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THE ROLE OF MICROBIOME IN PEDIATRIC CANCERS

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Abstract – *Objective:* Human microbiome as an important part of cancer development and treatment is actively investigated. However, most studies are based on adult patients, while in pediatric patients, only a few studies on this topic, with most of them focusing on pediatric blood diseases and hematopoietic stem cell transplantation, have been analyzed. Studies on gut microbiota in children with solid tumors are even scarcer.
Materials and Methods: We performed a literature review of studies on the role of microbiome in pediatric cancers and included studies published between 1st of March 2022 and 31st of March 2023. This review was performed according to the principles of preferred reporting items for systematic reviews and meta-analyses (PRISMA) statements. As the number of published studies on this topic is low, all studies (case reports, editorials, letters, systematic reviews and meta-analyses, case-control, cohort studies, randomized controlled trials) were included.
Results: Most studies focused on patients with hematological diseases and hematopoietic stem cell transplantation, and only two studies focused on pediatric patients with solid tumors. Data shows the importance of nutrition, antibiotic, and antifungal treatment on the differences in the composition of the microbiome. Also, there is a positive association between regular physical activity and diversity of microbiota; higher alpha- and beta-diversity was also associated with a shorter duration of febrile neutropenia.
Conclusions: Data on the gut microbiome in pediatric patients with cancer remains very scarce and more studies are needed.

Keywords: Microbiome, Pediatric oncology, Pediatric cancer, Leukemia, Solid tumors.

Abbreviations: ALL: Acute lymphoblastic leukemia; HSCT: Hematopoietic stem cell transplantation; FAP: Familial adenomatous polyposis; FN: Febrile neutropenia.

INTRODUCTION

Over three trillion bacteria might be found in the human body. A high number of microorganisms is related to the very high diversity of genetic information and has multiple functions[1]. The composition of human microbiome changes over time, influences the immune system, and is different in healthy humans and in patients with various diseases[2].

Changes in microbiome composition have been shown to increase the risk of various inflammatory diseases and related conditions, might stimulate the development of cancer, and might even determine the outcome of different diseases and their treatment[3]. The microbiome profile might also determine susceptibility or resistance to infectious diseases, the immune response to a tumor, and contribute to the tumor environment. It has been shown[4,5] that the microbiome profile can be altered by chemotherapy, biological therapy, and radiotherapy. Nonetheless, the

impact of nutrition and nutritional status on the diversity of microbiome in patients with cancer and especially in patients undergoing Hematopoietic stem cell transplantation (HSCT) is very important[6]. Immunotherapy in cancer treatment underwent a significant change in the last decade and is used as a treatment for various types of cancers. Especially treatments with check-point inhibitors in selected patients show promising results[7]. However, the effect of immunotherapy on the microbiome is not fully understood yet.

Human microbiome, as an important part of cancer development and treatment, is actively investigated. However, due to various reasons, most studies are based on adult patients[2,7,8]. A group from Hungary in 2022 performed a literature review with the aim of analyzing the influence of the gut microbiome in pediatric cancer origin and treatment[9]. Unfortunately, in pediatric patients, there have been only a few studies on this topic, with most of them focusing on pediatric blood diseases and hematopoietic stem cell transplantation[9]. The number of studies on microbiome in children with solid tumors is even lower and a lot of questions are still unanswered[4].

In the last year, several papers on gut microbiome in pediatric patients were published. Therefore, we performed a literature review on this topic with the aim to summarize the available data and to update the available knowledge.

MATERIALS AND METHODS

This review was performed according to the principles of preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement[10]. PubMed, Cochrane, and Lippincott Williams & Wilkins databases were searched for manuscripts from the 1st of March 2022 up to the 31st of March 2023. Our search included the following keywords and their combinations: microbiome, microbiota, pediatric cancer, pediatric tumor, solid pediatric tumors, and pediatric oncology. After checking titles and abstracts, inappropriate studies were excluded. The remaining full-text articles were reviewed carefully. Additionally, reference lists of selected articles were reviewed for eligible studies.

Inclusion criteria for selected studies were: (1) studies written in English language; (2) studies analyzing microbiome in pediatric oncology; (3) studies from the 1st of March 2022 up to 31st of March 2023. As the number of published studies on this topic is low, all studies (case reports, editorials, letters, systematic reviews and meta-analyses, case-control, cohort studies, randomized controlled trials) were included in the review. Ten (10) studies of various designs were included.

RESULTS

Children diagnosed with cancer not only suffer from the disease itself but also a significant impact is made by the multimodal treatment and its complications. One of the most affected factors is nutrition and nutritional status. Both undernutrition and overnutrition are observed in up to 70% of patients undergoing cancer treatment. Nutrition, together with the direct effect of chemotherapy, influences the composition of the microbiome. Pedretti et al[11] analyzed the role of nutrition in pediatric patients with cancer. According to the literature, in children with leukemia, the incidence of undernutrition at the diagnosis reaches up to 10%, in patients with neuroblastoma, up to 20-50%, and up to 30% in patients with other types of tumors. After performing an extensive literature review, the authors conclude that patients with an increased risk of malnutrition should be identified as early as possible. By using ready-to-use therapeutic foods or probiotics, a eubiosis of gut microbiota could be maintained. In this way, bacterial translocation, changes in immune system, and other infectious complications could be limited. As different types of nutrition might have different impact on the microbiota, oral nutrition should be preferred over the artificial one.

Muratore et al[12] performed a narrative review assessing the relationship between nutritional status and clinical outcomes and evaluated the effect of nutritional support ranging from specific diets to artificial feeding in pediatric patients undergoing HSCT. Data shows that malnutrition is a common complication in these patients, negatively affecting clinical outcomes and the composition of gut microbiota. A growing number of studies shows promising effects of prebiotics, probiotics and other compound in patients undergoing HSCT. However, data on pediatric patients

is still relatively scarce compared to adult patients. The safety of nutritional support, especially the use of newly developed compounds, also limits a broad implementation of it. Therefore, a multidisciplinary team consisting of a pediatric oncologist, gastroenterologist, dietitian, nurses, and physical therapists is expected to perform a structured nutritional assessment during HSCT and develop the best nutrition support plan for these patients.

Malnutrition as a consequence of cancer and cancer therapy was also analyzed in a study performed on 27 children who underwent chemotherapy because of solid tumors in the United States of America (USA)[13]. The selected group was compared to 22 healthy individuals, and differences in gut microbiome in stool were analyzed. The study group consisted of children who finished the chemotherapy within 1 year. Dietary intakes according to the Block Kids Food Screener were measured in both groups. Children with cancer were taking more calories and more macro- and micronutrients in. However, there was no difference in the alpha-diversity of the microbiome. Contrarily, the difference was found in beta-diversity and abundance analysis. The authors agree that the main limitations of this study were the small sample size, different types of tumors, and different types of chemotherapy. Therefore, bigger studies are needed to confirm the results.

Interesting findings on oral lactoferrin supplementation in pediatric patients diagnosed with hematologic malignancies and undergoing chemotherapy were shown in a study from Italy[14]. Lactoferrin has anti-inflammatory, immunomodulatory, and antimicrobial activity on gut microbiota. It was a multicenter double-blind, placebo-controlled study and included 34 pediatric patients with hematologic malignancy and undergoing induction chemotherapy. The first group received bovine lactoferrin (200 mg/day) for 2 months. The control group received placebo. From collected stool samples, microbial nucleic acids were extracted and 16S rRNA libraries were prepared. After analyzing the results, dysbiosis was shown already at the onset of the disease, and it worsened when induction chemotherapy was started. Accordingly, the increase of pathogens such as *Enterobacteria* and *Enterococcus* was observed. In the study group potential stabilizing effect of lactoferrin on the diversity of gut microbiota was shown. Significantly lower incidence of febrile neutropenia in the study group confirms the reduction of therapy-associated complications. In conclusion, supplementation of lactoferrin looks promising and, in the future, might be implemented in the treatment of pediatric patients with hematologic malignancies and undergoing induction chemotherapy.

Dunn et al[15] analyzed the impact of antibiotic and antifungal treatment in children diagnosed with leukemia and lymphoma. Antibacterial and antifungal treatment is regularly used in patients to prevent or treat infections and febrile neutropenia. However, it is known that especially antibacterial treatment has a negative effect on the diversity and structure of the microbiome. Data on antifungal treatment and especially in children is even scarcer. Authors investigated 134 stool samples from 47 children diagnosed with cancer (33 had acute lymphoblastic leukemia, 5 acute myeloid leukemia, 4 had Hodgkins lymphoma and 5 non-hodgkins lymphoma). Both the 16S rDNA amplicon sequence variants and metagenome sequence species were obtained from the samples. General agreement between these two datasets was found. Both antibacterial and antifungal treatments reduced the bacterial diversity (*e.g., Faecalibacterium, Anaerostipes, Dorea, Blautia*) in the stool samples. Additionally, the antifungal treatment increased the number of opportunistic pathogens. These findings confirm the fact that broad-spectrum antibacterial and antifungal treatment negatively impacts the treatment of pediatric cancer patients.

Children diagnosed with pediatric malignancies mostly are undergoing long and diverse treatment. Not only chemotherapy and immunotherapy, but also antibiotics, proton pump inhibitors and other immunosuppressive agents are given. Consequently, dysbiosis develops. Therefore, a group from Italy performed a literature review and analyzed the interaction between gut microbiome and drug pharmacokinetics and pharmacodynamics[16]. Thirty (30) studies published between 2008 and 2021 were included. Analysis showed that not only antibiotics, but also chemotherapeutic agents, immune checkpoint inhibitors (anti-PD-1/PD-L1 inhibitors), immunosuppressive agents (cyclosporine, tacrolimus), steroids, protonic pump inhibitors, ursodeoxycholic acid have a significant impact on the gut microbiome. All these agents might cause a disbalance of the microbiome and lead to dysbiosis. This knowledge is important when planning future trials and prescribing drugs – adjustment of initial and treatment doses might be needed. After treatment and dose adjustments, the treatment of each patient would be more individualized, more effective, and causing fewer complications.

TABLE 1. SUMMARY OF THE PUBLICATIONS FROM 2022-2023 WITH THE KEY POINTS RELATED TO THE ROLE OF MICROBIOTA IN PEDIATRIC CANCERS.

Nr.	Topic	Authors	Conclusions
1	Nutritional support in children undergoing hematopoietic stem cell transplantation	Muratore et al[6]	• Reduction in caloric intake and the catabolic effect of therapies and transplantation-related complications negatively influence nutritional status and gut microbiome • Nutritional support during the early post-transplantation period is essential
2	Gut Microbiome in Pediatric Cancer	Sági et al[9]	• In pediatric populations there have been only a few studies. • In patients with solid tumors only studies with low case numbers have been reported
3	Role of Nutrition in Pediatric Patients with Cancer	Pedretti et al[11]	• There is a lack of a standard recommendations for nutritional care in the pediatric cancer population • Regular nutritional monitoring should be performed a diagnosis, during treatment and during follow-up
4	Maintaining a healthy gut Microbiome in children with cancer	Zhou et al[13]	• Children with cancer exhibited a higher intake of daily calories, and thus had a higher intake of both macro- and micronutrients • No significant differences in alpha diversity were found between the cancer and control groups
5	Lactoferrin supplementation promotes gut microbiome in children with hematologic malignancies	D'Amico et al[14]	• Lactoferrin promoted gut microbiota homeostasis by favoring the maintenance of diversity and preventing the bloom of pathobionts
6	Antibiotic and antifungal use in pediatric leukemia and lymphoma patients	Dunn et al[15]	• The use of antibiotics and antifungals negatively influence the microbiome and may make the gastrointestinal barrier dysfunctional • The microbiome of children under 3 is less diverse
7	Pharmacomicrobiomics in Pediatric Oncology	Leardini et al[16]	• Gut microbiome has a significant role on drug pharmacokinetics and pharmacodynamics • In children the gut microbiome undergoes a constant multifactorial evolution following external stimuli
8	The composition of the fecal microbiota in children with acute lymphoblastic leukemia after hematopoietic stem cell transplantation	Ugrayová et al[17]	• Significant differences in microbiota composition were observed • Bacterial alpha diversity correlated with the exercise training characteristics
9	Impact of the duration of febrile neutropenia on the severity of gut microbiota	Masetti et al[19]	• More stable gut microbiota configuration over time is associated with a shorter duration of fever. • There is a possible association of the gut microbiota with the genesis and course of febrile neutropenia
10	Microbiome and pediatric familial adenomatous polyposis	Attard et al[20]	• The alpha- and beta-diversity shifted towards lower diversity in polyps' group

The effect of hematopoietic stem cell transplantation in children with acute lymphoblastic leukemia (ALL) on the diversity and composition of microbiome is an important topic to analyze. A team from Slovakia compared 16 patients with ALL after HSCT to a sample of 13 healthy controls[17]. Stool samples were collected, and the V1-V3 region of 16S rDNA was targeted. All patients got prophylactic antibacterial treatment. Antiviral and antifungal treatment was given according to the commonly accepted strategies. The negative effect of HSCT on the alpha diversity and composition of microbiota was shown on days 0, 7, 28 and 90. The relative abundance of pathogenic bacteria (*Enterobacter spp., Klebsiella spp.*) has increased in the follow-up period of

3 months after HSCT. However, there was an association between regular physical activity and the diversity of microbiota (Shannon and Simpson index). Some previous studies[18] have shown that physical activity might positively affect endothelial function. The regular physical activity was based on an individual training program during the hospital stay and included two sessions of 25-45 min training per week.

Association between the gut microbiome and several HSCT-related complications was identified in recent years. Febrile neutropenia, as one of these complications, was analyzed in an international multi-center study combing data from 3 European centers[19]. In the study, 37 patients undergoing HSCT for any underlying disease (up to 67% malignant) from Bologna (Italy), Verona (Italy) and Wroclaw (Poland) were included. Included patients were divided into two groups (FN ≤ 3 days *vs.* FN ≥ 3 days) and FN was treated with broad-spectrum antibiotics. This was the first study in pediatric patients to analyze the relationship between FN and changes in gut microbiota. The study showed that higher alpha- and beta-diversity was associated with a shorter duration of FN. In patients with longer duration of FN, higher levels of *Collinsella, Megasphaera, Prevotella* and *Roseburia* were observed in the pre-HSCT samples. Authors conclude that even if the association between the composition of gut microbiota and the course of FN seems possible, biological mechanism of this relationship are still not fully understood.

Data on microbiota in pediatric patients with solid tumors is very scarce. Attard et al[20] in 2022 published a manuscript on association of pediatric familial adenomatous polyposis (FAP) and microbiota. Even if FAP is not cancer, it harbors a high risk of early progression to colon cancer. Therefore, we decided to include this article in the review. Seven (7) patients were included together with 6 family members as controls. Stool (25) and tissue (25) samples were collected. Tissue samples consisted of polyp tissue and tissue of normal-appearing colon mucosa. Both, the alpha- and beta-diversity were significantly lower in a polyp group when compared to controls. Not only relative abundance of *Proteobacteria* and *Fusobacteria* was higher in the polyp group, but also differences in several types of bacteria were identified through the machine learning. Authors conclude that the diversity and concentration of selected bacteria might serve as adjunct biomarkers in colorectal cancer screening.

CONCLUSIONS

Studies in adult cancer patients show that changes in the microbiome influence the risk of developing cancer, the effectiveness and toxicity of chemotherapy and may even influence the effectiveness of the immune response. Unfortunately, there is a paucity of data on children. This review analyzed studies from the past year on microbiome in pediatric cancers and showed the importance of nutrition, impact of antibiotic and antifungal treatment. Differences in the composition of microbiome there also proven to be important. A positive association between regular physical activity and diversity of microbiota was also shown. One of the studies even showed that diversity and concentration of selected bacteria might serve as adjunct biomarkers in colorectal cancer screening. However, data on the impact of gut microbiome in pediatric patients with cancer remains very scarce and more studies are needed.

Conflict of Interest

The authors declare no conflict of interest.

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None.

Informed Consent

As it is a systematic review, informed consent is not applicable.

Author Contributions

JK, GR, RoK, RiK, PI concept development, critical review of the manuscript; JK, PI search and review of the literature; JK, PI writing of the manuscript.

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