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PhD THESIS SUMMARY

PECULIARITIES OF THE INTESTINAL MICROBIOTA IN INFANTS AND TODDLERS

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CURRENT STATE OF KNOWLEDGE

CHAPTER I. Introduction

The study of the human intestinal microbiota has become a topic of interest in recent years due to its role in maintaining health and in the pathogenesis of many diseases. (1) The intestinal microbiota influences the development of the immune system, metabolism and numerous physiological processes of the host, having effects that go beyond the gastrointestinal tract. (1) In pediatrics, the first 1000 days of life represent a critical window for the growth and development of the child; during this period, the intestinal microbiota is formed and stabilized, its balance having an important role in the determinism of health or disease. (2) An optimal intestinal microbial profile in the early years is associated with harmonious development, while the imbalance of microbial composition can predispose to various pathologies both in childhood and adulthood. (2)

CHAPTER II. Intestinal microbiota in the first 1000 days of life

In the early stages of life, the colonization of the gastrointestinal tract of the newborn is a dynamic and complex process, influenced by perinatal factors such as the type of birth and nutrition. (3) Natural nutrition favors the development of a beneficial microbiota, breast milk providing prebiotics and microorganisms with a protective effect, which promotes colonization with bifidobacteria and other commensal bacteria, contributing to the maturation of the immune system. (3) On the other hand, early exposure to antibiotics can disrupt the microbial balance, leading to intestinal dysbiosis and increased susceptibility to conditions such as allergies, metabolic or functional gastrointestinal disorders. (4)

The initial colonization of the intestine begins at birth and progresses rapidly; in just a few days after birth, the density of intestinal microorganisms reaches levels of 10^{10} per gram of feces. (5) In this early phase, facultative anaerobic bacteria, such as *Enterobacteriaceae*, predominate, which consume oxygen and create a favorable environment for the subsequent installation of beneficial microorganisms. (5) Subsequently, as the infant grows, the composition of the microbiota is dynamic, bifidobacteria become dominant in the first weeks, and with the

diversification of the diet, anaerobic bacterial families such as *Bacteroidaceae* appear in increasing numbers. (5) By the age of three years, the child's intestinal microbiota reaches a structure and diversity comparable to those of the adult. (6) This evolution underlines the importance of understanding the characteristics and variability of the microbiota in the window of the first 1000 days of life, a necessary foundation for the development of prophylactic and therapeutic interventions against pathologies associated with dysbiosis.

CHAPTER III. Environmental factors and the impact on the intestinal microbiota in the first 1000 days of life

External factors have a major influence on the composition of the infant's intestinal microbiota in the first months of life, a determining factor being the type of birth. (7) Infants born naturally acquire a microbiota dominated by bacteria from the maternal vaginal flora, such as *Lactobacillus*, while infants born by caesarean section show delayed and different colonization, the intestinal microbiota being predominantly composed of microorganisms of skin and environmental origin, such as *Staphylococcus* and *Corynebacterium*.(7-11) Thus, newborns by caesarean section frequently have lower microbial diversity and low levels of beneficial bacteria such as *Bacteroides* and *Bifidobacterium*, accompanied by a relatively high abundance of opportunistic bacteria, such as *Clostridium* species. (2,12,13) It has been observed that this colonization associated with cesarean delivery correlates with an increased risk of developing allergies and asthma in childhood, compared to children born naturally. (2.12)

The administration of antibiotics in the first months of life is another factor with a significant impact on intestinal microbiota. (14,15) Early exposure to antibiotics leads to a significant decrease in gut microbial diversity, accompanied by an increased abundance of bacteria in the phylum *Proteobacteria*, such as *Enterobacteriaceae*, and a reduction in populations of beneficial *Actinobacteria*, in particular *Bifidobacterium*. (14,15) Such an induced microbial imbalance may disrupt the normal modulation of the host's immune system and has been associated with an increased incidence of allergies, metabolic or inflammatory conditions. (7.16)

Diet also plays an important role in shaping the gut microbiota. The moment of dietary diversification, with the introduction of solids, causes significant changes in the intestinal microbial ecosystem, and with the transition from exclusively dairy to mixed food, there is a

decrease in the predominance of bifidobacteria and an increase in microbial diversity, including increases in bacterial groups such as *Bacteroidaceae*, *Lachnospiraceae* and *Ruminococcaceae.*(5.17)

CHAPTER IV. Composition of the intestinal microbiota and its implications in infants and toddlers allergies

The early gut microbiota plays an essential role in modulating the host's immune system, influencing susceptibility to allergic diseases in childhood. (18) Atopic dermatitis, one of the most common allergic diseases in infants, with a prevalence of more than 20% in children in developed countries, has been correlated with peculiarities of the intestinal microbiota. (18) Studies have shown that infants with atopic dermatitis have a reduced diversity of the gut microbiota, this decrease in biodiversity being associated with an increased severity of skin manifestations. (18) The hypothesis of the existence of a relationship between the gut-skin axis, according to which intestinal dysbiosis can contribute to the chronic inflammation of the skin characteristic of atopic dermatitis, has been outlined. (18,19) In support of this hypothesis, recent research has shown that pediatric patients with atopic dermatitis have specific changes in the composition of the intestinal flora, with a significant decrease in beneficial genera such as *Lactobacillus* and *Bifidobacterium*, while overcolonising opportunistic bacteria, such as *Escherichia coli* and *Clostridioides difficile*. (19,20)

In the case of allergies, the importance of the composition of the early intestinal microbiota has been demonstrated, meta-analyses highlighting the fact that infants and young children with allergies have a significantly lower intestinal microbial biodiversity, along with taxonomic profile differences, with an increased abundance of *Firmicutes* bacteria and the *Bacteroidaceae* family. (18,21) The occurrence of atopic dermatitis, asthma and food allergies has been associated with changes in the structure of the intestinal microbiota in the first years of life. (18,21,22) An important factor with a protective role against the development of allergies is natural nutrition, exclusive breastfeeding with breast milk, through the beneficial effects of breast milk on the infant's microbiota, being associated with a significantly lower risk of food allergies in children. (23) Overall, intestinal dysbiosis is increasingly incriminated as a contributing factor to the

increase in the incidence of pediatric allergies, although the exact causal relationship and mechanisms involved still remain incompletely elucidated. (18) Imbalance of the gut microbiota in the early years can negatively influence the maturation and regulation of the immune system, favoring an allergic response. (18) Certain commensal immunomodulatory bacteria, such as lactobacilli and bifidobacteria, are essential for the development of immunological tolerance; reducing the number of these beneficial microorganisms can favor the emergence of a pro-inflammatory environment that promotes allergic sensitization, through pro-inflammatory interleukins. (18,24)

CHAPTER V. Functional gastrointestinal disorders and intestinal microbiota in the first 3 years of life

Functional gastrointestinal disorders constitute a group of common conditions in pediatrics, characterized by chronic or recurrent digestive symptoms in the absence of identifiable organic abnormalities, according to the Rome IV criteria. (25) In the first months of life, up to half of infants may experience at least one episode of functional gastrointestinal disorder, the most common being infantile colic, gastroesophageal reflux and constipation. (25,26) The etiology of functional gastrointestinal disorders is recognized as multifactorial, including dietary factors, type of diet (breast milk or formula) and diversification, gastrointestinal motility and visceral sensitivity, as well as the composition of the gut microbiota. (26)

Infantile colic is manifested by paroxysmal episodes of intense crying and agitation in the first months of life. (27,28) Some studies have shown the association of colic with an imbalanced gut microbial profile; infants with colic often have reduced levels of beneficial bacteria, especially *Bifidobacterium* and *Lactobacillus*, and an increased abundance of potentially pathogenic coliform bacteria, such as *Escherichia coli* and *Klebsiella.*(26,27) In line with this hypothesis, the administration of specific probiotics, such as *Lactobacillus reuteri,* has been reported to have beneficial effects, reducing the duration and severity of crying episodes in infants with colic.(27) Thus, interventions aimed at microbiota balance can represent a therapeutic option in the management of infantile colic, the profile of the intestinal microbiota being important for the personalized therapeutic strategy.

Gastroesophageal reflux in infants is another common functional disorder, manifested by regurgitation of gastric content. (28,29) About 40% of infants experience recurrent regurgitation in the first 3–4 months, but the frequency of reflux episodes decreases considerably by the age of one year.(28,29) In addition to the mechanical factors involved (incomplete maturation of anti-reflux mechanisms), recent evidence suggests that the gut microbiota also plays an important role in the pathogenesis of reflux: abnormal intestinal colonization could contribute to the occurrence and severity of gastroesophageal reflux. (29) Research has indicated that oral supplementation with certain probiotic microorganisms according to the gut microbiota profile significantly reduces regurgitation episodes in infants and improves gastric emptying time by decreasing inflammation and modulating intestinal motility. (29,30,31) They emphasize the link between gut microbiota and reflux, paving the way for new therapeutic approaches to alleviate reflux symptoms in young children.

Functional constipation in the first years of life is also common, with an estimated prevalence of up to 30% in the pediatric population. (28,31,32) This condition is defined by infrequent and high-consistency stools in the absence of an organic cause, and is often aggravated by dietary factors such as insufficient fiber intake, behavioral or emotional problems. (31,32) Studies suggest that imbalances in the gut microbiota may contribute to the pathogenesis of chronic constipation in children. (32,33) Analyses of the composition of the intestinal microbiota have shown significant differences in children with constipation, with increased concentrations of bacteria in the phylum *Firmicutes*, including *Clostridium species*, concomitantly with a decrease in the level of *Bacteroidetes*.(33) They suggest the existence of dysbiosis associated with constipation, which could affect intestinal motility and stool consistency, but the precise role of the microbiota in pediatric functional constipation remains insufficiently clear, with some studies reporting conflicting results. Currently, the use of therapeutic interventions to modify the intestinal flora in constipation in children is being explored, aiming to improve intestinal transit and reduce symptoms. (32,33)

Functional diarrhea in children involves the frequent elimination of soft stools, which can be associated with changes in the intestinal microbiota. (34) Intestinal dysbiosis in children with diarrheal stools has been characterized by an increase in potentially pathogenic bacteria, such as *Escherichia coli*, enterococci, and a decrease in beneficial microorganisms, such as bifidobacteria and lactobacilli, being associated with inflammation of the intestine, mediated by proinflammatory

cytokines.(35,36) Oral supplementation with microorganisms such as *Lactobacillus* reduced diarrheal episodes by restoring the balance of the intestinal microbiota, decreasing intestinal inflammation and improving the barrier function of the intestinal epithelium.(37) However, the implications of the intestinal microbiota in functional diarrhea in children remain partially unelucidated, requiring further studies.

PERSONAL CONTRIBUTION

CHAPTER VI. Arguments and objectives of the research

At birth and in the first years of life, the colonization of the gastrointestinal tract forms the intestinal microbiota, with an impact on the immune system and the determinism of numerous pathologies in the first years of life. (7,38) The infant and toddler period, representing the first 1000 days of life, is a critical window for stabilizing the gut microbiota, its composition having dynamics and variability that significantly influences health status both in the short and long term. (6, 7, 38) Perinatal and environmental factors can modulate the microbial profile during this time window, such as birth type, diet type, antibiotic exposure, and other environmental factors. (7,38,39)

In this context, the research conducted analyzed the composition of the intestinal microbiota, in the first 3 years of life, in Dobrogea, in the south-eastern area of Romania, identifying the risk factors of intestinal dysbiosis and evaluating its implications in the common pathology of toddlers and infants, such as atopic dermatitis and functional gastrointestinal disorders. Also, the relationship between dysbiosis and intestinal inflammation was analyzed by determining fecal calprotectin and association with the intestinal flora index to establish the severity of dysbiosis and association with clinical manifestations.

The research included clinical studies, performed on pediatric patients, aged 0-3 years, from the south-eastern area of Romania (Dobrogea). Retrospective and prospective studies have been conducted, approved by Ethics Commission of the Faculty of Medicine, Ovidius University of Constanta. For the evaluation of the intestinal microbiota, samples were collected from feces, bacteriologically analyzed by cultures on selective media, following proteolytic bacteria, some of the Enterobacteriaceae family, such as *Escherichia coli, Proteus, Klebsiella, Enterobacter,* along with *Hafnia alvei, Serratia, Providencia, Morganella morganii, Kluyvera, Citrobacter,*

Pseudomonas and Clostridium, acidifying bacteria such as *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Bacteroides* and fungal species such as *Candida* and *Geotrichum*. The intestinal flora index was analyzed to evaluate the degree of dysbiosis and clinical manifestations, correlated with fecal calprotectin, a non-invasive marker of intestinal inflammation. Clinical and laboratory data were statistically analyzed, significantly considering the p-value below 0.05.

CHAPTER VII. Clinical study – Gut microbiota and dysbiosis risk factors in the first three years of life

The retrospective study, conducted between 2020 and 2022, included 82 children aged 0 to 36 months, diagnosed with intestinal dysbiosis, to identify factors influencing intestinal dysbiosis. The study showed that the gender distribution was approximately equal (48% male, 52% female), suggesting that the risk of dysbiosis is similar. Age significantly influenced the prevalence of intestinal dysbiosis, 63% of cases were in the 0-12 months group, compared to 37% at 1-3 years, indicating the higher susceptibility of infants. This predominance at young ages can be explained by the food transition around the age of 6 months, when the introduction of solid foods produces major changes in the composition of the microbiota. (38,40-42) Other studies have shown that dietary diversification decreases the abundance of beneficial bacteria, such as *Bifidobacterium*, and increases microbial diversity, favoring colonization with *Bacteroides.*(41,42)

Another significant factor associated with dysbiosis observed in the study was prematurity, with 70% of infants being born prematurely, this category frequently having an unbalanced microbiota, with reduced diversity and a deficiency of bifidobacteria, according to the literature. (43,44) Also, the type of birth influenced the composition of the intestinal microbiota, 72% of the children in the studied group being born by cesarean section, this high proportion being statistically correlated with intestinal dysbiosis ($p = 0.035$). Caesarean section deprives the newborn of exposure to maternal vaginal flora, which favors intestinal colonization with potentially pathogenic bacteria to the detriment of beneficial ones. (45) The literature confirms that caesarean section delivery is associated with the risk of intestinal dysbiosis, characterized by low abundance of *Bacteroidetes* and bifidobacteria and increased levels of *Firmicutes* in the first months of life, contributing to an increased risk of allergies and atopy. (46)

Another important factor that the study observed is nutrition in the first year: in the group studied, most infants (53%) were artificially fed, with milk formula, only 25% with breast milk and 22% mixed with milk formula and breast milk. Formula feeding in the first year was associated with more frequent dysbiosis, confirming the data that formula-fed infants have reduced microbial diversity and an increased load of potentially pathogenic bacteria, compared to those fed with breast milk. (47) Breast milk contains prebiotic factors that support the proliferation of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, which explain the predisposition to dysbiosis in formula-fed infants.(42,47) Antibiotic exposure was also common in the history of infants with dysbiosis (51% of cases received at least one antibiotic in the first year). In my study, I observed that antibiotic therapy, especially with cephalosporins or aminopenicillins, often preceded intestinal dysbiosis, with antibiotics causing a decrease in microbial diversity with a protective role such as bifidobacteria and the excessive proliferation of opportunistic genera, such as *Enterobacter* and *Enterococcus*, described in the literature. (48,49) In the group analyzed in my study, the weight status of infants with dysbiosis was predominantly eutrophic (54%), but in the 1-3 year age group it was observed an association between dysbiosis and overweight in 23% of cases, the literature suggesting the implications of the intestinal microbiota in the functions of metabolism and the risk of obesity. (50)

Clinically, intestinal dysbiosis in infants was manifested by a variety of gastrointestinal symptoms such as diarrheal stools or, on the contrary, constipation, the presence of mucus in the stool, abdominal pain and abdominal meteorism, and the severity of intestinal dysbiosis, assessed by the intestinal flora index, correlated with the intensity of these symptoms. In the study, 42% of the infants had moderate dysbiosis, with an intestinal flora index between 6-12, and 11% severe dysbiosis, with an average fecal pH value of 6.5, indicating a reduction in intestinal acidity. It was observed in the study that there was a significant increase in the concentration of the proteolytic bacteria *Escherichia coli*, *Klebsiella* and *Enterobacter,* and a decrease in the acidifying bacteria *Lactobacillus* and *Bifidobacterium* in children with severe dysbiosis, these changes being accompanied by alkalization of the intestinal environment.

Digestive symptoms such as diarrheal stools, constipation, abdominal bloating, and abdominal pain were more pronounced in patients with severe dysbiosis. One feature observed in my study was the association between dysbiosis and cow's milk protein allergy, 68% of infants with food allergy experienced dysbiosis, suggestive of acidic fecal pH and overpopulation with

proteolytic bacteria. This association is supported by studies that have shown in children with cow's milk protein allergy changes in the intestinal flora and impairment of the intestinal barrier. (51,52)

In conclusion, the study highlighted that perinatal factors such as prematurity, cesarean delivery, type of diet and early use of antibiotics play an important role in intestinal dysbiosis in infants. Intestinal dysbiosis in the early years manifests through varied digestive symptoms and can coexist with conditions such as food allergies, potentially impacting growth and development.

CHAPTER VIII. Clinical study – Gut Microbiota Profile and Atopic Dermatitis in the First Year of Life

The prospective study was conducted between April 2023 and May 2024, and analyzed the composition of the intestinal microbiota in infants with atopic dermatitis compared to healthy infants. The group included 121 cases aged between 1 month and 12 months, of which 91 were diagnosed with atopic dermatitis and 30 cases without atopic dermatitis. The inclusion criteria were the absence of acute infections or other chronic diseases and the lack of antibiotic or probiotic treatments in the last 4 weeks. Fecal samples were analyzed by bacteriological culture methods, quantifying proteolytic, acidifying and fungal bacterial species.

The general characteristics of the two groups showed notable differences: the percentage of infants fed naturally was lower in the group with atopic dermatitis than in controls, and the frequency of cesarean births was slightly higher in infants with atopic dermatitis. These observations suggest that factors that predispose to dysbiosis, such as cesarean section and artificial nutrition, could also contribute to the risk of atopic dermatitis, observations mentioned in the literature. (53,54)

Analysis of the composition of the intestinal microbiota showed significant differences between infants with atopic dermatitis and the control group; the group with atopic dermatitis showed an increased abundance of the phylum *Proteobacteria*, mainly the gram-negative *Enterobacteriaceae* family, facultative anaerobic and relatively low values for *Firmicutes* and *Actinobacteria*. Also, the study highlighted that infants with atopic dermatitis had significantly higher concentrations of proteolytic bacteria such as *Escherichia coli*, *Enterobacter* and *Klebsiella* ($p < 0.05$) and a significant increase in the genus *Bacteroides* ($p = 0.036$). On the other hand, the

levels of beneficial acidifying bacteria were significantly reduced in infants with atopic dermatitis: *Bifidobacterium*, *Lactobacillus* and *Enterococcus* showed significantly lower concentrations than healthy infants, with significant differences ($p < 0.05$). The study did not identify notable differences in commensal fungal flora (*Candida* spp., *Geotrichum* spp.), these microorganisms being present within normal limits in both groups.

The microbial profile that was observed in infants with atopic dermatitis highlighted a dysbiosis characterized by a deficiency of protective bacteria and overpopulated with opportunistic bacteria. The results obtained are consistent with some studies in the literature, which reported in children with atopic dermatitis a decrease in beneficial bacteria (bifidobacteria, lactobacilli) and an increase in some proteobacteria. (20) Other research has found correlations between atopic dermatitis and increased concentrations of *Bacteroides* and *Enterobacteriaceae*, supporting the hypothesis that certain microbial species such as *Bacteroides* spp. may contribute to the pathogenic mechanisms of atopy by producing proinflammatory lipopolysaccharides and affecting intestinal permeability.(53,55) In my study, microbial differences seen in infants with atopic dermatitis suggest that gut dysbiosis plays a role in this allergic condition. The absence of key beneficial microbial populations, such as bifidobacteria, in the first months of life could influence the development of the immune system, predisposing to atopy. Consequently, microbiota modulation interventions, by administering probiotic microorganisms depending on the microbiota profile, mentioned by other studies, could represent promising preventive or therapeutic strategies for atopic dermatitis. (56,57)

In conclusion, the study makes an original contribution by describing the microbial profile in infants with atopic dermatitis in the pediatric population of Dobrogea, in southeastern Romania and confirms the need for further research on the microbiota-host relationship in atopic pathology.

CHAPTER IX. Clinical Study – Gut Microbiota Profile and Functional Gastrointestinal Disorders in Infants

Functional gastrointestinal disorders include conditions such as abdominal colic, gastroesophageal reflux, functional constipation or functional diarrhea, in the absence of an organic cause. (58) In view of the hypothesis that gut microbiota might influence the pathophysiology of functional gastrointestinal disorders, I analyzed the gut microbial profile in

infants with such gastrointestinal disorders compared to healthy infants. (58) The study was prospective, conducted between April and September 2024 and included 134 infants aged 1 to 12 months, of whom 82 infants had functional gastrointestinal disorders diagnosed according to the Rome IV criteria and 52 infants were the control group, without functional gastrointestinal disorders. (59) The group with functional gastrointestinal disorders was subdivided into 4 subgroups: 23 infants with infantile colic, 21 cases with functional constipation, 20 cases with gastroesophageal reflux and 18 cases with functional diarrhea.

In the study, no significant differences were observed between the group with functional gastrointestinal disorders and the control group in terms of age, gestational age, birth weight or prematurity, but differences were observed in nutrition, with only 25.6% of infants with functional gastrointestinal disorders being breastfed, compared to 53.8% in the group without functional gastrointestinal disorders. Most infants were artificially fed, with low levels of acidifying flora for the species *Lactobacillus, Bifidobacterium, Bacteroides* and increased levels of proteolytic microorganisms such as *Escherichia coli* and *Klebsiella,* consistent with data in the literature.(60,61) In the study, the analysis of the gut microbiota in infants with functional gastrointestinal disorders revealed a profile characterized by a decrease in acidifying bacteria and an increase in opportunistic bacteria, compared to healthy infants.

Infants with abdominal colic showed a significant reduction in *Lactobacillus*, *Bifidobacterium*, *Bacteroides* and *Enterococcus* populations compared to the control group, concomitantly with increased levels of *Escherichia coli* and *Klebsiella*. The intestinal flora index in these infants predominantly indicated moderate dysbiosis. This feature, with a high abundance of proteobacteria and a deficiency of beneficial bacteria, has been reported by other studies. (62) The study revealed a significant association between *Escherichia coli* overpopulation and colic ($p = 0.037$), suggesting that this microorganism could contribute to colic symptomatology.

In infants with constipation, the study found lower levels of *Lactobacillus*, *Enterococcus* and *Bacteroides*, compared to those without constipation, and significant increases in *Escherichia coli* and *Klebsiella*. The dysbiosis index indicated moderate dysbiosis. These results suggest that moderate dysbiosis, with a reduction in bacteria that stimulate intestinal motility, such as lactobacilli, may be involved in functional constipation in the infants.

In infants with gastroesophageal reflux, it was observed a microbial profile characterized by a significant decrease in *Lactobacillus* and *Bacteroides*, along with increases in *Enterococcus*,

Escherichia coli and *Klebsiella*, with a moderate form of intestinal dysbiosis. Particular was the growth of *Enterococcus*, compared to the control group.

Infants with functional diarrhea had the most important changes, with important increases in *Escherichia coli*, *Enterobacter* and *Klebsiella*, concomitantly with decreases *in Bacteroides*, *Lactobacillus* and *Enterococcus*. In this subgroup the intestinal flora index indicated moderate dysbiosis.

In all cases, the species *Proteus*, *Pseudomonas*, *Serratia*, *Citrobacter*, *Clostridium* and fungi *Candida*, *Geotrichum* remained within normal values, with no differences between infants with functional gastrointestinal disorders and those without functional gastrointestinal disorders. Overall, the study showed that functional gastrointestinal disorders in infants are associated with some degree of intestinal dysbiosis. Abdominal colic, constipation, gastroesophageal reflux and functional diarrhea have in common a reduction in the protective flora represented by bifidobacteria and lactobacilli, and a relatively increased presence of *Enterobacteriaceae* (*Escherichia coli* and *Klebsiella*). According to the literature, infantile colic is characterized by a low-diversity microbiota, dominated by proteobacteria and with low levels of lactobacilli and bifidobacteria, and the administration of probiotics has shown beneficial effects in reducing symptoms. (62,63) In this research, the moderate dysbiosis found in many infants with colic suggests specific microbial differences, such as *Escherichia coli* overpopulation, rather than extensive dysbiosis. Similarly, in the case of agitation episodes it was not found significant microbial difference compared to infants without agitation episodes.

In conclusion, the study confirms a potential role of the gut microbiota in functional gastrointestinal disorders in infants and suggests that changes in specific species are involved rather than extensive microbiota imbalances.

CHAPTER X. Clinical study – Fecal Calprotectin and Clinical Manifestations of Intestinal Dysbiosis in Toddlers

The prospective study was conducted on 129 children, aged 1-3 years, which analyzed the relationship between intestinal dysbiosis and intestinal inflammation, determining fecal calprotectin, a non-invasive marker of intestinal inflammation. They were divided into 2 groups: group I with clinical manifestations of intestinal dysbiosis and group II without clinical

manifestations. It was analyzed clinical manifestations, such as abdominal colic, episodes of agitation, abdominal meteorism, constipation, the presence of mucus in the feces, changes in the color of the stool (green stools), watery stools, stools with blood or undigested food, as well as stools with a fetid odor. These manifestations were analyzed in relation to the profile of the intestinal microbiota and the level of fecal calprotectin. In the study, the average values of fecal calprotectin were within the normal range for this age group. It was compared the values according to the presence of clinical manifestations:

- Abdominal colic: In cases with abdominal colic, it was not observed a significant increase in fecal calprotectin compared to children without colic. It was observed the association of colic with a particular microbial profile, characterized by overpopulation with *Escherichia coli* and low concentrations of lactobacilli.
- Episodes of agitation: The study did not find significant differences in flora between children with frequent episodes of agitation and those without agitation, nor different values of fecal calprotectin. The initial hypothesis that dysbiosis could cause agitation has not been confirmed.
- Abdominal meteorism: Although some children with meteorism experienced slight increases in *Klebsiella* and reduction of *Enterococcus* in the intestinal flora, these variations were not significant, and calprotectin showed no differences between those with and without meteorism.
- Constipation: The microbial composition did not differ significantly from that of children without constipation, with only a tendency to reduce lactobacilli. Fecal calprotectin levels were similar, suggesting that functional forms of constipation are not associated with intestinal inflammation.
- Mucus in feces: The presence of mucus in feces has been correlated with severe forms of intestinal dysbiosis and overpopulation of *Enterobacteriaceae*. Fecal calprotectin has been increased in these cases.
- Green-colored stools: It was observed a significant association between green-colored stools and severe intestinal dysbiosis, with an intestinal microbiota profile dominated by opportunistic proteobacteria and low concentrations of beneficial bacteria. However, fecal calprotectin levels were not significantly different from children without green stools,

indicating that this clinical manifestation reflects an imbalance of the gut microbiota rather than intestinal inflammation.

- Watery stools: The presence of watery stools has been associated with important changes in the intestinal microbiota, especially with moderate or severe forms of intestinal dysbiosis and the increase of *Enterobacteriaceae*, confirming the link between dysbiosis and diarrheal stools. However, the average level of fecal calprotectin did not differ significantly between children with watery stools and those without watery stools. Analysis of the distribution of values showed great variability, suggesting that episodes of transient watery stools do not involve persistent intestinal inflammation.
- Bloody stools: It was noticed in the study that there was a tendency for higher levels of fecal calprotectin in children with bloody stools, without the difference reaching the threshold of statistical significance. This observation, however, raises the suspicion that episodes of hemorrhagic colitis in young children could involve intestinal dysbiosis associated with inflammation.
- Undigested food stools and foul-smelling stools: In the study it was not observed significant correlation between undigested food stools, foul-smelling stools, intestinal dysbiosis, and fecal calprotectin.

In conclusion, the study showed statistically significant correlations between the degree of intestinal dysbiosis, measured by the intestinal flora index, and the level of fecal calprotectin. Children with severe intestinal dysbiosis tend to have higher values of calprotectin, which supports the hypothesis that microbiota imbalance may be associated with an inflammatory bowel status. Overall, fecal calprotectin values in toddlers show high individual variability and may remain within normal limits even in the presence of dysbiosis or symptoms, indicating the need for careful interpretation of this biomarker in a clinical and microbiological context.

CHAPTER XI. Conclusions of the research and elements of originality

The research conducted, through the studies, highlights the importance of factors such as cesarean delivery, artificial nutrition and early exposure to antibiotics, involved in intestinal dysbiosis in the first three years of life. The research showed that the moment of dietary

diversification is a critical point, and dietary changes can disrupt the balance of the intestinal flora. Intestinal dysbiosis in the first year of life is clinically manifested by gastrointestinal symptoms such as diarrheal stools, stools with mucus, green stools, which reflect the severity of the microbial imbalance.

In infants with atopic dermatitis the research showed a particular microbial profile, with overpopulation of proteolytic bacteria, such as *Escherichia coli*, *Klebsiella*, *Enterobacter* and deficiency of beneficial flora such as *Lactobacillus* and *Bifidobacterium*.

In infants with functional gastrointestinal disorders, the research showed a reduced colonization of acidifying species (*Lactobacillus*, *Bifidobacterium*, *Enterococcus*) and an increase in *Enterobacteriaceae* (*Klebsiella*, *Escherichia coli*), which indicates the possible involvement of these microorganisms in the appearance of functional digestive symptoms. However, detailed analyses suggest that, for some manifestations such as abdominal colic, the microbial differences are relatively specific (excess of *Escherichia coli*) and do not involve the entire intestinal ecosystem. An association between intestinal dysbiosis and infant agitation episodes has not been confirmed. In contrast, the presence of mucus in the stool or green stools in toddlers has been correlated with marked microbial imbalances, which may indicate severe forms of intestinal dysbiosis. Regarding the relationship with intestinal inflammation, the study revealed that most children with intestinal dysbiosis do not show a significant inflammatory response, calprotectin presenting normal values, confirming the observations that fecal calprotectin values can vary widely at this age, without the presence of an organic pathology. However, the statistical association between an increased dysbiosis index and higher calprotectin values supports the existence of a link between microbiota imbalance and intestinal inflammation. This characteristic emphasizes the importance of maintaining an eubiotic intestinal microbiota for the prevention of inflammation.

The results obtained in the pediatric population of Dobrogea complement the limited studies so far on the characteristics of the intestinal microbiota of children in this geographical region of Romania and may have practical applications in guiding personalized medicine strategies. Children with risk factors for intestinal dysbiosis could benefit from monitoring and early prophylactic and therapeutic interventions to modulate the composition of the microbiota. Similarly, the identification of dysbiosis in infants with atopic dermatitis or gastrointestinal disorders could lead to targeted therapies on the gut microbiota. In conclusion, the research

presented brings new evidence on the role of intestinal microbiota in the first years of life, and highlights the need to integrate the evaluation of the intestinal microbiota in pediatric practice, opening perspectives for personalized therapeutic interventions.

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