student t-test in the case of a normal distribution and the Wilcoxon-Mann-Whitney test in the case of a non-normal distribution. Normality was established by the Shapiro-Wilk test. Discrete variables were compared by Pearson's chi-square test. However, if one of the four variables in 2X2 was less than a value of 5, the Fisher exact test was used. The results were expressed in probability with confidence intervals at 95% and were evaluated as statistically significant values of $p<0.05$. The survival of the individual patient was assessed based on the interval between the date of transplantation and the date of the last follow-up; the latter was made to coincide with the date of death in the case of a deceased patient. Survival curves were constructed using the Kaplan-Meier method and survival rates were compared by the Log-Rank test. The cumulative incidence curves of aGVHD were obtained by the Kalbfleisch and Prentice method and compared by Gray's test. Statistical surveys were conducted using the STATA 7.0 software (StataCorpVR, 2000, STATA Statistical Software: Release 7.0, Stata Corporation, College Station, TX).

d. Fecal Microbiota Transplantation

i. Aim

The aim was to evaluate the safety and effectiveness of FMT for pediatric patients with aGvHD or MDR colonization. Lastly, characterize the GM configuration after the FMT procedure.

ii. Study population and design

The study is a single-center prospective study enrolling pediatric patients (aged 0-18 years) undergoing allo-HCT for any clinical indication at IRCCS Azienda Ospedaliero-Universitaria di Bologna (Bologna, Italy) with either steroid-refractory/resistant gut GvHD or colonization by MDR strains, analyzed by means of anal swab. Each procedure was approved as individual compassionate use by the hospital Ethical Committee and the CNT (Centro Nazionale Trapianti).

iii. Endpoints

The endpoints were:

- clinical objective response of GvHD after FMT
- negative anal swab for MDR strains 6 weeks after FMT.
- Modification of the GM diversity and composition after FMT

iv. Eligibility criteria

Eligible patients were those: 1) aged between 0 and 18 years, 2) recipients of an allo-HCT, and 3) who signed informed consent. Exclusion criteria included patients who received an FMT before the allo-HCT.

v. Data collection

Information including 1) patient's characteristics, 2) details about the allo-HCT procedure, 3) GvhD features and 4) MDR colonization were gathered.

vi. Stool collection and analysis

Stool samples were collected from patients enrolled with a specific schedule. Specifically, stools were collected at days -1, +1, +2, +3, +4, +6, +7, +10, +14, +21 (with 1 day tolerance) from FMT procedure. Donor stools were also analyzed.

Microbial DNA was performed by extracting from fecal samples following the well-established protocol developed by Yu and Morrison (Yu and Morrison 2004) with few modifications (Casadei et al. 2024). Illumina MiSeq platform (Illumina, San Diego, CA, USA), is used to sequence the diluted samples which are prepared to obtain 16S rRNA gene amplicons, following guidelines outlined in the Illumina protocol "16S Metagenomic Sequencing Library Preparation.". Metagenomic DNA, extracted from the samples as detailed above, was prepared for sequencing using the IDT xGen DNA Library Prep EZ kit (Integrated DNA Technologies, Coralville, IA). Library preparation was automated on the Hamilton Microlab NGS Star platform (Hamilton Robotics, Reno, NV), following a custom protocol provided and optimized by the manufacturer. The resulting libraries were sequenced on the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA), with a 2x150bp approach generating paired end reads with a target yield of at least 10 Gb (giga bases) per sample.

vii. Definitions and statistical analysis

GvHD was diagnosed and classified according to the Glucksberg criteria[108]. Clinical response assessment of aGvHD was performed at 14 and 28 days after FMT respectively.

MRD decolonization was evaluated at 1 week (early-response) and 6 week (final response) after the FMT procedure.

Descriptive statistics were used to analyze the patients' cohort.

viii. FMT procedure

Indication for FMT was provided by the treating physician at the IRCCS Azienda Ospedaliero-Universitaria di Bologna (Bologna, Italy). Stools were donated by a third-party donor after a thorough eligibility check. The stools were resuspended and frozen, and then defrosted one hour before infusion, which was administered over a period of three hours via a naso-duodenal tube. The quantity of FMT used was 150 ml. The naso-duodenal tube was placed in a pediatric setting under sedation, and all FMT procedures were carried out in a pediatric environment.

5. RESULTS

a. Current practices for nutritional evaluation and care during the treatment of pediatric oncology patients

Twenty-one out of 49 (43%) centers invited to participate completed all items of the questionnaire. Detailed results of all the questions are reported in **Table 1**. About half of the of centers perform a routine nutritional evaluation of pediatric oncologic patients during the disease course. For nutritional evaluation, no specific protocol is applied in 76% of centers, and in only 5% a specific score is employed, namely STRONG kids and Subjective Global Nutrition Assessment Form. Nutritional assessment is performed based on clinical needs in 86% of the cases, and less frequently at specified time-points (**Table 1a**). Other indication reported by participating centers for the nutritional assessment include patients with head and neck tumors, obesity, or severe undernutrition at the time of diagnosis, and significant weight loss during treatment. Nutritional history and anthropometric measures are the more frequently used tools for nutritional evaluation, in 100% and 86% of participating centers, respectively. Biochemical markers are used in 71% of cases and include albumin, prealbumin, transferrin, retinol-binding protein, and vitamin levels. Specific dietary suggestions during the neutropenic phase after chemotherapy or autologous HCT are proposed in 90% of centers, and 81% employ a neutropenic diet, defined as in the Supporting Information. Dietary recommendations are conveyed through oral communications and/or written informative material. If oral nutrition is impossible for a prolonged period (e.g., mucositis), the considered strategies of nutritional support are parenteral nutrition in 95% of participating centers, specific food supplements in 57%, and enteral nutrition through a nasogastric tube in 29%. Answers related to allo-HCT were available for 11 centers (52%) which perform the follow-up of allo-HCT patients, the 73% of them also performing allo-HCT (Table 1b). In 45% of cases, specific nutritional guidelines or internal procedures are followed for patients receiving allo-HCT. Nutritional evaluation is provided in 36% of centers before and after HCT. Most of the centers monitor the caloric intake, in a qualitative (general description of food intake) and quantitative (exact number of caloric intake) way in 28% and 36% of cases, respectively. Plasmatic vitamin levels are monitored in 36% of the centers, being Vitamin D the most common. During aplasia, 72% of participating centers provide a neutropenic diet, while in 28% only a low-microbial diet with disinfected fresh vegetables and fruits. After the neutropenic phase, 28% of centers indicate to avoid lactose within 100 days after allo-HCT. Food supplements are employed as a part of nutritional support

of allo-HCT recipients in all centers, with most of them using energy-dense oral nutrition supplements

Table 1: Centers' responses on question on the general section and related to chemotherapy and/or autologous HCT (1a) and related to allogeneic HCT (1b). *: details are specified in the text

Table 1a: **Question**	**Centers** **(n=21)-n (%)**
Is a nutritional assessment routinary applied to patients?	
Yes	11 (52)
No	10 (48)
Are there specific protocols for the nutritional assessment/support?	
Yes, for support	2 (10)
Yes, for assessment	3 (14)
No	16 (76)
If yes, is a specific score applied?	
Yes*	1 (5)
No	10 (48)
When is nutritional assessment performed?	
Diagnosis	3 (14)
Relapse	3 (14)
Before HCT	4 (19)
After HCT	4 (19)
Before radiotherapy	2 (10)
After radiotherapy	1 (5)
Scheduled on clinical course	18 (86)
Others*	2 (10)
What kind of nutritional evaluation is performed?	
Alimentary history	21 (100)
Anthropometric measures	18 (86)
Biochemical markers*	15 (71)
Instrumental evaluation	1 (5)
Which health specialists are involved?	
Pediatric oncologist	15 (71)
Pediatric gastroenterologist	14 (67)
Pediatric endocrinologist	10 (48)
Dietitian	20 (95)
Do you provide specific dietary recommendations during the neutropenic phase?	
Yes*	19 (90)
No	2 (10)

If oral nutrition is impossible, which nutritional support is applied?	
Enteral nutrition with a nasogastric tube	6 (29)
Food supplements	12 (57)
Parenteral nutrition	20 (95)
How much do you think nutritional assessment is important in clinical practice?	
Very low	0 (0)
Low	0 (0)
Intermediate	2 (10)
High	8 (38)
Very high	11 (52)
Do you think that nutritional assessment is well implemented in your center?	
Very low	2 (10)
Low	5 (24)
Intermediate	7 (36)
High	5 (24)
Very high	2 (10)
Which is the main obstacle to the implementation of the nutritional support?	
Lack of dedicated specialist	12 (57)
Lack of training	8 (38)
Lack of evidence	4 (19)

Table 1b: Question	**Centers (n = 11) – n (%)**
Are there specific protocols for the nutritional assessment/support for patients receiving allo-HCT?	
Yes	5 (45)
No	6 (55)
Do you routinary monitor the caloric intake during allo-HCT?	
Yes, quantitative	3 (28)
Yes, qualitative	4 (36)
No	4 (36)
Do you routinary monitor the serum vitamin levels during allo-HCT?	
Yes*	4 (36)
No	7 (64)
Do you provide specific diet to patients during allo-HCT?	
Yes, neutropenic diet	8 (72)
Yes, low microbial diet*	3 (28)
No	0 (0)
Do you allow patients eat food prepared by caregivers at home?	
Yes	5 (45)

No	6 (55)
Is there a dedicated kitchen in the hospital to allow caregivers to prepare food?	
Yes	5 (45)
No	6 (55)
Do you recommend excluding lactose from the diet after allo-HCT?	
Yes	3 (28)
No	8 (72)
Do you provide specific dietary supplements during allo-HCT?	
Energy-dense oral nutrition supplements	8 (72)
Protein-rich anti-inflammatory oral nutrition supplements	2 (18)
Fiber-rich oral nutrition supplements	1 (9)
Vitamins*	10 (91)
Trace elements*	2 (18)
If oral nutrition is impossible, which nutritional support is applied?	
Enteral nutrition with nasogastric tube	1 (9)
Parenteral nutrition	10 (91)
What are the criteria to start nutritional support during allo-HCT?	
Inability to assume more than a specific amount of caloric intake	6 (55)
When aplasia occurs irrespectively of oral intake	3 (28)
Treating physician choice	2 (18)
Do you recommend specific dietary restrictions after discharge?	
Yes	11 (100)
No	0 (0)
For how long?	
1 month	0 (0)
3 months	3 (28)
6 months	7 (63)
1 year	1 (9)
Do you provide a specific diet when acute graft-versus-host disease occurs?	
Yes*	6 (55)
No	5 (45)
Do you routinary suspend oral intake when acute gut graft-versus-host disease occurs?	
Yes*	6 (55)
No	5 (45)

Vitamins are provided in 91% of centers, with vitamin D provided in 64% of cases, vitamin B12 in 28%, and in one center vitamin E and vitamin K are added. Trace elements, like zinc,

copper, and selenium are provided in 18%. During aplasia after allo-HCT, if the children are unable to eat orally, the type of nutritional support considered as a first line is parenteral nutrition in 91% of centers, while only 9% provide enteral nutrition through a nasogastric tube in the first-line setting. The criteria to start such nutritional support is when the child is unable to assume more than a specific amount of caloric intake in 55% of centers, whereas 28% start it as soon as the aplasia occurs irrespectively of oral intake, and 18% depending on treating physician choice. All the centers recommend dietary restrictions after discard, that the patients must follow for 3 months (28%), 6 months (63%), or 1 year (9%). Regarding the nutritional management of acute graft-versus-host disease (GvHD), 55% of centers provide a specific diet for these patients, including the use of anti-inflammatory oral nutrition supplements rich in TGF beta, hydrolyzed formula, and neutropenic diet. In the case of gut GvHD, 55% of centers interrupt the oral intake, and in one case minimal enteral feeding through a nasogastric tube is provided, and in another center, a gluten and lactose-free diet is employed.

b. Current Practices for Food Safety and Infection Prevention: a Study by the Infectious Diseases Working Group of AIEOP

21 out of 49 centers participated in the study, 14 of which performed allogeneic transplantation. 90% of centers considered dietary recommendations as very or extremely important. In 19 centers, these recommendations were provided through internal documents, primarily by pediatric oncologists (95%), followed by nutritionists (42%). A restrictive diet was recommended in 76% of cases during hospitalization and in 81% of cases at home (**Figure 9**). Foods commonly avoided included unpasteurized milk, mold-ripened cheeses, bakery/ice cream products, raw eggs and meat, cured meats, shellfish, and crustaceans. Sixty-six percent of centers recommended supplements, mainly trace elements, vitamin D, and caloric supplements. Twenty-eight percent of centers did not allow hospitalized patients to bring food from home, and 33% did not allow food delivery. Ninety-five percent of centers provided specific guidelines for food hygiene, mainly involving washing unpeeled fruits with specific disinfectants. Sixty-six percent of centers reported patients with foodborne infections, as detailed in **Table 2**, including 4 cases that led to death.

Sesso	M	M	M	M	M	F	F	M	F	M	F	M	F	F	F	F	F
Patologia	Ewing	LAL	Aplasia midollare	LAL	LAL	LAL	LAL	LH	LH	LLB	Ewing	LAL	Ewing	Ewing	K. Ovarico	ALL	ALL
Fase di terapia	Induzione	Induzione	Post 3° TCSE	Induzione	6 mesi post TCSE	3 mesi post TCSE	Reinduzione	Induzione	Induzione	Induzione	Induzione	Reinduzione	Induzione	Induzione	Palliazione	3 mesi post TCSE	Reinduzione
Patogeno	Clostridium difficile	Clostridium difficile	Geotricum	Salmonella tipo B	Listeria	Norovirus	Bacillus Cereus	Norovirus	Salmonella tipo B	Salmonella tipo E	Adenovirus	E. Coli	Rotavirus	Clostridium difficile	Clostridium difficile	Norovirus	Bacillus Cereus
Cibo sospetto	No	No	Patatine al formaggio	No	Pizza	No	No	No	Verdura del proprio orto	Acciughe sottolio	No	No	No	No	No	No	No
Campione	Feci	Feci	Feci	Feci	Sangue	Feci	Sangue + liquor	Feci	Feci	Feci	Feci	Feci	Feci	Feci	Feci	Feci	Sangue + liquor
Esito	Risoluzione	Risoluzione	Risoluzione	Risoluzione	Decesso in seguito all'infezione	Risoluzione	Decesso in seguito all'infezione	Risoluzione	Risolto	Risoluzione	Risoluzione	Non noto	Decesso per progressione di malattia	Decesso in seguito all'infezione	Decesso per progressione di malattia	Risoluzione	Decesso in seguito all'infezione

Table 2: main characteristics of the patients with reported foodborne infections

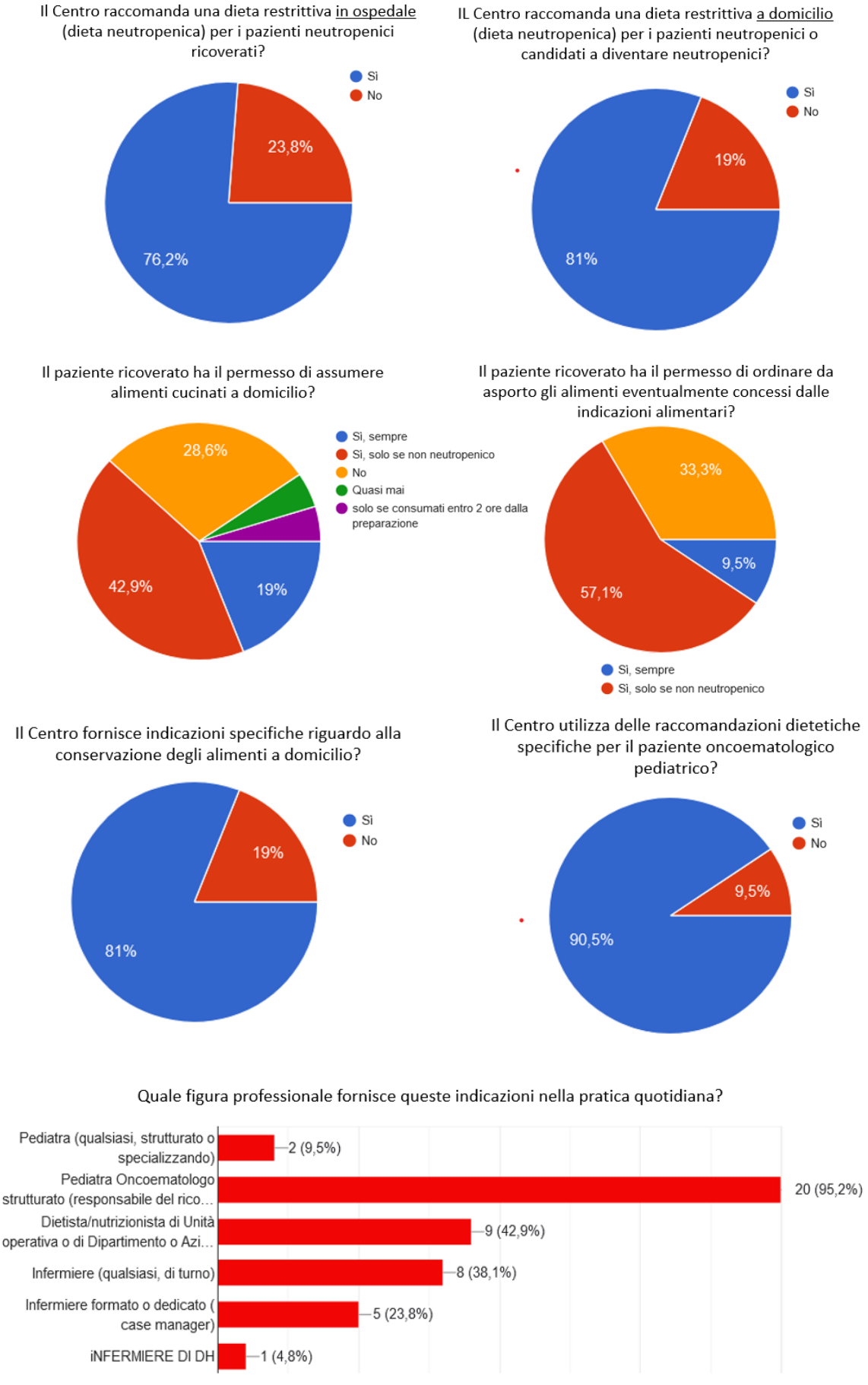

Figure 9: Pie charts and bar graph of relevant answers to the survey

c. Impact of Enteral Nutrition (EN) versus Parenteral Nutrition (PN) on nutritional and allo-HCT outcomes

96 patients submitted to allogeneic TCSE were enrolled consecutively between 1 January 2016 and 13 September 2023 at the onco-hematology Pediatric of the IRCCS University of Bologna. The median age of the patients included in the study was 9.71 years and the hospitalization period was 48.5 days.

Acute GvHD occurred in 43 patients (45.74%); among them, 11 (25.58%) developed a severe GvHD and 9 (10.93%) developed a severe gut GvHD, defined by the "Glucksberg Criteria". Only 19 patients (20.65%) developed chronic GvHD. Bloodstream Infections (BSI) were recorded in 38 patients (39.58%).

Patients who received NE were 63 (65.63%), while those who received NP were 68 (70.83%). Of these, only 33 patients (48.53%) received exclusive NP. Of the 63 patients undergoing NE, 28 (44.44%) received exclusive NE, while the remaining 35 (55.56%) received both types of nutritional support. Of the 33 patients who underwent exclusive NP, 22 were from January 2016 to November 2017, when NP was still used as the first line of nutritional support. Thus, only 13 patients have been submitted to exclusive NP since January 2018, with the change of indications in the ESPEN guidelines. When confronting patients receiving EN versus PN, no statistical significance was found among allo-HCT related clinical outcomes (**Table 3).**

Variable	Cases EN (n, % or median)	Casi PN (n, % or median)	P value
GVHD			
GVHD	29 (47,54%)	14 (42,42%)	0,64
GVHD II-IV	22 (36,07%)	8 (24,24%)	0,24
GVHD III-IV	6 (9,84%)	5 (15,15%)	0,44
Gut GVHD III-IV	4 (6,56%)	5 (15,15%)	0,27
2nd line therapy gut GVHD	7 (11,67%)	5 (15,15%)	0,63
cGVHD	11 (18,33%)	8 (25%)	0,45
Infections			
BSI	23 (36,51%)	15 (45,45%)	0,40
NHSN 1	9 (40,91%)	8 (53,33%)	0,14
NHSN 2	12 (54,55%)	4 (26,67%)	0,14
NHSN 3	1 (4,55%)	3 (20%)	0,14
BSI with resistant strains	12 (66,67%)	4 (26,67%)	0,04
CMV reactivation	26 (45,61%)	14 (42,42%)	0,77
Allo-HCT complications			

Mucositis	48 (84,21%)	32 (96,97%)	0,07
Severe Mucositis (III-IV)	15 (26,32%)	4 (12,12%)	0,07
Duration mucositis	13,00	11,50	0,98
VOD	9 (14,52%)	3 (9,09%)	0,53
PRES	4 (6,45%)	1 (3,03%)	0,66
Other endothelial complications	2 (3,28%)	1 (3,03%)	1
ICU	12 (19,67%)	5 (15,15%)	0,59
Hospitalization period	49,00	47,00	0,83

Table 3: Clinical outcome post allo-HCT for patients receiving EN vs PN.

Albumin at day +14 was found to be significantly higher in the EN group (**Table 4**)

Variable	Casi EN (n, % or median)	Cases PN (n, % or median)	P value
Nutrition			
Duration PN	18,00	21,00	0,08
Strat oral nutrition	24,00	22,00	0,31
Duration nutritional support	22,00	21,00	0,66
Body Weight (kg)			
Hospitalization	41,00	26,80	0,25
d+0	41,30	25,30	0,22
d+7	40,40	25,50	0,34
d+14	34,50	25,60	0,61
d+21	33,23	27,95	0,61
d+28	30,50	26,80	0,79
d+35	25,90	27,40	0,87
Discharge	35,70	25,00	0,31
Weigh loss>10%	30 (50%)	10 (30,30%)	0,06
BMI			
BMI hospitalization	18,27	17,53	0,48
BMI d+0	18,36	17,71	0,30
BMI d+7	17,98	17,43	0,42
BMI d+14	17,54	17,75	0,89
BMI d+21	17,56	17,25	0,92
BMI d+28	17,15	17,13	0,79
BMI d+35	16,86	16,65	0,92
BMI discharge	17,19	16,44	0,48
z-score			

z-score hospitalization	0,36	0,19	0,74
z-score d+0	0,28	0,30	0,79
z-score d+7	0,00	-0,01	0,96
z-score d+14	0,15	0,03	0,58
z-score d+21	-0,25	-0,04	0,56
z-score d+28	-0,20	-0,14	0,50
z-score d+35	-0,71	-0,50	0,68
z-score discharge	-0,73	-0,71	0,96
Albumin			
albumina hospitalization	4,15	4,05	0,72
albumina d+0	3,39	3,29	0,27
albumina d+7	3,25	3,21	0,63
albumina d+14	***3,36***	***3,23***	***0,03***
albumina d+21	3,40	3,34	0,48
albumina d+28	3,42	3,52	0,24
albumina d+35	3,55	3,51	0,82
albumina discharge	3,62	3,68	0,30
Hypoalbuminemia	54 (88,52%)	31 (93,94%)	0,49

Table 4: nutritional outcome in the pre-and post-HCT for patients receiving EN vs PN.

We further divided the cohort into patients who received >50% of the max prescribed dose of EN (n=46) versus those who received < 50% of the max prescribed dose of EN (n=50) (**Table 5**).

Variable	Cases EN>50% (n, % or median)	Cases EN<50% (n, % o median)	P value
GVHD			
GVHD	23 (50%)	20 (44,44%)	0,60
GVHD II-IV	17 (36,96%)	13 (28,89%)	0,41
GVHD III-IV	4 (8,70%)	7 (15,56%)	0,35
Gut GVHD III-IV	2 (4,35%)	7 (15,56%)	0,09
2nd line therapy gut GVHD	4 (8,70%)	8 (17,78%)	0,23
cGVHD	9 (19,57%)	10 (22,73%)	0,71
Infections			
BSI	***13 (27,66%)***	***23 (50%)***	***0,03***
NHSN 1	4 (33,33%)	11 (47,83%)	0,56
NHSN 2	7 (58,33%)	9 (39,13%)	0,56
NHSN 3	1 (8,33%)	3 (13,04%)	0,56

BSI with resistant strains	6 (66,67%)	9 (40,90%)	0,25
CMV reactivation	20 (45,45%)	19 (43,18%)	0,83
Allo-HCT complications			
Mucositis	37 (84,09%)	41 (93,18%)	0,11
Severe Mucositis (III-IV)	12 (27,27%)	7 (15,91%)	0,11
Duration mucositis	13,00	12,50	0,71
VOD	6 (12,77%)	6 (13,04%)	0,97
PRES	1 (2,17%)	4 (8,70%)	0,36
Other endothelial complications	2 (4,44%)	1 (2,17%)	0,62
ICU	9 (19,57%)	8 (17,39%)	0,79
Hospitalization period	50,00	47,00	0,32

Table 5: nutritional outcome in the pre-and post-HCT for patients receiving EN vs PN.

This analysis shows a significance (p= 0.03) for bacteriaemia. The number of bacteriaemia in the first group (n=13) is almost half that in the second group (n=23), with percentage values of 27.66% and 50%, respectively. Again, there are no statistically significant differences between the two groups regarding the type of infection classified according to the NHSN system and the presence of possible antibiotic resistance.

In the group that carried out NE<50% the infections were prevalent, according to the NHSN classification, those of type NHSN 1 (47.83%), sustained by pathogens of both intestinal origin such as *Escherichia Coli*, *Enterococcus faecalis*, *Klebsiella Pneumoniae* both oral such as *Streptococcus oralis*, *Streptococcus Mitis* or *Leptotrichia Buccalis*. In the group that performed NE>50% infections were prevalent NHSN type 2, supported by pathogens such as *streptococci* (eg *S. Maltophila* and S. *Vestibularis*) *Pseudomonas Aeruginosa* and *staphylococci* (eg S. *Epidermidis*, S. *Aureus*, S. *Hominis*, S. *Hemoliticus*), with a percentage of 58.33%. This percentage is higher than the percentage of NHSN2-class infections in the group that performed NE<50%. Again, NHSN3 infections are under-represented in both groups. There is also a trend towards significance (p=0.09) regarding cases of severe GVHD at the level of the digestive tract, which is lower in the first group (4.35% against 15.56% in group 2).

d. Fecal Microbiota Transplatation

FMT was performed in three patients with steroid-refractory gut aGvHD, two patients with colonization by an MDR bacteria, and two for both indications. In the 7 patients, 16 FMT infusions were performed via upper GI, a median of 150 ml of fecal material from unrelated donors. Characteristics of patients are reported in **Table 6**.

	Patients n=7/ infusions n=16
N°. of infusions per patient, median (range)	2 (1-4)
Age, median (range)	3,7 (2-16,4)
Type of allo-HCT Haplo PTCy/MUD, n	4/3
Indication SR-GvHD/MDR decolonization/both, n	3/2/2
N.° of previous lines of GvHD therapy, median (range) Ruxo/anti-TNF/ECP/Aba/Ustek/Ibrut/Vedo	4 (3-7) 3/3/2/1/3/1/1
SAE, n	0
AE n grade	 3 I,I,II

Table 6: clinical characteristics of pediatric patients undergoing FMT

All patients have an objective response at 28 days of follow-up for both indications (**Figure 10**).

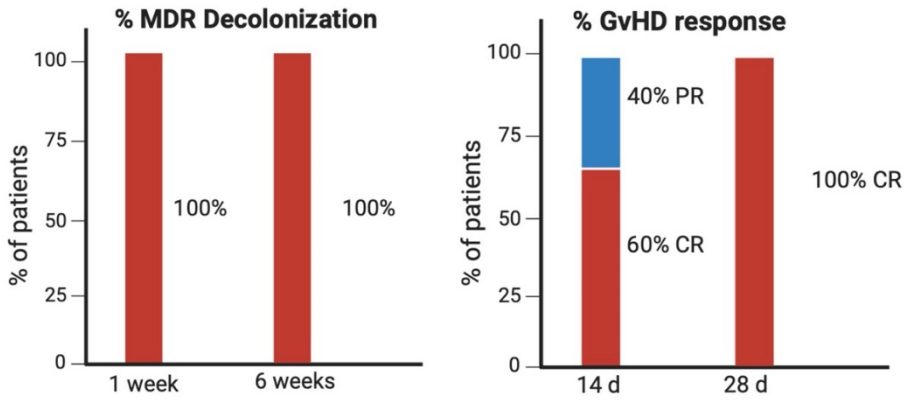

Figure 10: MRD decolonization (left) and GvHD response (right) at two distinct time points.

GM profile after FMT was analyzed for one patient who received FMT for steroid-resistant gut GvHD. Two FMT infusions of 150 ml of thawed, emulsified fecal material were administered via a naso-duodenal tube on day +224 and on day +227 post-allo-HCT, sourced from two different healthy unrelated donors screened according to the national protocol. Both procedures were well tolerated without significant adverse effects attributable to the FMT, with only transient abdominal pain following the infusions and a single episode of emesis. In this patient, alpha diversity slowly and progressively increased after the two infusions (**Figure 11**).

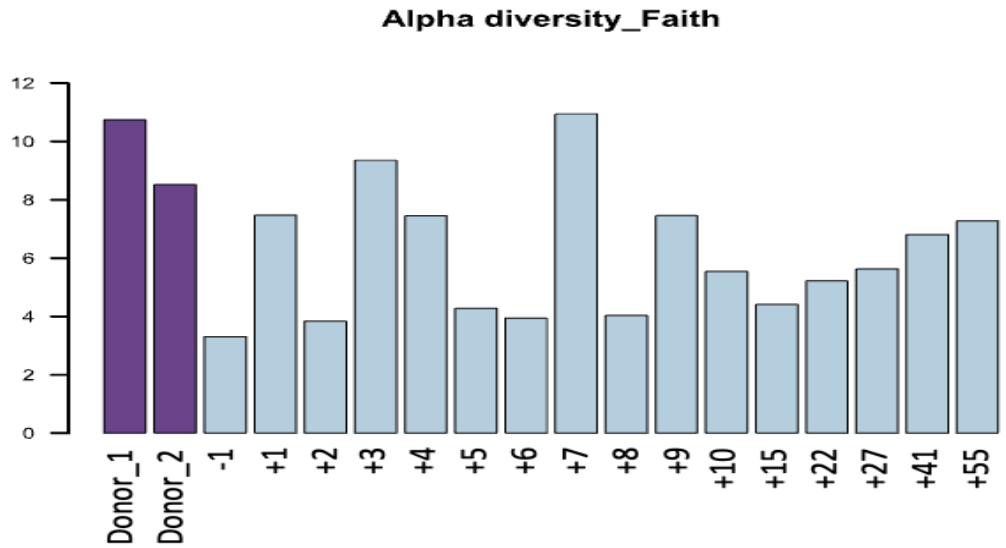

Figure 11: Alpha-diversity after FMT.

Interestingly, a marked enrichment of commensals and a loss of pathobionts was observed. In particular, the enrichment in *Bacteroidetes* and the reduction of *Escherichia/Shigella* were observed (**Figure 12)**.

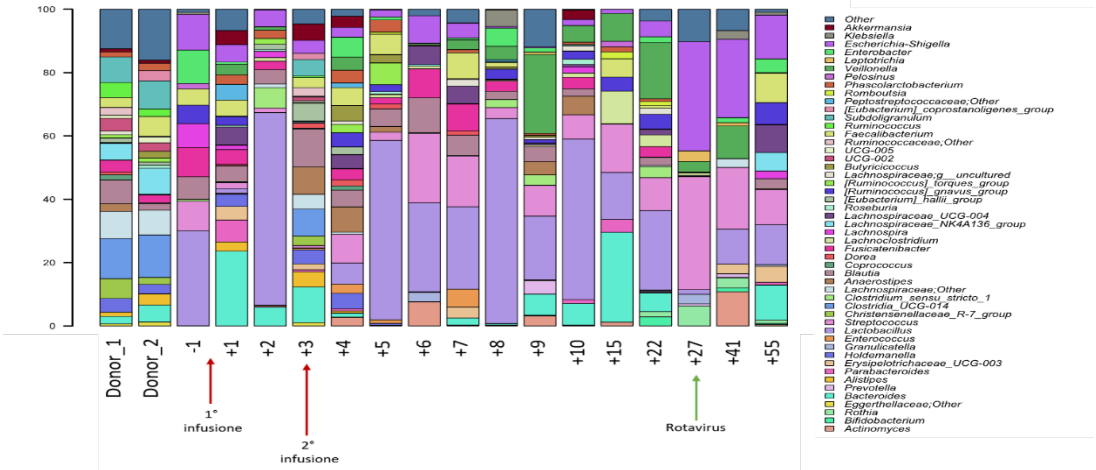

Figure 12: Genus-level composition of GM after FMT.

Immunological monitoring post-FMT from peripheral blood samples revealed an expansion of regulatory T cells, myeloid-derived suppressor cells, and dendritic cells up to day +21 after the FMT procedure. These markers indicated an increase in the immune tolerance between donor and recipient and a more anti-inflammatory immune system configuration (**Figure 13**).

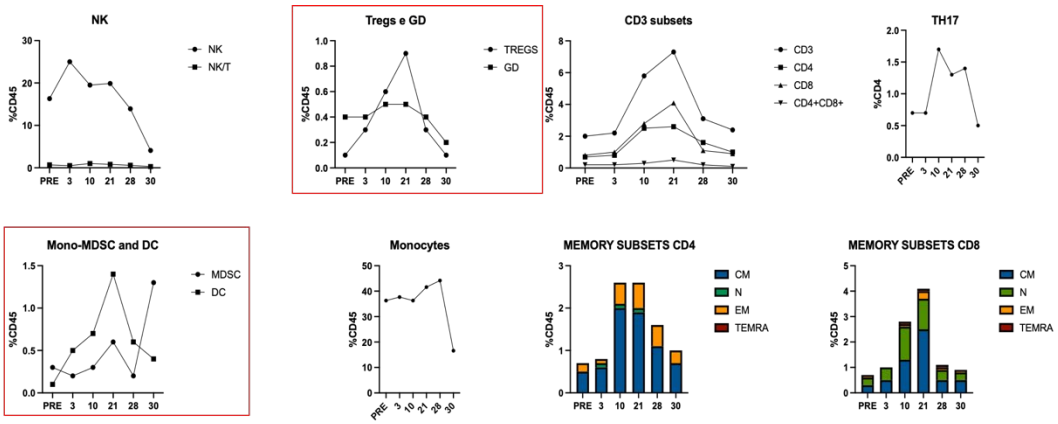

Figure 13: Trend in the peripheral blood of (from left to right): NK cells, Treg and GD, CD3+ subset, Th17, MDSC, DC, Monocytes, Memory CD4, Memory CD8.

6. DISCUSSION

a. Current practices for nutritional evaluation and care during the treatment of pediatric oncology patients

Despite the growing awareness of the importance of nutritional assessment and support, our data show high variability in nutritional approach among Italian pediatric oncological centers. Moreover, only half of the involved centers provide routine nutritional evaluations to all patients. This possibly reflects the lack of solid evidence and shared guidelines that should be certainly implemented in the next few years. Moreover, many barriers to the implementation of a standardized nutritional approach exist, and the participating physicians underline how the lack of dedicated specialists and focused training are the two main obstacles to the proper application of up-to-date nutritional care. Indeed, there is a high heterogeneity in the timing of nutritional assessment among the centers. Recently, a consensus statement published in collaboration with AIEOP suggests performing nutritional assessment in all patients at diagnosis and repeating it periodically during treatment and follow-up, which may help to standardize the approach[8]. Regarding nutritional interventions, our survey highlights how a neutropenic diet is still widely employed in neutropenia after chemotherapy or HCT. On this topic, the Pediatric Diseases Working Party of the European Society for Blood and Marrow Transplantation (EBMT) suggested replacing it with safe food handling procedures and further evidence of its safety may enhance the modification of the approach[8,109]. Nutritional support during aplasia after HCT represents another key area of inconsistency. The European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines indicate that artificial nutrition should be provided when oral caloric intake is below 60–70% of calculated requirements for 3 days[23], making it necessary to measure caloric intake quantitatively. Our results show how only 54% of centers provide nutritional support based on the monitoring of caloric intake, with only 28% performing quantitative measures. Moreover, only one center provides enteral nutrition via a nasogastric tube as first-line nutritional support during the neutropenic period, despite the international recommendations[23] and the recent literature showing clinical benefits compared to parenteral nutrition[84,110]. Our results further advocate the implementation of shared guidelines to guide nutritional evaluation and care during the treatment of pediatric oncology patients. This is of utmost importance, considering that nutritional status is a potentially addressable risk factor to improve survival in pediatric cancer patients[111], and nutritional support could be possibly employed to improve clinical outcomes.

b. Current Practices for Food Safety and Infection Prevention: a Study by the Infectious Diseases Working Group of AIEOP

Most participating centers provide recommendations on dietary management of oncohematological patients, although with significant heterogeneity. The reported cases of foodborne infections are high, with four cases leading to death. A detailed analysis of the recommendations based on each food type is ongoing.

This survey is a first step in the attempt to standardize and improve food safety practices at the AIEOP level and lays the foundations for shared recommendations.

c. Impact of Enteral Nutrition (EN) versus Parenteral Nutrition (PN) on nutritional and allo-HCT outcomes

The comparison between patients receiving EN versus NP did not show any statistically significant difference regarding the possible impacts of the two nutritional support strategies.

The comparison between patients that received NE>50% versus <50% of the maximum dose showed a reduction in the incidence of BSI in the group that received NE>50%. This suggests a possible protective role of NE for BSIs, which are one of the main causes of post-transplant mortality. This is consistent with the report of Zama et al. in a previous 2020 study on pediatric patients undergoing allogeneic transplantation of CSE[84].

The potential protective role of NE is certainly due to the trophic effect exerted at the level of the digestive tract. The cells of the gastroenteric tract gather their nutrients from the foods present within the intestinal lumen. Due to the atrophy of the intestinal mucosa caused by a possible NP, the function of the mucosa barrier is lost, which facilitates the movement of pathogens from the lumen through the intestinal wall and, ultimately, into the bloodstream.

Furthermore, Ikeda et al. reported an increase in Toll-like receptors (TLR) at the level of intestinal cell membranes in patients undergoing total NP[112]. As a result of the increased expression of TLR and also the increased expression of inflammatory cytokines, such as IFN- and TNF- observed in this study, there is a dysregulation of the immune response at the intestinal level[112].

In addition, NE formulations are enriched with fibers from which, thanks to certain bacterial species included in the GM, SCFA are produced. These metabolites have an immunoregulatory function and act on chemotaxis, differentiation, and activation of the cells of the immune system. The SCFA formed by the NE therefore could play a crucial role in immune defense and this would further explain the protective role of the NE against BSI[113,114].

One SCFA in particular, butyrate, also has a local effect; it is a source of nutrition for enterocytes and helps maintain the integrity of tight junctions by promoting the integrity of the intestinal barrier.

Several studies have evaluated the effects of NE on GM. Among these, Andersen et al. observed in an adult population that NE can determine better diversity and ensure an optimal profile at the level of the intestinal microbiota, with a higher abundance of species such as Blautia and Faecalibacterium capable of producing SCFA[115]. Tvedt et al., on the other hand, observed a difference between NE and NP patients at the metabolomic level, with lower SCFA concentration in the second group[116].

The positive effects of an enteral type support at the intestinal level are important given the close correlation between intestinal microbiota and episodes of bacteriemia and sepsis that has been observed in several studies[117,118].

Another result is a trend toward statistical significance concerning the lower number of cases of gastrointestinal aGVHD in the group that performed NE>50% compared to the group that performed NE<50%. This would confirm the lower tendency to develop intestinal aGVHD in patients undergoing NE observed in other studies conducted both in the pediatric and adult population[84,119,120]. These, together with further studies on the pediatric population, have also seen a reduced number of cases of aGVHD in general (and not only at the gastrointestinal level) in patients undergoing NE[84,119].

The possible protective role of NE may be due to its trophic and immunoregulatory effect at the level of the gastrointestinal barrier. The production of SCFA from the fibers contained in enteral formulations leads to a greater expansion of regulatory T cells.

No statistically significant differences were found in the impact of NE performed for less time and at lower doses and NE performed for more time and at higher doses on the different nutritional aspects examined in this thesis. This result shows the nutritional non-inferiority of enteral support compared to NP and confirms the feasibility of the NE as a nutritional support. Despite the recent guidelines, only a few centers of Pediatric Oncohematology have adopted changes in their clinical practice as shown by several analyses, including our AIEOP survey[121]. Most centers continued to offer NP as the first choice of nutritional support.

d. Fecal Microbiota Transplantation

In our limited cohort of patients, we evaluated the FMT, focusing on clinical and microbiological outcomes. Data on pediatric patients in this context are extremely limited, with most studies focusing on decolonization from MDR bacteria[122]. Notably, ours represents the largest cohort reported in the literature for the treatment of GvHD. Notably, in our cohort, the response rate to FMT for GvHD was excellent, reaching 100% at 28 days post-infusion. The same applies to decolonization. No severe adverse events were reported. Interestingly, our FMTs were administered via naso-duodenal tube, reducing the invasiveness of the procedure compared to what is commonly reported in the literature[110]. The treated patients showed an increased alpha diversity, and their microbiome composition shifted toward that of the donor, restoring eubiosis. An immune-modulatory effect was observed following FMT, as expected. This data demonstrates that FMT is a safe and effective procedure for treating intestinal aGvHD without significant risks. Further prospective studies will help define the clinical and microbiological factors associated with better responses and determine the optimal procedure.

7. CONCLUSIONS

To date, we struggle to meet the nutritional needs of pediatric patients undergoing cancer treatment. Scientific evidence demonstrates that nutritional status has a great impact on the overall survival of specific pediatric cancers, as well as a great impact on the morbidity of pediatric cancer survivors. The lack of shared, simple, and cost-effective guidelines regarding best practices for nutritional assessment and care is the main limiting factor to a standardized approach to the nutrition of pediatric patients undergoing cancer treatment.

We also know that nutritional status has an impact on specific allogeneic HCT complications, such as GvhD and BSI. Furthermore, nutritional strategies can be utilized to modulate the GM, with the aim of reducing the risk (or even treating) specific allogeneic HCT complications. Among others, nutritional support with enteral nutrition seems to reduce the risk of BSI and GvHD compared to parenteral nutrition.

Finally, our preliminary data shows that fecal microbiota transplantation (the newest GM- GM-modulation strategy) has a good safety profile with good clinical efficacy for the treatment of steroid-refractory gut GvHD and bacterial MDR decolonization, and metagenomic characterization confirms the restoration of the donor-derived eubiosis in the recipient. Indeed, more data from larger international clinical trials is needed to confirm our reports.

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