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THE EFFECT OF CANCER TREATMENT ON THE GUT MICROBIOTA OF PEDIATRIC PATIENTS

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Gut microbiota's implications on health has been extensively studied during the recent decade. There is growing evidence that gut microbiota has both facilitating and protecting effect against the development of cancer, but exact mechanisms are still not well known. Existing studies on the impact of cancer treatments on the gut microbiota, particularly in pediatric patients, is still rather scarce. The topic is important, as survivors of childhood cancer have been shown to suffer from increased morbidity and mortality compared to regular population even after decades after the diagnosis. Typical long-term health implications include obesity, metabolic syndrome and cardiovascular diseases. Also, a higher risk of cancer has been reported.

Early childhood is vital in terms of the gut microbiota development. Gut microbiota develops in parallel with the immune system in a continuous crosstalk. Disruptions of the microbiota due to, for instance, pre-term birth, birth via cesarean section, or early use of antibiotics, may result in gut microbiota dysbiosis and induce a lasting impact on the immune system. Dysbiosis has been linked to a higher risk of obesity, allergies, autoimmune diseases, infectious diseases, cardiovascular diseases, type 2 diabetes mellitus and cancer.

According to existing preclinical and clinical studies, chemotherapy, radiation therapy, hematopoietic stem cell transplantation as well as immunotherapy may influence the gut microbiota of childhood cancer patients. Such treatments have an impact on the microbial abundance, diversity, and relative proportions, increase the inflammation inducing bacteria, decrease the beneficial bacteria, have metabolic interactions with gut microbes and change the effect of microbial products, such as endotoxins. Typically, the patients treated with chemotherapy or radiation therapy suffer from diarrhea, which has been linked to altered microbiota. Furthermore, microbiota alterations caused by chemotherapy play a role in gastrointestinal mucositis, bloodstream infections (BSI) and C. difficile infections (CDI).

The existing studies relating to pediatric cancer demonstrate that the gut microbiota of pediatric cancer patients is different from healthy controls already prior to chemotherapy treatments. Probiotic/prebiotic intervention may alleviate the adverse effects of chemotherapy.

Key words: Childhood cancer, gut microbiota

Table of contents

The symbiosis of microbes and humans has been a subject of intense research during the last decades, and it has been found to have a strong linkage to several physiological and pathophysiological processes occurring in a human body. In the recently updated Hallmarks of Cancer review, distinctive microbiomes in gut as well as in other organs have been recognized as promoting and inhibiting agents in cancer development, progression and responsiveness to different therapies. However, the understanding of the exact mechanisms of this interaction is still to be studied. For instance, the interlinkage between specific gut microbiota constitution and variables in cancer progression and development is an important topic for the future research. (Hanahan 2022).

This paper discusses the effect of cancer treatment on the gut microbiota of pediatric patients. The knowledge on this topic is currently insufficient; fairly few studies discuss the effect of cancer diagnosis on gut microbiota (e.g. Van Vliet et al. 2009, Huang et al. 2012, Rajagopala et al. 2016, Bai et al. 2017, Chua et al. 2020, Liu et al. 2020,) and the effect of cancer treatments of gut microbiota (Van Vliet et al. 2009, Huang et al. 2012, Cozen et al. 2013, Rajagopala et al. 2016, Hakim et al. 2018, Niering et al. 2019, Thomas et al. 2020) in pediatric patients. Furthermore, there are several studies discussing the gut microbiota implications of diverse cancer treatments in adults, such as chemotherapy (Zwielehner et al. 2011, Stringer et al. 2013, Montassier et al. 2015, Wang et al. 2016), radiotherapy (Johnson et al. 2004, Manichanh et al. 2008, Nam et al. 2013 and Wang et al. 2015), and immunotherapy (Vétizou et al. 2015, Gopalakrishnan et al. 2018). The effect of hematopoietic stem-cell transplantation on gut microbiota has been studied in pediatric patients (Biagi et al. 2015) as well as in adults (Holler et al. 2014). However, the existing knowledge on the impact of cancer treatments on gut microbiota is still insufficient, and more research is needed particularly in the pediatrics. The purpose of this literature review is to provide a structured view on the topic and analyze the previous studies on the topic in a systematic manner.

1. Gut microbiota of healthy adults

Gut microbiota, or microbiome, refers to the variety of micro-organisms colonizing the mammalian gastrointestinal tract (Sekirov et al. 2010). Terms microbiota and microbiome are often used interchangeably, but microbiota can more specifically be associated to the taxa in the human body, whereas microbiome refers to their genetic information (Ursell et al. 2012).

99 percent of the microbial mass of the human body is found in the gastrointestinal tract, and therefore the gut microbiota has an essential role in maintaining human health (Schwabe and Jobin 2013). According to estimates, human body contains 10^{14} bacterial cells, and 70 percent of these are found in colon alone (Ley et al. 2006). Every person has a unique microbiota profile, which is separable from others (Claesson et al. 2011) and rather stable over time (Kundu et al. 2017).

Gut microbiota can be considered as an “organ”, which, among others, regulates immunity, processes nutrients, produces vitamins, stimulates angiogenesis, regulates fat storage and maintains healthy gut epithelium (Eckburg et al. 2005, Palmer 2007, Shin et al. 2015). The colonization of gut microbiota in the early childhood is vital in the development of immune system and so called “colonization resistance”, which refers to symbiotic bacteria attaching to the gut surface and protecting it against pathogen penetration (Weng and Walker 2013).

The gastrointestinal tract of adult humans is predominantly inhabited by two bacterial phyla, *Firmicutes* and *Bacteroidetes*, but also *Actinobacteria, Proteobacteria, Fusobacteria, Verrucomicrobia, Tenericutes, Lentisphaerae and Spirochaetes* are found (Eckburg et al. 2005, Tremaroli and Bäckhed 2012, Rea et al. 2018). The main genera belonging to *Firmicutes* are *Ruminococcus*, *Clostridium* and *Lactobacillus* as well as butyrate-producing *Faecalibacterium* and *Roseburia*. Glycan-degrading *Bacteroides*, *Prevotella* and *Xylanibacteria* belong to Bacteroidetes; *Collinsella* and *Bifidobacterium* to Actinobacteria; and *Escherichia* and *Desulfovibrio* are common Proteobacteria. Some strains of *Lactobacillus* and *Bifidobacterium* are probiotic. (Tremaroli and Bäckhed 2012). It is also worth noting that gut microbiota includes not only bacteria, but also virus, protozoa, yeasts and helminth, though any research data on these is very scarce (Rizzatti et al. 2018) and excluded from this paper.

Different sites of gastrointestinal tract have varying colonization of microbiota; *Streptococcus* subgroup of *Firmicutes* as well as *Actinobacteria* are found specifically in small intestine, whereas *Bacteroidetes* are found in colon (Frank et al. 2007). Furthermore, the composition of microbiota varies between the epithelium, mucosal layer and lumen of the intestine (Sekirov et al. 2010).

Arumugam et al. (2011) have further classified the composition of gut microbiota and identified three clusters of microbiota named as enterotypes, which can be identified by the varying abundance of different genera, *Bacteroides*, *Prevotella* and *Ruminococcus*. According to Arumugam et al. (2011), the microbial composition of the gut is affected by selective pressure caused by the host and competing microbial species, and it typically leads to homeostasis. However, while certain species of microbes are typically much more abundant in the gut than others, the less common species may perform essential functions and thus they have a significant impact on the functional complexity of the microbial community (Arumugam et al. 2011).

1.1. Gut microbiota and immune system

As stated earlier, microbe-colonized gastrointestinal tract is a prerequisite to the function of both innate and adaptive immune functions (Walker 2013). Research on germ-free mice shows that the absence of gut microbes leads to many immune system defects, such as lower levels of $CD4^+$ and $CD8^+$ T cells, fewer proliferating dendritic and T cells and a lower expression of antimicrobial peptides compared to mice with normal gut microbiota (Chung et al. 2012). Gut microbiota, gut-associated immune components and intestinal barrier consisting of epithelial cells and mucus,

layered by mucins, antimicrobial peptides and immunoglobulin A (IgA), preserve homeostasis and result in a functional immune phenotype via cross-talk (Weng & Walker 2013).

Gut microbiota affects the first line of defense, the intestinal barrier, in numerous ways: It increases the secretion of mucin and activates the production of pathogen-fighting antimicrobial peptides and IgA. Correspondingly, the secretion of antimicrobial peptides and IgA regulate the microbial community and its functions to maintain homeostasis (Weng and Walker 2013).

1.1.1. Toll-like receptor signaling pathways

Both commensal and pathogenic bacteria can activate signaling pathways related to immune reactions, which are recognized by pattern-recognition receptors, such as toll-like receptors (TLRs) located on the surface of epithelial and dendrite cells (Round et al. 2011, Weng and Walker 2013).

Immune reactions mediated by TLRs are dependent on an adaptor protein MyD88. Pathogens use a MyD88-dependent pathway to trigger inflammation reactions, whereas commensal microbes use it to suppress immune reactions by producing cytoprotective factors (Rakoff-Nahoum et al. 2004, Round et al. 2011). A study of Rakoff-Nahoum et al. (2004) showcases the importance of gut microbes as regulators of immune reactions via adaptor proteins and receptors: It was demonstrated that mice short of the adaptor protein MyD88 as well as receptors TLR2 and TLR4 suffered from severe epithelial cell damage and increased mortality following the treatment with Dextran Sulfate Sodium (DSS), which is an epithelium-irritant substance. Wild-type mice suffered from the same condition only when they were treated with vancomycin, neomycin, metronidatzole, and ampicilline, which depleted commensal bacteria prior to DSS treatment. (Rakoff-Nahoum et al. 2004).

Gut microbiota also regulates the immune response of T-helper (Th) cells via cross-talk with the TLRs of the intestinal dendritic cells. This cross-talk results in a cytokine secretion needed for the maturation of Th cells, which mediate e.g. cellular immunity, humoral immunity (i.e. antibody production), tissue inflammation and clearance of extracellular pathogens. The same pathway is also involved in the development of oral tolerance, which refers to the immune system’s capability to reduce the immune reaction against harmless microbes and antigens received orally. (Walker 2013).

2. Development of gut microbiota during lifespan

In the western countries, the immune-mediated diseases, such as allergies and autoimmune diseases, have grown in number in the recent decades. This has been linked to the changed lifestyle including improved sanitation, immunizations, antibiotics and diet changes, which in turn have an impact on the gut microbiota and possible dysbiosis. (Walker 2013). In this chapter, I first address the development of the gut microbiota across lifespan, and in the next chapter I discuss the development of gut microbiota dysbiosis, or imbalance, more specifically.

2.1. Neonatal period and early childhood

Earlier it was believed that humans are born germ-free, first suggested by Tissier in 1900s, and the initial contact to microbiota happens during birth (Kundu et al. 2017). However, this view has been challenged in the more recent research, as meconium, amniotic fluid, placenta and umbilical cord have been found to contain diverse microbes, suggesting that the exposure to microbes occurs already before birth (Collado et al. 2015).

After birth, the colonization of microbiota in a newborn's gut includes the following steps: (1) acquisition of mother's vaginal, colonial and skin microbiota at delivery (2) starting of either breastfeeding or formula feeding (3) weaning and (4) achieving the adult microbiota (Walker 2013). Full-term, vaginally born children are colonized by over 1000 microbial species during their first year of life (Weng and Walker 2013).

2.1.1. Mode of delivery

At the first step, the infant is exposed to maternal microbiota during birth, which has been regarded as the most important phase of the initial gut microbiota colonization (Weng and Walker 2013). Here, the mode of delivery has an impact: During vaginal delivery, the child is exposed to vaginal microbes, dominantly *Lactobacillus* and *Prevotella*, whereas during caesarian section, the newborn is exposed to typical skin microbiome including *Staphylococcus*, *Corynebacterium* and *Propionibacterium* (Dominguez-Bello et al. 2010).

These differences may have an impact on the infant's later gut microbiota composition and possible implications on health (Dominguez-Bello et al. 2010), which will be discussed later in the 'Gut microbiota dysbiosis and health' chapter.

2.1.2. Breastfeeding vs. formula-feeding

At the second step, the early feeding by breastfeeding or formula milk impacts the developing gut microbiota of the infant. The development of the gut microbiota in infancy seems to be a process started prenatally and continued after birth by breastfeeding, as in 3-4 days from birth the gut microbiota of a baby starts resembling that of colostrum (Collado et al. 2015). Human breastmilk contains hormones, antibodies, antimicrobials, nutrients and other bioactive molecules, which support the development of a healthy gut microbiota (Pacheco et al. 2014). The colonization of the gut microbes is supported by the prebiotic influence of the undigestible polysaccharides of the breast milk, which provide substrates for production of short-chain fatty acids and lead to the proliferation of health-supporting bacteria such as *Bifidobacteria* and *Lactobacillus* (Weng & Walker 2013). Also, the oligosaccharides induce a stimulus to mucosal immune defense (Walker 2013).

Compared to milk formulas, breast milk is more complex, and it is considered as the optimal nutrition to the infant during the first 6 months (Le Huërou-Luron 2010). Compared to formula-fed

children, the breastfed children had more *Bifidobacteria* in their gut, whereas formula-fed babies have a more diverse gut microbiota, though also they had a *Bifidobacteria* dominance (Bezirtzoglou 2011). Formula-fed infants also had higher counts of *Bacteroides*, *Atopobium* and *C. difficile*, which has a pathogenic association to enteric and atopic diseases (Bezirtzoglou 2011, Azad et al. 2013a). However, the clinical significance of microbial variation requires further study (Azad et al. 2013a).

2.1.3. Weaning

Upon withdrawing from breastfeeding or formula feeding and moving to solid food, the gut microbiota of a child transfers from a rather simple microbiome acquired from mother to a more complex and taxonomically diverse, mature microbiome. At the same time, the immune system of a child develops rapidly. The transition is believed to take up to three years. It is noteworthy that during the first 2 to 3 years of life, the gut microbiota goes through “the basic maturation”, and it can impact the development and functionalities of several organs and systems, such as enteric nervous system, which communicates bi-directionally with the central nervous system. (Kundu 2017).

According to Bergström et al. (2014), notable changes in the gut microbiota occur especially at the 9-18 months of age, when the weaning and complementary feeding are started. The counts of *Bifidobacteria*, *Lactobacilli* and *Enterobacteriaceae* diminish and are replaced by *Bacteroides spp*. and *Clostridium spp*. Also, the enterotypes are established between 9 and 36 months of age. (Bergström et al. 2014). The shift to solid food improves the pathogen resistant ability of the gut, as more commensal bacteria attach to the epithelial surface (Weng & Walker 2013).

2.2. Puberty and adulthood

Puberty is characterized by sexual maturation driven by hormones and cross-talk between brain, skin and genitalia (Kundu 2017). During this period, the gut microbiota starts to change to gender-specific (Markle et al. 2017). Compared to adults’ microbiota, the microbiota of adolescents seems to differ in terms of population abundance and functional potential. The gut microbiota of a pre-adolescence cohort (7-12 years) seemed to express genes relating to development, whereas adults’ gut microbiota was more associated to obesity, inflammation and adiposity. The microbiota of children was dominated by *Bifidobacterium* spp., *Faecalibacterium* spp., and members of the *Lachnospiraceae*, whereas adult gut had a greater abundance of *Bacteroides* spp. (Hollister et al. 2015). Claesson et al. (2011) suggest that the microbiota composition of elderly people shifts towards the dominance of *Bacterodetes* from the earlier dominance of *Firmicutes* in young adulthood; however, the significant inter-individual variation in the microbiota composition remains (Claesson et al. 2011).

3. Gut microbiota dysbiosis and health

3.1. What is dysbiosis

Dysbiosis refers to the imbalance in the counts of microbiota species (Shin et al. 2015) when “good” microbes no longer control “bad” ones (Schippa and Conte 2014). Dysbiosis has been connected with numerous diseases including autoimmune and autoinflammatory diseases, such as allergies, obesity and enteric diseases. Its origin has been associated with the Western lifestyle including a diet with lots of sugar, animal fat and red meat, sedentary lifestyle, low consumption of dietary fibers and high use of antibiotics. The dysbiosis may result in a chronic disease especially in genetically susceptible hosts. (Schippa and Conte 2014). A study has shown the interconnection of gut microbiota, host genotype and chronic inflammation on gnotobiotic mice (Eun at al. 2014).

In adults, the gut microbiota contains a unique core set of microbes, which are preserved over time, and environmental factors, such as stress, nutritional changes, use of antibiotics and travelling seem to have a limited impact on it. However, as time passes, the relative richness of microbiota species changes significantly: The most stable component of microbiota consists of mainly anaerobic bacteria, whereas the aerobic bacteria such as *Lactobacillus spp*. seem to be more unstable. (Rajilic´-Stojanovic´ et al. 2012). It was reported that enterotypes have a strong association with long-term diets, high consumption of animal protein and fat leading to the dominance of *Bacteroides*, and consumption of carbohydrates to the dominance of *Prevotella* (Wu et al. 2011). Schippa et al. (2014) state that the core set of microbial genes relate to degradation of polysaccharides and synthesis of short-chain fatty acids and nutrients, whereas the variable microbiota entails a smaller set of genes. The variable microbiota depends more on host-specific factors, such as diet, lifestyle, environment and physiological status, than the core microbiota (Schippa and Conte 2014).

Shin et al. (2015) suggest that the relative dominance of *Proteobacteria* can be used as a clinical criterion for dysbiosis: The *Proteobacteria* phyla has been found to be the most unstable of microbial phylas, assumingly because it is more sensitive to environmental changes such as diet. In a healthy gut, *Proteobacteria* is found in a minor fraction, but in dysbiosis it is increased. (Shin et al. 2015). Gao et al. (2018) found that the microbiota composition and function differed significantly between obese, overweight and healthy patients: The abundance of *Proteobacteria* was elevated in the obese group, whereas *Ruminococcus*, which has an ability to degrade starch particles and improve insulin resistance, was less abundant. Also, *Bifidobacterium* and anti-inflammatory *Faecalibacterium* were decreased in the obese group, whereas *Bacillus* as well as potentially opportunistic pathogens, such as *Fusobacterium* and *Escherichia-Shigella,* had significantly increased. The results indicate that the microbiota composition has an important role in the development of metabolic disorders related to obesity (Gao et al. 2018).

3.2. Development of gut microbiota dysbiosis in children

On the contrary to adults' mature gut microbiota, which is believed to be rather stable, the infants' developing gut microbiota is constantly changing and vulnerable to external disturbances (Gibson et al. 2015).

As cited earlier in Chapter 2, the gut microbiota of children is strongly affected by the microbiota of the pre-birth conditions such as placenta and amniotic fluid, the choice of delivery method, and early breastfeeding or formula-feeding (Walker 2013). Also, environmental factors such as the microbiota of other family members and pets may have an impact on a child's gut microbiota (Azad et al. 2013b). Nutrition has a drastic impact on children's gut microbes: It was reported that the relative richness of gut microbes of children aged 1-6 from rural Africa differs from that of European children and reflects the differences in diets based on starch, fiber and plant polysaccharides in African children vs. high amount of sugar and animal protein in European children (de Filippo et al. 2010). *Prevotella* and *Xylanibacteria* found in considerable amounts in African children are efficient in degrading cellulose and xylans, which makes it possible to harvest more energy from a starch-based diet (Tremaroli and Bäckhed 2012).

Early infancy is a critical period in terms of gut microbiota development, and therefore disturbances delay the complete colonization of the gut by 4-6 years despite of oral feeding type and weaning. During the recovery from the disturbance, the child is more prone to both systemic and intestinal infections, and if the inadequate colonization persists, there is a higher risk for the occurrence of immunity-mediated disease. Usually, inadequate colonization relates to premature delivery, cesarean section, or excessive antibiotic usage in the perinatal period. (Weng and Walker 2013).

Studying the mechanisms and health implications of pediatric dysbiosis is complicated, as it is affected by multiple and complex factors, and it includes interactions between the microbiota and the immune system (Vangay et al. 2015). Vangay et al. (2015) suggest several interdependent frameworks for understanding aspects of pediatric dysbiosis: *Dysbiosis-centric* view characterized four diverse types of dysbiosis caused by an outer stimulus. For instance, broad-spectrum antibiotics may destabilize the microbiota by 1) eradicating essential taxa maintaining homeostasis or affecting host development (e.g. immune system), 2) causing overall loss of microbial biodiversity 3) eradicating niche taxa which are then replace by pathogens, or 4) causing a shift in functional capability of the microbiota (e.g. energy harvesting) due to incomplete microbiota recovery. On the contrary, *Disease-centric* approach classifies the health consequences of dysbiosis by disease class including the mechanisms and interactions with other systems of the host, such as immune system or microbiota. The authors also mention *Age-centric, Response-centric* and *Recovery-centric* views, which focus on the development stage, the phase of the antibiotic treatment, and speed of the recovery as the causes of dysbiosis. Even though all frameworks have their strengths and weaknesses, the combination of dysbiosis-centric and

disease-centric views can sufficiently help to summarize the current knowledge about potential dysbiosis-induced disease mechanisms. (Vangay et al. 2015).

In the following, I will discuss dysbiosis in children both from the dysbiosis-centric view explaining the chains of events leading to different dysbiosis types as well as examining the most common diseases associated to dysbiosis.

3.2.1. Pre-term birth and caesarian section

Hill et al. (2017) found that pre-term babies had a significantly greater number of *Proteobacteria* compared to full-term babies after one week of life, and different metabolite profile compared to full-term babies at 4 weeks. Also, at the age of 24 weeks, differences in the microbiota compared to full-term babies remained. These findings may be associated to different feeding, hospitalization and use of antibiotics in pre-term babies. (Hill et al. 2017).

Azad et al. (2013a) found that especially those infants, who were born through elective cesarean delivery, had a low diversity and abundance of gut microbes (Azad et al 2013a). Hill et al. (2017) observed that full-term infants born through cesarean section had increased counts of *Firmicutes* and lower number of *Actinobacteria* compared to full-term, vaginally born infants after the first week of life; however, the difference was gradually narrowed down in 24 weeks. Prolonged breastfeeding had an impact on the gut microrbiota of caesarian-born children, but not on vaginally-born children. Therefore, breastfeeding is recommended particularly to babies born through caesarian section to balance out the gut microbiota. (Hill et al. 2017).

Marcotte et al. (2018) found a connection between birth by caesarian section and an increased risk of acute lymphoblastic leukemia (ALL) in childhood. Changed microbial colonization and its implications on the immune system development is offered as a possible explanation for the finding (Marcotte et al. 2018).

3.2.2. Antibiotics

It has been estimated that by the age of two, and average child in the United States has been prescribed three antibiotics courses, number rising to ten by the age of 10 years and to 17 by the age of twenty (Cox and Blaser 2015). Overuse or misuse of antibiotics has been reported around the world, also in the Nordic countries (Rún Sigurðardóttir et al. 2015). In addition to altered microbiota and health consequences, the risks of excessive use of antibiotics include the rising number of antibiotic resistance genes (Gibson et al. 2015).

Cox et al. (2014) state that even if the microbiota composition returns to normal after a course of antibiotics, the metabolic changes persist, because gut microbiota oversees the long-term programming of metabolic functions (Cox et al. 2014). The magnitude of disturbance caused by antibiotics and the speed of recovery significantly varies between people, and it is assumed that

unknown biotic or abiotic factors affect the resilience of microbial communities (Ursell et al. 2012). Also, the type, dosage, duration, pharmacokinetics and pharmacodynamics of prescribed antibiotics play a role in gut microbiota recovery (Rizzatti et al. 2018).

Research conducted on neonatal mice suggests that streptomycin treatment had a negligible effect on gut microbiota and incidence of allergic asthma on mice, but vancomycin reduced microbial diversity of the intestine and enhanced the severity of asthma (Russell et al. 2012). Finnish researchers found out that the use of macrolides (azithromycin and clarithromycin) was associated with long-term (up to 2 years) changes in gut microbiota composition and richness, whereas the changes caused by penicillin-type of antibiotics (amoxicillin with or without clavulanic acid and penicillin V) were more rapidly recovered and did not induce a large long-term effect on microbiota. A full recovery of gut microbiota may take longer than the average time between antibiotics courses, and therefore the antibiotics-induced microbiota may remain, if antibiotics are used every year or even more frequently. (Korpela et al. 2016).

It has also been suggested that perhaps antibiotics themselves are not the reason to antibiotics-associated diseases and health consequences, such as obesity, but they are merely a marker of an underlying infection leading to the condition. This suggestion needs to be further evaluated on animal models and epidemiological research. (Turta and Rautava 2016).

3.3. Health implications of dysbiosis

Just like in adults, gut microbiota dysbiosis is related to numerous diseases in pediatric patients, including the above-mentioned obesity, but also allergy, atopy, autoimmune diseases and infectious diseases (Vangay et al. 2015). In the following, I will discuss some important dysbiosis-associated disease classes. I include diseases that are more typical in adults, such as T2DM, as the microbiota disruption in the childhood may have long term implications.

3.3.1. Obesity

Incomplete recovery from an antibiotic course can cause a long-lasting metabolic change in the gut microbiota, and lead to increased adiposity and obesity (Vangay et al. 2015). Low dose of antibiotics on young mice before weaning increased their body mass in adulthood suggesting that gut microbiota affects the early programming of the metabolic system. Furthermore, the antibiotics increased the effect of a fat rich diet. (Cox et al. 2014).

The increase of certain species of gut microbiota is linked to more effective energy harvesting and absorption from carbohydrates (Tremaroli and Bäckhed 2012). Research comparing obese and lean adults confirm that obesity is associated with reduced bacterial richness, lower proportion of *Bacteroides* and higher proportion of *Actinobacteria* (Turnbaugh et al. 2009). In children, an increased number of *Firmicutes* and lower number of *Bacteroides* has been connected to obesity (Orbe-Orihuela et al. 2018). In the contrary to Turnbaugh et al. (2009), Bai et al. (2019) found that

the abundance of *Actinobacteria* is decreased in children with high BMI, while the number of *Proteobacteria* is increased. Obese children also had higher number of pathogenic species in their gut. (Bai et al. 2019). Also, according to Bai et al. (2019), *Lactobacillus* and *Bifidobacterium* were connected to higher BMI, which is interesting as they are also known as health-promoting and probiotic (Tremaroli and Bäckhed 2012, Weng & Walker 2013).

3.3.2. Allergy and atopy

Vangay et al. (2015) suggest that allergy and atopy can derive specifically from two dysbiosis types, low number of keystone taxa and increase in pathogen counts (Vangay et al. 2015). Research shows evidence for this, as Abrahamsson et al. (2012) found that lower gut microbiota diversity during the first month of life was related to later occurrence of atopic eczema development. The possible explanation to this is that the repeated exposure to new bacterial antigens helps the development of immune regulation (Abrahamsson et al. 2012). According to another study, the bacterial composition of patients with allergic diseases differs from that of the healthy control group showing lower counts of *Bifidobacterium* and *Lactobacilli* and higher abundance of *Staphylococcus* and *Bacteroides* (Björkstén et al. 1999).

3.3.3. Autoimmune diseases

Autoimmune diseases include among others type 1 diabetes mellitus (T1DM), rheumatoid arthritis, multiple sclerosis, Crohn's disease and celiac disease (Giongo et al. 2011, Vangay et al. 2015), and they are typically diagnosed on young children (Giongo et al. 2011). Vangay et al. (2015) hypothesize that the linkage between autoimmune diseases and microbiota resembles that in allergy and atopic diseases, and the occurrence of diseases derive from the loss of keystone taxa and surge of pathogens resulting from e.g. antibiotics, though the evidence is still insufficient (Vangay et al. 2015). Animal models highlight the importance of commensal microbiota in the development of autoimmunity diseases, as germ-free animals are not able to develop many of the autoimmune diseases (Lerner et al. 2016). Vaarala et al. (2008) state that the onset of T1DM and many other autoimmune diseases depends on an interplay between abnormal gut microbiota, a "leaky" intestinal mucosal barrier, and intestinal immune responsiveness, which ultimately results in autoimmunity in T1DM (Vaarala et al. 2008).

3.3.4. Infectious diseases

The time between a disturbance of the gut microbiota, such as an antibiotics course, and the recovery opens a door for the bloom of pathogens, especially antibiotic-resistant strains, and exposes the host to infections (Vangay et al. 2015). Typical example of an antibiotic-induced infection is the *Clostridium difficile* infection, which causes symptoms ranging from mild diarrhea to severe colitis (Ferreyra et al. 2014). *Clostridium difficile* infection is believed to be caused by gut microbiota alterations, and it is specifically connected to broad-spectrum antibiotics clindamycin,

cephalosporins, penicillins, and fluoroquinolones (Rizzatti et al. 2018). Gut microbiota disturbance by antibiotics is also associated to necrotizing enterocolitis, which is a severe illness sometimes occurring in low weight, pre-term babies (Warner et al. 2016).

3.3.5. Cardiovascular diseases

Cardiovascular diseases (CVDs) are the main cause of death in the Western countries, and they are strongly associated to obesity, metabolic syndrome and type 2 diabetes mellitus (Miele et al. 2015). This provides an indirect link between gut microbiota and CDVs, as microbiota is associated to the risk factors of CVDs (Larsen et al. 2010, Cox et al. 2014). Furthermore, there is increasing evidence on the linkage of the microbiota induced immune system activation and CVD risk: Wang et al. (2011) found that gut flora dependent metabolism of phosphatidylcholine is linked to the pathogenesis of CVD on animal models (Wang et al. 2011). In another clinical study by Tang et al. (2013), it was found that increased plasma levels of trimethylamine-*N*-oxide (TMAO), a metabolite of phosphatidylcholine, were associated to higher incidence of adverse cardiovascular events. The role of gut microbiota in the formation of TMAO was tested by suppressing the gut microbiota by antibiotics and measuring the TMAO levels during and after the antibiotics course. (Tang et al. 2013).

3.3.6. Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is believed to derive from multiple causes, such as age, genetics, obesity as well as lifestyle and dietary factors (Han and Lin 2014). Recently, the pathogenesis of T2DM has been linked to gut microbiota: Larsen et al. (2010) have found that adults with T2DM had a lower proportion of phylum *Firmicutes* and genera *Clostridia* compared to the control group. Also, the relative abundance of *Bacteroidetes* compared to *Firmicutes* and *Prevotella* correlated significantly with plasma glucose concentration, but not with BMIs (Larsen et al. 2010).

3.3.7. Cancer

The risk factors for cancer in adults are like those for obesity, T2DB and cardiovascular diseases, which have been shown to be associated to gut microbiota. Thus, changes in microbiota inducing metabolic syndrome are also potentially risk factors for certain cancers at least in adults (Zitvogel et al. 2017). Gut microbiota affects the cancer progression and tumorigenesis by modulating inflammation, deregulating signaling pathways of the host's cells and interacting with nutrients or host metabolites increasing the growth of pathogens or causing a risk of DNA damage (Rea et al. 2018). For instance, gut microbiota metabolizes particles of red meat and highly processed food, which can be mutagenic (Rea et al. 2018). The relationship between cancer and microbiota is complex and bi-directional, as the changes in microbiota may be resulted by the disease, but they may further affect the disease progression (Zitvotel et al. 2017). Changes in gut microbiota has been linked to several adult-type cancers such as colorectal, gastric, pancreas, liver, prostate and

breast cancers (Wong et al. 2018). Recently, Vicente-Dueñas et al. (2020) found out that in genetically predisposed mice, an antibiotic-induced gut microbiota disturbance was sufficient to induce leukemia.

3.4. Prevention and treatment of dysbiosis

Especially long-term diets are known to change the gut microbiota both in humans and mice (Tremaroli and Bäckhed 2012). For instance, diet therapy in obese patients increases the abundance of *Bacteroides* (Ley et al. 2006b), suggesting that *Bacteroides* may be responsible for calorie intake (Tremaroli and Bäckhed 2012). In young children born through caesarian section, breastfeeding can help narrow the difference in the gut microbe composition compared to vaginally-born children (Hill et al. 2017). The longer duration of breastfeeding can also have a protective effect against obesity later in life, whereas too early introduction of solid foods (child younger than 15 weeks) is associated to increased adiposity (Farrow et al. 2013).

Probiotics are used to treat several conditions, also other than gastrointestinal related diseases (Rizzatti et al. 2018). Probiotics are defined as living microbial food ingredients having a favorable effect on health. Probiotic potential differs between different strains of even the same microbial genera. (Isolauri et al. 2004). For example, certain strains of *Lactobacillus* and *Bifidobacteria* have been associated with anti-inflammatory properties (Tremaroli and Bäckhed 2012). Also certain strains of *Enterococcus*, *Streptococcus* and *Leuconostoc* genera have probiotic properties (Butel 2014). *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* have been a subject of several studies, as they show probiotic potential in several diseases, such as obesity and inflammatory bowel disease (Rizzatti et al. 2018).

Probiotics improve the intestine's barrier against pathogens through metabolites and mechanisms affecting microbial competition or adhesion and modulate the immune system (Butel 2014). Research suggests that microbiota modulation by probiotics can have a counteracting effect to pregnancy complications or allergic and inflammatory symptoms of the child, and probiotics intake of pregnant or breastfeeding mothers and their babies promote the development of a healthy immune system of the offspring. According to research evidence, adding probiotics to milk formulas can have beneficial effects on the baby's health, but the intervention is the most efficient, if the use of probiotic supplements begins already during pregnancy. (Berti et al. 2017).

Prebiotics are typically non-digestible fiber substances, such as phytoestrogens (e.g. resveratrol) found in certain berries, fibers received from plants and vegetables and herbal supplements, which support the growth and function of commensal gut microbiota. Some of them have been found to have beneficial health effects in the interaction with gut microbiota. (Rea et al. 2018). Certain antibiotics may also have a beneficial effect on gut microbiota; for example, nonabsorbable intestinal antibiotic rifaximin has been shown to increase the number of several commensal gut bacteria, such as *Lactobacillus*. Furthermore, gut microbiota dysbiosis can be treated by fecal

microbiota transplant (FMT), which is now an established treatment against *C. difficile* infection and under investigation in many other conditions, such as ulcerative colitis. (Rizzatti et al. 2018).

4. Gut microbiota and cancer treatment

Cancer is one of the major causes of death in the developed world, and its occurrence has been linked to aging, nutritional choices, tobacco smoking, alcohol consumption and carcinogenic substances (Rea et al. 2018). Worldwide, 3.6 percent newly diagnosed cancers were related to high BMI in 2012 (Arnold et al. 2015). As many as 20 percent of cancers are caused by microbes, such as Helicobakter pylori, Fusobacterium nucleatum, Ebstein-Barr virus and human papilloma virus (Zitvogel et al. 2017). The most common cancers among adult men in Finland are prostate, lung/trachea and colorectum cancers, and among women breast, colorectum and corpus uteri cancers (Ferlay et al. 2013).

The pediatric cancers add up to only for 1 percent of all cancers, but despite their rareness and good prognosis, they are the major cause of disease-related deaths in children in the developed world (Ching-Hon et al. 2016). The most common pediatric cancers include leukemia, lymphoma and brain/CNS tumors (Prusakowski and Cannone 2014). Acute lymphoblastic leukemia (ALL) is the most common childhood cancer totaling 25 percent of childhood cancers (Bhojwani et al. 2015). The pathogenesis of childhood cancers has been linked for example to genetic alterations, such as translocations, hyperdiploidy and hypodiploidy (Bhojwani et al. 2015, Ching-Hon et al. 2016); however, the exact mechanism behind ALL outburst is not clear (Wen and Chen 2019).

4.1. Health implications of childhood cancer survivors

The occurrence of cancer in the population of age below 20 was 20/100.000 and the total number of cases numbered 229 in year 2019 (Suomen syöpärekisteri 2021). The intensive treatment of cancer including chemotherapy and prophylactic or therapeutic antibiotics can have a potential long-term effect on gut microbiota (Rajagopala 2016); however, the existing knowledge on the gut microbiota of children with cancer is by far scarce (Bai et al. 2018).

Survivors of childhood cancer suffer from markedly higher morbidity and mortality than the population in general, even after decades from the diagnosis. In the short-term, the deaths are usually related to relapses of the original cancer, whereas in the long-term occurrence of second cancer and non-cancer related causes become more common. (Garwicz et al. 2012). Childhood cancer survivors suffer from an elevated risk of long-term health effects, such as pulmonary, auditory, endocrinal, cardiac and neurocognitive conditions (Hudson et al. 2013). Deaths due to both recurrence of the primary cancer and other health-related reasons has decreased in the last decades, partly thanks to the development of cancer treatments (Armstrong et al. 2016). However, the threat of serious illnesses and health conditions of cancer survivors remains elevated compared to healthy controls, and by the age of 50, about half of the childhood cancer survivors suffer from a severe or even life-threatening health condition. Despite age-relating, accumulating

health conditions in the general population, the mortality of childhood cancer survivors remains increased across the whole lifespan. (Armstrong et al. 2014).

Obesity, diabetes mellitus and hyperlipidemia are among the top long-term health effects of childhood cancer survivors (Barnea et al. 2015). Conditions such as increased weight (Breene et al. 2010), insulin resistance (Tonorezos et al. 2012) and other metabolic syndrome symptoms, such as hypertriglyceridemia and low HDL (Oudin et al. 2017), poor physical fitness and low physical activity (Järvelä et al. 2010, Winter et al. 2010) have been reported. Even though there is evidence of changes in microbiota of pediatric cancer patients, it is still unclear whether the changes are due to the malignancy, cancer specific treatments, or other factors, such as the use of antibiotics (Nycz et al. 2018). Along with gut microbiota composition changes, physical, cognitive and social limitations of cancer survivors have been suggested as reasons for the increased occurrence of adverse health effects, and these mechanisms require further investigation (Barnea et al. 2015).

In the short term, pediatric patients with cancer often suffer from C. difficile (CDI) and bloodstream infections (BSI), which result in higher morbidity and longer hospitalization of patients (Nycz et al. 2018). Moreover, a typical complication of cancer treatments, occurring in 50 percent of all cancer patients, is gastrointestinal mucositis, which causes inflammation or ulcers in the gastrointestinal tract with symptoms including diarrhea, fatigue, abdominal pain, bleeding and electrolyte imbalance, and sometimes has severe complications, such as bacteremia and sepsis (Kuiken et al. 2014, Touchefeu et al. 2014). Mucositis in pediatric patients is still not well studied, but the clinical presentation in children resembles that in adults (Kuiken et al. 2014). Recently, there has been increasing evidence on the connection of microbiota dysbiosis in pediatric patients and the development of CDI and BSI (Nycz et al. 2018, Chen et al. 2019). Also, the development of gastrointestinal mucositis has been connected to the dysbiosis induced by cancer treatments; however, it is still to be confirmed, whether the microbiota disturbance is the cause or effect of intestinal inflammation (Touchefeu et al. 2014).

4.2. Effect of cancer treatment on gut microbiota

Research on the effect of cancer treatments on pediatric patients' gut microbiota is still in an early phase despite of growing interest in the topic during the recent years (e.g. Van Vliet et al. 2009, Huang et al. 2012, Rajagopala et al. 2016, Bai et al. 2017, Bai et al. 2018, Hakim et al. 2018, Nycz et al. 2018, Nearing et al. 2019, Chua et al. 2020, Liu et al. 2020, Thomas et al. 2020). However, the topic has been previously researched with pre-clinical models and clinical studies in adults (Touchefeu et al. 2014). In the following, I will review existing studies on the impact of cancer treatments on the gut microbiota of adult cancer patients. I discuss chemotherapy, radiation therapy, stem-cell transplants and immunotherapy, as these therapy types also apply to pediatric patients. After that, I will review the current literature on the impact of cancer treatments on the gut microbiota of pediatric patients.

4.2.1. Chemotherapy

Chemotherapy drugs are cytotoxic, and they include alkylating agents, heavy metals such as platinum, cytotoxic antibiotics, antimetabolites and spindle poisons. Their antitumor activity is usually based on targeting DNA integrity, cell division of cancerous cells, mitochondria and cell membranes. Gut microbiota has been demonstrated to influence carcinogenesis and interact with chemotherapy drugs affecting their metabolism and efficiency. (Roy and Trinchieri 2017, Wong et al. 2018). However, because of the limitation of the scope of this paper, I will not discuss these mechanisms in detail.

Chemotherapy has been shown to have a drastic impact on gut microbiota: A recent preclinical study on gemcitabine chemotherapy in xenografted mice revealed a decrease in *Firmicutes* and *Bacteroidetes* following the treatment, and they were replaced by *Proteobacteria*. An overall increase of inflammation inducing bacteria was observed. (Panebianco et al. 2018). In another preclinical study it was also found that rats treated with a common chemotherapy drug 5-Fluorouracil (5-FU) had significant increases in feces of *Clostridium spp*., *Staphylococcus spp*. and infection-inducing *E. coli*, whereas *Bacteroides spp*. and *Lactobacillus spp*. decreased (Stringer et al. 2009). Furthermore, a study on 5-FU-treated rats found that the drug shifted the balance in the small intestine from gram-positive cocci to gram-negative rods. In the colon, the gram-negative facultatives increased while the gram-positive facultatives decreased, whereby the total amount of facultative anaerobes increased. (Von Bültzingslöwen et al. 2003). The shift in bacterial balance may result in overgrowth of infectious bacterial species, and the loss of species affects their function in the gut, such as immune development and protection (Stringer et al. 2009).

Changes in bacterial balance have also been associated to the chemotherapy substance irinotecan in animal models (Stringer et al. 2007, Stringer et al. 2008). The metabolite of irinotecan, SN-38 glucuronide, can be converted to toxic SN-38 by b-glucuronidase, which is produced by several gut microbes such as *Enterobacteriaceae* including *E. Coli*. Counts of *E. Coli* were observed to increase in rats after a single intraperitoneal dose of irinotecan. (Stringer et al. 2007, Stringer et al. 2008).

Wang et al. (2016) found that patients treated with doxorubicin had higher levels of endotoxin and TNFα in their serum compared to healthy individuals and state that the endotoxin is derived from gut microbiota and leaked to circulation due to epithelial damage. According to them, that is a compelling cause of adverse effects of doxorubicin, such as systemic inflammation and multiorgan damages (Wang et al. 2016). Stringer et al. (2013) found that patients with a common side effect of chemotherapy, diarrhea, had decreased counts of *Lactobacillus spp*., *Bifidobacterium* spp., *Bacteroides* spp. and *Enterococcus* spp., and increased number of *Escherichia coli* and *Staphylococcus* spp. Decrease was shown also in Methanogenic archaea. (Stringer et al. 2013). Also, studies of Zwielehner et al. (2011) and Montassier et al. (2014) found that patients

receiving chemotherapy had a disrupted microbiota following the treatment, characterized by reduced counts and richness of the microbiota (Zwielehner et al. 2011, Montassier et al. 2014). Zwielehner et al. (2011) observed decreased in *Bacteroides*, *Bifidobacteria*, and *Clostridium cluster IV* and *XIVa*. In some patients, these changes coincided with the growth of *C. difficile* (Zwielehner et al. 2011). Montassier et al. (2015) found that fecal samples collected after chemotherapy treatment showed significant decreases in *Firmicutes* and *Actinobacteria*, and significant increases of *Proteobacteria* (Montassier et al. 2015).

4.2.2. Radiation therapy

Radiation therapy is used widely to treat diverse types of cancers; however, its gastrointestinal (GI) toxicity limits its use. Especially the small intestine is sensitive to the effects of radiation. (Johnson et al. 2004). GI radiation-induced toxicity is a major complication of radiation treatment of cancer. As the symptoms of toxicity may be severe and develop even decades after the treatment, the attempts to understand the mechanisms of toxicity development is vital. (Fuccio et al. 2015).

In a preclinical study on mice, it was found that radiation reduced the intestinal microbiota of the irradiated mice with a significant decrease in aerobic, anaerobic, *Enterobacteriaceae* and *Lactobacillus* counts in two hours after the radiation, and after 16 hours of the treatment, the aerobic, *Enterobacteriaceae* and *Lactobacillus* were still reduced in the radiated animals compared to non-radiated ones. However, 24 hours after radiation there the difference between the groups ceased. (Johnson et al. 2004).

Fatigue and diarrhea are common side effects of radiotherapy (Wang et al. 2015). In the clinical studies, Nam et al. (2013) found that patient receiving pelvic radiotherapy due to gynecological cancer had a significantly different microbiota composition compared to healthy individuals in terms of counts and relative diversity. *Firmicutes* significantly decreased and *Fusobacterium* increased following the radiation, and most patients suffered from post-radiation diarrhea (Nam et al. 2013). Also, Wang et al. (2015) found that fatigue, diarrhea and increasing inflammatory markers, α1-antitrypsin, TNF-α and lipopolysaccharides in serum, correlate in patients receiving pelvic radiotherapy. Most interestingly, the gut microbiota of patients developing diarrhea had significantly lower alpha diversity and higher *Firmicutes/Bacteroides* ratio compared to patients without diarrhea prior to radiotherapy. Especially *Clostridia* cluster XVIII and *Faecalibacterium* genus, which have been linked to protection from allergy and colitis, were significantly decreased in diarrhea patients prior to radiotherapy, while the *Clostridium* cluster XI, which includes the pathogenic *C. difficile*, was increased post-radiotherapy. (Wang et al. 2015). Also, Manichanh et al. (2008) found in their study that healthy controls and patients, who did not develop diarrhea during 5 weeks of abdominal radiotherapy, had a stable gut microbiota over 7 weeks, while the patients with developing diarrhea had an increased count of *Actinobacteria* and *Bacilli*, and decreased count of *Clostridia* (Manichanh et al. 2008). According to studies, use of probiotics, such as VSL #3 and Lactobacillus

casei DN-114 001 may reduce the severity and incidence of post-radiotherapy diarrhea (Visich and Pluth Yeo 2010).

4.2.3. Hematopoietic stem-cell transplantation (HSCT)

Zama et al. (2017) point out that before stem-cell transplantation, the gut microbiota composition of the patients is similar to that of healthy individuals. This reflects the microbiota's ability to recover after chemotherapy and antibiotic treatments. (Zama et al. 2017). Biagi et al. (2015) followed the changes in gut microbiota in pediatric patients undergoing HSCT and found that the transplantation causes a temporary disruption in the microbiota in terms of diversity, variability and short-chain fatty acid production. The alpha diversity decreased by 30 percent after the treatment and less than 10 percent of the pre-HSCT species were conserved. There was a significant drop in health-promoting species, such as *Faecalibacterium* and *Ruminococcus*. However, the gut microbiota recovered in approximately 2 months in richness and functionality. (Biagi et al. 2015). According to another study, patients developing Graft-versus-host disease (GvHD), which is the main complication of HSCT, have a more prominent shift to *Enterococci* after the transplantation, and this shift was also associated to antibiotic prophylaxis and treatment of neutropenic infections (Holler et al. 2014). It was also found out that patients suffering from GvHD had a different gut microbiota already prior to HSCT, compared to patients, who did not develop GvHD. Non-GvHD patients had a higher number of *Bacteroidetes* phylum, which are assumed to have an essential role in the gut microbiota balance restoring after the treatment. (Biagi et al. 2015).

4.2.4. Immunotherapy

Cancer immunotherapy strategies are among the most novel therapies against cancer. These therapies augment a patient's own immune surveillance system and help it detect and eradicate cancer cells. (Patel and Crawford 2018). Research findings on the effect of immunotherapy on gut microbiota are very scarce, but it was found that ipilimumab, which is a monoclonal antibody against CTLA-4, a negative T-cell regulator, can cause "subclinical colitis", as ipilimumab was able to alter microbiota composition at the genus level increasing *Bacteroides app*. This was found to be essential for the anticancer effects of the treatment. (Vétizou et al. 2015). Gut microbiota composition has been proved to have an impact on the clinical response also to PD-1 blockade immunotherapy (Gopalakrishnan et al. 2018). Gopalakrishnan et al. (2018) found that patients responding to treatment had higher diversity of gut microbiota and more *Ruminococcaceae/Faecalibacterium* (Gopalakrishnan et al. 2018).

4.3. Effect of cancer diagnosis and treatment on gut microbiome of children

In the following, I will discuss the findings of the existing studies regarding the effect of cancer diagnosis and treatment on gut microbiome in pediatric patients. The existing literature has discussed the topic from a few different angles, which are listed below. In ***Table 1***, the most relevant findings on the topic are summarized.

4.3.1. Differences between newly diagnosed ALL patients and healthy controls

Several studies confirm that the microbiota profile of pediatric cancer patients differs from healthy controls already before the start of the chemotherapy. Van Vliet et al. (2009) found that the bacterial counts of AML patients were decreased before chemotherapy, and during the chemotherapy, they further decreased compared to healthy controls. These findings are similar to those of Huang et al. (2012), who found that the amount of gut microbiota was about 30 percent lower in children with ALL compared to the controls prior to chemotherapy, and the total counts of *Bifidobacteria, Lactobacillus* and *E. coli* were reduced compared to the healthy control group (Huang et al. 2012). Chua et al. (2020) found out that ALL patients had higher levels of *Bacteroides* and lower level of *Firmicutes* and *Actinobacteria* compared to healthy controls prior to chemotherapy. There was also a trend of lower alpha diversity in ALL patients prior to chemotherapy. Most patients in this cohort had had a course of antibiotics prior to diagnosis. (Chua et al. 2020).

The research of Rajagopala et al. (2016) found that ALL patients and their healthy siblings had a similar microbial profile consisting dominantly of *Bacteroides*, *Prevotella* and *Faecalibacterium* before chemotherapy, but the microbial alpha diversity of siblings was significantly better than that of ALL patients prior to the treatments (Rajagopala et al. 2016). In the contrary, Liu et al. (2020) found no differences between alpha diversity of newly diagnosed ALL patients and healthy controls. Instead, they found that species *Edwardsiella tarda* and *Prevotella maculosa* were decreased in ALL patients and this had a positive correlation with interleukin-10 levels, which were found to be significantly lower among pediatric ALL patients compared to healthy controls suggesting that gut dysbiosis may be a factor behind the ALL pathogenesis. ALL patients also had a decreased number of *Roseburia faecis* and *Fusobacterium naviforme* and enriched amount of *Bacteroides clarus*. (Liu et al. 2020).

Bai et al. (2017) and Hakim et al. (2018) agree with the decreased microbial diversity of ALL patients compared to healthy controls (Bai et al. 2017, Hakim et al. 2018). Bai et al. (2017) also point out that beta diversity is independent of prior antibiotic use, unlike alpha diversity. ALL patients also had lower *Firmicutes/Bacteroides* ratio compared to healthy controls prior to any cancer treatments, which is a sign of dysbiosis. *Bacteroidales* and *Enterococcaceae* could even serve as ALL biomarkers, as their abundance was significantly increased in ALL patients (Bai et al. 2017).

4.3.2. Gut microbiota alterations due to chemotherapy

The microbiota seems to alter further during the chemotherapy, but it can also be restored during and after chemotherapy: Huang et al. (2012) noted that the chemotherapy further decreased the total number of gut bacteria in children, especially the counts of *Bifidobacteria* and *Lactobacillus*, but the amount of *E. coli* increased. After the treatment cycles, the diversity was nevertheless partially restored. Rajagopala et al. (2016) found out that ALL patients had a reduction in *Lachnospiraceae* and *Roseburia* and increase in *Bacteroides* compared to the controls after the chemotherapy. Chua et al. (2020) observed a decrease in the relative abundance of *Bacteroides* during chemotherapy, and up to 9 months post-chemotherapy, the numbers of *Bacteroides, Atopobium, Fusobacterium, Prevotella*, and *Corynebacterium* genera were lower than in healthy controls. One OTU belonging to *Bifidobacterium* was at a higher level compared to healthy controls after chemotherapy. (Chua et al. 2020).

Hakim et al. (2018) observed a decrease of anti-inflammatory *Faecalibacterium* species and *Ruminococcaceae* in ALL patients during the chemotherapy compared to the pre-chemotherapy situation. Thomas et al. (2020) found out that this change may last for years: They observed a decreased number of *Faecalibacterium* in the stool of ALL survivors after one or more years of chemotherapy compared to their healthy siblings. There were also differences in multiple OTUs; however, they found no statistically significant differences in alpha and beta diversity. This is in concordance with the earlier findings of Cozen et al. (2013), who studied young Hodgkin lymphoma survivors and their healthy twins 1-3 decades after the diagnosis and treatments. They found out that the controls had significantly more unique OTUs (operational taxonomic units) in stool and the survivors had somewhat lower diversity of microbiota. However, the difference in the abundance or alpha diversity was not statistically significant. (Cozen et al. 2013).

Chua et al. (2017) studied 18-35-year-old ALL survivors, who had the last chemotherapy at least 5 years prior to the stool sample collection (median time from the treatment cessation was 18.5 years) and compared them to healthy controls. They found that ALL survivors had a significantly reduced alpha diversity of gut microbiota compared to healthy controls. As for phylas, ALL survivors had slightly higher number of Actinobacteria and slightly lower number of Bacteroides and Proteobacteria. In the Firmicutes group, there was an increase of *Tissierellaceae* and *Staphylococaceae,* and reduction of *Ruminococaceae*, *Lachnospiraceae and Faecalicabacterium,* which are lower in abundance also in e.g. inflammatory bowel diseases. They conclude that there seems to be a persistent dysbiosis in the gut of ALL survivors. (Chua et al. 2017).

4.3.3. Gut microbiota and chemotherapy complications

A few recent studies have focused on the connection between microbiota and infectious complications of cancer treatments on children. Nycz et al. (2018) compared the gut microbiota

between pediatric cancer patients, who received CDI or BSI with those patients who did not. According to them, alpha diversity was related to the development of BSI on patients, but not to CDI development or chemotherapy status. No taxa differences were related to the development of CDI, whereas patients who developed BSI had a lower count *of Bacteroides* and higher count of *Enterobacteriaceae* compared to those who did not develop BSI. (Nycz et al. 2018). Furthermore, Nycz et al. (2018) found a significant association between microbiota and cancer type: Microbiota of ALL patients differed substantially from those of AML and HSCT patients, which in turn differed from the microbiota of solid tumor patients. The greatest differences were noted in alpha-diversity and complexity, but not in the total bacterial count. (Nycz et al. 2018).

Nearing et al. (2019) observed a difference in the alpha diversity of microbiota in those pediatric ALL patients, who had an infectious complication (BSIs, GI infections or febrile neutropenia) during the first 6 months of chemotherapy, to those who did not. In general, the alpha diversity of the microbiota decreased as the chemotherapy was commenced. The baseline phylogenetic diversity did not predict the probability of infectious complications, but the phylogenetic distance, i.e. beta diversity, had a significant association to the occurrence of infections along with sex (all patients with infections were female), use of vancomycin, duration of the chemotherapy and use of antifungals. Lower phylogenetic diversity associated specifically to BSIs. As of phylas, *Bacteroidetes* were significantly increased and *Proteobacteria* significantly decreased in patients without infections, whereas *Proteobacteria* were increased in patients with infections. (Nearing et al. 2019) Hakim et al. (2018) ended up with a similar finding, associating the increased number of *Proteobacteria* to the higher risk of febrile neutropenia (Hakim et al. 2018). As of bacterial species, anti-inflammatory *Faecalibacterium prausnitzii* was found the be more numerous in patients without infections and even completely absent in patients with infections (Nearing et al. 2019). According to Hakim et al. (2018), the microbiota composition changes during chemotherapy, and increased abundance of Proteobacteria, *Streptococcus* species, and *Enterococcus* species, predicted subsequent infections (Hakim et al. 2018).

Also, Nearing et al. (2019) found a connection between a decrease of phylogenetic diversity and the use of vancomycin. However, this association was not found when any other antibiotics were used. All antibiotics except from piperacillin-tazobactam were highly connected to infectious complications, but the use of antibiotics did not seem to be related to the phylogenetic diversity. (Nearing et al. 2019).

Previous studies on the gut microbiota of childhood cancer survivors have included rather small cohorts, and more research is needed in terms of gut microbiota effects of both the malignancy and its treatment. Long-term consequences of cancer treatment on the young patients' gut microbiota need more investigation, and more knowledge is needed on the possible differences between distinct types and duration of cancer treatment on microbiota and the possible microbiota-

induced mechanisms causing complications, such as CDI and BSI. This knowledge can help prevent the complications and improve the further health of childhood cancer survivors.

Table 1: Summary of the gut microbiota implications of cancer diagnosis and treatment in pediatric patients

	Gut microbiota amount	Diversity of microbiota	Increased microbes	Decreased microbes	Effect of antibiotics	Complications and microbiota	Effect of probiotics/ prebiotics used
Prior to chemotherapy	Lower (29.6%) in patients compared to healthy controls (Huang et al. 2012) Lower compared to healthy controls (Van Vliet et al. 2009)	Lower alpha diversity compared to healthy controls (Chua et al. 2020) No differences in alpha diversity compared to healthy controls (Liu et al. 2020) Lower in patients compared to healthy controls (Bai et al. 2017) Lower in patients compared to healthy controls (Rajagopala et al. 2016)	More *Bacteroides* in patients compared to healthy controls (Liu et al. 2020) More *Bacteroides* in patients compared to healthy controls (Chua et al. 2020) More *Bacteroide*s in patients compared to healthy controls (Bai et al. 2017) More *Bacteroidales* and *Entero coccaceae* in patients compared to healthy controls (Bai et al. 2017) More *Bacteroides* in patients compared to healthy controls (Rajagopala et al. 2016)	Less *Edwardsiella tarda*, *Prevotella maculosa*, *Roseburia faecis* and *Fusobacterium naviforme* compared to healthy controls (Liu et al. 2020) Less *Firmicutes* and *Actinobacteria* compared to healthy controls (Chua et al. 2020) Less *Firmicutes* in patients compared to healthy controls (Bai et al. 2017) Less *Lachnospiraceae and Roseburia* in patients compared to healthy controls (Rajagopala et al. 2016) Less *Anaerostipes*, *Coprococcus*, *Roseburia*, and *Ruminococcus2* compared to healthy controls (Rajagopala et al. 2016)	Antibiotics have an adverse effect on alpha diversity, but do not affect beta diversity (Bai et al. 2017) Microbiota diversity of patients was significantly lower compared to healthy controls independent of antibiotic use in one-month period before the first stool samples (Rajagopala et al. 2016)	No significant differences in alpha diversity in patients with infectious complications and those with not (Nearing et al. 2019) *Burkholderiales* increased in patients with future infectious complications (Nearing et al. 2019) No significant differences in microbiota diversity in baseline samples with patients who developed febrile neutropenia and those who did not (Hakim et al. 2018)	

				Less *Bifidobacterium, Lactobacillus, E. Coli*, compared to healthy controls (Huang et al. 2012)			
During, or short-term (less than a year) after chemotherapy	Lower (26.26%) in patients compared to pre-chemotherapy and healthy controls (Huang et al. 2012) Lower in patients compared to healthy controls throughout the treatment but restored after 6 weeks of treatment. No difference in early and late samples	Lower alpha and beta diversity in patients developing infectious complications, alpha diversity decreased during the chemotherapy (Nearing et al. 2019) Diversity increased over the course of chemotherapy (Hakim et al. 2018) No significant difference between pre-chemotherapy situation and after the first dose of chemotherapy in patients, but increase over the course of chemotherapy (Rajagopala et al. 2016) Significantly lower in patients compared to healthy controls at the start of chemotherapy. Diversity was somewhat restored prior to another course of chemotherapy, but low similarity in	More *Bifidobacterium* compared to healthy controls up to 9 months post-chemotherapy (Chua et al. 2020). *Streptococcaceae, Clostridiaceae, Enterococcaceae, Lactobacillaceae,* and other *Firmicutes* increased after the chemotherapy compared to pre-chemotherapy situation (Hakim et al. 2018) More *Peptostreptococcaceae* in patients in chemotherapy compared to patients without chemotherapy (Nycz et al. 2018) More *E. Coli* after 7 days of chemotherapy in patients compared to the situation pre-	Relative decrease of *Bacteroides* during chemotherapy and lower number of Bacteroides, Atopobium, Fusobacterium, Prevotella, and Corynebacterium c ompared to healthy controls up to 9 months post chemotherapy. Chua et al. (2020) Less *Bacteroidetes, Actinobacteria, Faecalibacteria, Verrucomicrobia* and *Ruminococcaceae i*n post-chemotherapy samples compared to pre-chemotherapy situation (Hakim et al. 2018) Less *Streptococcus, Parabacteroides, Prevotella, Subdoligranulum,* compared to patients	No significant impact on diversity followed by antibiotics (Hakim et al. 2018) Significant decrease of bacterial phylogenetic diversity in those patients using vancomycin, all antibiotics except from piperacilling-tazobactam associated to infectious complications. (Nearing et al. 2019)	More *Bacteroides, Proteobacteria* and specifically *Faecalibacterium prausnitzii* on patients without infectious complications (Nearing et al. 2019) *Proteobacteria* increased in patients with infectious complications (Nearing et al. 2019) Patients with infectious complications have more gut microbe genome virulence factors	Not possible to define due to a small group using probiotics (Hakim et al. 2018) More *Enterobacteriaceae* in placebo group compared to a group using probiotics, both groups undergoing chemotherapy (Wada et al. 2010) No significant difference in facultative aerobes and anaerobes between patient groups using probiotics and placebo (Wada et al. 2010)

	(Van Vleit et al. 2009)	microbiota between early and late samples compared to healthy controls. Lowest diversity between chemotherapy cycles I and III, related to highest incidence of intestinal toxicity (Van Vliet et al. 2009)	chemotherapy (Huang et al. 2012) More *Enterococci* in patients during and after chemotherapy compared to healthy controls (Van Vliet et al. 2009) More Lactobacilli in the group receiving fructooligosaccharide supplement compared to placebo, both groups undergoing chemotherapy (Zheng et al. 2006)	without chemotherapy (Nycz et al. 2018) Less *Bifidobacterium, Lactobacillus (*after 3 & 7 days), *E. Coli* (after 3 days), compared to healthy controls (Huang et al. 2012) Less *Enterococcus* and *bacillus* in the group using probiotics compared to placebo group, both groups undergoing chemotherapy (Wada et al. 2010) Less *Bacteroides, Clostridium XIVa, Faecalibacterium and Bifidobacterium* during the chemotherapy, Bifidobacterium, Bacteroides and Streptococci still after 6 weeks compared to controls (Van Vliet et al. 2009)	Bacterial diversity differed in early and late samples despite of continuous use of prophylactic antibiotics, and did not differ depending on whether broad-spectrum antibiotics against febrile neutropenia were used (Van Vliet et al. 2009)	(Nearing et al. 2019) Post-infection samples of patients with infectious complications differed significantly from the samples of patients with no complications (Nearing et al. 2019) Incidence of C. difficile not associated to microbiota changes (Nycz et al. 2018) Bloodstream infections associated to lower microbiota diversity (Nycz et al. 2018) Proteobacteria increased in patients with an outburst of febrile	Less fevers and shorter duration of antibiotics in the probiotic group compared to group receiving placebo (Wada et al. 2010) Decreased inflammatory and nutritional index (PINI), higher hemoglobin and hematocrit and lower sedimentation rate in the group receiving fructo-oligosaccharide supplement compared to placebo, decreased morbidity and mortality index (Zheng et al. 2006) Less side effects in use of co-trimoxazole with Lactobacilli for neutropenia

						neutropenia, also increases risk of diarrhea and infections (Hakim et al. 2018) Dominance of Enterococcaceae and Streptococcaceae connected to risk of infections (Hakim et al. 2018)	compared to alternative regimen (Ekert et al. 1980)
Long-term (years) after chemotherapy		No statistically significant differences in alpha or beta diversity between survivors and healthy controls (Thomas et al. 2020) Lower alpha diversity compared to healthy controls (Chua et al. 2017) Survivors had less unique OTUs (operational taxonomic units) compared to controls, alpha-diversity differences not statistically significant (Cozen et al. 2013)	ALL survivors had slightly higher number of *Actinobacteria* compared to healthy controls. In the *Firmicutes* group, there was an increase of *Tissierellaceae* and *Staphylococcaceae* (Chua et al. 2017)	Slightly lower number of *Bacteroides* and *Proteobacteria*, reduction of *Ruminococcaceae, Lachnospiraceae* and *Faecalicabacterium* in the *Firmicutes* group (Chua et al. 2017) Decreased number of *Faecalibacterium* in the stool of ALL survivors after one or more years of chemotherapy compared to their healthy siblings (Thomas et al. 2020) Less *Actinobacteria collinsella* in patients			

				compared to healthy controls (Cozen et al. 2013)			

4.4. Effect of probiotics and prebiotics on dysbiosis in children with cancer

Fever and diarrhea are typical chemotherapy-induced complications. Fever can be the only sign of a possibly severe infection in patients who suffer from neutropenia caused by cytotoxic chemotherapy. Diarrhea can be caused by e.g. chemotherapy-induced mucositis, antibiotics or infectious diarrhea on a neutropenic patient. (Wada et al. 2010).

Only a few studies have investigated the use of prebiotics or probiotics in children with cancer: Ekert et al. (1980) studied children with leukemia or solid tumors and found that the combined use of Co-trimoxazole (trimethoprim and sulfamethoxazole) and *Lactobacilli* for neutropenia were better tolerated and had less side effects, such as vomiting and diarrhea, than framycetin, colymycin, nystatin, and metronidazole (Ekert et al. 1980).

Wada et al. (2010) investigated pediatric cancer patients going through chemotherapy randomized in two groups, one receiving probiotic *Bifidobacterium breve,* and one receiving placebo. According to their findings, the frequency and duration of fevers were less in the probiotic group than in the placebo group. Also, the duration of parenteric antibiotic therapy was shorter in the group using probiotics compared to the placebo group. The probiotic groups had lowering counts of *Enterococcus* (from 0 to 2 weeks) and Bacillus (from 2 to 5 weeks) post chemotherapy, whereas the *Enterobacteriaceae* counts of the Placebo group increased at 3 and 4 weeks. The concentrations of organic acids and short chain fatty acids (SCFA) were mostly maintained at an acceptable level only in the probiotic group, although both groups had a decrease in SCFAs during chemotherapy, and recovery afterwards. (Wada et al. 2010).

Zheng et al. (2006) examined the effect and tolerance of a prebiotic fructo-oligosaccharide (FOS)-containing enteral formula in pediatric patients with cancer going through chemotherapy. The FOS-group had a significantly more *Lactobacilli* on the day 30. Similar but less significant trend was noted in *Bifidobacteria*. There was no significant difference between the groups in *Enterobacteria* or *C. perfringens* at any time during the trial. The FOS group also showed decreased inflammatory and nutritional index (PINI) at day 30, had higher hemoglobin and hematocrit as well as lower sedimentation rate than the placebo group. No differences between the immunologic parameters of the groups were found during the study. According to the study, the use of FOS can decrease the morbidity and mortality index of the patients ($p<0.05$). (Zheng et al. 2006).

In a summary, the use of probiotic and prebiotic supplements has a potentially favorable effect on the gut microbiota of the pediatric cancer patients. However, also adverse effects of probiotic use have been reported in pediatric cancer patients suffering from febrile neutropenia, such as a Lactobacillus bacteremia in a 2-year-old girl (Chi-Wai Lee et al. 2011). There are also reports on probiotic-induced bacteremia in adult cancer patients (Redman et al. 2014). The existing research

on the topic is scarce, and more knowledge is required on the topic covering the potential risk of probiotic-induced infections or other adverse effects in cancer-suffering patients.

5. Conclusions

The impact of cancer treatments on the gut microbiota has been studied on children and adults, but the knowledge is still preliminary. **Table 1** summarizes the most relevant findings relating to pediatric patients.

There are several mechanisms by which the cancer treatments may have an adverse effect on gut microbiota and thus on the general health of the patient. Firstly, chemotherapy, radiotherapy and HSCT are associated with lower microbiota counts, reduced richness and disturbed relative abundance of bacteria (e.g. Johnson et al. 2004, Zwielehner et al. 2011, Huang et al. 2012, Van Vliet et al. 2009, Nam et al. 2013, Biagi et al. 2015).

Secondly, an increase of inflammation-inducing bacteria has been observed in the gut of chemotherapy treated mice. *Proteobacteria*, which has been linked to dysbiosis (Shin et al. 2015), increased significantly, while normal flora *Firmicutes* and *Bacteroidetes* decreased (Panebianco et al. 2018). Similar findings have been made in clinical studies (Montassier et al. 2015). An increase of infection-inducing *E. Coli* after chemotherapy has been observed in both clinical and preclinical studies (Stringer et al. 2009, Huang et al. 2012, Stringer et al. 2013), whereas the increase of *Enterococci* was observed in patients developing GvHD after HSCT (Holler et al. 2014).

Thirdly, cancer treatment has been shown to reduce bacteria, which are considered as health-supporting or probiotic (Weng and Walker 2013), such as *Bifidobacterium* and *Lactobacillus* in chemotherapy studies (Van Vliet et al. 2009, Zwielehner 2011, Huang et al. 2012, Stringer et al. 2013), *Faecalibacterium* and *Ruminococcus* in a HCST study (Biagi et al. 2015), and *Clostridia cluster XVIII* and *Faecalibacterium*, which have been linked to the protection from allergy and colitis, in a radiotherapy study (Wang et al. 2015). Because of the disturbed microbiota, several side effects of cancer treatments may occur, such as GI mucositis, *C. difficile* infections and bloodstream infections in chemotherapy treated patients (Nycz et al. 2018), diarrhea and fatigue after radiotherapy (Nam et al. 2013) and GvHD in HSCT patients (Holler et al. 2014, Biagi et al. 2015).

Fourthly, cancer treatment drugs may have metabolic interactions with gut microbiota, which makes some drug metabolites more toxic and harmful (Stringer et al. 2007, Stringer et al. 2008). Fifthly, they can also change the distribution of bacterial products; Wang et al. (2016) found that endotoxins produced by gut microbes were released into the circulation because of a damaged epithelium caused by doxorubicin, which results in systemic inflammation and damage in many organs (Wang et al. 2016). In addition, in immunotherapy, monoclonal antibodies have been

reported to impact on the genus level composition of gut microbiota, which increases the anticancer effects of the treatment (Vétizou et al. 2015).

It is also noteworthy that the gut microbiota variations between cancer patients and healthy individuals do not solely depend on cancer treatments, but the malignancy itself seems to influence the gut microbiota (Huang et al. 2012, Rajagopala et al. 2018, Hakim et al. 2018, Liu et al. 2020). The microbiota seems to recover after the cancer treatments (Cozen et al. 2013, Zama et al. 2017) and at least short-term side effects of cancer therapies may be alleviated by probiotics (Zheng et al. 2006, Wada et al. 2010). However, it has been assumed, that metabolic changes remain because of the long-term programming induced by gut microbiota changes (Cox et al. 2014). Therefore, it is important to conduct further studies on the impact of cancer treatments on the gut microbiota, particularly in the pediatric patients. If the links of gut microbiota changes to long-term adverse health implications are found, it is possible to develop more effective interventions and treatments to diminish or avoid the increased morbidity.

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