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PH.D THESIS

"Functional Gastrointestinal Disorders in Children: New insights in Pathogenesis, Prevalence, Clinical and Therapeutic Management".

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Chapter 1

-Background and Aims of the Study Project-

Functional gastrointestinal disorders (FGIDs) are defined as a combination of chronic or recurrent symptoms interesting the gastrointestinal tract, that cannot be explained by structural or biochemical abnormalities (1,2).

One of the difficulties for clinicians in dealing with FGIDs is the lack of biochemical or structural markers that can be used to diagnose or monitor progression of these disorders.

In children, FGDI are currently diagnosed according to symptom-based Rome criteria. The Rome criteria find their origins in 1990, when a group of gastroenterology experts created a classification system with diagnostic criteria for FGIDs in adults. In 1999, with the first update, specific standardized criteria were established for FGIDs in children (3). They were revised in 2006, with the introduction of a distinction between FGIDs in younger children (neonate/toddler) and older children (child/adolescent) (4,5), and recently, in 2016 (1,2). The complete Rome IV criteria are provided in Table 1 and 2.

FGIDs are very common in children of all ages. Recently, several studies attempted to evaluate the epidemiology of DFGI in Europe and US. Kortenik et al. reported that worldwide prevalence of Pediatric Functional Abdominal Pain Disorders was 13.5% (6), while van Tilburg et al showed that 27% of infants/toddler group (7) and 23.1% of children/adolescent group qualified for FGIDs (8). In 2018, a large study investigating prevalence of FGIDs was conducted in nine countries in the Mediterranean Region, showing that the overall prevalence in the group from 4 to 10 years was 20.7%, while in the group from 11 to 18 years was 26.6%. However, significant differences in the prevalence of specific disorders were found among different countries (9).

Symptoms related to FGIDs have an high impact on families and patient's quality of life, healthcare utilization and related costs (10,11). The advantage of using the new Rome IV criteria is that they allow a "positive" approach to the

patient, avoiding unnecessary tests to rule out an organic cause, with beneficial effect on patient's health and costs. However, a recent study from Hoekman et al. showed that FGIDs, and in particular functional abdominal pain disorders, still represent a huge economic burden for the society with an estimate annual cost/patient of $2.512, with half of the costs consisting of inpatient/outpatient healthcare use, and one-fourth of the costs related to parental productivity loss (12).These data suggest that, in spite of the Rome criteria, diagnostic approach to FGIDs still varies widely among different countries, due to several factors, including cultural customs. Currently, in many countries the diagnosis of FGDIs is still considered an exclusion diagnosis, which come after a lot of investigations and medical visits, with high burden in terms of patient's anxiety and healthcare costs.

FGDIs are multifactorial conditions and several pathophysiological mechanisms appear to contribute to their onset. Nowadays, the most important pathophysiological hypothesis is the "biopsychosocial model". Early in life, genetics, sociocultural influences, and environmental factors may affect one's psychosocial development and influence the susceptibility to gut dysfunction (abnormal motility or sensitivity, altered mucosal, immune dysfunction or inflammation), via the gut-brain axis. Visceral hyperalgesia is shown as the final outcome of sensitizing medical factors (infections, allergies), motility disorders and anxiety or mood disorders, that are superimposed on a background of genetic predisposition and early life events (1,13).

Recently, the interest regarding the role of the gut microbiota in health and disease has increased. Changes in the gut microbiota have been demonstrated in several organic and functional disorders.

For these reasons, it is not surprising that the gut microbiome has been implicated in pathogenesis of functional gastrointestinal disorders (FGIDs), given its role in modulating physiological processes such as immune development, gastrointestinal motility and secretion, epithelial barrier integrity, and brain-gut communication (14). This has led to an increased interest in ways to alter the gut microbiota and in the therapeutic value of prebiotics, probiotics and synbiotics. Currently, there is a limited amount of published data available for their use in pediatric FGIDs. The evidence

suggests that specific probiotic strains may be beneficial in children with Functional Abdominal Pain Disorders (FAPDs) (15), but for other FGIDs such as infant colic and functional constipations the evidence is still ambiguous (16,17).
A better understanding of the role of the gut microbiota in FGIDs is required in order to develop novel tailored therapeutic approaches aimed at modulating the gut microbiota that may benefit patients with FGIDs.

Given these premises, the main aims of the present Ph.D. thesis were:

1. To define the prevalence of FGIDs
2. To investigate physiopathology of FGID;
3. To evaluate the current diagnostic-therapeutic approach to FGDIs, focusing on changing in microbiota and therapeutic value of probiotics.

Table 1. Rome IV classification of Childhood Functional Gastrointestinal Disorders

FUNCTIONAL GASTROINTESTINAL DISORDERS: NEONATE AND TODDLER
Infant regurgitation Infant rumination syndrome Cyclic vomiting syndrome Infant colic Functional diarrhea Infant dyschezia
FUNCTIONAL GASTROINTESTINAL DISORDERS: CHILD AND ADOLESCENT
Functional nausea and vomiting disorders Cyclic vomiting syndrome Functional nausea and functional vomiting Rumination syndrome Aerophagia **Functional abdominal pain disorders** Functional dyspepsia Irritable bowel syndrome Abdominal migraine Functional abdominal pain-not otherwise specified **Functional defecation disorders** Functional constipation Non retentive fecal incontinence

Chapter 2

- Definition and Diagnosis of Functional Gastrointestinal Disorders –

2.1 Functional Chronic Constipation: Rome III criteria versus Rome IV

Functional Constipation (FC) is very common FGID especially in children and adolescent, it is characterized by painful, difficult and infrequent evacuations of hard stools (1). A recent systematic review showed that the reported prevalence of functional constipation in children ranged widely from 0.5% to 32.2%, with a high prevalence in the Americas and Europe and a low prevalence in Asia (18).

FC is responsible of 3% of visits to pediatric clinics and even 10% to 25% of visits to pediatric gastroenterology clinics (19). Symptoms starts early in life, with a median age of onset of 2.3 years (20) and could lead to a reduction in quality of life, school performance and social interaction (21). For this reasons, a timely and correct diagnosis is very important to establish early therapeutic intervention.

Nowadays, the diagnosis of FC is based on Rome IV criteria (1,2), which, as reported before (Chapter 1), are an update of the previous Rome III (5).

Concerning the chapter of Functional Constipation only minor changes have been made: a) in the neonate/toddler group the age limit for the diagnosis of dyschezia has been moved up to 9 months, and typical symptoms of infant dyschezia have been associated also with unsuccessful passage of stools and not only with successful defecations; b) in the child/adolescent group, the duration of symptoms needed for the diagnosis has been decreased from 2 to 1 month in order to harmonize with the European and the North American Societies for Pediatric Gastroenterology, Hepatology and Nutrition constipation guidelines, which suggested that the 2-month interval listed in the Rome III criteria for older children may delay treatment ; c) a

differentiation between children who are / are not toiled trained has been introduced , to help the recognition of fecal incontinence even in toddlers.

Taking account of this change in Rome IV criteria, we decided to perform a prospective study in order to evaluate the diagnostic agreement between the Rome III and the Rome IV criteria and to establish the prevalence of constipation. We enrolled 214 children between infancy and 17 years old, dived in 2 age groups: 81 neonates/ toddlers aged <4 years (mean age 18,1 +- 14,8 months), and 133 children/adolescents aged 4-17 years (mean age 113,8 +- 43,8 months). We observed that, despite the reduction in the symptoms duration, the Rome IV criteria have the same applicability of the Rome III criteria for the diagnosis of functional defecation disorders (18.2% vs 17.3%; P = 0.62) respectively, Cohen‘s k test agreement k 0.72. The prevalence of FC, assessed through the questions on defecation frequency, stool consistency, painful defecation, stool withholding behavior, large diameter stools and fecal incontinence, was 18.2 % with Rome IV criteria and 17.3% with Rome III (p=62). These results showed that we did not miss a significant number of diagnosis using the new criteria.

In the neonate/toddler group, we found that 2/81 (2.4%) patients showed a bowel frequency ≤ 2 times/week (1 according to Rome III and1 according to Rome IV criteria). Regarding painful defecations 23/81 (28.4%) patients were identified (17 according to both Rome III and Rome IV criteria, 2 according to Rome III and 4 according to Rome IV questionnaire). Moreover, 4/81 (4%) had large diameter stools according to both questionnaires. Hard stool were reported in 16/81 (19.7%), 8 according to both questionnaires, 1 according to Rome III and 7 according Rome IV criteria.

In the child and adolescent group (4-17 years old), 10/133 (7.5%) subjects showed a bowel frequency ≤ 2 times/week according to both Rome III and Rome IV criteria, while 3 according to Rome III criteria. Forty-five/133 (33.8%) children reported painful defecation (35 were identified according to Rome III and Rome IV criteria, 5 were positive to Rome III and 5 to Rome IV criteria). Moreover, 12/133 (9.0%) had large diameter stools (10 according to both Rome III and Rome IV criteria, 1 according only to Rome III and 1 according to Rome IV criteria). Finally,

39/133 (29.3%) reported hard stool, 18 according to both questionnaires, 13 according to Rome III and 8 according to Rome IV criteria (Table 3). Fecal Incontinence was present in 9/133 children (6.7%), 5 according to both questionnaires, 3according to Rome III and 1 according Rome IV criteria.

In conclusion, our results suggest that the new Rome IV criteria has a good agreement with the Rome III criteria, moreover the new Rome IV criteria does not increase the number of false positive diagnoses of FC, despite the reduction in the symptoms duration. However, it may improve the outcome in the treatment of FC due to an earlier diagnosis.

The results of this study on the agreement between Rome III and Rome IV criteria for the diagnosis of FC and the role of the related questionnaires in the evaluation of its prevalence have been published in *Journal of Neurogastroenterology and Motility* in 2019.

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Original Article

Functional Chronic Constipation: Rome III Criteria Versus Rome IV Criteria

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Background/Aims
Functional constipation (FC) is aa frequent functional gastrointestinal disorder, diagnosed according to the Rome criteria. In this study, we compared Rome III and Rome IV criteria for the diagnosis of FC, and determined the prevalence of FC according to these criteria.

Methods
Consecutive children between infancy and 17 years old were recruited for the study, excluding those with a known organic gastrointestinal disease. A prospective longitudinal design has beenused. For the diagnosis of FC, questionnaires on Pediatric Gastrointestinal Symptoms (QPGS) based on the Rome III and Rome IV criteria (QPGS-RIII and QPGS-RIV) were used. The agreement between these 2 questionnaires was measured by Cohen's kappa coefficient.

Results
Two hundred fourteen children (mean age, 77.4 ± 59.5 months; 103 males) were screened. There was no statistically significant difference in the prevalence of FC evaluated using the QPGS-Rome IV vs the QPGS-Rome III in the overall sample (39/214 [18.2%] vs 37/214 [17.3.0%]; $P = 0.831$) as well as in any of the groups. The Cohen's kappa test showed a good agreement between the 2 criteria ($\kappa = 0.65$; 95% CI, 0.51 to 0.78).

Conclusion
Our study demonstrates that the new Rome IV criteria have a good agreement with the Rome III criteria for the diagnosis of FC, without an increase in the number of potential diagnoses, despite the reduction in the duration of the symptoms. This conclusion is important in the management of childhood FC, since a late diagnosis negatively affects the prognosis.
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Key Words
Functional constipation; Pediatric; Questionnaires

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Introduction

Functional constipation (FC) is frequent in children. It is characterized by abdominal pain , evacuation of hard stool emission and reduced bowel movements.

The prevalence of pediatric FC ranges between 0.7-29.6% and it has a high impact on healthcare costs.[1,2] Symptoms often appear early in life. A study conducted by Malowitz et al[3] has shown that the median age of onset of FC is 2.3 years. Constipation symptoms may lead to reduction in health-related quality of life, poor school performances and difficult social interactions at a time that the child is known to lay social and educational foundations for its future.[4] Since FC is one of the most frequent diseases in very early age, it is essential to diagnose this functional disorder promptly

Perhaps, a delay in the diagnosis and in the treatment is negatively correlated with the recovery of childhood FC, therefore it is fundamental a correct diagnosis and a previous intervention.[5]

Currently, the diagnosis of FC is based on the new Rome IV criteria,[6,7] which are the updated version of the Rome III criteria.[8,9] Only minor changes have been made in the Rome IV diagnostic criteria compared to the previous Rome III criteria.[10] In the group of neonate/toddlers, considering that the majority of toddlers aged less than 2.5 years are not toilet trained, a differentiation between toilet-trained and not toilet-trained children has been included. In fact, it is not possible to recognize fecal incontinence (FI) in a child wearing a diaper. In the group of child/adolescent the only modification is the decrease from 2 months to 1 month in the duration of symptoms needed to fulfill the criteria for the diagnosis. In accordance with the latest European and North American Societies for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN/NASPGHAN) constipation guidelines,[11] a shorter duration of symptoms is needed for the definition of FC in the child/adolescent group.

Our objective is to evaluate the agreement between Rome III and Rome IV criteria for the diagnosis of FC and the role of the related questionnaires in the evaluation of its prevalence.

Materials and Methods

The prevalence of FC was assessed based on the questions regarding defecation frequency (< 2 times per week), stool consistency, painful defecation, stool withholding behavior, large diameter stools and FI.

Subjects

Study subjects were consecutively recruited among children between infancy and 17 years old attending their general pediatrician for growth monitoring. Patients with a clinical history of organic gastrointestinal disease (eg, celiac disease, inflammatory bowel disease, food allergy, and surgery of the gastrointestinal tract) were excluded "a priori" and were not offered to participate in the study.

Ethical Considerations

Written informed consent was obtained from participants' parents, and the assent was obtained for all patients older than 10 years. The study was approved by the Institutional Review Board of the University of Naples "Federico II" (No. 58/18).

Measures and Procedures

The Questionnaire on Pediatric Gastrointestinal Symptoms (QPGS) is a validated instrument designed to classify gastrointestinal symptoms associated with functional gastrointestinal according to the Rome III and Rome IV criteria (QPGS-RIII and QPGS-RIV).[6-10] Each questionnaire is presented in 3 forms, due to the fact that diagnostic criteria are different for infants and toddlers and for children and adolescents: form A, for parents of infants and toddlers up to the age of 4 years; form B, for parents of children and adolescents between 4 and 10 years of age; and form C, for children and adolescents aged between 10 and 17 years. These 3 forms also collect sociodemographic and medical/developmental information.

Parents of all children younger than 10 years and adolescents aged 10 to 17 years old completed the age-related QPGS-RIII and QPGS-RIV helped by a research assistant. Compilation of both questionnaires took about 10 minutes.

The main characteristics of children's bowel habits were investigated: frequency of bowel movements, consistency of stools, onset of constipation symptoms, hard or painful bowel movements, family history of constipation, urgency or feeling of an unfinished bowel movement, mucus in stool, history of large-diameter stools that may block up the toilet, withholding stools for fear of pain, squeezing the legs or buttocks together (retentive posturing), FI (staining or soiling) during the day and/or night, large fecal mass in the rectum, and presence of associated symptoms.

All data were analyzed to establish how many patients met the Rome III, the Rome IV or both criteria.

Statistical Methods

Sample size was calculated separately with Epi-Info Statistical

Calculator (Division of Health Informatics and Surveillance, Center for Surveillance, Epidemiology, and Laboratory Services, Clifton Road Atlanta, GA, USA) based on the estimated prevalence of functional constipation derived from the available literature, which is about 20%. We considered a confidence interval (CI) of 95% and a precision error of 2%. Assuming this, the sample size was 262 children between 0 and 17 years.

Numerical data are expressed as mean ± SD, while categorical variables were expressed as absolute frequencies and percentages.

Difference in prevalence of FC according to the 2 criteria was assessed using the McNemar test for paired samples and the agreement was globally measured using the Cohen's kappa coefficient κ statistics (0-0.4, poor agreement; 0.4-0.6, good agreement; 0.6-0.8, very good agreement; 0.8-1, excellent agreement) with the correspondent 95% CI.

Statistical analyses were performed using SPSS 22.0 (IBM Corporation, Armonk, NY, USA). A 2-sided *P*-value < 0.05 was considered statistically significant.

Results

Between January and May 2017, 220 children were consecutively screened. The parents of 6 children could not answer the questionnaires, therefore these patients were excluded from the study.

Clinical and demographic characteristics of the 214 children enrolled, are shown in Table 1. No statistically significant differences were found in the prevalence of FC estimated using the Rome III versus the Rome IV criteria: 37/214 (17.3%) vs 39/214 (18.2%), respectively ($P = 0.831$).

The Cohen's κ test showed a good agreement between the Rome III and Rome IV criteria, for the definition of FC ($\kappa = 0.65$; 95% CI, 0.51 to 0.78).

The distribution of FC in the 214 subjects according to the Rome III and Rome IV criteria, stratified for the 2 age groups, is shown in Table 2. In particular, in the sub-group < 4 years, 11/81 children (13.6%) fulfilled the Rome IV criteria while only 8/81 subjects (9.9%) fulfilled the Rome III criteria ($P = 0.508$). In the subgroup > 4 years, 28/133 children (21.0%) fulfilled the Rome IV criteria while 29/133 subjects (21.8%) fulfilled the Rome III criteria ($P > 0.99$).

Twenty-seven subjects out of 214 (12.6%) fulfilled both the Rome III and the Rome IV criteria for FC, 10/214 (4.7%) fulfilled only the Rome III, whereas 12/214 were positive according to Rome IV criteria. These subjects were mainly from the group aged 4 to 17 years (Table 2).

The Cohen's κ test between the 2 questionnaires regarding defecation frequency ≤ 2 times/week, painful defecation, history of large diameter of stools, and FI showed a very good agreement (0.79 [0.61 to 0.97], 0.81 [0.73 to 0.9], 0.93 [0.83 to 1.03], 0.61 [0.35 to 0.87], respectively), while for hard stools the agreement was good (0.56 [0.41 to 0.7]).

Table 1. Demographical Characteristics of the Study Subjects According to Age Group

Subjects	All subject	4 to 17 yr	< 4 yr	*P*-value
Number of subjects	214	133	81	NA
Age (mo)	79.9 ± 58.5	113.8 ± 43.8	18.1 ± 14.8	NA
Females	111 (51.9)	75 (56.4)	36 (44.4)	0.590

NA, not available.
Data are presented as mean ± SD or n (%).

Table 2. Distribution of the 214 Subjects According to Rome III and Rome IV Criteria, Stratified According to the Age Group

Rome criteria	Age group		
	< 4 yr (n = 81)	4-17 yr (n = 133)	Total (N = 214)
Rome III−/Rome IV−	67 (82.7)	98 (73.7)	165 (77.1)
Rome III−/Rome IV+	6 (7.4)	6 (4.5)	12 (5.6)
Rome III+/Rome IV−	3 (3.7)	7 (5.3)	10 (4.7)
Rome III+/Rome IV+	5 (6.2)	22 (16.5)	27 (12.6)

−, negative diagnosis; +, positive diagnosis.
Data are presented as n (%).

Subgroup Analysis

Infants and toddler group

Looking at the clinical characteristics of the infants and toddler group (0-4 years old) we found that 2/81 (2.4%) patients showed a bowel frequency ≤ 2 times/week (1 according to Rome III and 1 according to Rome IV criteria). Regarding painful defecations, 23/81 (28.4%) patients were identified (17 according to both Rome III and Rome IV criteria, 2 according to Rome III and 4 according to Rome IV questionnaire). Moreover 4/81 had large diameter stools according to both questionnaires. Hard stool were reported in 16/81 (19.7%), 8/81 according to both questionnaires, 1 according to Rome III and 7 according Rome IV criteria (Table 3).

In this group of patients, 17/81 (21.0%) had acquired toilet training skills at a median age of 27.6 months. FI was present in 4/17 (23.5%): 1 according to both questionnaires, 3 according to Rome III criteria.

Child and adolescent group

In the child and adolescent group (4-17 years old), 10/133 (7.5%) subjects showed a bowel frequency ≤ 2 times/week according to both Rome III and Rome IV criteria, while 3 according to Rome III criteria. Forty-five/133 (33.8%) children reported painful defecation (35 were identified according to Rome III and Rome IV criteria, 5 were positive to Rome III and 5 to Rome IV criteria). Moreover, 12/133 (9.0%) had large diameter stools (10 according to both Rome III and Rome IV criteria, 1 according only to Rome III and 1 according to Rome IV criteria). Finally, 39/133 (29.3%) reported hard stool, 18 according to both questionnaires, 13 according to Rome III and 8 according to Rome IV criteria (Table 3). FI was present in 9/133 (6.7%) 5 according to both questionnaires, 3

Table 3. Agreement Between the Rome III and Rome IV Questionnaires Regarding Defecation Frequency, Painful Defecation, Large-diameter Stools, Hard Stools, and Fecal Incontinence

Rome criteria	Age group		
	< 4 yr (n = 81)	4-17 yr (n = 133)	Total (N = 214)
Defecation frequency ≤ 2/wk			
Rome III−/Rome IV−	79 (97.5)	120 (90.2)	199 (93)
Rome III−/Rome IV+	1 (1.2)	0 (0.0)	1 (0.5)
Rome III+/Rome IV−	1 (1.2)	3 (2.2)	4 (1.9)
Rome III+/Rome IV+	0 (0.0)	10 (7.5)	10 (4.7)
Painful defecation			
Rome III−/Rome IV−	58 (71.6)	88 (66.1)	145 (68.1)
Rome III−/Rome IV+	4 (4.9)	5 (3.8)	9 (4.2)
Rome III+/Rome IV−	2 (2.5)	5 (3.8)	7 (3.3)
Rome III+/Rome IV+	17 (21.0)	35 (26.3)	52 (24.4)
Large-diameter stools			
Rome III−/Rome IV−	77 (95.1)	121(91)	198 (92.5)
Rome III−/Rome IV+	0 (0.0)	1 (0.7)	1 (0.5)
Rome III+/Rome IV−	0 (0.0)	1 (0.7)	1 (0.5)
Rome III+/Rome IV+	4 (4.5)	10 (7.5)	14 (6.5)
Hard stools			
Rome III−/Rome IV−	65 (80.2)	94 (70.7)	159 (74.3)
Rome III−/Rome IV+	7 (8.6)	8 (6.0)	15 (7.0)
Rome III+/Rome IV−	1 (1.2)	13 (9.8)	14 (6.5)
Rome III+/Rome IV+	8 (9.9)	18 (13.5)	26 (12.1)
Fecal incontinence			
Rome III−/Rome IV−	13 (76.4)	124 (93.2)	137 (91.3)
Rome III−/Rome IV+	0 (0.0)	1 (0.7)	1 (0.6)
Rome III+/Rome IV−	3 (17.6)	3 (2.3)	6 (4.0)
Rome III+/Rome IV+	1 (5.8)	5 (3.8)	6 (4.0)

–, negative diagnosis; +, positive diagnosis.
Data are presented as n (%).

according to Rome III and 1 according Rome IV criteria.

Discussion

Our study shows good agreement between the Rome III and the Rome IV criteria for the definition of FC, despite the symptoms are observed for a shorter time. This is very important because it is well known that an earlier diagnosis improves the outcome of children with FC. In a study by Bongers et al,[12] it has been demonstrated that a delay between the onset of symptoms and the first visit was one of the factors related to constipation's recurrence in adulthood. We showed that the reduction in the symptoms' duration did not increase the number of false positive diagnoses. Indeed, we found that the overall prevalence of constipation was 17.3% according to the Rome III criteria and 18.2% according to the Rome IV criteria.

A systematic review of the available literature reported that the global prevalence of childhood FC ranges from 0.7% to 30%. According to this review Asian countries have a lower prevalence of constipation (median 10.8%),[1] compared to North America (16%), Europe (19.2%), and Oceania (19.7%). The authors suggested that this discrepancy could be due to cultural, dietary, genetic, environmental, and socioeconomic conditions, and to the different healthcare systems. Although, there is also a lack of uniformity in the criteria used for the diagnosis, since not all of these studies adopted the Rome III criteria. In our study, we found a fair agreement between the Rome IV and the Rome III criteria in establishing the prevalence of FC.

Concerning the prevalence of functional gastrointestinal disorders in infants and toddlers, only limited studies using the Rome III criteria have been published. In these studies,[11] FC was more frequent in toddlers than in infants, according also to recent findings from a retrospective chart review study, which described that the median age of onset of FC in children was 2.3 years.[13] We found that in the sub-group < 4 years, 11/81 children (13.6%) fulfilled the Rome IV criteria while only 8/81 subjects (9.9%) were positive according to Rome III criteria, unfortunately we do not have the prevalence of FC in the infants group.

In the Rome IV questionnaires, another change has been the introduction of the Bristol stool form scale for the assessment of stool consistency in the group of children and adolescents. Indeed, we found a different percentage of patients reporting hard stools according to Rome III and Rome IV questionnaires. However, this difference did not reach statistical significance regarding the prevalence of FC. Therefore, our results are in accordance with the study from Koppen et al,[14] which demonstrated that the agreement between the Bristol stool form scale and the parental report for assessing the prevalence of FC is excellent. As the previous authors reported, we also demonstrated that the introduction of the Bristol stool form did not influence the evaluation of the prevalence of FC. Indeed, the Rome criteria encompasses many elements, not only stool consistency. Indeed, the Rome III criteria for hard and painful stools is a combined criterion, and children fulfill these criteria if they have either hard stools or painful defecation. This decreases the impact of stool consistency alone in diagnosing FC.

Finally, in the Rome IV criteria a last differentiation has been made between children who are toilet trained and children who are not, expecting this to be important for the definition of FI. In our study, 20% of children with an age between 0-4 years had acquired toilet training skills in accordance with previous literature.[14] However, in this group of patients FI was reported only in 4 children, with a prevalence of FI of 23.5%. This is in line with the observation that FI is reported only in 20% of children with FC treated in primary care .[15,16]

The strength of our study is that we have recruited these children from a large general outpatient clinic sample, therefore an unselected population, and had an adequate number of cases to have a good statistical power and draw conclusions. Furthermore, we "diagnosed" FC not only on the basis of a questionnaire, but we also included a physical examination. In fact, a questionnaire is only a screening tool and the physician's assessment is always necessary to properly diagnose FC.

In summary, this study highlights that the new Rome IV criteria has a good agreement with the Rome III criteria. We conclude that the new Rome IV criteria does not increase the number of false positive diagnoses of FC, despite the reduction in the symptoms duration. However, it may improve the outcome in the treatment of FC due to an earlier diagnosis.

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Conflicts of interest: None.

Author contributions: Annamaria Staiano is the guarantor of the article: designed the research study, critically revised the manuscript, and approved the final version and the submission; Marina Russo and Caterina Strisciuglio performed data acquisition, wrote the first draft of the manuscript, and approved the final version of the paper; Elena Scarpato critically revised the manuscript and approved the final version of the paper; Dario Bruzzese analyzed the data, critically revised the manuscript, and approved the final

version of the paper; and Marianna Casertano performed clinical assessment and data acquisition and approved the final version and the submission.

References

1. Mugie SM, Benninga MA, Di Lorenzo C. Epidemiology of constipation in children and adults: a systematic review. Best Prac Res Clin Gastroenterol 2011;25:3-18.
2. Rajindrajith S, Devanarayana NM. Constipation in children: novel insight into epidemiology, pathophysiology and management. J Neurogastroenterol Motil 2011;17:35-47.
3. Malowitz S, Green M, Karpinski A, Rosenberg A, Hyman PE. Age of onset of functional constipation. J Pediatr Gastroenterol Nutr 2016;62:600-602.
4. Bongers ME, van Dijk M, Benninga MA, Grootenhuis MA. Health related quality of life in children with constipation-associated fecal incontinence. J Pediatr 2009;154:749-753.
5. Chogle A, Saps M. Yield and cost of performing screening tests for constipation in children. Can J Gastroenterol 2013;27:e35-e38.
6. Benninga MA, Faure C, Hyman PE, St James Roberts I, Schechter NL, Nurko S. Childhood functional gastrointestinal disorders: neonate/toddler. Gastroenterology 2016;150:1443-1445, e2.
7. Hyams JS, Di Lorenzo C, Saps M, Shulman RJ, Staiano A, van Tilburg M. Childhood functional gastrointestinal disorders: Child/Adolescent. Gastroenterology 2016;150:1456-1468.
8. Hyman PE, Milla PJ, Benninga MA, Davidson GP, Fleisher DF, Taminiau J. Childhood functional gastrointestinal disorders: neonate/toddler. Gastroenterology 2006;130:1519-1526.
9. Rasquin A, Di Lorenzo C, Forbes D, et al. Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterology 2006;130:1527-1537.
10. Schmulson MJ, Drossman DA. What is new in Rome IV. J Neurogastroenterol Motil 2017;23:151-163.
11. North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. Evaluation and treatment of constipation in children: summary of updated recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 2006;43:405-407.
12. Bongers ME, van Wijk MP, Reitsma JB, Benninga MA. Long-term prognosis for childhood constipation: clinical outcomes in adulthood. Pediatrics 2010;126:e156-e162.
13. Devanarayana NM, Rajindrajith S. Association between constipation and stressful life events in a cohort of Sri Lankan children and adolescents. J Trop Pediatr 2010;56:144-148.
14. Koppen I.JN, Velasco-Benitez CA, Benninga MA, Di Lorenzo C, Saps M. Using the bristol stool scale and parental report of stool consistency as part of the Rome III criteria for functional constipation in infants and toddlers. J Pediatr 2016;177:44-48, e1.
15. Schum TR, Kolb TM, McAuliffe TL, Simms MD, Underhill RL, Lewis M. Sequential acquisition of toilet-training skills: a descriptive study of gender and age differences in normal children. Pediatrics 2002;109:E48.
16. Loening-Baucke V. Prevalence rates for constipation and faecal and urinary incontinence. Arch Dis Child 2007;92:486-489.

Chapter 3

-The pathogenesis of Functional Gastrointestinal Disorders-

3.1 High-resolution anorectal manometry in children with functional constipation with or without fecal incontinence

Functional constipation (FC) is the most common DFGI in children/ adolescent, as reported in chapter 1. The triggering event is most likely the universal instinct to avoid defecation because of pain or social reasons (eg, school, travel). Because of withholding, the colonic mucosa absorbs water from the stool that become progressively more difficult to evacuate. This process leads to a vicious cycle of stool retention in which the rectum is increasingly distended, resulting in overflow fecal incontinence, loss of rectal sensation, and ultimately, loss of the normal urge to defecate. Increasing fecal accumulation in the rectum also causes decreased motility in the fore gut, leading to anorexia, abdominal distention and pain (1).

Anorectal manometry (ARM), introduced in the 1970s, is the most common motility test performed in children to evaluate anorectal sensorimotor mechanisms that are responsible for fecal continence and defecation. Moreover, this test evaluates anal sphincter length, tone, function and anal reflexes (21)

High-resolution anorectal manometry (HR-ARM), introduced in 2008, better evaluates anorectal pressures compared to conventional ARM and better explains mechanisms that are involved in fecal continence and defecation (22).

According to Rome criteria, diagnosis of constipation is symptom based, while the main indication to perform anorectal manometry in the evaluation of intractable constipation is to assess the presence of the Recto-Anal Inhibitory Reflex (RAIR), in order to exclude Hirschsprung disease. However, pathophysiologic mechanisms of functional constipation have already being studied by anorectal

manometry (23). In children with chronic functional constipation, the major manometric findings are: anal hypertonia (24,25), anal hypotonia (26,27,28), paradoxical contraction of the external anal sphincter (29), decreased ability of the internal anal sphincter to relax during rectal distension, increased rectal compliance, decreased conscious rectal sensitivity, and decreased rectal contractility (24). Nevertheless, few studies specifically addressing the use of HR-ARM in pediatric patients with defecation disorders have been published. Ambartsumyan et al, in 2013 (30) characterized intra-anal pressure profiles in children during rest and squeeze, showing that 3D topographic pressure measurements demonstrate longitudinal and radial asymmetry of the anal canal, as already reported in adults (31). To the best of our knowledge, there are no studies comparing HR-ARM parameters in constipated children with and without Fecal Incontinence (FI). Therefore, we decide to perform this study, in order to characterize and compare anorectal pressures detected by HR-ARM in children with FC with or without FI; and as secondary objective, compare anorectal pressures of our FC population with a control group of children without lower gastrointestinal symptoms (32). Twenty-nine consecutive children (M/F: 21/8; mean age ± SD: 9.5 ± 3.1 years;range 4-15), of whom 21 affected by FC without FI (mean age ± SD: 9.3 ± 3.23 years) and 8 affected by FC with FI (mean age ± SD: 10.2 ± 3.08 years), were enrolled. No significant differences were found regard to gender and age. The analysis of HR-ARM3D plots demonstrated asymmetry of the anal canal, with higher pressures in distal halves. Comparing pressures between the two groups, we found lower values in FC with FI than in FC without FI group, with a statistically significance for maximum and mean resting pressures (P = .032 and P = .008, respectively). When evaluating our study population respect to asymptomatic children (32), we found no differences in High Pressure Zone (HPZ) length (2.45 cm vs 2.6 cm); however, the maximum and mean resting pressures and the maximum squeeze pressure of our population study were lower (76 mm Hgvs 100 mm Hg; 61 mm Hg vs 83 mm Hg; and 137.9 mm Hg vs191 mm Hg, respectively); the mean RAIR values resulted higher (32.6 mL vs 15.7 mL, respectively) resting pressures, lower maximum squeeze pressure, and higher RAIR values.

In conclusion, our data demonstrate lower HR-ARM pressures in children affected by FC with FI compared to subjects diagnosed by FC without FI, particularly in anteroposterior quadrants. These findings highlight how, in these cases, FI represents a result of chronic FC, most probably due to a weakening of anorectal musculature secondary to prolonged stools' retention. Interestingly, anteroposterior musculature seems to be more affected by this process.

Further studies are needed to confirm the results of this work. Moreover, efforts should be made to standardize the execution of HR-ARM with regard to the equipment to be used, the procedure to be followed, and the reference values in children.

The results of this study on the evaluation of HR-ARM pressures in children with functional constipation (FC), with or without fecal incontinence (FI) have been published in *Neurogastroenterology and Motility in 2020*

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ORIGINAL ARTICLE

High-resolution anorectal manometry in children with functional constipation with or without fecal incontinence

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Abstract

Background: High-resolution anorectal manometry (HR-ARM) is expected to be better than conventional manometry. Our aim was to characterize HR-ARM pressures in children with functional constipation (FC), with or without fecal incontinence (FI).

Methods: Children with diagnosis of FC, with or without FI, according to Rome-IV criteria, were enrolled. All patients underwent HR-ARM using 24-channel water-perfused catheter.

Results: Twenty-nine consecutive children (M/F: 21/8; mean age ± SD: 9.5 ± 3.1 years; range 4-15), of whom 21 affected by FC without FI (mean age ± SD: 9.3 ± 3.23 years) and 8 affected by FC with FI (mean age ± SD: 10.2 ± 3.08 years), were enrolled. No significant differences were found regard to gender and age. The analysis of HR-ARM 3D plots demonstrated asymmetry of the anal canal, with higher pressures in distal halves. Comparing pressures between the two groups, we found lower values in FC with FI than in FC without FI group, with a statistically significance for maximum and mean resting pressures (P = .032 and P = .008, respectively). When evaluating our study population respect to asymptomatic children, we found lower resting pressures, lower maximum squeeze pressure, and higher rectoanal inhibitory reflex (RAIR) values.

Conclusions: Our data demonstrate that HR-ARM pressures at rest and during squeezing in FC with FI children are lower than FC without FI subjects, particularly in anteroposterior quadrants. Compared to children without lower gastrointestinal symptoms, children with FC with or without FI show lower pressures and higher values of RAIR.

KEYWORDS

fecal incontinence, functional constipation, high-resolution anorectal manometry, pediatrics

1 | INTRODUCTION

Functional constipation (FC) and fecal incontinence (FI) are very common defecation disorders, ultimately impairing personal and social life of children and adults.[1,2] It is well known that half of constipated children develop fecal incontinence; however, the pathophysiology is not yet well understood. Abnormal anal sphincter function, decreased ability of internal sphincter to relax during rectal distension,

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abnormalities in rectal and/or colonic sensitivity, and weak muscle contractions during defecation have been suggested as possible pathogenetic mechanisms involved in this process.[3-6]

Anorectal manometry (ARM), introduced in the 1970s, is the most common motility test performed in children. This test evaluates anal sphincter length, tone, function, anal reflexes, and defecation process. High-resolution anorectal manometry (HR-ARM), introduced in 2008, better evaluates anorectal pressures compared to conventional ARM and better explains mechanisms that are responsible for fecal continence and defecation.[7,8] Recently, 3D high-definition anorectal manometry (3D HD-ARM), an advanced version of the high-resolution system, was introduced into clinical practice. High-definition catheters show pressures recorded by the individual sensors present along the catheter's circumference, allowing highlighting any axial asymmetry.[8]

According to the North American Motility Society (ANMS) and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN), the main indications for ARM in children are the following: the assessment of RAIR in order to exclude Hirschsprung's disease; the evaluation of patients with anorectal malformations and persistent defecation problems after surgical repair; the defecation dynamics in patients with chronic constipation.[9] Nevertheless, few studies specifically addressing the use of HR-ARM in pediatric patients with defecation disorders have been published. Ambartsumyan et al, in 2013,[10] characterized intra-anal pressure profiles in children during rest and squeeze, showing that 3D topographic pressure measurements demonstrate longitudinal and radial asymmetry of the anal canal, as already reported in adults.[11] To the best of our knowledge, there are no studies comparing HR-ARM parameters in constipated children with and without FI. Therefore, the primary aim of this study was to characterize and compare anorectal pressures detected by HR-ARM in children with FC with or without FI; as secondary objective, we aimed to compare anorectal pressures of our FC population with a control group of children without lower gastrointestinal symptoms.[12]

Key Points

- FC with FI children showed lower pressures than FC without FI group, with a statistically significance for maximum and mean resting pressures, and with a major involvement of antero-posterior quadrants.
- Compared to children without lower gastrointestinal symptoms, children with FC with or without FI show lower pressures and higher values of RAIR.
- These results could have impact in the future evaluation of patients with incontinence, and may improve therapeutic options.

2 | METHODS

2.1 | Population

This was a single-center, prospective, observational study. All children with a diagnosis of FC with or without FI, according to Rome IV criteria,[13] referred to the Department of Translational Medical Science, Section of Pediatrics of the University of Naples "Federico II," were consecutively enrolled between January 2017 and June 2019. Medical records were reviewed to obtain demographic characteristics and blood tests. In order to be included in the analysis, all the children had to fulfill the following inclusion criteria: diagnosis of functional chronic constipation with or without fecal incontinence based on Rome IV criteria; negative blood tests including celiac serology, thyroid function test, and calcemia; failure of the first line optimized treatment of FC with polyethylene glycol. Exclusion criteria were as follows: age < 4 years; diagnosis of anorectal or spinal malformations; muscular or neurological disease; inflammation of the anorectal area; patients who underwent surgery due to anorectal malformations; celiac disease, hypothyroidism, and hypercalcemia. All the enrolled patients underwent HR-ARM. In order to identify age-related differences, we divided the children in 3 different age groups: 4-8 years; 8-11 years; and 11-15 years. For the primary aim of the study, all the recruited children were divided in 2 groups: those with FC without FI versus children affected by FC with FI. In addition, considering the absence of HR-ARM anorectal parameters normal values, we compared the overall results of our study population with a group of children without lower gastrointestinal symptoms previously reported by Banasiuk et al.[12]

2.2 | Equipment

The HR-ARM is composed by a catheter attached to a record system, which is connected to the computer. We used a 24-channel silicone water-perfused (WP) multi-directional high-resolution catheter, which allows visualization of the entire length and dynamics of the anal canal. At the end of the catheter, there is a silicon balloon. Thanks to an air channel linked to this catheter, it is possible the administration of crescent air bolus in the balloon by a 60-mL syringe. The software transforms pressure recording in 2D or 3D images. With QuickView software *(MMS Investigation & Diagnostic Software Version 9.3)*, HR-ARM data were displayed in a three-dimensional plot, allowing recognition of asymmetry of the anal canal. Pressures' regions delineating rest and squeeze area are displayed on the PC monitor. Visualization was made relative to atmospheric pressure set from 0 to 150 mm Hg and with a range of colors from yellow-green for low pressure to red-purple for high pressure. Resting pressure was defined as the mean maximal pressure recorded by channels during the 20- to 30-second rest period. In details, maximum resting pressure is the highest pressure at any instant, whereas mean resting pressure is averaged across the duration of the maneuver. Maximum squeeze pressure was defined as the highest pressure recorded in the anal canal during a voluntary squeeze achieved during

a 20-second period of voluntary squeeze. The RAIR was evaluated by inflation of the balloon with incremental volume of air from 10 to 60 mL. RAIR was considered to be present if the balloon inflation elicited a pressure decrease of at least 25%.

2.3 | Procedure

According to the ANMS-NASPGHAN consensus document on anorectal manometry in children,[9] we fulfilled the following protocol: We prescribed an enema the night before and another one in the morning just before the manometric procedure. No sedative drugs were administered before undergoing manometry. A digital rectal examination was required before starting the examination. Patients were placed in a supine position. Probe was inserted gently in the anorectum after lubrication. The depth of the probe was established so that the proximal and distal margins of the high-pressure zone (HPZ) were clearly identified: In this way, anal canal length can be established. Measurements were performed during rest and squeeze for 20 second each. Resting pressure was recorded after about 3 minutes from the insertion of catheter. Break period between measurements was about 30 seconds. The RAIR and the anal canal length were analyzed. In the absence of reference values of HR-ARM in pediatric age, despite some differences in population and equipment used, we compared our findings with the results of Banasiuk et al, which performed HR-ARM in children without lower gastrointestinal symptoms.[12] Although they cannot be considered as normal values, this comparison may at least give us some insights on the differences with constipated children.

2.4 | Statistical analysis

Variables were screened for their distribution, and appropriate parametric or non-parametric tests were adopted as necessary. The Student's *t* test, the ANOVA test, and the Mann-Whitney test for continuous variables, and the chi-square and Fisher's exact tests for categorical variables were used where appropriate. Statistical significance was predetermined as $P < .05$. Percentages were rounded to the nearest whole numbers. SPSS version 20.0 was used for all statistical analyses.

TABLE 1 Baseline characteristics of 29 children affected by functional constipation with or without fecal incontinence

Characteristics	FC[c] without FI[d] (n = 21)	FC[c] with FI[d] (n = 8)	*P*[a]
Age (mean ± SD[e], years)	9.3 ± 3.23	10.2 ± 3.08	.483[a]
Male gender (n, %)	16 (76.2%)	5 (62.5)	.646[b]

[a]Student's *t* test;
[b]Fisher's exact test;
[c]functional constipation;
[d]fecal incontinence;
[e]standard deviation.

3 | RESULTS

Twenty-nine consecutive children (M/F: 21/8; mean age ± SD: 9.5 ± 3.1 years; range 4-15), of whom 21 (72.4%) affected by FC without FI (mean age ± SD: 9.3 ± 3.23 years) and 8 (27.6%) affected by FC with FI (mean age ± SD: 10.2 ± 3.08 years), were enrolled. The baseline characteristics of the study population are shown in Table 1.

We firstly compared anorectal pressures based on age and sex. No significant differences were observed among the 3 different age groups for maximum and mean resting pressures and for maximum squeeze pressure ($P = .798$, $P = .959$, and $P = .839$, respectively) (Table 2). No significant gender differences were observed in terms of maximum resting pressure (M: 75.4 mm Hg ± 19.8 vs F: 77.5 mm Hg ± 26.4; $P = .518$), mean resting pressure (M: 61.4 mm Hg ± 17.9 vs F: 60 mm Hg ± 18.5; $P = .943$), and maximum squeeze pressure (M: 140.4 mm Hg ± 38.8 vs F: 131.25 mm Hg ± 51.6; $P = .684$).

In FC with FI group, maximum and mean resting pressures were significantly decreased when compared with FC without FI group ($P = .032$ and $P = .008$, respectively; Mann-Whitney test). The maximum squeeze pressure was also decreased in FC with FI group, but the difference was not statistically significant ($P = .401$) (Table 3). No statistical significant differences were found concerning HPZ length and mean anal relaxation rate between the two groups ($P = .943$ and $P = .720$, respectively) (Table 3). RAIR was elicited in all children, and no difference was found between the two groups ($P = .935$) (Table 3). The use of QuickView software allowed displaying HR-ARM data in three-dimensional plots. Anorectal pressures of anterior, posterior, right, and left quadrants in proximal and distal halves are shown in Table 4. We reported significant higher pressures in distal versus proximal halves in FC children with and without FI in most of the analyzed quadrants (Table 4). In addition, we found that anteroposterior (AP) pressures were significantly higher in the FC without FI group respect to the FC with FI group for maximum and mean resting pressures and for maximum squeeze pressure ($P = .032$, $P = .006$, and $P = .048$, respectively) (Figure 1). No differences were found comparing right-left (RL) maximum and mean resting pressures and maximum squeeze pressure in FC with and without FI groups ($P = .549$, $P = .218$, and $P = .457$, respectively) (Figure 1).

Considering the absence of HR-ARM anorectal parameters reference values, we compared our results with a group of children described by Banasiuk et al[12] without lower gastrointestinal symptoms. We observed no differences in HPZ length (2.45 cm vs 2.6 cm); however, the maximum and mean resting pressures and the maximum squeeze pressure of our population study were lower (76 mm Hg vs 100 mm Hg; 61 mm Hg vs 83 mm Hg; and 137.9 mm Hg vs 191 mm Hg, respectively); the mean RAIR values resulted higher (32.6 mL vs 15.7 mL, respectively) (Table 5).

TABLE 2 Anorectal pressures based on different age groups at the enrollment

HR-ARM[b] pressures (mean ± SD[c], mm Hg)	4-8 years (n = 11)	8-11 years (n = 9)	11-15 years (n = 9)	P[a]
Maximum resting pressure	77 ± 18.9	78.7 ± 19.1	72 ± 27.3	.798
Mean resting pressure	60.6 ± 10.6	62.3 ± 19.9	59.9 ± 23.6	.959
Maximum squeeze pressure	144.9 ± 37.2	137.44 ± 46.6	134 ± 43.4	.839

[a]ANOVA test.
[b]high-resolution anorectal manometry;
[c]Standard deviation.

TABLE 3 HR-ARM parameters in FC without FI and FC with FI groups

Variables	FC[b] without FI[c] (n = 21) (mean ± SD[d])	FC[b] with FI[c] (n = 8) (mean ± SD[d])	P[a]
Maximum resting pressure (mm Hg)	81.04 ± 20.50	62.75 ± 18.47	.032
Mean resting pressure (mm Hg)	65.62 ± 16.56	48.88 ± 15.49	.008
Maximum squeeze pressure (mm Hg)	142.19 ± 36.75	126.63 ± 54.53	.401
HPZ length (cm)	2.42 ± 0.35	2.51 ± 0.17	.943
Squeezing duration (s)	5.48 ± 2.10	6.25 ± 4.37	.649
Mean anal Relaxation rate (%)	13.71 ± 9.99	22.13 ± 23.27	.720
Residual Anal Pressure (mm Hg)	58.00 ± 14.94	45.88 ± 21.16	.200
RAIR (mL)	32.5 ± 12.1	32.9 ± 11.1	.935

[a]Mann-Whitney test.
[b]Functional constipation;
[c]Fecal incontinence;
[d]Standard deviation.

4 | DISCUSSION

This study provides anal HR-ARM values in constipated children with and without FI, investigating sphincter function and anal pressure profiles. We found out that maximum and mean resting pressures and maximum squeeze pressure were lower in FC with FI children when compared to FC without FI group. More specifically, topographic pressure plots allowed us to demonstrate that AP pressures are significantly decreased in FC with FI. In addition, we showed an asymmetry of the anal canal with higher pressures in distal halves. When comparing our findings with the pediatric study population without lower gastrointestinal symptoms reported by Banasiuk et al,[12] we noticed lower values of pressures at rest and during squeezing and higher values of RAIR.

Although normal values for ARM and 3D HR-ARM in adults have already been published,[14,15] there are only few studies reporting manometric data of normal and constipated children.[10,12] Tang et al reported normal values of HR-ARM in a large cohort of newborns using WP catheter.[16] The more recent study by Banasiuk et al provides range values from 3D HR-ARM in 61 children without lower gastrointestinal symptoms, using solid-state (SS) catheter.[12]

Our study provides anorectal pressures in constipated children with and without FI. The comparison of anorectal pressures between the two groups showed lower values in FC with FI than FC without FI group, with a statistically significance for maximum and mean resting pressures, but not for maximum squeeze pressure. Mion et al evaluated 3D HR-ARM results in asymptomatic subjects, FI and chronic constipated (CC) patients, and they demonstrated how anal resting and squeeze pressures were lower in FI women than in asymptomatic or CC ones. No differences were found in anal pressures between asymptomatic subjects and CC patients.[17] These findings are not confirmed in pediatric population, where previous studies showed similar values of resting and squeeze pressures in healthy children and in those with constipation and FI.[4,6,18-20] According to Mion et al,[17] we demonstrated that patients affected by FC with FI have lower values of anorectal pressures at rest and during squeezing compared to children diagnosed by FC without FI. It is already known that primary cause of FI in constipation is fecal retention. Fecal retention is common in children with stool withholding behavior and painful defecation. Withholding behavior determines prolonged total and segmental colonic transit times, accumulating stools in the entire colon.[21] Rectum and sigmoid colon gradually dilate causing megarectum and megacolon, which determine decreased propulsive contractile forces of the rectal musculature.[22] Finally, semi-liquid stools slide between the fecal mass and rectal wall and escape through the anal canal when the sphincter muscles are relaxed.[21] According to these pathogenetic mechanisms, the presence of FI in patients affected by FC can be considered as a subsequent step respect to chronic constipation, appearing when rectum and sigmoid colon dilation determine an impairment

TABLE 4 Anorectal pressures of anterior, posterior, right, and left quadrants in FC without FI and FC with FI groups

HR-ARM[b] pressures (mean ± SD[c], mm Hg)	Proximal Half	Distal Half	P[a]
FC[d] without FI[e] group			
Maximum resting pressure			
Anterior	68.05 ± 34.56	82.10 ± 28.37	.163
Posterior	66.95 ± 24.08	79.62 ± 22.45	.071
Right	68.86 ± 28.52	83.33 ± 24.94	.066
Left	68.52 ± 25.69	82.05 ± 24.44	.049
Mean resting pressure			
Anterior	53.43 ± 27.12	64.76 ± 22.75	.114
Posterior	51.19 ± 20.57	67.52 ± 19.54	.007
Right	53.14 ± 16.90	68.43 ± 19.34	.007
Left	51.81 ± 21.03	68.43 ± 20.91	<.001
Maximum squeeze pressure			
Anterior	115.86 ± 50.71	150.29 ± 49.72	.004
Posterior	122.76 ± 47.29	147.62 ± 40.28	.006
Right	130.33 ± 48.17	146.67 ± 42.80	.037
Left	122.38 ± 51.75	150.81 ± 50.84	<.001
FC[d] with FI[e] group			
Maximum resting pressure			
Anterior	47.38 ± 26.63	62.50 ± 21.53	.173
Posterior	49.38 ± 23.32	53.50 ± 26.31	.051
Right	56.25 ± 29.71	78.25 ± 34.91	.075
Left	65.00 ± 29.31	73.13 ± 30.79	.065
Mean resting pressure			
Anterior	35.13 ± 18.20	46.25 ± 17.17	.349
Posterior	36.75 ± 18.62	41.75 ± 20.62	.063
Right	40.38 ± 16.83	62.25 ± 32.81	.069
Left	49.25 ± 14.91	59.88 ± 22.66	.041
Maximum squeeze pressure			
Anterior	96.25 ± 45.30	116.00 ± 61.44	.411
Posterior	98.25 ± 41.84	107.88 ± 35.42	.274
Right	114.63 ± 63.36	129.38 ± 52.78	.081
Left	112.88 ± 50.83	153.50 ± 59.08	.101

[a]Student's *t* test;
[b]High-resolution anorectal manometry;
[c]standard deviation;
[d]Functional constipation;
[e]Fecal incontinence.

of musculature tone, demonstrated by lower HR-ARM pressures at rest and during squeezing in FC with FI children than those with FC without FI.

It is well known how intra-anal pressure is determined by three different muscles, intra-anal sphincter (IAS), external anal sphincter (EAS), and puborectalis muscle (PRM). Studies of correlation between 3D HR-ARM, 3D ultrasound (3D-US), and magnetic resonance imaging (MRI) have shown how the EAS is the main contributor to the pressure in the distal anal canal, while the pressure in the middle anal canal is probably determined by the contributions of EAS and IAS. Differently, the IAS and PRM muscle are the main contributors to the pressures in the proximal anal canal.[11,23,24] Analysis of 3D topographic pressure plots in our patients demonstrated an asymmetry of the anal canal with higher pressures in distal halves in most of quadrants in both groups. Ambartsumyan et al showed similar findings in 30 constipated children.[10] Based on previous studies, the asymmetry of the anal canal reflects the contribution of different muscles to determine intra-anal pressures.

Topographic pressure plots showed also that AP pressures were significantly higher in the FC without FI group respect to the FC with

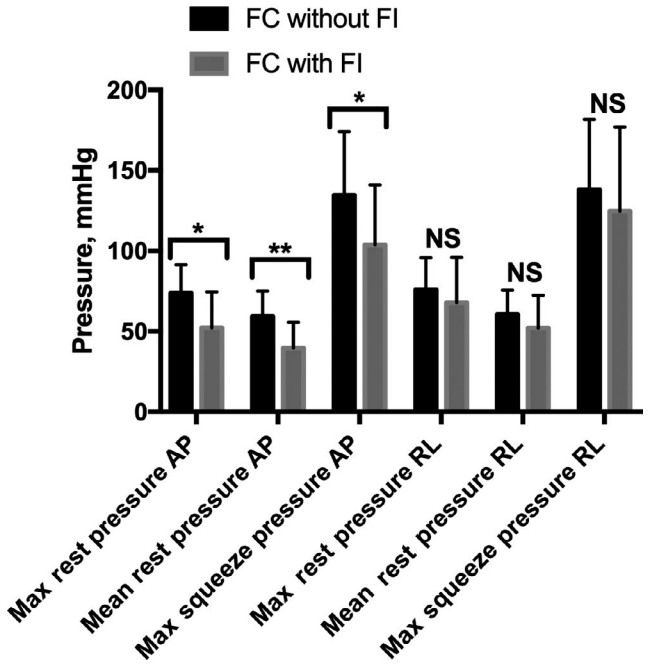

FIGURE 1 High-resolution anorectal manometry (HR-ARM) pressures in children affected by functional constipation (FC) without fecal incontinence (FI) versus pediatric patients diagnosed by FC with FI. When looking more specifically at the topographic pressures, we demonstrated that anteroposterior (AP) pressures were significantly higher in the FC without FI group respect to the FC with FI group for maximum and mean resting pressures and maximum squeeze pressures ($P = .032$, $P = .006$, and $P = .048$, respectively). No differences were found comparing right-left (RL) maximum and mean resting pressures and maximum squeeze pressure in FC without FI and FC with FI group ($P = .549$, $P = .218$, and $P = .457$, respectively). Mann-Whitney test, $^{*}P < .05$; $^{**}P < .01$; NS, Not significant

FI group for maximum and mean resting pressures and for maximum squeeze pressure ($P < .01$, $P < .01$, and $P < .01$, respectively). Overall, patients affected by FC with FI have significant lower pressures particularly in anteroposterior quadrants probably due to the distinctive contribution of each muscle in different sections of the anal canal. Surely, further detailed study of correlation between HR-ARM, 3D-US, and MRI is needed to establish the contribution of different muscles in determining anal canal pressures.

Considering the absence of HR-ARM anorectal parameters reference values in children, we compared our results with a group studied by Banasiuk et al[12] The authors performed 3D HR-ARM in children without lower gastrointestinal symptoms. The comparison with our study population demonstrated no differences in anal canal length, lower maximum and mean resting pressure and lower maximum squeeze pressure; mean RAIR values were higher in our population than in asymptomatic children.[12] Once again, these differences can be explained by the underlying pathogenetic mechanism of FC. Rectal ampulla full of stools determines dilation of colon with weakening of anorectal muscles.[21] It has to be noted that there are several limitations in our comparison, due to consistent

TABLE 5 Comparison of HR-ARM[a] parameters between our study population and control group by Banasiuk et al

Variables	Study population (mean ± SD[b])	Banasiuk et al (mean ± SD[b])
Maximum resting pressure (mm Hg)	76 ± 21	100 ± 27
Mean resting pressure (mm Hg)	61 ± 18	83 ± 23
Maximum squeeze pressure (mm Hg)	138 ± 42	191 ± 64
HPZ[c] length (cm)	2.5 ± 0.31	2.6 ± 0.68
RAIR[d] (mL)	32.6 ± 11.6	15.7 ± 10.9

[a]High-resolution anorectal manometry;
[b]Standard deviation;
[c]High-pressure zone;
[d]Rectoanal inhibitory reflex.

differences between the two studies, such as (a) number of subjects; mean age and ranges; (b) manometry system: MMS vs Manoscan; (c) type of catheter: water-perfused vs solid-state; (d) channels/sensors: 24 vs 256; (e) accommodation period: 3 minutes vs 2 minutes; and (f) break between measurements: 30 seconds in both studies. These dissimilarities may certainly affect the validity of the comparison, and therefore, the differences between the two populations in resting and squeeze pressure and in RAIR values need to be interpreted with caution.

We evaluated the mean threshold volume to elicite RAIR, and it was 32.6 mL. This result is consistent with previous studies in FC patients that reported a range of volume between 11 and 50 mL.[25-31]

In literature, there is significant discrepancy in methods for data acquisition, analysis, and interpretation of ARM. There is a great variability in the equipment used to perform ARM. Some authors used WP catheter, some others SS type. Furthermore, some studies performed conventional manometry, while others HR-ARM or 3D HR-ARM.[32] In 2017, Rasijeff et al compared SS and WP HR-ARM employing equivalent catheter configurations. They found no differences at rest between the two types of catheter, while anal sphincter pressure measurements during squeeze ($P < .0.01$) and cough ($P < .001$) were significantly higher using SS than WP catheter.[33] It seems evident that a better standardization of the procedures is needed in order to obtain comparable values.

Our study has certainly several limitations. For a good evaluation of fecal incontinence, several other measures were needed to be evaluated, such as maximal squeeze pressure increment, urge pressures, and maximal rectal capacity. These ARM measures are able to assess the resistive system (anal sphincters) and the capacitive system (rectum); both are crucial for anal continence.[34] Moreover, we acknowledge that there may be differences after balloon distention and sensation between healthy controls and constipated children, but those are not the parameters we studied in this study. Our sample size was too small, particularly for FC

with FI group, so larger studies are needed to confirm and improve our results, specifically regard to comparison between patient diagnosed by FC with and without FI. Our equipment consisted of high-resolution 24-channels WP catheter that allowed 360 degrees measurements, but we know that more detailed results could be obtained with 3D high-definition probe, consisting in a SS catheter with a total of 256 sensors. Finally, the comparison of our results with those by Banasiuk et al have some limitations due to consistent differences between the two studies, as listed above. So, the results related to the comparison between the two populations need to be interpreted with caution.

In conclusion, our data demonstrate lower HR-ARM pressures in children affected by FC with FI compared to subjects diagnosed by FC without FI, particularly in anteroposterior quadrants. These findings highlight how, in these cases, FI represents a result of chronic FC, most probably due to a weakening of anorectal musculature secondary to prolonged stools' retention. Interestingly, anteroposterior musculature seems to be more affected by this process. Further studies are needed to confirm the results of this work. Moreover, efforts should be made to standardize the execution of HR-ARM with regard to the equipment to be used, the procedure to be followed, and the reference values in children.

CONFLICT OF INTEREST

The authors have no competing interests to disclose regarding this paper. AS is clinical investigator for Janssen Biologics B.V. and PAREXEL International Srl, was clinical investigator for Aboca, was consultant for Aboca, D.M.G. Italy and Nestlè, was data safety monitoring board member for Sucampo AG and speaker for Aboca, Angelini, D.M.G. Italy and Valeas. EM served as speaker, as investigator and member of advisory board for the following companies: Bioprojet, Ferring, and Takeda.

AUTHOR CONTRIBUTIONS

AA and RT contributed to conception and design of the study, collection, analysis and interpretation of data, drafting the article and final approval of the version to be published; MR contributed design of the study, collection, analysis and interpretation of data, drafting the article and final approval of the version to be published; AP contributed to collection of data, drafting the article and final approval of the version to be published; EM and AS contributed to conception and design of the study, interpretation of data, revising the article critically for important intellectual content, and final approval of the version to be published.

ETHICAL APPROVAL

The study was approved by Federico II Ethic Committee, with registration number 271/17. Parents and children, when required, signed an informed consent before starting the study.

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REFERENCES

1. van derWal MF, Benninga MA, Hirasing RA. The prevalence of encopresis in a multicultural population. *J Pediatr Gastroenterol Nutr*. 2005;40:345-348.
2. JoinsonC, Heron J, Butler U, et al. Psychological diff erences between children with and without soiling problems. *Pediatrics*. 2006;117:1575-1584.
3. SutphenJ, Borowitz S, Ling W, Cox DJ, Kovatchev B. Ling W e t al. Anorectal manometric examination in encopretic-constipated children. *Dis Colon Rectum*. 1997;40:1051-1055.
4. RaghunathN, Glassman MS, Halata MS, et al. Anorectal motility abnormalities in children with encopresis and chronic constipation. *J Pediatr*. 2011;158:293-296.
5. KaurG, Gardiner A, Duthie GS. Rectoanal reflex parameters in incontinence and constipation. *Dis Colon Rectum*. 2002;45:928-933.
6. FeinbergL, Mahajan L, Steffen R. Steff en R. The constipated child: is there a correlation between symptoms and manometric findings? *J Pediatr Gastroenterol Nutr*. 2008;47:607-611.
7. BharuchaAE. Pelvic floor: anatomy and function. *Neurogastroenterol Motil*. 2006;18(7):507-519.
8. LeeTH, Bharucha AE. How to perform and interpret a high-resolution anorectal manometry test. *J Neurogastroenterol Motil*. 2016;22(1):46-59.
9. RodriguezL, Sood M, Di Lorenzo C, et al. An ANMS-NASPGHAN consensus document on anorectal and colonic manometry in children. *J Neurogastroenterol Motil*. 2017;29(1):e12944.
10. AmbartsumyanL, Rodriguez L, Morera C, et al. Longitudinal and radial characteristics of intra-anal pressures in children using 3D high-definition anorectal manometry: new observations. *Am J Gastroenterol*. 2013;108(12):1918-1928.
11. RaizadaV, Bhargava V, Karsten A, et al. Functional morphology of anal sphincter complex unveiled by high definition anal manometry and three dimensional ultrasound imaging. *Neurogastroenterol Motil*. 2011;23(11):1013-1019, e460.
12. BanasiukM, Banaszkiewicz A, Dziekiewicz M, et al. Values from three-dimensional high-resolution anorectal manometry analysis of children without lower gastrointestinal symptoms. *Clin Gastroenterol Hepatol*. 2016;14(7):993-1000.e3.
13. Hyams JS, Di Lorenzo C, Saps M, Shulman RJ, Staiano A, van Tilburg M. Childhood functional gastrointestinal disorders: child/adolescent. *Gastroenterology*. 2016.150(6):1456–1468.e2.
14. Coss-AdameE, Rao SSC, Valestin J, et al. Accuracy and reproducibility of high-definition anorectal manometry and pressure topography analyses in healthy subjects. *Clin Gastroenterol Hepatol*. 2015;13(6):1143-1150.e1.
15. LiY, Yang X, Xu C, et al. Normal values and pressure morphology for three-dimensional high-resolution anorectal manometry of asymptomatic adults: a study in 110 subjects. *Int J Colorectal Dis*. 2013;28(8):1161-1168.
16. TangY-F, Chen J-G, An H-J, et al. High-resolution anorectal manometry in newborns: normative values and diagnostic utility in Hirschsprung disease. *Neurogastroenterol Motil*. 2014;26(11):1565-1572.
17. MionF, Garros A, Brochard C, et al. 3D High-definition anorectal manometry: values obtained in asymptomatic volunteers, fecal incontinence and chronic constipation. Results of a prospective multicenter study (NOMAD). *Neurogastroenterol Motil*. 2017;29(8):e13049.
18. KeshtgarAS, Ward HC, Clayden GS. Pathophysiology of chronic childhood constipation: functional and morphological evaluation by anorectal manometry and endosonography and colonic transit study. *J Pediatr Surg*. 2013;48(4):806-812.
19. LiZH, Dong M, Wang ZF. Functional constipation in children: investigation and management of anorectal motility. *World J Pediatr*. 2008;4(1):45-48.

20. MartinacM, Ebling B, Dujsin M, et al. Clinical and anorectal motility features in chronically constipated children. *Coll Antropol*. 2011;35(2):505-512.
21. RajindrajithS, Devanarayana NM, Benninga MA. Review article: faecal incontinence in children: epidemiology, pathophysiology, clinical evaluation and management. *Aliment Pharmacol Ther*. 2013;37(1):37-48.
22. DinningPG, Bampton PA, Andre J, et al. Abnormal predefecatory colonic motor patterns define constipation in obstructed defecation. *Gastroenterology*. 2004;127(1):49-56.
23. PaddaBS, Jung S-A, Pretorius D, et al. Effects of pelvic floor muscle contraction on anal canal pressure. *Am J Physiol Gastrointest Liver Physiol*. 2007;292(2):G565-G571.
24. JungS-A, Pretorius DH, Weinstein M, et al. Closure mechanism of the anal canal in women: assessed by three-dimensional ultrasound imaging. *Dis Colon Rectum*. 2008;51(6):932-939.
25. MeunierP, Marechal JM, de Beaujeu MJ. Rectoanal pressures and rectal sensitivity studies in chronic childhood constipation. *Gastroenterology*. 1979;77(2):330-336.
26. CorazziariE, Cucchiara S, Staiano A, et al. Gastrointestinal transit time, frequency of defecation, and anorectal manometry in healthy and constipated children. *J Pediatr*. 1985;106(3):379-382.
27. BenningaMA, Wijers OB, van der Hoeven CWP, et al. Manometry, profilometry, and endosonography: normal physiology and anatomy of the anal canal in healthy children. *J Pediatr Gastroenterol Nutr*. 1994;18(1):68-77.
28. KumarS, Ramadan S, Gupta V, et al. Manometric tests of anorectal function in 90 healthy children: a clinical study from Kuwait. *J Pediatr Surg*. 2009;44(9):1786-1790.
29. BenningaMA, Omari TI, Haslam RR, et al. Characterization of anorectal pressure and the anorectal inhibitory reflex in healthy preterm and term infants. *J Pediatr*. 2001;139(2):233-237.
30. MolnarD, Taitz LS, Urwin OM, et al. Anorectal manometry results in defecation disorders. *Arch Dis Child*. 1983;58(4):257-261.
31. WaldA, Chandra R, Chiponis D, et al. Anorectal function and continence mechanisms in childhood encopresis. *J Pediatr Gastroenterol Nutr*. 1986;5(3):346-351.
32. CarringtonEV, Heinrich H, Knowles CH, et al. International Anorectal Physiology Working Party Group (IAPWG). Methods of anorectal manometry vary widely in clinical practice: Results from an international survey. *Neurogastroenterol Motil*. 2017;29(8):e13016.
33. RasijeffAMP, Withers M, Burke JM, et al. High-resolution anorectal manometry: A comparison of solid-state and water-perfused catheters. *Neurogastroenterol Motil*. 2017;29(11):e13124.
34. YeapZH, Simillis C, Qiu S, et al. Diagnostic accuracy of anorectal manometry for fecal incontinence: a meta-analysis. *Acta Chir Belg*. 2017;117(6):347-355.

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Chapter 4

-Treating Functional Gastrointestinal Disorders-

4.1 *Efficacy of a partially hydrolysed formula, with reduced lactose content and with Lactobacillus reuteri DSM 17938 in infant colic: A double blind, randomised clinical tria*

Infant colic (IC) defined as recurrent and prolonged periods of infant crying, fussing, or irritability reported by caregivers, that occur without obvious cause and cannot be prevented or resolved by caregivers, without any alarm symptoms. IC is responsible of 25 % of pediatric consultations in the first 3-4 months of life affecting from 5% to 30% of infants between 2 weeks and 3 months of life (33-36). Moreover, in the United Kingdom, the annual total cost to the National Health Service for infant crying and sleeping problems in the first 12 weeks after birth, has been estimated at £65 million.

Different theories on the pathogenesis of IC exist (37-40) and currently there isn't an uniform therapeutic approach. If no alarm symptoms are presents, fist line recommendation is reassurance of parents. Several investigators have examined the effect of nutrition on IC and the evidence is often contrasting. For the breast-fed infants with IC, clinicians should advise mothers to continue breast-feeding but can sometimes recommend that the mothers avoid cow's milk from their own diet for a minimum of two weeks. For the formula-fed IC, the use of a time-limited empiric trial of an extensively hydrolyzed formula may be considered (34). Partially hydrolyzed formula (PHF) has been considered by experience, but not evidences, as a valid alternative, especially when extensive hydrolyzed would be too expensive or not available, and cow's milk protein allergy is not a potential cause of IC (41-43). In some cases, these formulas are lactose-reduced or lactose-free and contain prebiotics or probiotics causing a reduction in the number of crying episodes (34). Pharmacological therapy (e.g. proton pump inhibitors, simethicone) is not effective, and may cause serious adverse reactions.

During the last years, the composition of intestinal microbiome has been addressed as an independent risk factor for IC. Several studies indicate that inadequate lactobacilli in the first few months of life may affect intestinal fatty acid profile and cause the development of IC (44). Coliform bacteria have also been found more in colicky infants and the hypothesis is that altering the intestinal microbiota composition may positively influence the management of affected infants. Chau et al. showed that administration of Lactobacillus reuteri DSM 17938 significantly improved colic symptoms by reducing crying and fussing times in breastfed Canadian infants with colic (45). In contrast, a double blind, placebo controlled randomized trial on the same probiotic strain L. reuteri DSM 17938 showed that it did not benefit a community sample of Australian breast-fed infants and formula-fed infants with IC (46).

We decided to perform this study to compare the efficacy of a partially PHF with reduced lactose content (40%) with addition of maltodextrine (60%) and L. reuteri DSM 17938 with a standard formula on reducing the infant crying duration in IC. We also evaluated the effects of these two formulas 1) on prolonging the duration of sleeping period and 2) on parents' and infants' quality of life and 3) on parents' perception of colic severity and sleep quality. Moreover we detected the L.reuteri stool colonization and we evaluated its relationship with the crying time.

We enrolled 233 full term infants aged <4 months and randomly divided into two groups: Group A (124 infants) treated with partially hydrolyzed formulas with reduced lactose content, addition of maltodextrins and L. reuteri DSM 17938, (coded 3B8 formula); Group B (117 infants) treated with standard formula (coded 7T2 formula) for 4 weeks. We perform two follow up at 4 and 8 weeks. When the whole follow up period was analyzed using Linear mixed model (LMM) it emerged that mean daily crying time at 28th day was significantly lower in Group B when compared to Group A (-41.8, 95% C.I.: -66.5 to -17.1, p=0.001). A significantly higher diurnal sleep duration and improvement of pediatric quality of life score was reported in Group B when compared to Group A. A significant improvement of mothers' quality of life and of mothers' perception of severity of colic and of sleep quality of infants was detected in Group B respect to Group A. The detection and,

marginally, the density of L. reuteri in stools were both significantly associated with a reduction of crying time, irrespective of the treatment group. No adverse effects were reported.

In conclusion, in our study, standard formula showed a lower overall crying time, a longer duration of daily sleep in the first two weeks, an overall better perception of mothers' quality of life, severity of colic and of sleep pattern of infants respect to a PHF with reduced lactose content, maltodextrins and L. reuteri DSM 17938. Since the presence of L. reuteri seems to be associated with a reduction of crying time, irrespective of the formula groups, the reasons of these findings are likely to be found in the differences of formula matrix and/or the low colonization rate of the probiotic in a population of infants harboring a high endemic level of closely related L.reuteri strains. Considering our results and the self-limiting condition of IC, probably based on a spontaneous change of intrinsic microbioma, reassurance of the parents continue to be the milestone of infant colic management while the use of supplemented formula should be further investigated.

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Randomized Control Trials

Efficacy of a partially hydrolysed formula, with reduced lactose content and with Lactobacillus reuteri DSM 17938 in infant colic: A double blind, randomised clinical trial

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SUMMARY

Background & aims: We aimed to compare the efficacy of a partially hydrolysed formula (pHF) with reduced lactose content and *Lactobacillus reuteri DSM 17938 (L. reuteri)* with a standard formula in infant colic (IC).

Methods: We performed a double blind, parallel-group randomized active-controlled. Inclusion criteria were: exclusively formula fed, full term infants, aged <4 months, diagnosis of IC. All the enrolled infants were randomized to receive either pHF with reduced lactose content and *L. reuteri* (Group A) or standard formula (Group B). The treatment duration was 4 weeks and children were followed-up to 8 weeks. The primary outcome was the mean infant crying duration at 28 days.

Results: Two-hundred-forty-one children were randomized to the treatments' group (Group A = 124; Group B = 117). Mean daily crying time at 28th day was significantly lower in Group B when compared to Group A [104.7 (87–122.4) versus 146.4 min (129.2–163.7), treatment effect −41.8 (95% C.I.: −66.5 to −17.1), $p = 0.001$]. No significant adverse event was reported in both groups.

Conclusions: Standard formula showed a lower overall crying time respect to the intervention formula (*ClinicalTrials.gov NCT02813772*).

1. Introduction

Infant colic (IC) is responsible of 25% of paediatric consultations in the first 3–4 months of life affecting from 5% to 30% of infants between 2 weeks and 3 months of life [1–4]. The pathogenesis remains elusive, and although benign and self-limiting, IC is associated with maternal depression, early breastfeeding cessation, and shaken baby syndrome [5–7]. The mainstay of the treatment is still limited to the reassurance of the parents, but these findings underline the need for new therapeutic strategies [3]. Indeed, a number of treatments have been tried, including dietary modifications, complementary and alternative therapies, behavioural interventions, drugs and more recently probiotics, with conflicting results [8–12]. Concerning nutritional attempts, partially hydrolysed formulas (pHF) have been considered on expert opinion, as a valid alternative especially when extensive hydrolysed would be too expensive or not available, and cow's milk protein allergy is not a potential cause of IC [13]. In a double-blind, placebo-controlled trial, pHF with high β-palmitate content and a specific prebiotics mixture with galacto and fructo-oligosaccharides resulted in a significant decrease of crying episodes in infants with colic after 7 and 14 days when compared with a standard formula [14]. During the last years, the composition of intestinal microbiome has been suggested as an independent risk factor for IC. Several studies indicate that inadequate lactobacilli in the first few months of life may affect intestinal fatty acid profile favouring IC development, while coliform bacteria have been found more abundantly in colicky infants [15]. On the basis of this rationale 3 different trials and 2 metanalyses showed that administration of *Lactobacillus reuteri DSM 17938 (L. reuteri)* improved colic symptoms by reducing crying and fussing times in breastfed colicky infants [16–20]. In contrast, one double blind, placebo controlled randomized trial on

Abbreviations: pHF, partially hydrolysed formula; IC, Infant colic; FGIDs, functional gastrointestinal disorders; *Lactobacillus reuteri DSM 17938*, *L. reuteri*; VAS, visual analogue scale.

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the same probiotic strain showed no efficacy in a community sample of Australian breast-fed infants and formula-fed infants with IC [12]. The primary aim of this study was to compare the efficacy of a pHF with reduced lactose content (40%) with addition of maltodextrins (60%) and *L. reuteri DSM 17938* with a standard formula with intact protein body formula (70% of whey protein, 30% of casein), with 100% of lactose content and not containing *L. reuteri*, on reducing the infant crying duration in IC.

2. Methods

2.1. Trial design

This prospective, double-blind, parallel-group randomised active-controlled (allocation ratio 1:1) trial was coordinated by the Department of Translational Medicine, Section of Pediatrics, University of Naples "Federico II", while infants were recruited by 23 general paediatricians, belonging to the Paediatrics Investigator Committee of Campania Region, Italy. Paediatricians were also chosen evenly over the Region to cover the needs of the entire Campania's paediatric population.

2.2. Participants

Inclusion criteria were: full term infants ($\geq$37 weeks gestation at birth; 5-min Apgar score $\geq$7; Birth weight $\geq$2500 g), aged <4 months, diagnosed with IC according to Rome III criteria [1], exclusively formula fed. Parents of infants participating in the study were supplied with the milk formula by Nestec Ltd. Infants with consumption of formula containing probiotics, pHF or with reduced lactose content at time of enrolment, with major medical problem or acute illness, history of antibiotic treatment or probiotic supplementation before or within the study, history of allergies to any of the ingredients in the probiotic *L. reuteri*, concurrent participation in another clinical trial, birth weight <2500 g, breastfed infants, formula-fed infants with formula containing *L. reuteri* were all excluded. The Institutional Review Board of the participating paediatricians approved the research protocol, which was subsequently registered in the Clinical Trials Protocol Registration System *(ClinicalTrials.gov)* with the identifier NCT02813772. All parents gave written informed consent.

2.3. Intervention

Enrolled infants were randomly divided into 2 groups: Group A treated with pHF with reduced lactose content, addition of maltodextrins and *L. reuteri DSM 17938* (coded 3B8 formula); Group B treated with standard formula (coded 7T2 formula) for 4 weeks (Supplementary Table 1). At enrolment, clinical and dietary history, obstetrical data and anthropometry were recorded. The subjects were classified as having IC based on their parents' responses to the validated questionnaires regarding IC according to Rome III criteria. Parents were also asked to fulfil the following questionnaires: 1) Baby's Day Diary on daily cry and infant sleep duration [21]; 2) a scale (visual analogue scale, VAS, 0–10) for parents' quality of life, a questionnaire on infant's quality of life; 3) a form for stool frequency and consistency; 4) a scale for parental perception of colic severity (VAS 0–10) and 5) a scale for parental perception of sleep quality (VAS 0–10). Infants were evaluated by a physician for follow-up visits at weeks 4 and 8. During the visits physical examination was performed and information regarding drugs administration, number and site of infections and eventual adverse medical events were recorded. Moreover, parents had to answer to: 1) the Baby's Day Diary on daily cry and infant sleep duration; 2) the VAS Questionnaire for parents 'quality of life; 3) the questionnaire on infant's quality of life; 4) a review of stool frequency and consistency; 5) the parental perception of colic severity (VAS 0–10) and 6) the parental perception of sleep quality (VAS 0–10). All the authors had access to the study data. Compliance was assessed by evaluating the diary provided by the parents.

2.4. Outcomes

The primary endpoint was the infant crying duration at 28 days. Infant crying duration was also compared between groups using a dichotomous outcome defined as at least 25% reduction in crying time from baseline. As supportive analysis we also considered a more pronounced reduction corresponding to a decrease, with respect to baseline, of 50% or more in the crying time duration. All these results were confirmed on an intention to treat analysis where missing values were imputed using Multiple Imputation Method (data not shown). Secondary endpoints included: infant sleep duration at 7, 14, 28 days, and 8 weeks post-intervention, mean scores on a standardized measure of parents' and infants' quality of life, changes in stool consistency, number of episodes of infant colic per day, parental perception of colic severity (VAS 0–10), parental perception of sleep quality (VAS 0–10), relationship between *L. reuteri* stool colonization and crying time at 28 days of intervention. Faecal samples were collected from the diapers and stored at −80 °C. Quantification of faecal DSM 17938 *L. reuteri* was performed at Microsynth AG (Balgach, Switzerland). Total DNA was extracted using the QIAamp DNA StoolMini Kit (QIAGEN) with the addition of a series of mechanical disruption steps (4 × 45 s) in Lysing Matrix B tubes and a FastPrep apparatus at speed 4 (MP Biochemicals). Real-time PCR analysis was performed as previously described [22] with the addition of a hydrolysis probe to improve the specificity of the assay (5′-GGTCGCTGACGACGGGACGGCAACGATCCTGTATCCAGACGGCTC -3′). Serial dilutions of genomic DNA from *L. reuteri* DSM 17938 were used to establish a standard curve. They were calibrated by digital PCR (Kantonales Labor Zürich, Switzerland) to evaluate the absolute copy number of the targeted gene encoding the cell surface protein Lr1694. The limit of quantification was defined at $10^{4.5}$gene copies per gram of stool. Quantifications were performed in triplicates for each infant faecal sample and expressed as medians of log transformed gene copies per gram of stool.

2.5. Sample size

This study was designed as a superiority trial with a null hypothesis of there being no difference between the two treatments with respect to the primary outcome; the sample size was established considering a 90% power to detect a difference of at least 25% in infant crying time between the two groups, with an alpha of 0.05, and assuming that the crying duration of studied infants in the control treatment is of 180 min/day with a standard deviation of 105 min [21].

2.6. Randomisation

The randomisation procedure was blinded and treatment randomisation codes were kept in order to respect blindness with regard to treatment assignment. The randomisation of participants into treatment groups was maintained through all steps of data processing up until the decision to break the code was formally taken. Breaking the treatment code was possible at any time according to the clinical rules adopted in the trial to stop treatment and information including date, reasons for, and name of the individual breaking the study code was documented.

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None of the people involved in the study accessed to the codes before the treatment code was broken for statistical analysis. Copies of the treatment code were available to the investigators at the end of the study after the database was 'locked'. Formula cans were labelled with codes to mask identity of the study feedings. Neither the parents nor the physicians were aware of the composition of the formula until the entire study was completed.

2.7. Sequence generation

A fixed block design with block size equal to 4 (two test treatment and two comparators) was used. Random permutations were generated by computer.

2.8. Allocation concealment

Infants were equally allocated to one of the 2 study groups by centralized randomisation.

2.9. Implementation

Nestec Ltd, Switzerland generated the random allocation sequence, while general paediatricians enrolled the participating subjects. All eligible infants were randomized to one of the treatment groups by a centralized computerized system, accessible to the paediatricians through an electronic eCRF. The system indicated which treatment to be assigned to each patient, through a corresponding code present on the product label.

2.10. Blinding

The involved company attempted all the procedures of blinding, labelling and packaging according to requirements of Directive 2001/20/EC, of current EU GMP. Blinding was performed having the pHF formula and the standard formula with the code kept by the company until the study was completed and data analysed. The taste and smell of the formulas were not readily identifiable. The pharmacist dispensed the study products to the research coordinator after each infant's initial visit. All the investigators, including the general paediatricians, were blinded.

2.11. Statistics analysis

The difference in crying duration at week 4 was compared between treatment groups using a general linear model with treatment group as main factor and baseline crying duration as covariate. Logistic regression with the same covariates was instead used in case of dichotomic response variable. This approach was also used when comparing groups defined on the basis on the colonization status. Longitudinal trajectories of primary and secondary outcomes during the whole follow up period were analysed using linear mixed models with random term for intercept. The fixed effects of the model included the group factor, the time coded as a categorical factor and the interaction between them. Association between density of *L. reuteri* and crying time was assessed with a linear regression model (assumptions of normality and homoscedasticity of the residuals met), median regression, and Spearman's correlation coefficients. Unadjusted comparisons were based on the Mann–Whitney test or the Fisher exact test as appropriate. All analyses were based on a per-protocol principle but for the assessment of the primary outcome a Multiple Imputation approach was also used.

3. Results

3.1. Baseline characteristics

From November 2015 to November 2016, 246 infants were consecutively enrolled. Fig. 1 shows the subjects' progression through the study. One infant discontinued before randomisation; four infants were excluded for protocol violation by the investigators. Two-hundred-forty-one infants were randomly assigned to receive the 3B8 formula (124 infants, Group A) and the 7T2 formula (117 infants, Group B). Eight infants (three from Group A and five from Group B) dropped out of the study for parental withdrawal due to different reasons such as persisting/worsening of crying, lack of appetite, constipation, diarrhoea and consent withdrawal. Two-hundred-thirty-three out of 246 (94.7%) infants completed the whole follow-up.

Baseline clinical and demographic characteristics of the enrolled infants are showed in Table 1, Table 2 and Supplementary Table 2.

3.2. Primary outcomes

Generalized linear model, adjusted for baseline crying duration, showed that mean daily crying time at day 28th was significantly lower in group B when compared to group A: −41.8, 95% C.I.: −66.5 to −17.1, p = 0.001 (Table 1). When the whole follow up period was analysed using Linear mixed model (LMM) it emerged that the differences between the two formula became statistically significant at day 14th and remained significant at any subsequent follow up, albeit to a different extent (Fig. 2a). Ninety out of 124 (76.9%) infants in Group A experienced a 25% of crying reduction with respect to baseline, compared with a hundred out of 117 infants (90.1%) of Group B (OR: 2.78, 95% C.I.: 1.3 to 5.94, $p = 0.008$). The two formulas did not show a significant difference when a reduction in crying duration of at least 50% was considered (Table 1).

3.3. Secondary outcomes

Regarding the sleep duration, we found a significantly higher diurnal sleep in Group B when compared to Group A at day 7th (26.6, 95% C.I. 0.4 to 52.9, $p = 0.047$) and at day 14th (27.0, 95% C.I. 0.7 to 53.2; $p = 0.044$) (Fig. 2b). The difference did not persist at day 28th or 8 weeks. No significant differences in nocturnal sleep were detected at any time points (Fig. 2c). We observed a significant higher improvement of parental quality of life score at 60th day of intervention, in Group B when compared to Group A (92.3 vs 88.4, 95% C.I. 0.4 to 4.7; $p = 0.031$) (Fig. 2d). In addition, a significant improvement of mothers' VAS, was detected in Group B when compared to Group A at 28th day. A significant difference was observed for the fathers' VAS at the same time point only with respect to the colic severity (Table 2). With regards to the stool consistency (watery, soft, formed, hard) we didn't find any statistical significant difference at 28th day of intervention. Differently, at 8 weeks we observed that group B showed a higher frequency of watery and soft stool when combined together [2 (1.7) vs 6 (5.3) and 39 (33.1) vs 57 (50)] than Group A ($p = 0.012$).

3.4. Adverse events

No severe adverse event was reported in both groups. Among mild symptoms including diarrhoea, constipation, worsening of crying and sleeping disorders were reported in a minority of children without significant differences between the 2 treatment groups.

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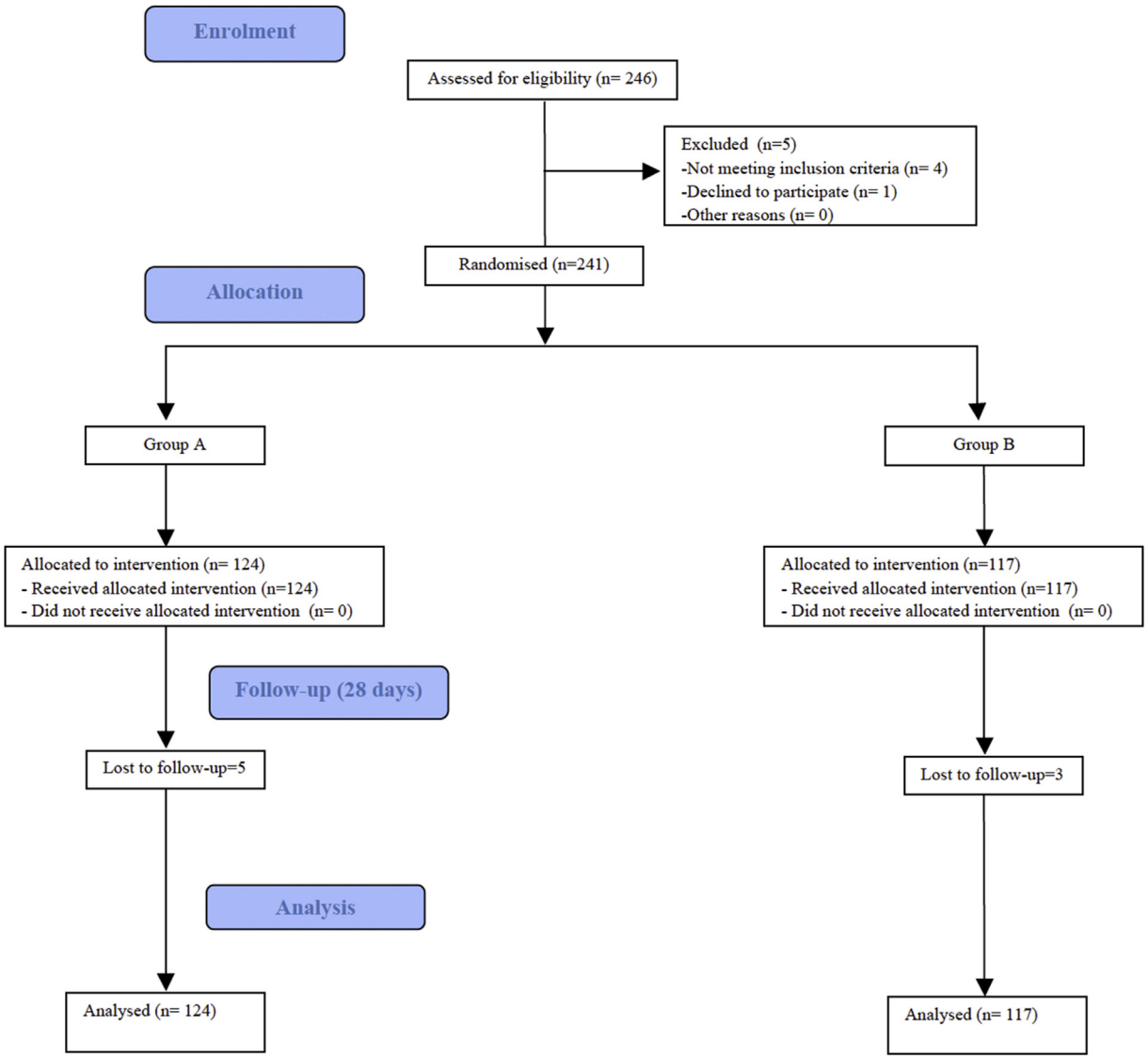

Fig. 1. Flow diagram of the subjects' progression through the study.

Table 1

	Group A	Group B	Treatment effect	p
Criyng/fussing time at 28 days #	146.4 [129.2 to 163.7]	104.7 [87 to 122.4]	−41.8 [-66.5 to −17.1]	0.001
At least 25% reduction in criyng/fussing time from baseline ◦	90 (76.9)	100 (90.1)	2.78 [1.3 to 5.94]	0.008
At least 50% reduction in criyng/fussing time from baseline ◦	57 (48.7)	65 (58.6)	1.52 [0.9 to 2.58]	0.117

◦ Treatment effect is reported as Odds Ratio with 95% Confidence Interval and it was obtained using a logistic regression model with treatment group as the main predictor adjusted by crying/fussing time at baseline.
Treatment effect is reported as Estimated Marginal Means of a general linear model (Nornal distribution, Identity link) with treatment group as the main predictor adjusted by crying/fussing time at baseline

Table 2
Mother's and Father' VAS score after 4 weeks on their quality of life and their perception of colic severity and sleep quality. Data are reported as median [25th; 75th percentile] (min; max).

	Mother			Father		
	Group A	Group B	p value	Group A	Group B	p value
VAS qol	4 [2; 6] (0; 10)	2 [2; 4] (0; 8)	0.003	2 [0; 4] (0; 10)	2 [0; 4] (0; 8)	0.052
VAS colic	4 [2; 4] (0; 10)	2 [0; 4] (0; 6)	0.002	2 [0; 4] (0; 10)	2 [0; 2] (0; 6)	0.008
VAS sleep	2 [2; 4] (0; 10)	2 [0; 4] (0; 6)	0.040	2 [0; 4] (0; 10)	2 [0; 2] (0; 8)	0.067

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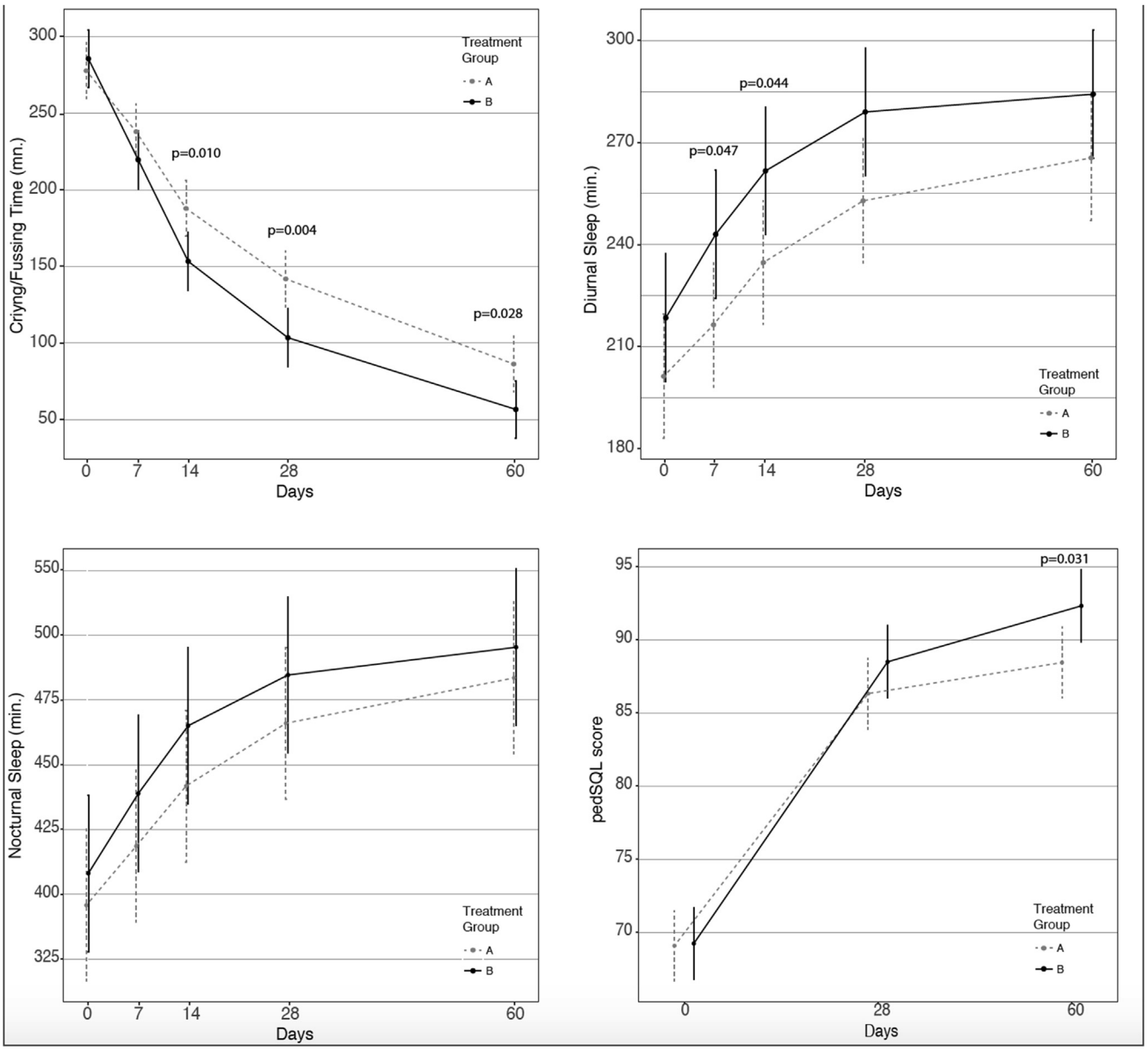

Fig. 2. Longitudinal trajectories of daily crying time (a), diurnal (b) and nocturnal (c) sleep and parental quality of life score (d) among the 2 different treatment's groups during the follow-up period, analysed using random-intercept linear mixed model. Dots represent Estimated Marginal Means with the corresponding 95% Confidence Intervals.

3.5. *L. reuteri* colonization

Collected stool samples were considered suitable for microbiome analysis in a subset of 182 infants and their faecal density of DSM 17938 *L. reuteri* was measured by quantitative PCR. At baseline, 25% of the infants were positive, irrespective of the treatment groups (24/93 = 26% in group A, 22/89 = 25% in group B), with a median density of $10^{7.8}$*L. reuteri* per gram of stool in the positive samples. These results showed that the DSM 17938 strain quantification assay was not sufficiently specific in this population of infants likely harbouring a high prevalence of endemic closely related *L. reuteri* strains. Consequently, we thereafter refer to *L. reuteri* quantification assay. Of the 93 infants that received the probiotic, 41 (44%) were colonized with *L. reuteri* at day 28th, whilst 21 of the 89 infants in group B (24%) were still positive ($p = 0.005$). The median density of *L. reuteri* in the positive samples was also higher in group A ($10^{7.8}$/g stool) than in group B ($10^{6.6}$/g stool; $p = 0.003$).

3.6. Outcomes at day 28th according to *L. reuteri* colonization status

To investigate the relationship between *L. reuteri* colonization and the clinical outcomes, we performed an analysis according to treatment groups considering the colonization status of the infants. We first verified that the 182 infants subset returned the same clinical outcomes than the whole studied population when comparing groups A and B (Table 3). The mean daily crying time at day 28th, adjusted for baseline crying duration, was significantly reduced in colonized infants: −41.3, 95% C.I.: −71.6 to −10.9, $p = 0.008$ (Table 3). Thirty-nine (66.1%) *L. reuteri* colonized infants experienced a 50% of crying reduction with respect to baseline, compared with 52 (44%) for the*L. reuteri* negative infants (OR: 2.9, 95% C.I.: 1.45 to 5.8, p = 0.003). A similar trend was observed when a reduction in crying duration of at least 25% was considered (Table 3).

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Table 3
Outcomes at day 28th according to *L. reuteri* colonization status.

	Treatment effect				Colonization effect			
	Group A	Group B	Estimate	Pvalue	Not Colonized	Colonized	Estimate	pvalue
Crying/fussing time at 28 days #	152.5 [132.9 to 172.1]	93.5 [72 to 115]	−59 [-87.5 to −30.5]	<0.001	143.6	102.3	−41.3 [-71.6 to −10.9]	0.008
At leas 25% reduction in crying/fussing time from baseline ◦	67 (73.6)	78 (89.7)	3.6 [1.5 to 8.6]	0.003	94 (79)	51 (86.4)	2.3 [0.9 to 5.6]	0.08
At leas 50% reduction in crying/fussing time from baseline ◦	42 (46.15)	49 (56.3)	1.91 [1.01 to 3.6]	0.047	52 (43.7)	39 (66.1)	2.9 [1.45 to 5.8]	0.003

Treatment/Colonization effect is reported as difference (with the corresponding 95% CI) in the Estimated Marginal Means of a general linear model (Normal distribution, Identity link) with treatment group as the main predictor adjusted by crying/fussing time at baseline.
◦ Treatment/Colonization effect is reported as Odds Ratio with 95% Confidence Interval and it was obtained using a logistic regression model with treatment group as the main predictor adjusted by crying/fussing time at baseline.

3.7. Effect of L. reuteri density on crying time

In an attempt to reproduce previous investigations performed on samples from another trial with the same probiotic, we unsuccessfully (linear regression model: $p = 0.313$) tried to correlate the crying time with the density of *L. reuteri*, focusing on the stools of infants who receiving the *L. reuteri*DSM 17,938 and were colonized at day 28th. In contrast, a low correlation was observed between the percentage of crying time reduction (day 28th vs. baseline) and the density of *L. reuteri* at day 28th, when considering all infants (linear regression model: 1.45% of reduction per *L. reuteri* log with $p = 0.036$; median regression: −2.84 (95% CI: −5.12 to −1.15), $p = 0.0153$; Spearman rho = −0.17, $p = 0.023$).

4. Discussion

To the best of our knowledge this is the first RCT demonstrating that infants with colic fed with a standard formula show a significant improvement, defined by the decrease of mean daily crying time at day 28th, when compared to a pHF with reduced lactose content, with addition of maltodextrins and *L. reuteri DSM 17938*. The difference in crying time was even more significant after 2 weeks of intervention. In particular, 90% infants fed for 28 days with the standard formula experienced a 25% of crying reduction with respect to baseline, compared with 76.7% infants fed for 28 days with a pHF with reduced lactose content, addition of maltodextrins and *L. reuteri DSM 17938*. These findings overall confirm the benign and self-limiting nature of IC, not supporting the need for special formulas in colicky infants.

Up to now the use of pHF in the management of IC has been reported in few studies. Savino et al. in two different papers evaluated the efficacy of a pHF with prebiotic oligosaccharides and low lactose level in IC [14] or in minor gastrointestinal problems [23], respectively, finding a good response in reducing crying episodes. Similar results were reported analysing the effect of extensively hydrolysed formulas [24–26]. Differently, from the previous reports our data seem to exclude that a partial hydrolysis of cow's milk proteins may be efficacious in the management of IC. With regards to probiotics, the use *L. reuteri* DMS 17938 in exclusively breastfed colic infants is supported by RCTs and metanalysis [16–20,27–30]. Nevertheless, its usefulness in the treatment of formula fed colic infants still leaves many doubts [19,31]. In particular, Sung et al., clearly demonstrated that *L. reuteri* did not improve the symptoms of colicky infants [12]. However, differently from previously published RCTs, Sung et al. included in the analysis also formula-fed infants [12]. The most recent published metanalysis confirmed that the efficacy of *L. reuteri* might be limited to the breastfed infants [19]. Data coming from our study are completely in agreement, since the adjunct of *L. reuteri* in the intervention formula did not help managing IC symptoms.

Lactose intolerance is particularly suspected in infants with colic and the transient relative lactase deficiency is still a current theory about the pathogenesis of IC. Recently, different low lactose formulas have been marketed, but data were not very promising [32,33]. Considering that the etiology of colic is multifactorial the efficacy of a treatment is likely due to the colic's trigger. Thus, a formula with low dose of lactose could be effective in this specific subgroup of infants whose pathogenesis of colic is related to low relative lactase activity and this could explain why in our study it was barely efficacious in a small percentage of infants.

In our study adding maltodextrins to the formula didn't seem to have beneficial on the crying duration of IC. Maltodextrins are considered as FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) [34]. Despite their health effects, it's known that FODMAPs can exacerbate symptoms in Functional gastrointestinal disorders (FGDIs) [35]. As a matter of fact, the use of a low FODMAPs diet as a therapeutic management in subjects with FGIDs has recently widespread [36]. Hereby, we speculate that the addition of maltodextrins may represent one of the main factors leading to the unsatisfactory results of the intervention formula, due to its negative effects on infants' intestinal distension with consequent pain.

It has been widely reported how stressing and depressing the infant colic could be for parents and for the perception of their babies' health and quality of life [37]. As a matter of fact, mothers of our infants showed a better perception of their infants' quality of life, severity of colic and of sleep quality at the end of the intervention in the group on standard formula compared with the group on pHF with *L. reuteri DSM 17938*.

Our findings showed that at 28th day the group on standard formula had softer and watery stools (when the two categories were grouped together) than the group on pHF. It is known that the composition and the type of protein, the dose of lactose and the use of probiotics may influence the stool consistency. A previous study testing 3 different formulas (pHF, pHF with *Bifidobacterium lactis* and intact protein formula) found that the stools were softer with the intact protein formula than with the other formulas [38], thus confirming our results. However recent analysis showed that infants fed with a pHF had a significantly higher probability of soft stools when compared to infants fed with a standard formula [39–41]. Whether probiotics have a significant effect on stool frequency and consistency is still debated [42–44]. Moreover it is known that lactose has a positive influence on intestinal motility [45]. This may partially explain why a standard dose in formula fed infants may lead to softer stools.

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Since the PCR primers used in our study to quantify the presence of *L. reuteri* DSM 17938 in stools targets a gene encoding a cell surface protein [22], the specificity of the assay was not expected to be at strain level, but to encompass closely related *L. reuteri* strains. Surprisingly a quarter of the infants in our trial harboured endemic *L. reuteri* strains positive in our assay. Therefore, we could not differentiate between the infants colonized by the probiotic or the endemic strains. Despite this limitation, we could observe a low level of colonization by *L. reuteri* DSM 17938 since less than half of the infant receiving the formula with the probiotic were positive, in agreement with previous results [12]. Recently, the same researchers evaluated the relationship between the *L. reuteri* and *E. coli* colonization, microbial diversity and crying time in a subset of infants with colics, showing a decrease of crying time from day 0 to day 28 regardless of *L. reuteri* colonization status [46]. In contrast, irrespective of the treatment groups, our subset re-analyses according to the *L. reuteri* colonization status at day 28th, showed that the presence of *L. reuteri* was consistently associated with a reduction of crying time of the same magnitude as the treatment effect.

This RCT is not without limitations. The main limitation, similar to previous studies on IC, is represented by the difficulties in assessing with an objective measure the duration of infant crying/fussing. As a consequence, the primary outcome only relies on parents' report and possible recall biases may occur. Nevertheless, as recently described by Steutel et al., who published a core outcome set for IC, infant crying duration is still considered as the main outcome in colicky infants management by both parents and health-care professionals [47]. Despite this clear evidence, the authors do not comment on the lack of objective measures to better assess this peculiar outcome. In a similar way the assessment of compliance might be considered another potential limitation, since it fully relied on parent reports. Despite these limitations, the strengths of our study are represented by the large sample size and the detailed daily diaries.

In conclusion, in our study standard formula showed a lower overall crying time, a longer duration of daily sleep in the first two weeks, an overall better perception of mothers' quality of life, severity of colic and of sleep pattern of infants respect to a pHF with reduced lactose content, maltodextrins and *L. reuteri DSM 17938*. Since the presence of *L. reuteri* seems to be associated with a reduction of crying time, irrespective of the formula groups, the reasons of these findings are likely to be found in the differences of formula matrix and/or the low colonization rate of the probiotic in a population of infants harbouring a high endemic level of closely related *L. reuteri* strains. Considering our results and the self-limiting condition of IC, probably based on a spontaneous change of intrinsic microbiome, reassurance of the parents continue to be the milestone of infant colic management. The use of different therapeutic strategies, including nutritional attempts, have to be eventually further investigated by well-designed trials. In addition, efforts should be made in order to define new diagnostic measures to better assess IC treatment outcomes.

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The authors declare no conflict of interest to disclose regards to this paper.

Author contributions to manuscript

Rossella Turco-substantial contributions to conception and design, acquisition of data, analysis and interpretation of data, drafting the article and final approval of the version to be published. Marina Russo-acquisition of data, interpretation of data, final approval of the version to be published. Dario Bruzzese-responsible for the statistical design, the analysis plan and oversight of the data analysis, final approval of the version to be published. Annamaria Staiano-substantial contributions to conception and design, acquisition of data, analysis and interpretation of data, drafting the article and final approval of the version to be published. All the authors approved the final version of the article.

Conflicts of interest

AA received consult fee from AbbVie and research grants from AbbVie and Janssen. The other authors declare no conflict of interest to disclose regards to this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2020.05.048.

References

[1] Hyman PE, Milla PJ, Benninga MA, Davidson GP, Fleisher DF, Taminiau J. Childhood functional gastrointestinal disorders: neonate/toddler. Gastroenterology 2006;130:1519–26.
[2] Vandenplas Y, Gutierrez-Castrellon P, Velasco-Benitez C, Palacios J, Jaen D, Ribeiro H, et al. Practical algorithms for managing common gastrointestinal symptoms in infants. Nutrition 2013;29:184–94.
[3] Cohen-Silver J, Ratnapalan S. Management of infantile colic: a review. Clin Pediatr 2009;48:14–7.
[4] Iacono G, Merolla R, D'Amico D, Bonci E, Cavataio F, Di Prima L, et al. Gastrointestinal symptoms in infancy: a population- based prospective study. Dig Liver Dis 2005;37:432–8.
[5] Petzoldt J, Wittchen HU, Wittich J, Einsle F, Höfler M, Martini J. Maternal anxiety disorders predict excessive infant crying: a prospective longitudinal study. Arch Dis Child 2014;99:800–6.
[6] Lee C, Barr RG, Catherine N, Wicks A. Age-related incidence of publicly reported shaken baby syndrome cases: is crying a trigger for shaking? J Dev Behav Pediatr 2007;28:288–93.

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[7] Howard CR, Lanphear N, Lanphear BP, Eberly S, Lawrence RA. Parental responses to infant crying and colic: the effect on breastfeeding duration. Breastfeed Med 2006;1:146-155.
[8] Gordon M, Biagioli E, Sorrenti M, Lingua C, Moja L, Banks SS, et al. Dietary modifications for infantile colic. Cochrane Database Syst Rev 2018;10:CD011029.
[9] Martinelli M, Ummarino D, Giugliano FP, Sciorio E, Tortora C, Bruzzese D, et al. Efficacy of a standardized extract of Matricariae chamomilla L., Melissa officinalis L. and tyndallized Lactobacillus acidophilus (HA122) in infantile colic: an open randomized controlled trial. Neuro Gastroenterol Motil 2017;29. https://doi.org/10.1111/nmo.13145.
[10] Dobson D, Lucassen PL, Miller JJ, Vlieger AM, Prescott P, Lewith G. Manipulative therapies for infantile colic. Cochrane Database Syst Rev 2012;12:CD004796.
[11] Lucassen P. Colic in infants. BMJ Clin Evid 2010;2010:0309.
[12] Olsen MF, Abdissa A, Kæstel P, Tesfaye M, Yilma D, Girma T, et al. Treating infant colic with the probiotic Lactobacillus reuteri: double blind, placebo controlled randomised trial. BMJ 2014;348:210.
[13] Vandenplas Y, Latiff AHA, Fleischer DM, Gutiérrez-Castrellón P, Miqdady M-IS, Smith PK, et al. Partially hydrolyzed formula in non-exclusively breastfed infants: a systematic review and expert consensus. Nutrition 2019;57:268–74.
[14] Savino F, Palumeri E, Castagno E, Cresi F, Dalmasso P, Cavallo F, et al. Reduction of crying episodes owing to infantile colic: a randomized controlled study on the efficacy of a new infant formula. Eur J Clin Nutr 2006;60:1304–10.
[15] Lucassen PL, Assendelft WJ, van Eijk JT, Gubbels JW, Douwes AC, van Geldrop WJ. Systematic review of the occurrence of infantile colic in the community. Arch Dis Child 2001;84:398–403.
[16] Savino F, Cordisco L, Tarasco V, Palumeri E, Calabrese R, Oggero R, et al. Lactobacillus reuteri DSM 17938 in infantile colic: a randomized, double-blind, placebo-controlled trial. Pediatrics 2010;126:e526–33.
[17] Szajewska H, Gyrczuk E, Horvath A. Lactobacillus reuteri DSM 17938 for the management of infantile colic in breastfed infants: a randomized, double-blind, placebo-controlled trial. J Pediatr 2013;162:257-262.
[18] Chau K, Lau E, Greenberg S, Jacobson S, Yazdani-Brojeni P, Verma N, et al. Probiotics for infantile colic: a randomized, double-blind, placebo-controlled trial investigating Lactobacillus reuteri DSM 17938. J Pediatr 2015;166:74–8.
[19] Sung V, D'Amico F, Cabana MD, Kim Chau K, Koren G, Savino F, et al. *Lactobacillus reuteri* to treat infant colic: a meta-analysis. Pediatrics 2018;141:e20171811.
[20] Dryl R, Szajewska H. Probiotics for management of infantile colic: a systematic review of randomized controlled trials. Arch Med Sci 2018;14:1137–43.
[21] St James-Roberts I, Halil T. Infant crying patterns in the first year: normal community and clinical findings. JCPP (J Child Psychol Psychiatry) 1991;32:951–68.
[22] Mobini R, Tremaroli V, Ståhlman M, Karlsson F, Levin M, Ljungberg M, et al. Metabolic effects of Lactobacillus reuteri DSM 17938 in people with type 2 diabetes: a randomized controlled trial. Diabetes Obes Metab 2017;19:579–89.
[23] Savino F, Cresi F, Maccario S, Cavallo F, Dalmasso P, Fanaro S, et al. 'Minor' feeding problems during the first months of life: effect of a partially hydrolysed milk formula containing fructoand galacto-oligosaccharides. Acta Paediatr 2003;91:86–90.
[24] Lucassen PL, Assendelft WJ, Gubbels JW, van Eijk JT, Douwes AC. Infantile colic: crying time reduction with a whey hydrolysate: a double-blind, randomized, placebo-controlled trial. Pediatrics 2000;106:1349-1354.
[25] Jakobsson I, Lothe L, Ley D, Borschel MW. Effectiveness of casein hydrolysate feedings in infants with colic. Acta Paediatr 2000;89:18–21.
[26] Estep DC, Kulczycki Jr A. Treatment of infant colic with amino acid-based infant formula; a preliminary study. Acta Paediatr 2000;106:1349–54.
[27] Anabrees J, Indrio F, Paes B, AlFaleh K. Probiotics for infantile colic: a systematic review. BMC Pediatr 2013;13:186.
[28] Harb T, Matsuyama M, David M, Hill RJ. Infant colic-what works: a systematic review of interventions for breast-fed infants. J Pediatr Gastroenterol Nutr 2016;62:668–86.
[29] Xu M, Wang J, Wang N, Sun F, Wang L, Liu XH. The efficacy and safety of the probiotic bacterium Lactobacillus reuteri DSM 17938 for infantile colic: a meta-analysis of randomized controlled trials. PloS One 2015;10:e0141445.
[30] Schreck Bird A, Gregory PJ, Jalloh MA, Risoldi Cochrane Z, Hein DJ. Probiotics for the treatment of infantile colic: a systematic review. J Pharm Pract 2017;30:366–74.
[31] St James-Roberts I. Is it time to recommend *Lactobacillus* for colic? Not necessarily. Pediatrics 2018;141:e20173445.
[32] Miller JJ, McVeagh P, Fleet GH, Petocz P, Brand JC. Effect of yeast lactase enzyme on "colic" in infants fed human milk. J Pediatr 1990;117:261–3.
[33] Kanabar D, Randhawa M, Clayton P. Improvement of symptoms in infant colic following reduction of lactose load with lactase. J Hum Nutr Diet 2001;14:359–63.
[34] Shepherd SJ, Lomer MC, Gibson PR. Short-chain carbohydrates and functional gastrointestinal disorders. Am J Gastroenterol 2013;108:707–17.
[35] Turco R, Salvatore S, Miele E, Romano C, Marseglia GL, Staiano A. Does a low FODMAPs diet reduce symptoms of functional abdominal pain disorders? A systematic review in adult and paediatric population, on behalf of Italian Society of Pediatrics. Ital J Pediatr 2018;44:53.
[36] Chumpitazi BP, Cope JL, Hollister EB, Tsai CM, McMeans AR, Luna RA, et al. Randomised clinical trial: gut microbiome biomarkers are associated with clinical response to a low FODMAP diet in children with irritable bowel syndrome. Aliment Pharmacol Ther 2015;42:418–27.
[37] Wake M, Morton-Allen E, Poulakis Z, Hiscock H, Gallagher S, Oberklaid F. Prevalence, stability, and outcomes of cry-fuss and sleep problems in the first 2 years of life: prospective community-based study. Pediatrics 2006;117:836–42.
[38] Ziegler EE, Jeter JM, Drulis JM, Nelson SE. Formula with reduced content of improved, partially hydrolyzed protein and probiotics: infant growth and health. Monatsschr Kinderheilkd 2003;151:S65–71.
[39] Czerkies LA, Kineman BD, Cohen SS, Reichert H, Carvalho RS. A pooled analysis of growth and tolerance of infants exclusively fed partially hydrolyzed whey or intact protein-based infant formulas. Int J Pediatr 2018;2018:4969576.
[40] Baldassarre ME, Di Mauro A, Tafuri S, Rizzo V, Gallone MS, Mastromarino P, et al. Effectiveness and safety of a probiotic-mixture for the treatment of infantile colic: a double-blind, randomized, placebo-controlled clinical trial with fecal real-time PCR and NMR-based metabolomics analysis. Nutrients 2018;10:195.
[41] Skórka A, Pieścik-Lech M, Kołodziej M, Szajewska H. To add or not to add probiotics to infant formulae? An updated systematic review. Benef Microbes 2017;8:717–25.
[42] Mugambi MN, Musekiwa A, Lombard M, Young T, Blaauw R. Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review. Nutr J 2012;11:81.
[43] Papagaroufalis K, Fotiou A, Egli D, Tran LA, Steenhout P. A randomized double blind controlled safety trial evaluating d-lactic acid production in healthy infants fed a Lactobacillus reuteri-containing formula. Nutr Metab Insights 2014;7:19–27.
[44] Petschow BW, Figueroa R, Harris CL, Beck LB, Ziegler E, Goldin B. Effects of feeding an infant formula containing Lactobacillus GG on the colonization of the intestine. J Clin Gastroenterol 2005;39:786–90.
[45] Roggero P, Mosca F, Motta G, Mangiaterra V, Perazzani M, Offredi ML, et al. Sugar absorption in healthy preterm and full-term infants. J Pediatr Gastroenterol Nutr 1986;5:214–9.
[46] Nation ML, Dunne EM, Joseph SJ, Mensah FK, Sung V, Satzke C, et al. Impact of Lactobacillus reuteri colonization on gut microbiota, inflammation, and crying time in infant colic. Sci Rep 2017;7:15047.
[47] Steutel NF, Benninga MA, Langendam MW, Korterink JJ, Indrio F, Szajewska H, et al. Developing a core outcome set for infant colic for primary, secondary and tertiary care settings: a prospective study. BMJ Open 2017;7:e015418.

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4.2 *Efficacy of a mixture of probiotic agents as complementary therapy for chronic functional constipation in childhood*

Definition and pathophysiology of Functional Constipation (FC) has been already discussed in the previous chapters. Currently, the treatment of FC in children consists of non-pharmacological interventions (e.g., education and demystification, toilet training, a reward system and a defecation diary) and pharmacological treatment with oral laxatives, especially polyethylene glycol (PEG) (47) that should be the laxative of first choice in pediatrics (48-50). Long-term treatment with PEG is believed to be safe (51,52). Nevertheless, over the last years several studies showed that PEG might change the intestinal milieu by accelerating the passage of luminal contents and by increasing the luminal water content, possibly leading to a change in the intestinal microflora (53). Changes in microbial community structure related to PEG-induced osmotic diarrhea are profound and show similarities to those observed in other GI disorders including inflammatory bowel diseases (54). Recently some adverse events (hypersensitivity and anaphylaxis) have been reported after the use of PEG. Furthermore, despite the acknowledged short-term efficacy of the available treatments, about 30% of constipated children continue to struggle with constipation beyond puberty (55). Therefore, growing interest has recently raised on the use of probiotics as complementary therapy for FC. Modulating the GI flora, as a means of improving symptoms and increasing PEG efficacy, may possibly be an attractive treatment option.

Following this new field of research, we decide to perform a randomized, open-label, controlled trial to evaluate the efficacy of a probiotic mixture (PM), including Bifidobacteria breve, Infantis, and Longum added to oral PEG compared to the traditional therapy with PEG alone on childhood FC, assessing safety and tolerability of the study products for short-term treatment. We enrolled fifty-five children (26 boys;mean age ± SD: 7.2 ± 2.3 years; age range: 4.1–11.8 years).

Patients were randomized into two groups: Group A consisting of 28 children (13 boys) treated with PEG and group B consisting of 27 children (13 boys) treated with an oral combination of PEG plus the probiotic mixture (PEG + PM).The final data set of patients completing the study consisted of a total of 50 children (25 in the PEG group and 25 in the PEG + PM group).

Two-week follow-up findings

An overall improvement of constipation was reported for 72% of children in the PEG group and 59% of children in the PEG + PM group (p:0.02). In children of both groups bowel movement frequency increased and stool consistency decreased significantly from baseline

Four-week follow-up findings

The 4-week outcome data were not significantly different between the two treatment groups (p:0.27). In particular, improvement of constipation was reported for 80% of children in the PEG group and 63.6% of children in the PEG + PM group ($p < 0.05$ and $p < 0.05$ respectively,compared with the initial data).

Eight-week follow-up findings

After one month, children who experienced improvement in the PEG and in the PEG + PM group were 88 and 81.8%, respectively ($p= 0.24$).

Twelve-week follow-up findings

All children returned for the last assessment or were, alternatively, contacted by telephone. Among the PEG group 13/25 children (52%) were off therapy compared to 16/25 children (64%) within the PEG + PM group (p:0.28).

In conclusion, our study showed that PEG and PEG + PM are equally effective and safe in the treatment of children with chronic constipation. Moreover, after one month from the end of the study treatment, a positive trend towards a higher rate of clinical remission was observed within children treated with PEG and PM compared to those who took only PEG (p:0.28). Nevertheless, further studies are needed to show if adding Bifidobacteria strains to conventional therapy may lead to a better long-term outcome.

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Italian Journal of Pediatrics

RESEARCH Open Access

Efficacy of a mixture of probiotic agents as complementary therapy for chronic functional constipation in childhood

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Abstract

Background: About 30% of constipated children continue to struggle with constipation beyond puberty. Growing interest has recently raised on the use of probiotics as complementary therapy for FC, in order to prevent the possible PEG-related intestinal dysbiosis. Our study aimed at evaluating the effect on childhood FC of a probiotic mixture (PM), including Bifidobacteria breve M-16 V®, infantis M-63®, and longum BB536®.

Methods: Fifty-five consecutive children suffering from FC were randomly assigned into two groups: group A received a daily oral combination of PEG plus PM and group B received oral PEG only. Physical and clinical data were collected from each patient at week-1, week-2, week-4, and week-8.

Results: After 1 month, children who experienced improvement in the PEG and in the PEG + PM group were 88 and 81.8%, respectively ($p = 0.24$). After 1 month from the end of the study treatment, a positive trend towards a higher rate of clinical remission was observed within children treated with PM compared to those who took only PEG (percentage of children off therapy: 64 vs 52, respectively; $p = 0.28$).

Conclusions: PEG and PEG + PM are equally effective and safe in the treatment of children with chronic constipation. Nevertheless, further studies are needed to show if adding Bifidobacteria strains to conventional therapy may lead to a better long-term outcome.

Keywords: Constipation, Probiotics, Polyethylene glycol

Background

Functional constipation (FC) is one of the most common gastrointestinal (GI) disorders in childhood, with a reported prevalence of 3% in Western countries [1]. FC accounts for about 95% of pediatric chronic constipation, whereas an organic cause, such as structural, endocrine or metabolic disease, can be found in a small minority of patients. The pathophysiology underlying FC is multifactorial and currently not fully understood, even if a withholding behavior following painful defecation is considered one of the main factors leading to the onset of the disorder [2].

The recommended treatment for FC includes a combination of dietary interventions, toilet training and oral laxatives [3]. Although multiple laxatives have been routinely used in the treatment of childhood constipation, recent evidence suggests that polyethylene glycol (PEG) should be the laxative of first choice in pediatrics [4–6]. PEG is a soluble, inert polymer that is not absorbed and acts by osmosis and volume expansion in the large intestine. Long-term treatment with PEG is believed to be safe [7, 8]. Nevertheless, over the last years several studies showed that PEG may change the intestinal milieu by accelerating the passage of luminal contents and by increasing the luminal water content, possibly leading to a change in the intestinal microflora [9]. Changes in microbial community structure related to PEG-induced osmotic diarrhea are profound and show similarities to those observed in other GI disorders including inflammatory bowel diseases [10]. Furthermore, despite the acknowledged short-term efficacy of the available treatments, about 30% of constipated children continue to struggle with constipation beyond

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puberty [11]. Therefore, growing interest has recently raised on the use of probiotics as complementary therapy for FC. Modulating the GI flora, as a means of improving symptoms and increasing PEG efficacy, may possibly be an attractive treatment option.

The main aim of the present study was to evaluate the efficacy of a probiotic mixture (PM), including Bifidobacteria breve, infantis, and longum added to oral PEG compared to the traditional therapy with PEG alone on childhood FC. Secondary aims were to assess safety and tolerability of the study products for short-term treatment.

Methods

All consecutive children aged 4–12 years suffering from FC were enrolled from January 2014 to December 2014 at the Gastrointestinal Endoscopy and Motility Unit of the Department of Translational Medical Science, Section of Pediatrics, University of Naples "Federico II", Italy, until reaching the planned sample size. FC was diagnosed according to the Rome III Criteria as having at least two of these symptoms: < 3 defecations per week; history of excessive stool retention and painful or hard bowel movements; faecal incontinence >2 times/week; withholding behaviour; presence of a large fecal mass in the rectum; history of large-diameter stools [2]. Children with suspected or proved organic causes of constipation, such as Hirschsprung's disease, spinal bifida, hypothyroidism or other metabolic or renal abnormalities, and mental retardation were excluded from the study. An informed consent was obtained at enrollment from parents of all children younger than 10 years and from both parents and children, if older than 10 years. The study was approved by the Independent Ethics Committee of the "Federico II" University of Naples (reference number: 107/13).

At enrollment, frequency of bowel movements, stool consistency according to the Bristol stool form scale (BSFS) [12], presence of fecal incontinence, abdominal pain, painful defecation, and rectal bleeding were accurately recorded. A thorough medical history was collected by one of the authors and all patients underwent a clinical evaluation, including anorectum digital examination, in order to evaluate whether an abdominal or rectal fecal mass was present. All the enrolled children were then randomly assigned into two groups according to an automatically generated randomization list: group A received a daily oral combination of PEG 4000 (Pergidal® sachets 3.6 g) plus a PM including Bifidobacteria breve M-16 V®, infantis M-63®, and longum BB536® (Tribif® sachets 3 g) (Valeas®Spa, Milan, Italy) and group B received oral PEG only (Pergidal® sachets 3.6 g). The starting dose of PEG was 0.4 g/kg/day for both groups. Increased doses up to 0.8 g/kg body weight daily were allowed by the authors for children not improving after at least 3 days of treatment. The duration of the treatment was 8 weeks. The investigators, the children, and their parents were aware of the study group assignment. Children of both groups underwent rectal disimpaction by rectal enema (120 mL sodium-dioctylsulfosuccinate and sorbitol) on three consecutive days to achieve an empty rectum before starting the treatment trial. The use of other laxatives was not allowed during the study period, whereas enemas were permitted only when there was no defecation for >3 days, as a rescue therapy. A proper toilet training, with regular stool sittings for 5–10 min after each meal, was required. During the 8 weeks of study treatment, the patients and their parents were asked to keep a stool diary, which weekly reported frequency of bowel movements, stool consistency measured through the BSFS, episodes of fecal incontinence, abdominal pain, painful defecation, rectal bleeding, and possible use of enemas.

Follow-up visits were scheduled at week 1 (T1), week 2 (T2), week 4 (T3), week 8 (T4), and week 12 (T5). At each visit, the interim history was assessed, stool diaries were reviewed and discussed, and a further physical evaluation was performed. Clinical progress and compliance with the treatment program were assessed from the stool diaries and history. Week-1 follow-up visit served only to check children's compliance to the assigned treatment and to allow eventual dose modifications. At week 12 children were re-assessed in order to investigate whether they were still on- or off-therapy. If a child did not return for a planned follow-up visit, follow-up data were obtained through a telephone call by the authors, who gave advice regarding dose adjustment and toilet sitting, and encouraged the parents to come for a follow-up visit if the child had not already recovered.

Primary outcome measures were frequency of bowel movements per week, stool consistency, presence of abdominal pain, faecal incontinence, painful defecation, and rectal bleeding. Treatment success was defined as ≥3 defecation per week, stool consistency ≥ grade 3 on BSFS, and no episodes of abdominal pain, faecal incontinence, painful defecation, and rectal bleeding. Secondary outcome measures were safety and tolerability of the study products evaluated through the incidence of adverse effects such as vomiting, nausea or meteorism, flatulence, and diarrhea.

Data were entered into Excel (Microsoft, Redmond, WA) and analyzed with SPSS software, version 8.0 (SPSS, Chicago, Illinois). Efficacy analyses, bowel movement frequency, stool consistency, and presence/absence of abdominal pain, pain on defecation, fecal bleeding, and fecal incontinence were calculated from the available follow-up data. Our hypothesis was that PEG + PM would have been more successful tham PEG alone in treating chronic FC. Comparisons were made between

the initial data and the 2-, 4-, and 8-week follow-up data within each group, and between the two study groups. Statistical analyses included determination of means and SDs, t test, $\chi 2$ test, and Fisher's exact test, with significance accepted at the 5% level. Results are expressed as mean ± SD or percentage. The power evaluation for both univariate and multivariate tests has been computed with the SPSS Multivariate Anova: population rate, 2.9%; smallest difference, 15%; first type error, 0.05; second type error, 0.05; $p < 0.05$; power, 85%; case/control, 1/1.

Results

Initial patient characteristics

A total of 62 children and their families were asked to participate in the study. Fifty-five children (26 boys; mean age ± SD: 7.2 ± 2.3 years; age range: 4.1–11.8 years) and their families agreed to participate and were enrolled in the study. According to the randomization list, 28 children (13 boys) were randomly assigned to receive PEG and 27 children (13 boys) to receive an oral combination of PEG plus the probiotic mixture (PEG + PM). Initial patient characteristics of the children who received PEG and PEG + PM are shown in Table 1. The baseline characteristics of the two groups were not statistically different, with respect to demographic features and examined parameters of constipation. During the treatment period, 5/55 (9.1%) children dropped out from the study due to different reasons. A detailed flow diagram of the children's progress throughout the study with time and reasons for the dropouts is presented in Fig. 1. The final data set of patients completing the study consisted of a total of 50 children (25 in the PEG group and 25 in the PEG + PM group).

Two-week follow-up findings

In the PEG group 2 children refused to take PEG due to its bad taste. In the PEG + PM group 1 children discontinued participation in the study because of his refusal to take the drug and one child was lost to follow-up. Data of the 2-week follow-up visits are shown in Table 2.

Table 1 Baseline features of the enrolled children

	PEG ($n = 28$)	PEG + PM ($n = 27$)	P
Age, mean ± SD, y	7.1 ± 2.5	7.4 ± 2.8	NS
Male, n (%)	13 (46.4)	13 (48.1)	NS
Bowel movements, mean ± SD, episodes per week	2.5 ± 1.1	2.3 ± 0.7	NS
Stool consistency, mean ± SD, BSFS grade	2.6 ± 0.6	2.5 ± 0.7	NS
Presence of fecal incontinence, n (%)	4 (14)	5 (18.5)	NS
Presence of abdominal pain, n (%)	17 (60.7)	15 (55.6)	NS
Presence of rectal bleeding, n (%)	7 (25)	6 (22)	NS

An overall improvement of constipation was reported for 72% of children in the PEG group and 59% of children in the PEG + PM group (p:0.02) (Fig. 2). In children of both groups bowel movement frequency increased and stool consistency decreased significantly from baseline (Table 2).

Four-week follow-up findings

One child from the PEG group was lost to follow-up, whereas all children from the PEG + PM group returned for the 4-week follow-up visit. Results of the 4-week follow-up visit are shown in Table 2. The 4-week outcome data were not significantly different between the two treatment groups (p:0.27) (Table 2). In particular, improvement of constipation was reported for 80% of children in the PEG group and 63.6% of children in the PEG + PM group ($p < 0.05$ and $p < 0.05$ respectively, compared with the initial data) (Fig. 2).

Eight-week follow-up findings

In both the PEG and PEG + PM group all children returned for the follow-up visit. The percentages of children who experienced improvement in the PEG group and the PEG + PM group were 88 and 81.8%, respectively (p:0.24) (Fig. 2). The 8-week data for frequency of bowel movements, stool consistency, fecal incontinence, percentage of children with abdominal pain, rectal bleeding, were not significantly different between the PEG and PEG + PM groups (Table 3). Compared to baseline, both bowel movement frequency and stool consistency improved significantly in children of both groups (Table 2).

Twelve-week follow-up findings

All children returned for the last assessment or were, alternatively, contacted by telephone. Among the PEG group 13/25 children (52%) were off therapy compared to 16/25 children (64%) within the PEG + PM group (p:0.28).

Treatment doses

The mean PEG treatment dose in the PEG group was 0.69 g/kg body weight daily at the 2-week follow-up evaluation, 0.73 g daily at the 4-week follow-up evaluation, and 0.71 g/kg daily at the 8-week final evaluation. The mean PEG doses were similar for children who had and had not experienced improvement.

The mean PEG in the PEG + PM group treatment dose was 0.74 g/kg daily at the 2-week follow-up evaluation, 0.77 g/kg body weight daily at the 4-week follow-up evaluation, and 0.75 g/kg at the 8-week final evaluation. The mean PEG + PM doses were similar for children who had and had not experienced improvement. During the study period none of the children needed an enema as rescue therapy.

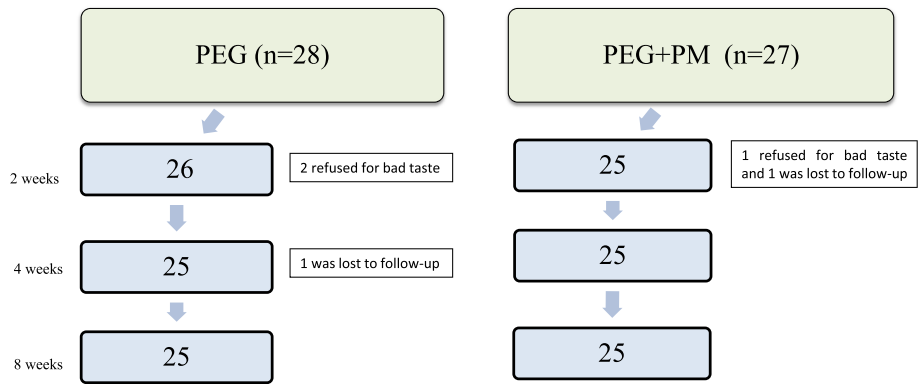

Fig. 1 Flow-chart of the study

Adverse effects

No significant clinical adverse effects were reported with either PEG or PEG + PM except for transient diarrhea, which disappeared with dose reduction. There were no complaints of abdominal distention, increased flatus, or new onset of abdominal pain. The children in both groups who came for follow-up evaluations continued to grow in weight and height, along their growth curves, during the entire study period. There were no new abnormal physical findings on examination.

Patient acceptance

Several children complained about the taste of PEG and PEG + PM. Nevertheless, only 2/28 (7.1%) and 1/27 (3.7%) children definitely refused to take PEG and PEG + PM, respectively. (p: 1) (Fig. 1).

Discussion

In this prospective, randomized study we found that the efficacy of PEG + PM and PEG alone in the short-term treatment of children with chronic FC did not differ significantly. At week-2, week-4, and week-8 follow-up evaluations, similar improvement rates were seen in the PEG + PM and PEG groups, with a significant increase in bowel movement frequency, a significant decrease in stool consistency, and a significant resolution of abdominal pain, painful defecation, rectal bleeding, and fecal incontinence, compared to baseline.

In both groups improvement rates increased steadily during the study period, although children treated with PEG + PM experienced benefit slightly more slowly in the first 2 weeks. Nevertheless, no statistically significant difference in any of the measured outcomes within the two groups was reported at the end of the study treatment period nor at the further assessment after 4 weeks from the end of the study treatment. At this point, the number of children who were off-therapy was higher in the PEG + PM group compared to the PEG group. Even if the difference did not reach a statistical significance, we may hypothesize a possible long-term positive effect on constipation of the PM which deserves further attention. However, according to our overall data, in this study we could not definitely demonstrate the superior efficacy of one treatment option over the other for any of the measured outcomes.

Although constipation is a common clinical problem, reports on the efficacy of probiotics for this disorder are still rather contradictory. *Coccorullo* et al. reported a significant improvement of bowel frequency after the administration of Lactobacillus (L.) reuteri DSM 17938 in infants with chronic FC [13]. In addition, *Sadeghzadeh* et al. showed that a mixture of seven probiotic bacteria, including L. casei, L. rhamnosus, Streptococcus thermophiles, B. breve, L. acidophilus, B. infantis, and L. bulgaricus, had a positive role in increasing the frequency and improving the consistency after 1-month treatment [14].

Table 2 Frequency of bowel movements and stool consistency at each follow-up

Time points	Bowel movement frequency, mean ± SD, episodes per wk			Stool consistency, mean ± SD, BSFS grade		
	PEG	PEG + PM	*P*	PEG	PEG + PM	*P*
Enrollment	2.5 ± 1.1	2.3 ± 0.7	0.344	2.6 ± 0.6	2.5 ± 0.7	0.395
2-week follow-up visit	5.9 ± 1.3	5.4 ± 1.4	0.168	4.2 ± 0.5	3.9 ± 1.0	0.271
4-week follow-up visit	6.3 ± 0.9	6.0 ± 1.2	0.659	4.4 ± 0.5	4.1 ± 0.6	0.267
8-week follow-up visit	6.3 ± 0.9	6.3 ± 1.0	0.924	4.2 ± 0.5	4.2 ± 0.5	0.857

Both bowel movement frequency and stool consistency were improved significantly at each time point in the PEG and PEG + PM groups, compared with the enrollment values ($p < 0.05$)

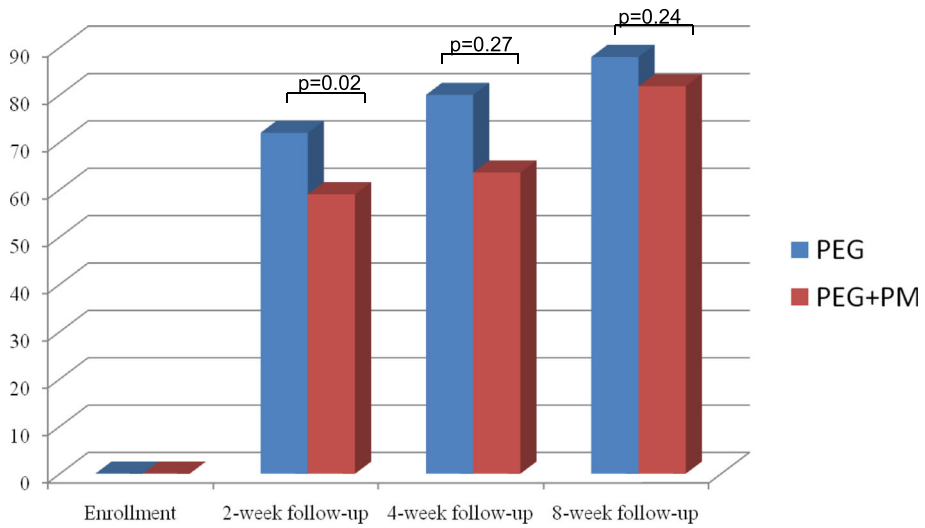

Fig. 2 Efficacy of the study treatments (percentages)

In another study by *Bu* et al. children with constipation were allocated into three groups, receiving L. casei plus L. rhamnosus, magnesium oxide, and placebo. The results of this study showed that the probiotics were as effective as the magnesium oxide, without entailing its possible side-effects [15]. In a study carried out by *Khodadad* et al. children with constipation received paraffin (1.5 ml/kg/day), a mixture of Lactobacilli and Bifidobacteria, or a combination of the two probiotics plus paraffin [16]. According to their findings, defecation frequency increased significantly in children assuming the probiotic mixture. Nevertheless, no beneficial effects were observed on stool form, fecal incontinence, and painful defecation. In contrast with the previous studies, in 2005 a double-blind, placebo-controlled, randomized trial by *Banaszkiewicz* showed that L. rhamnosus was not effective when added to lactulose in the treatment of children with FC [17]. More recently, *Tabbers* et al. showed that in constipated children the fermented dairy product containing B. lactis strain DN-173 010 did increase stool frequency, but this increase was comparable in the control group [18]. The same results were reported by another open trial which showed, in addition, the efficacy of B. breve in improving stool consistency as well [19]. Finally, a recent review by *Vandenplas* et al. concluded that, although some probiotic strains may be helpful in the treatment of childhood constipation, the design of existing trials has been too heterogeneous to allow strong recommendations and that there is a lack of well-designed high-quality randomized controlled trials concerning probiotic treatment of pediatric FC [20].

The authors of the present study are well aware of some methodological drawbacks. In our opinion the main shortcoming is that we did not perform a blinded study because both investigators and patients were aware of the assigned medication. A blinded design would have been hard to carry out because of the need to increase doses differently between the study medications. Furthermore, we lacked to measure the biochemical profiles of children because mandatory blood testing would have affected recruitment and not all children were brought in for the agreed-upon follow-up visits.

Besides efficacy, we've also studied the possible adverse effects and patient acceptance to the proposed drugs, which are two further important issues for an appropriate treatment. Both PEG + PM and PEG were not associated with any significant clinical adverse effects and appeared to be safe for oral use in children. Indeed, this finding has already been reported about PEG and PM alone [11, 14]. Compliance with taking the prescribed

Table 3 Percentages of abdominal pain, fecal incontinence, and rectal bleeding at each follow-up

	Abdominal pain, %			Fecal incontinence %			Rectal bleeding, %		
	PEG	PEG + PM	*p*	PEG	PEG + PM	*p*	PEG	PEG + PM	*p*
Initial visit	60	56	0.778	14	18.5	0.215	25	22	0.86
2-week visit	16	13	0.534	8	12	0.533	10	8	0.671
4-week visit	12	10	0.778	4	8	0.351	3	3	0.949
8-week visit	8	4	0.369	4	6	0.65	1	2	0.505

compounds was similar for children treated with PEG (92.8%) compared with children treated with PEG + PM (96.2%) during the entire 8-week study period. Both medications were administered orally in the form of soluble powder that could be mixed in a beverage of the patient's choice. Compliance rates are of paramount importance since patient acceptance is a crucial factor for successful long-term resolution of constipation.

Conclusions

In conclusion, this prospective, randomized, controlled trial showed that adding a mixture of B. breve, B. infantis and B. longum to PEG as complementary therapy for childhood FC confers no additional short-term effect on the main complained symptoms. According to our findings, neither bowel frequency nor stool consistency were significantly altered by the assumption of the PM. Nevertheless, we may hypothesize that adding Bifidobacteria strains to conventional therapy may lead to a better long-term outcome and a possible longer treatment with PM could optimize the efficacy of PEG therapy. In addition, symptoms related to constipation, such as fecal incontinence, abdominal pain, painful defecation, and rectal bleeding, decreased similarly in children assuming PEG with or without PM. As previously mentioned, there are currently conflicting evidence in literature about the use of probiotics for FC. Although experimental models have shown that Bifidobacteria improve colonic peristalsis thus having potential utility for constipation treatment, we have reported that they lack a clinical impact in the short-term therapy of constipated children. Many factors could be involved in their poor efficacy, most of which are yet to be understood. Nevertheless, in our opinion, one of the main issues accounting for our finding concerns the low prevalence of slow transit constipation in pediatric age. Indeed, most constipated children have been shown to have a normal transit time, being rectal obstruction the cause of their disorder. These patients are likely to be less affected by the assumption of probiotics which act by decreasing luminal pH and enhancing colonic transit. Moreover, other factors which could have been involved in the lack of probiotic supplementation efficacy are: the optimal achieving of the primary outcome in the both groups, the lack of fecal microbiota assessment, and the short-term follow-up. Further studies evaluating possible PEG-induced changes in fecal microbiota with longer patient follow-up are welcomed in order to clarify the true role of PEG dysbiosis and possible probiotic treatment.

Abbreviations

FC: Functional constipation; GI: Gastro-intestinal; PEG: Polyethylene glycol; PM: Probiotic mixture

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Availability of data and materials

Data will not be shared in accordance to the Department's policy.

Authors' contributions

MR performed data acquisition, wrote the first draft of the manuscript, and approved the final version of the paper. FPG and PQ analyzed the data, critically revised the manuscript and approved the final version of the paper. VM performed data acquisition, critically revised the manuscript and approved the final version of the paper. ES designed the research study, analyzed the data, and approved the final version of the paper. AS designed the research study, critically revised the manuscript and approved the final version and the submission.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was approved by the Independent Ethics Committee of the "Federico II" University of Naples (reference number: 107/13).

Financial disclosure

The authors have no financial relationships relevant to this article to disclose.

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References

1. van den Berg MM, Benninga MA, Di Lorenzo C. Epidemiology of childhood constipation: a systematic review. Am J Gastroenterol. 2006;101:2401–9.
2. Rasquin A, Di Lorenzo C, Forbes D, Guiraldes E, Hyams JS, Staiano A, et al. Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterology. 2006;130:1527–37.
3. Benninga MA, Voskuijl WP, Taminiau JA. Childhood constipation: is there new light in the tunnel? J Pediatr Gastroenterol Nutr. 2004;39:448–64.
4. Baker S, Liptak G, Colletti R, Croffie J, Di Lorenzo C, Ector W, et al. Evaluation and treatment of constipation in children: summary of updated recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr. 2000;30(1):109.
5. Staiano A. Use of polyethylene glycol solution in functional and organic constipation in children. Ital J Gastroenterol Hepatol. 1999;31 Suppl 3:S260–3.
6. Pashankar DS, Bishop WP, Loening-Baucke V. Long-term efficacy of polyethylene glycol 3350 for the treatment of chronic constipation in children with and without encopresis. Clin Pediatr. 2003;42:815–9.
7. DiPalma JA, Cleveland MV, McGowan J, Herrera JL. A randomized, multicenter, placebo-controlled trial of polyethylene glycol laxative for chronic treatment of chronic constipation. Am J Gastroenterol. 2007;102:1436–41.
8. Pashankar DS, Loening-Baucke V, Bishop WP. Safety of polyethyleneglycol 3350 for the treatment of chronic constipation in children. Arch Pediatr Adolesc Med. 2003;157:661–4.
9. Cuperus FJ, Iemhoff AA, van der Wulp MY, Havinga R, Verkade HJ. Acceleration of the gastrointestinal transit by polyethylene glycol effectively treats unconjugated hyperbilirubinemia in Gunn rats. Gut. 2010;59:373–80.
10. Gorkiewicz G, Thallinger GG, Trajanoski S, Lackner S, Stocker G, Hinterleitner T, Gülly C, Högenauer C. Alterations in the colonic microbiota in response to osmotic diarrhea. PLoS One. 2013;8(2):e55817.
11. van Ginkel R, Reitsma JB, Buller HA, van Wijk MP, Taminiau JA, Benninga MA. Childhood constipation: longitudinal follow-up beyond puberty. Gastroenterology. 2003;125:357–63.
12. Koh H, Lee MJ, Kim MJ, Shin JI, Chung KS. Simple diagnostic approach to childhood fecal retention using the Leech score and Bristol stool form scale in medical practice. J Gastroenterol Hepatol. 2010;25(2):334–8.

13. Coccorullo P, Strisciuglio C, Martinelli M, Miele E, Greco L, Staiano A. Lactobacillus reuteri (DSM 17938) in infants with functional chronicconstipation: a double-blind, randomized, placebo-controlled study. J Pediatr. 2010;157:598–602.
14. Sadeghzadeh M, Rabieefar A, Khoshnevisasl P, Mousavinasab N, Eftekhari K. The effect of probiotics on childhood constipation: a randomized controlled doubleblind clinical trial. Int J Pediatr. 2014;2014:937212.
15. Bu LN, Chang MH, Ni YH, Chen HL, Cheng CC. Lactobacillus caseirhamnosus Lcr35 in children with chronic constipation. Pediatr Int. 2007;49:485–90.
16. Khodadad A, Sabbaghian M. Role of synbiotics in the treatment of childhood constipation: a double-blind randomized placebo controlled trial. Iran J Pediatr. 2010;20:387–92.
17. Banaszkiewicz A, Szajewska H. Ineffectiveness of Lactobacillus GG as an adjunct to lactulose for the treatment of constipation in children: a double-blind, placebo-controlled randomized trial. J Pediatr. 2005;146:364–9.
18. Tabbers MM, Chmielewska A, Roseboom MG, Crastes N, Perrin C, Reitsma JB, et al. Fermented milk containing Bifidobacterium lactis DN-173 010 in childhood constipation: a randomized, double-blind, controlled trial. Pediatrics. 2011;127(6):e1392–9.
19. Tabbers MM, de Milliano I, Roseboom MG, Benninga MA. Is Bifidobacterium breve effective in the treatment of childhood constipation? Results from a pilot study. Nutr J. 2011;10:19.
20. Vandenplas Y, De Greef E, Devreker T, Veereman-Wauters G, Hauser B. Probiotics and prebiotics in infants and children. Curr Infect Dis Rep. 2013;15:251–62.

Chapter 5

-Conclusive Remarks-

Our studies have confirmed that FGDIs are very common in children of all ages, causing significant reduction in children and parents quality of life.

Novel insights into the pathophysiology, evaluation and management of FGIDs have led to the new Rome IV criteria.

These criteria have encouraged health-care workers to make symptom-based approach to DFGI, avoiding expensive and exhaustive investigations to exclude an underlying organic cause and have advanced empirical research in childhood FGIDs.

Increased knowledge of the pathophysiology has led to a biopsychosocial model, in which genetic, physiological and psychological factors interplay. Potential targets for pharmacological and non pharmacological therapy are arising from this model.

However, the use of Rome diagnostic criteria is not sufficiently widespread among pediatricians and large variability remains in the management of FGIDs. Educational efforts are required to ensure a “positive” approach to functional disorders, in order to avoid inappropriate use of healthcare resources and excessive treatment of overall benign conditions.

Chapter 6

-References-

1. Benninga MA, Faure C, Hyman PE, et al. Childhood Functional Gastrointestinal Disorders: Neonate/Toddler. Gastroenterology.150(6), 1443–1455.e2 (2016).** The Rome IV criteria for neonates/toddlers.

2. Hyams JS, Di Lorenzo C, Saps M, et al. FunctionalDisorders: Children and Adolescents. Gastroenterology. 150(6), 1456–68 (2016).** The Rome IV criteria for children/adolescents.

3. Rasquin –Weber A, Hyam PE, Cucchiara S, et al. Childhood functional gastrointestinal disorders, Gut 1999; 45 (Suppl II) : II 60-68

4. Hyman PE, Milla PJ, Benninga MA, et al. Childhood functional gastrointestinal disorders: neonate/toddler. Gastroenterology 2006;130(5):1519-26

5. Rasquin A, Di Lorenzo C, Forbes D, et al. Childhood functional gastrointestinal disorders : chil/ adolescent. Gastroenterology 2006 Apri ; 130 (5) :1527-37 (2015).

6.kortenik JJ et al. PLoS one. 2015 May20;10(5):30126982

7. Miranda A L van Tilburg, Paul E Hyman , Lynne Walker , et al Prevalence of functional gastrointestinal disorders in infants and toddlers. J Pediatr. 2015 Mar;166(3):684-9.

8. Lewis ML, Palsson OS, Whitehead WE, van Tilburg MAL. Prevalence of Functional Gastrointestinal Disorders in Children and Adolescents.J Pediatr. 2016 Oct;177:39-43.e3

9. Elena Scarpato, Sanja Kolacek, Danijela Jojkic-Pavkov et al.Prevalence of Functional Gastrointestinal Disorders in Children and Adolescents in the Mediterranean Region of Europe

Clinical Gastroenterology and Hepatology 2018; 16:870-876

10. Varni JW, Bendo CB, Nurko S, et al. Health-related quality of life in pediatric patientswith functional and organic gastrointestinal diseases. J. Pediatr. 166(1), 85–90

11. Hoekman DR, Rutten JMTM, Vlieger AM, et al. AnnualCosts of Care for Pediatric Irritable Bowel Syndrome, Functional Abdominal Pain, andFunctional Abdominal Pain Syndrome. J. Pediatr. 167(5), 1103–8.e2 (2015).

12. Hoekman DR, Rutten JM, Vlieger AM, et al. Annua Costs of Care for Pediatric Irritable Bowel Syndrome, Functional Abdominal Pain, and Functional Abdominal Pain Syndrome. J Pediatr 2015; 167 (5) :1103-8

13. Mayer EA, Tillisch K. The brain-gut axis in abdominal pain syndromes. Annu. Rev.Med. 62, 381–96 (2011).

14. Shin A et al The Gut Microbiome in Adult and Pediatric Functional Gastrointestinal Disorders. Clin Gastroenterol Hepatol. 2019 January ; 17(2): 256–274.

15. Korterink JJ, Ockeloen L, Benninga MA, et al. Probiotics for childhood functional gastrointestinal disorders: a systematic review andmeta-analysis. Acta Paediatr. 103(4), 365–72 (2014).

16. Koppen IJN, Benninga MA, Tabbers MM. Is there a role for pre-, pro- and synbiotics inthe treatment of functional constipation in children? A systematic review. J. Pediatr.Gastroenterol. Nutr. 63 Suppl 1, S27-35 (2016).

17. Anabrees J, Indrio F, Paes B, et al. Probiotics for infantile colic: a systematic review. BMC Pediatr. 13, 186 (2013).

18. Mugie SM, Benninga MA, DiLorenzo C. Epidemiology of constipation in children and adults: a systematic review. Best Pract Res Clin Gastroenterol 2011;25:3–18.

19. Loening-Baucke VA. Chronic constipation in children. Gastroenterology 1993;105:1557-64.

20.Bongers MEJ, van Dijk M, Benninga MA, et al. Healt related quality of life in children with constipation associated fecal incontinence. J Pediatr 2009; 154_749-53

21. Chogle A and Saps M. Yield and cost of perfoming screening tests for constipation in children.Can J Gastrenterol 2013; 27(12):e35-8.

22.Bharucha AE. Pelvic floor: anatomy and function. Neurogastroenterol Motil. 2006 Jul; 18(7):507–19.

23. Lee TH, Bharucha AE. How to perform and interpret a high-resolution anorectal manometry test. J Neurogastroenterol Motil. 2016 Jan 31; 22(1):46–59. [PubMed: 26717931]

24. Meunier P, Marechal JM, De Beaujeu MJ. Rectoanal pressures and rectal sensitivity studies in chronic childhood constipation. Gastroenterology 1979;77:330-6.
25. Molnar D, Taitz LS, Urwin OM,et al. Anorectal manometry results in defecationdisorders. Arch Dis Child 1983;58:257-61.
26. Loening-Baucke VA, Younoszai MK. Abnormal and sphincter response in chronically constipated children. J Pediatr 1982;100:213-8.
27. Loening-Baucke VA. Abnormal rectoanal function in children recovered from chronic constipation and encopresis. Gastroenterology 1984;87:1229-304.
28. Loening-Baucke VA, Younoszai MK. Effect of treatment on rectal and sigmoid motility in chronic constipated children. Pediatrics 1984;73:199-205.
29. Loening-Baucke VA, Cruikshank, BM. Abnormal defecation dynamics in chronically constipated children with encopresis. J Pediatr 1986;108:562-6.
30. AmbartsumyanL, Rodriguez L, Morera C, et al. Longitudinal andradial characteristics of intra-anal pressures in children using 3Dhigh-definition anorectal manometry: new observations. Am JGastroenterol. 2013;108(12):1918-1928.
31. RaizadaV, Bhargava V, Karsten A, et al. Functional morphology ofanal sphincter complex unveiled by high definition anal manometryand three dimensional ultrasound imaging. Neurogastroenterol Motil. 2011;23(11):1013-1019, e460.
32. BanasiukM, Banaszkiewicz A, Dziekiewicz M, et al. Values fromthree-dimensional high-resolution anorectal manometry analysisof children without lower gastrointestinal symptoms. ClinGastroenterol Hepatol. 2016;14(7):993-1000.e3.
33. Hyman PE, Milla PJ, Benninga MA, et al. Childhood functional gastrointestinal disorders: neonate/toddler. Gastroen- terology 2006;130:1519e26.
34. Vandenplas Y, Gutierrez-Castrellon P, Velasco-Benitez C, et al. Practical algorithms for managing common gastrointestinal symptoms in infants. Nutrition 2013;29:184e94.
35. Cohen-Silver J, Ratnapalan S. Management of infantile colic: a review. Clin Pediatr 2009;48:14e7.
36. Iacono G, Merolla R, D'Amico D, et al. Gastrointestinal symptoms in infancy: a population- based prospective study. Dig Liver Dis 2005;37:432e8.

37. Petzoldt J, Wittchen HU, Wittich J, et al. Maternalanxiety disorders predict excessive infant crying: a prospective longitudinal study. Arch Dis Child 2014;99:800e6.
38. Lee C, Barr RG, Catherine N, et al. Age-related incidence of publicly re- ported shaken baby syndrome cases: is crying a trigger for shaking? J Dev Behav Pediatr 2007;28:288e93.
39. Howard CR, Lanphear N, Lanphear BP, et al. Parental re- sponses to infant crying and colic: the effect on breastfeeding duration. Breastfeed Med 2006;1:146-155.
40. Gordon M, Biagioli E, Sorrenti M, et al. Dietary modifications for infantile colic. Cochrane Database Syst Rev 2018;10: CD011029.
41. Martinelli M, Ummarino D, Giugliano FP, et al. Efficacy of a standardized extract of Matricariae chamomilla L., Melissa offi- cinalis L. and tyndallized Lactobacillus acidophilus (HA122) in infantile colic: an open randomized controlled trial. Neuro Gastroenterol Motil 2017;29. https://doi.org/10.1111/nmo.13145.
42. Dobson D, Lucassen PL, Miller JJ, et al. Manipu- lative therapies for infantile colic. Cochrane Database Syst Rev 2012;12: CD004796.
43. Lucassen P. Colic in infants. BMJ Clin Evid 2010;2010:0309.
44. Vandenplas Y, Latiff AHA, Fleischer DM, et al. Partially hydrolyzed formula in non-exclusively breastfed infants: a systematic review and expert consensus. Nutrition 2019;57: 268e74.
45. Savino F, Palumeri E, Castagno E, et al. Reduction of crying episodes owing to infantile colic: a randomized controlled study on the efficacy of a new infant formula. Eur J Clin Nutr 2006;60:
46. Szajewska H, Gyrczuk E, Horvath A. Lactobacillus reuteri DSM 17938 for the management of infantile colic in breastfed infants: a randomized, double- blind, placebo-controlled trial. J Pediatr 2013;162:257-262.
47. Benninga MA, Voskuijl WP, Taminiau JA. Childhood constipation: is there new light in the tunnel? J Pediatr Gastroenterol Nutr. 2004;39:448–64.
48. Baker S, Liptak G, Colletti R, et al. Evaluation and treatment of constipation in children: summary of updated recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr. 2000;30(1):109.

49. Staiano A. Use of polyethylene glycol solution in functional and organic constipation in children. Ital J Gastroenterol Hepatol. 1999;31 Suppl 3:S260–3.
50. Pashankar DS, Bishop WP, Loening-Baucke V. Long-term efficacy of polyethylene glycol 3350 for the treatment of chronic constipation in children with and without encopresis. Clin Pediatr. 2003;42:815–9.
51. Di Palma JA, Cleveland MV, McGowan J, et al. A randomized, multicenter, placebo-controlled trial of polyethylene glycol laxative for chronic treatment of chronic constipation. Am J Gastroenterol. 2007;102:1436–41.
52. Pashankar DS, Loening-Baucke V, Bishop WP. Safety of polyethyleneglycol 3350 for the treatment of chronic constipation in children. Arch Pediatr Adolesc Med. 2003;157:661–4.
53. Cuperus FJ, Iemhoff AA, van der Wulp MY, et al. Acceleration of the gastrointestinal transit by polyethylene glycol effectively treats unconjugated hyperbilirubinemia in Gunn rats. Gut. 2010;59:373–80.
54. Gorkiewicz G, Thallinger GG, Trajanoski S, et al. Alterations in the colonic microbiota in response to osmotic diarrhea. PLoS One. 2013;8(2):e55817.
55. van Ginkel R, Reitsma JB, Buller HA, et al. Childhood constipation: longitudinal follow-up beyond puberty. Gastroenterology. 2003;125:357–63.

Chapter 7
-Other publications-

The Changing Face of Pediatric Ulcerative Colitis: A Population-based Cohort Study

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ABSTRACT

Objectives: The aims of this retrospective study were to describe ulcerative colitis (UC) phenotype at diagnosis and follow-up and to identify possible predictors of severe disease course.

Methods: This was a retrospective, single-center study. We reviewed the charts of patients with UC diagnosed between 2 and 18 years at our referral center from January 2007 to January 2016. Laboratory and clinical features at diagnosis, such as disease extent, atypical phenotypes, extraintestinal manifestations, and therapies, and pattern changes during the follow-up, including relapse rate, disease extension, and the cumulative risk for colectomy were collected.

Results: One hundred eleven patients were enrolled. Atypical phenotypes were identified at diagnosis in 55 out of 111 patients (49.5%). Extraintestinal manifestations were detected in 16 out of 111 (14.4%) at the diagnosis. During the follow-up 60 out of 111 (54%) patients needed to start azathioprine, 9 out of 111 (8.1%) patients started biologic therapy and 10 out of 111 (patients underwent surgery, resulting in a cumulative risk of 8% at 5 years and 16% at 10 years. Steroid refractoriness (hazard ratio: 13.9) and starting of biologic therapy (hazard ratio: 25.3) represented the best predictors for surgery. The cumulative probability of first relapse was 47% at 6 months and 63% at 1 year. Disease extension was reported in 21 out of 70 patients (30%).

Conclusion: Pediatric UC is associated with a severe phenotype and a high percentage of atypical features. Surgery rate seems to be decreased from early reports.

Key Words: atypical phenotypes, inflammatory bowel disease, pediatrics, ulcerative colitis

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What Is Known

- The diagnosis of pediatric onset ulcerative colitis may be more challenging due to the existence of atypical phenotypes.
- Ulcerative colitis onset during childhood has a different disease pattern and a more aggressive development compared to adult onset.

What Is New

- This is the largest study assessing the prevalence of ulcerative colitis atypical features at diagnosis, after the publication of the revised Porto criteria.
- Although associated with a higher percentage of atypical features and with a more severe phenotype, we observed a decrease in the surgery rate during the last decade in comparison with the early studies.

Inflammatory bowel disease (IBD), including Crohn disease (CD) and ulcerative colitis (UC), is a group of life-long chronic and relapsing inflammatory disorders of the gastrointestinal tract whose pathogenesis is still unknown (1,2). Although the incidence and prevalence of IBD in pediatric population differ among countries, the general trend shows an overall increase over the past few decades especially in adolescence and young adulthood (3,4). Differently from most studies reporting a predominance of CD incidence over UC (5), in Italy it has been described an opposite trend in children, with UC exceeding CD diagnoses (52% vs 40%) (6). UC is typically characterized by a chronic mucosal inflammation, which involves the intestinal tract starting from the rectum and proceeding continuously toward proximal segments. Nevertheless, the diagnosis of pediatric-onset UC may be more challenging due to the existence of atypical phenotypes (7). In particular, 6 different atypical UC phenotypes have been recently identified in the revised Porto criteria for the diagnosis of pediatric IBD: rectal sparing, short duration, cecal patch, upper gastrointestinal (UGI) findings, acute severe colitis (ASC) and backwash ileitis (8). The incidence of atypical features has been poorly described. It is well known that childhood-onset UC is characterized by a more extensive intestinal involvement and a more rapid progression when compared to adult UC, including a higher risk of corticosteroids dependency, an earlier immunosuppressive therapy introduction, and surgery occurrence (9,10). In 2009 Gower-Rousseau et al (11) reported the data from EPIMAD registry and found that pediatric UC is characterized by widespread localization, a high rate of disease extension, and a colectomy rate of 20% after 5 years of follow-up. As well stated in a

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A.S. served as investigator and member of advisory board for the following companies: D.M.G, Valeas, Angelini, Miltè, Danone, Nestlé, Sucampo, Menarini. E.M. served as speaker, as investigator, and member of advisory board for the following companies: Abbvie, Angelini, Bioprojet, Ferring, Menarini, Milte, Valeas. The remaining authors report no conflicts of interest.

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recent systematic review (12), limited studies are, however, available in the era of immunosuppressants and biological therapies. More recent data are needed to evaluate the impact of new treatment strategies on the natural history of pediatric UC and to define possible risk factors for a poor outcome of disease.

Therefore, the aims of this retrospective study were to describe UC phenotype at diagnosis and follow-up and to identify possible predictors of severe disease course in a large cohort of pediatric patients.

MATERIALS AND METHODS

This was a retrospective, single-center study. We performed a paper chart review of children and adolescents diagnosed with UC between 2 and 18 years at the pediatric IBD referral center of the Department of Translational Medical Science, University of Naples "Federico II" from January 2007 to January 2016. The inclusion criteria were a confirmed diagnosis of UC; age at diagnosis of 18 years or younger; a clinical follow-up of at least 12 months. We excluded from the analysis children affected by CD and IBD unclassified and patients with an initial diagnosis of UC, switching to CD during the follow-up. The diagnosis of UC was established on the basis of clinical, endoscopic, radiological, and histological criteria according to the Porto criteria until 2014 (13). Revised Porto criteria were used after their publication in June 2014 (8). At diagnosis, all patients underwent colonoscopy with mucosal biopsies and small bowel imaging, including abdominal ultrasound and magnetic resonance enterograhy or small bowel follow through. Not all, but the majority of the patients was also investigated with UGI endoscopy. The period between the first onset of IBD symptoms and diagnosis date was defined as diagnostic delay. Patients with a diagnosis at an age $\leq$6 years were defined as very early onset (VEO) IBD, as previously reported (14). For the purpose of this manuscript, disease extent was characterized on the basis of Paris classification (15). Disease extension was defined as the endoscopic and histologic involvement of at least 1 additional segment during the follow-up. In addition, we retrospectively identified at diagnosis the following atypical phenotypes on the basis of the revised Porto criteria (8): rectal sparing, defined as the absence of macroscopic disease in rectum or rectosigmoid; short duration or patchiness of disease, characterized by patchy involvement in biopsies or lack of typical architectural distortion in pathological specimens; cecal patch, represented by the presence of left-sided colitis with an area of cecal inflammation; UGI findings, characterized by erosions or small ulcers in stomach, neither serpiginous nor linear; ASC, characterized by transmural inflammation and deep ulcers, contiguously from the rectum; backwash ileitis, represented by macroscopic, short-segment, nonstenosing terminal ileitis, without granulomata (8). Clinical activity of the disease was evaluated at time of diagnosis using the Pediatric Ulcerative Colitis Activity Index (PUCAI) with the previously validated cut-off values for remission (<10 points), mild disease (<35), moderate disease (<65), and severe disease ($\geq$65) (16). In addition, timing of first clinical relapse, defined as the occurrence or worsening of symptoms accompanied by an increase of PUCAI >10 points, was also recorded. A relapse occurring within 12 months from the diagnosis was defined as an early relapse. Family history was defined as positive if at least one first-degree relative was diagnosed with IBD. Symptoms and laboratory parameters at diagnosis including erythrocyte sedimentation rate (mm/h), C-reactive protein (CRP, mg/dL), hemoglobin concentration (g/dL), platelets count ($\times 10^3/\mu$L) and fecal calprotectin (μg/g of stool) were also collected. Extraintestinal manifestations (EIMs) at diagnosis included skin, joints, ocular manifestations, pancreatic involvement, and primary sclerosing cholangitis. Two expert pediatric gastroenterologists (A.S. and E.M.) made all decisions regarding therapeutic interventions, in line with the validated international guidelines (17). The followed strategy was a classical step-up approach. Oral steroid treatment was generally used as induction therapy in patients with moderate to severe disease. Steroid dependency was defined as remission with corticosteroids but recurrence of symptoms when the dose was lowered or within 3 months following complete taper, or if steroids could not be stopped within 14 to 16 weeks; steroid refractoriness was defined as a nonresponse at oral steroids within 7 to 14 days (17). Aminosalicylates (5-ASA) were used as induction therapy in patients with mild to moderate disease and as a standard maintenance therapy. Patients in whom induction therapy had failed or patients with early relapse were treated with azathioprine (AZA). Methotrexate (MTX) was used as second-line immunosuppressant in those patients intolerant or refractory to AZA. Infliximab (IFX) (5 mg/kg per dose at weeks 0, 2, and 6 and then 8 weekly) was given as first biologic agent in patients refractory or intolerant to steroids and immunomodulators. Patients refractory or intolerant to IFX therapy were treated with adalimumab (loading dose: 160/80 or 80/40 mg at weeks 0 and 2, respectively in patients weighing $\geq$40 or <40 kg; maintenance dose: 40 and 20 mg every 2 weeks respectively in patients weighing $\geq$40 or <40 kg). In line with UC guidelines surgery was taken into considerations in patients with active or steroid-dependent UC despite maximal treatment with 5-ASA, thiopurines, and anti-tumor necrosis factor therapy or in case of ASC, not responding to the induction treatments. Elective subtotal colectomy with ileoanal pouch anastomosis in 2 to 3 stages was considered the preferred option (17).

Outcome Measures

In line with the objectives of the study, we identified the need for surgery as the primary outcome, defining a severe disease course. We considered early relapse within the first year and disease extension as the secondary outcomes. In addition, we compared VEO disease group to the remaining patients to distinguish the factors associated with a UC onset before 6 years of age.

Statistical Analysis

Variables were screened for their distribution, and appropriate parametric or nonparametric tests were adopted as necessary. The Student t test, the analysis of variance test and the Mann-Whitney test for continuous variables and the χ^2 and Fisher exact tests for categorical variables were used where appropriate. The Kaplan-Meier method was used to estimate the cumulative risk of surgery at 1, 2,5, and 10 years from the diagnosis and the cumulative risk of first relapse during the whole follow-up. Cox regression models were used to compute the risk of colectomy for baseline and follow-up patients' characteristics. Univariate and multivariate logistic regression analysis were used to explore the association between baseline characteristics and the risk of a relapse at 12 months, disease extension, and VEO disease. In multivariate modeling, all the factors associated at univariate analysis were entered using a stepwise approach. Statistical significance was predetermined as $P < 0.05$. Percentages were rounded to the nearest whole numbers. SPSS version 15 was used for all statistical analyses.

Ethical Considerations

The Institutional Review Board of the University of Naples "Federico II" approved the study protocol with the registration number 176/16. Written, informed consent was obtained from all parents and also from children, where appropriate.

RESULTS

One hundred eleven patients with a confirmed diagnosis of UC were identified between January 2007 and January 2016

TABLE 1. Baseline characteristics of 111 ulcerative colitis children at the time of diagnosis

Median age at enrolment (y, range)	16.5 (6–27.9)
Median age at diagnosis (y, range)	12 (2.1–17.5)
Sex (n, %)	
Male	51 (45)
Female	60 (55)
Median diagnostic delay (mo, range)	3 (0–80)
Diagnostic delay>6 mo (n, %)	38 (34.2)
Very early onset IBD (<6 y) (n, %)	23 (20.7)
Family history (n, %)	17 (15.3)
Symptoms (n, %)	
Abdominal pain	80 (72.1)
Bloody diarrhea	70 (63.1)
Rectal bleeding	32 (28.8)
Tenesmus	22 (19.8)
Weight loss	32 (28.8)
Median PUCAI (range)	40 (15–75)
Disease activity based on PUCAI	
Severe	4 (3.6)
Moderate	69 (62.2)
Mild	38 (34.2)
Disease extent (Paris classification) at diagnosis (n, %)	
Proctosigmoiditis (E1)	24 (21.6)
Left-sided colitis (E2)	21 (18.9)
Extensive colitis (E3)	25 (22.5)
Pancolitis (E4)	41 (36.9)
Extraintestinal manifestations (n, %)	16 (14.4)
Skin	2 (1.8)
Joints	3 (2.7)
Pancreatic involvement	6 (5.4)
Sclerosing cholangitis	5 (4.5)
Induction therapy at diagnosis (n, %)	
Steroids	60 (54.1)
Mesalazine	92 (82.9)
Immunosuppressants	17 (15.3)
Topic steroids	25 (22.5)
Topic mesalazine	24 (21.6)

IBD = Inflammatory bowel disease; UC = ulcerative colitis, UGI = upper gastrointestinal involvement.

(median age: 16 years; range 6–27.9; M/F: 50/61). All the patients were followed-up with a median of 51 months (range 12–185 months).

Demographic and Clinical Data at Diagnosis

Baseline characteristics at the time of diagnosis are reported in Table 1. Atypical phenotypes were identified at diagnosis in 55 out of 111 patients (49.5%). A patchy colonic inflammation was found in 22 out of 111 patients (19.8%). At diagnosis only 68 out of 111 (61.2) underwent UGI endoscopy and among them 20.5% (14/68) presented with UGI findings. Cecal patch was identified in 7 of 111 (6.3%) patients and rectal sparing in 5 of 111 (4.5%). Four out of 111 (3.6%) patients presented with ASC and 3 of 111 (2.7%) with backwash ileitis. Regarding induction therapy at the diagnosis, 54.1% (n = 60) received oral or intravenous corticosteroids, whereas 92 patients (82.9%) started 5-ASA or sulfasalazine. Twenty-five out 111 children (22.5%) needed a treatment with topical steroids and 24 of 111 (21.6%) with topical mesalazine. Seventeen out of 111 patients (15.3%) started AZA as maintenance therapy from the diagnosis (Table 1).

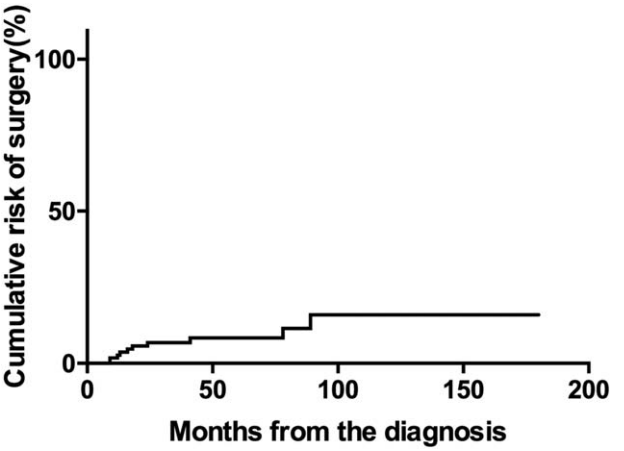

FIGURE 1. Cumulative risk of surgery in 111 children affected by ulcerative colitis. The cumulative probability of surgery was 0.03 (95% CI: 0.02–0.28) at 1 year, 0.07 (95% CI: 0.06–0.211) at 2 years, 0.08 (95% CI: 0.07–0.2) at 5 years, and 0.16 (95% CI: 0.1–0.3) at 10 years from time of diagnosis.

Medical Therapy Received During the Follow-up

During the follow-up steroid dependency was 23.4% and steroid refractoriness 7.2%. At the last follow-up 60 out of 111 (54%) patients needed to start second-line therapy with AZA. The median time to start AZA was 7 months (range 0–110 months). Among the 60 patients starting AZA, 51 (85%) achieved and maintained clinical remission, whereas 9 (15%) had to switch therapy due to side effects (3/60 [5%]) or lack of response (6/60 [10%]). Among patients failing AZA therapy, 8 switched to MTX and 1 to IFX as a rescue therapy for ASC. All the patients starting MTX switched to IFX therapy with a median time of 6.5 months (range: 1–26) for persistent activity (7/8 [87.5%]) or (1 [12.5%]) for the onset of side effects related to the therapy. Overall, 9 out of 111 patients (8.1%) started IFX. Two of these 9 children (22.2%) switched to adalimumab for loss of response. All the children starting biologic therapy were subsequently referred for surgery with a median time of 12 months (range 0–28 months).

Surgery

At the maximal follow-up, 10 out of 111 patients underwent surgery, resulting in a crude colectomy rate of 9%. Among children undergoing surgery, 4 out 10 (40%) were referred for ASC, whereas the remaining 6 (60%) were steroid-dependent UC, resistant to all medical treatments. The cumulative probability of surgery was 0.03 (95% CI: 0.02–0.28) at 1 year, 0.07 (95% CI: 0.06–0.211) at 2 years, 0.08 (95% CI: 0.07–0.2) at 5 years, and 0.16 (95% CI: 0.1–0.3) at 10 years from time of diagnosis (Fig. 1). Patients undergoing surgery had significantly higher values of PUCAI score and decreased hemoglobin values at diagnosis when compared to the remaining children (mean ± standard deviation: 48.7 ± 12.1 vs 37.6 ± 12.5, $P = 0.01$; 10.1 ± 1.8 vs 11.9 ± 1.8, $P = 0.001$, respectively). At univariate analysis, PUCAI ≥35 at diagnosis (hazard ratio [HR] = 1.1), early relapse within the 1 year (HR = 5.6), steroid refractoriness (HR = 28.8), need to start MTX (HR = 20.9), and biologic therapy (HR = 34.5) during the follow-up were associated with an increased risk of colectomy (Table 2). At the multivariate analysis, the only variables independently associated with an

TABLE 2. Risk factors associated with colectomy in 111 children with a diagnosis of ulcerative colitis at univariate and multivariate analysis

Variables	HR	95% CI	*P*
Univariate analysis			
Disease onset before 6 years of age	1.01	0.99–1.03	0.2
Sex	0.98	0.28–3.42	0.9
Family history of IBD	3.1	0.78–12.5	0.1
Diagnostic delay >6 mo	0.78	0.2–3.03	0.7
Disease extent at diagnosis (Paris classification)			
E1	0.86	0.24–3.08	0.8
E2	1.38	0.19–9.8	0.7
E3	1.41	0.2–9.7	0.7
E4	1.62	0.3–8.04	0.5
EIM at diagnosis	1.53	0.3–7.2	0.5
PUCAI ≥35 at diagnosis	1.11	1.05–1.17	**<0.001**
ASC at diagnosis	2.87	0.3–22.6	0.3
Positive CRP at diagnosis	5.45	0.67–44.4	0.1
Positive ESR at diagnosis	4.52	0.9–22.5	0.06
Fecal calprotectin >200 at diagnosis	30.5	0.02–540	0.3
Steroids at diagnosis	1.04	0.2–3.7	0.9
Immunosuppressants at diagnosis	3.08	0.8–11	0.08
Early relapse<12 mo	5.65	1.19–26	**0.03**
Steroid refractoriness	28.8	5.6–147.7	**<0.001**
Need to start methotrexate therapy	20.9	5.2–84.5	**<0.001**
Need to start biologic therapy	34.5	7.14–167	**<0.001**
Multivariate analysis			
Steroid refractoriness	13.9	2.2–1009	**0.01**
Need to start biologic therapy	25.3	5–127.6	**<0.001**

Values presented in bold are statistically significant.

ASC = acute severe colitis; 5% CI = 95% confidence intervals; CRP = C-reactive protein; EIM = extraintestinal manifestations; ESR = erythrocyte sedimentation rate; HR = hazard ratio; IBD = Inflammatory bowel disease; PUCAI = Pediatric Ulcerative Colitis Activity Index.

increased risk of surgery were steroid refractoriness (HR = 13.9) and the need of biological therapy during the follow-up (HR = 25.3) (Table 2).

Early Relapse

The rate of first relapse from the diagnosis was 24.3% (27/111) at 3 months, 47.7% (53/111) at 6 months, 61.3% (68/111) at 12 months, and 68.5% (76/111) at 24 months. The median time from the first relapse was 6 months (range 1–110). Based on Kaplan-Meyer analysis, the cumulative probability of first relapse was 0.47 (95% CI: 0.4–0.56) at 6 months, 0.63 (95% CI: 0.58–0.74) at 1 year, 0.73 (95% CI: 0.7–0.82) at 2 years, and 0.77 (95% CI: 0.72–0.9) at 3 years from the time of diagnosis (Fig. 2). At the univariate analysis a PUCAI ≥35 at (odds ratio [OR] = 1.04, 95% confidence intervals [CI] 1.01–1.08, $P = 0.04$), a positive CRP (OR = 2.89, 95% CI 1.27–6.56, $P = 0.02$) and the presence of pancolitis (OR = 3.1, 95% CI 1.07–9.3, $P = 0.03$) at diagnosis were significantly associated with an increased risk of early relapse within the first year. At the multivariate analysis, only a PUCAI ≥35 (OR = 1.05, 95% CI 1.01–1.09, $P = 0.01$) and a positive CRP (OR = 2.75, 95% CI 1.12–6.78, $P = 0.02$) confirmed to be independently associated with an increased risk of early relapse.

Disease Extension

Seventy out of 111 (61.3%) patients received an endoscopic follow-up. Among them, disease extension was reported in 21 patients (30%). In details, among the patients with E1 at diagnosis 4.1% (n = 1), 8.3% (n = 2), and 20% (n = 5) progressed to E2, E3, and E4, respectively; among children with an initial E2 location 14.2% (n = 3) advanced to E3 and 23.8% (n = 5) to E4; among UC children with E3 at diagnosis 20% (n = 5) progressed to E4. At maximal follow-up, disease location was E4 in 45 out of 111 patients (40%), E3 in 12.6% (n = 14), left-sided colitis (E2) in 8.1% (n = 9), and E1 in 18.9% of patients (n = 21); only 5 patients were in remission of disease. None of the analyzed variables resulted to be associated with disease extension both at the univariate and multivariate analysis.

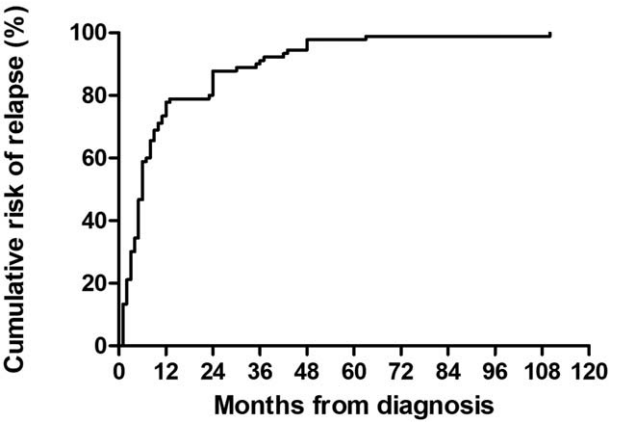

FIGURE 2. Cumulative risk of first relapse in 111 children affected by ulcerative colitis. The cumulative probability was 0.47 (95% CI: 0.4–0.56) at 6 months, 0.63 (95% CI: 0.58–0.74) at 1 year, 0.73 (95% CI: 0.7–0.82) at 2 years, and 0.77 (95% CI: 0.72–0.9) at 3 years from time of diagnosis.

Very Early Onset Inflammatory Bowel Disease

Twenty-three out of 111 patients (20.5%) were classified as VEO-IBD (onset <6 years) (Table 1). VEO-IBD children showed significant decreased values of hemoglobin and a significant higher platelets' count when compared with the remaining patients (mean ± standard deviation: 10.9 ± 2.2 vs 11.9 ± 1.7, $P = 0.03$; 525 ± 179 vs 354 ± 116, $P = 0.002$, respectively). At the univariate analysis the presence at diagnosis of EIMs (OR = 5.3, 95% CI 1.7–16.4, $P = 0.003$), ASC (OR = 13, 95% CI 1.2–132, $P = 0.02$) and a positive erythrocyte sedimentation rate (OR = 4, 95% CI 1.3–12.3, $P = 0.01$) resulted to be associated with the VEO children. VEO-IBD children did not show a higher risk of early relapse and surgery when compared to the remaining children (14/23 [60.9%] vs 54/88 [61.4%], $P = 0.9$; 2/23 [8.7%] vs 8/80 [9.1%], $P = 1$, respectively). At the multivariate analysis only the presence of EIMs at diagnosis resulted independently associated with VEO group (OR = 4.8, 95% CI 1.8–14.3, $P < 0.001$).

DISCUSSION

The present retrospective study describes the clinical features at diagnosis and the disease course of pediatric UC over the last 10 years at a referral center in Southern Italy. To the best of our knowledge this is the largest study assessing the prevalence of atypical features at diagnosis, after the publication of the revised Porto criteria. Furthermore our cohort provides useful insights about possible predictive factors for surgery and early relapse. Although confirming that pediatric UC is associated with a more severe phenotype, we demonstrated

a significant reduction of surgery rate at 5 and 10 years when compared with early reports.

As finally stated in the revised Porto criteria (8), atypical phenotypes represent a peculiarity of pediatric UC and are associated with relevant diagnostic challenges (18). Nevertheless, few studies have assessed their actual prevalence. Earlier in 2013, the Eurokids registry study tried to give an answer to this question (7). However, these data were published before the proper classification of Porto criteria and the authors only investigated some of these phenotypes, including UGI findings, rectal sparing, and backwash ileitis (7). In our study population, we demonstrated that the overall prevalence of atypical phenotypes at diagnosis in pediatric UC is rather high, reaching the 49.5% of our cohort. UGI findings resulted the most frequent atypical UC phenotype, being diagnosed in 20.5% of children. Levine et al (7) found that 4.2% of UC children presented UGI findings. Previously, Tobin et al (19) described histological gastritis in up to 50% of UC children. Not all our population was screened at diagnosis with a UGI endoscopy and in addition we do not routinely repeat it during the follow-up in patients with UC, unless symptomatic. Therefore, larger prospective studies are needed to establish the effective incidence of UGI findings in pediatric UC and clarify the need for further UGI control endoscopies. Patchy histologic inflammation, or short disease duration, was the second most frequent atypical feature. It seems to be the result of an early diagnosis, immediately after the starting of symptoms (8,20). Our prevalence of 19.8% is in agreement with the previous data from Aloi et al (21).

It is well known that UC onset during childhood has a different disease pattern and a more aggressive development compared to adult onset (22,23). Already in 2008, Van Limbergen et al (10) comparing a cohort of children with UC with an adult population demonstrated that 74.5% of pediatric patients were diagnosed with an extensive colitis based on the Montreal classification. These data were even worst at the maximal follow-up (82.2%) and significantly higher than adult patients with UC (47%) (10). The largest pediatric cohort data using Paris classification derives again from Eurokids registry (7). The authors reported that almost 78% of the enrolled children were affected by extensive colitis or pancolitis (7). The severity of UC pediatric phenotype was confirmed in our cohort, as demonstrated by the high percentage of pancolitis/extensive colitis at diagnosis (60%), the disease extension during the course of disease (30%), and the early relapse rate (61.3% within the first year from diagnosis). The identification of markers able to predict the disease course is strongly needed to stratify patients and orientate the treatment strategies. Many recent articles assessed the value of PUCAI at diagnosis and at 3 months as the best predictor of sustained steroid-free remission, ASC, and early need for rescue therapy (24–26). According with this literature, we demonstrated that a PUCAI $\geq$35 and a positive CRP at diagnosis are the most significant predictive factors for an early relapse within the first year. These data reinforce the importance of PUCAI monitoring and suggest a possible role for CRP at diagnosis, as reliable indicator of severe disease course in pediatric UC. Another marker of phenotype severity is usually represented by EIMs, which have been reported over a broad range from 3% to 30% (27–29). Aloi et al (30) more recently demonstrated in another Italian pediatric UC cohort that EIMs may be detected in at least the 16% of patients at diagnosis. In our population EIMs reach the percentage of around 14.4%, confirming the previous data. In addition, EIMs at diagnosis resulted in the only independent significant factor associated with the VEO-IBD group. This finding is in line with the consolidated hypothesis that VEO-IBD is usually associated with a more severe phenotype, including the possible involvement of other organs and systems (21,31).

In accordance with the current therapeutic management of pediatric UC (17), the majority of our children received 5-ASA both as first-line therapy for induction and maintenance of remission in children with mild to moderate UC, whereas 54.1% of children needed to receive a cycle of steroids for the induction of remission. As reported in other cohorts (32), up to 54% of children needed to start AZA and very early during the course of disease, with a median time of 7 months. These data are strikingly different from the initial percentages reported 2 decades ago from Hyams et al (9), and it is reflective of what it was anticipated from the Scottish cohort and from the EPIMAD registry (10,11). We use more immunosuppressive and biologics drugs and we use them earlier. This tendency to introduce earlier immunosuppressive drugs may be partly responsible of the decreased risk of surgery, more recently reported (33). Indeed, surgery is widely recognized as the main indicator to define the severe course of pediatric UC. In 2008 and in 2009, Van Limbergen et al (10) and Gower-Rousseau et al (11) described a cumulative risk of surgery of 26.1% and 20% at 5 years, respectively. In 2013 Aloi et al (30) reported a cumulative risk at 5 years of 14%; more recently Malmborg et al (34) and Rinawi et al (35) found that the risk of surgery was only 8% at 5 years and 17% at 10 years, respectively. Data of our study-population are perfectly in line with this recent literature with a crude colectomy rate of 9%. The Kaplan-Meyer analysis confirmed that the cumulative risk of surgery at 5 and 10 years were only 8% and 16%, respectively. These findings confirm a decreased surgery rates when comparing with the earlier studies. One could question whether surgery is merely postponed or if more aggressive strategies may actually modify the course of disease. With regards to the factors associated with the risk of surgery, previous studies identified EIMs and disease extension as the main risk factors (10,11). At the multivariate analysis, the need to start biologic therapy and the steroid refractoriness resulted in the only independent predictive factors for surgery in our cohort. These results stress the importance of steroid refractoriness, as negative prognostic factors during pediatric UC course. Differently, the data of biologics are strongly reflective of our therapeutic strategy. Indeed, although introducing earlier immunosuppressive drugs, we tend to use biologics only in those patients nonresponding to conventional therapies. Hence, this association may probably represent the selection of more aggressive phenotypes.

It is acknowledged that the present study is not without limitations. The main limitation is of course related to the retrospective nature, and therefore the concrete possibility of recall biases needs to be taken into account. In addition, this was a single-center study. The main strength lies in the availability of detailed medical records of a well-defined large cohort of pediatric patients with UC with a median follow-up duration of 4 years.

CONCLUSIONS

In conclusion this retrospective, single-center study in Southern Italy confirmed that pediatric UC represent both a diagnostic and a therapeutic challenge. Although associated with a higher percentage of atypical features and with a more severe phenotype, we observed a decrease in the surgery rate at 5 and 10 years during the last decade. Steroid refractoriness and the need to start of biologic therapy represented the most predictive factors for surgery, whereas a PUCAI $\geq$35 and a positive CRP at diagnosis were associated with an increased risk of early relapse. Further prospective, multicenter, longitudinal studies, are needed to better identify predictive factors of severe disease course and to clarify whether more aggressive medical strategies may be able to modify the natural history of pediatric UC.

REFERENCES

1. Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol* 2006;3:390–407.
2. Rosen MJ, Dhawan A, Saeed SA. Inflammatory bowel disease in children and adolescents. *JAMA Pediatr* 2015;169:1053–60.
3. Virta LJ, Saarinen MM, Kolho KL. Inflammatory bowel disease incidence is on the continuous rise among all paediatric patients except for the very young: a nationwide registry-based study on 28-year follow-up. *J Crohns Colitis* 2017;11:150–6.
4. Martín-de-Carpi J, Rodríguez A, Ramos E, et al. SPIRIT-IBD Working Group of SEGHNP (Sociedad Española de Gastroenterología Hepatología Nutrición Pediátrica). The complete picture of changing pediatric inflammatory bowel disease incidence in Spain in 25 years (1985–2009): the EXPERIENCE registry. *J Crohns Colitis* 2014;8:763–9.
5. Sawczenko A, Sandhu BK, Logan RF, et al. Prospective survey of childhood inflammatory bowel disease in the British Isles. *Lancet* 2001;357:1093–4.
6. Castro M, Papadatou B, Baldassare M, et al. Inflammatory bowel disease in children and adolescents in Italy: data from the pediatric national IBD register (1996–2003). *Inflamm Bowel Dis* 2008;14: 1246–52.
7. Levine A, De Bie CI, Turner D, et al. EUROKIDS Porto IBD Working Group of ESPGHAN. Atypical disease phenotypes in pediatric ulcerative colitis: 5-year analyses of the EUROKIDS Registry. *Inflamm Bowel Dis* 2013;19:370–7.
8. Levine A, Koletzko S, Turner D, et al. European Society of Pediatric Gastroenterology, Hepatology, and Nutrition. ESPGHAN revised porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. *J Pediatr Gastroenterol Nutr* 2014;58:795–806.
9. Hyams JS, Davis P, Grancher K, et al. Clinical outcome of ulcerative colitis in children. *J Pediatr* 1996;129:81–8.
10. Van Limbergen J, Russell RK, Drummond HE, et al. Definition of phenotypic characteristics of childhood-onset inflammatory bowel disease. *Gastroenterology* 2008;135:1114–22.
11. Gower-Rousseau C, Dauchet L, Vernier-Massouille G, et al. The natural history of pediatric ulcerative colitis: a population-based cohort study. *Am J Gastroenterol* 2009;104:2080–8.
12. Fumery M, Duricova D, Gower-Rousseau C, et al. Review article: the natural history of paediatric-onset ulcerative colitis in population-based studies. *Aliment Pharmacol Ther* 2016;43:346–55.
13. IBD Working Group of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition. Inflammatory bowel disease in children and adolescents: recommendations for diagnosis—the Porto criteria. *J Pediatr Gastroenterol Nutr* 2005;41:1–7.
14. Uhlig HH, Schwerd T, Koletzko S, et al. COLORS in IBD Study Group and NEOPICS. The diagnostic approach to monogenic very early onset inflammatory bowel disease. *Gastroenterology* 2014;147:990.e3–1007.e3.
15. Levine A, Griffiths A, Markowitz J, et al. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis* 2011;17:1314–21.
16. Turner D, Otley AR, Mack D, et al. Development, validation, and evaluation of a pediatric ulcerative colitis activity index: a prospective multicenter study. *Gastroenterology* 2007;133:423–32.
17. Turner D, Levine A, Escher JC, et al., European Crohn's and Colitis Organization; European Society for Paediatric Gastroenterology, Hepatology, and Nutrition. Management of pediatric ulcerative colitis: joint ECCO and ESPGHAN evidence-based consensus guidelines. *J Pediatr Gastroenterol Nutr* 2012;55:340–61.
18. Glickman JN, Bousvaros A, Farraye FA, et al. Pediatric patients with untreated ulcerative colitis may present initially with unusual morphologic findings. *Am J Surg Pathol* 2004;28:190–7.
19. Tobin JM, Sinha B, Ramani P, et al. Upper gastrointestinal mucosal disease in pediatric Crohn disease and ulcerative colitis: a blinded, controlled study. *J Pediatr Gastroenterol Nutr* 2001;32:443–8.
20. Robert ME, Tang L, Hao LM, et al. Patterns of inflammation in mucosal biopsies of ulcerative colitis: perceived differences in pediatric populations are limited to children younger than 10 years. *Am J Surg Pathol* 2004;28:183–9.
21. Aloi M, Lionetti P, Barabino A, et al., SIGENP IBD Group. Phenotype and disease course of early-onset pediatric inflammatory bowel disease. *Inflamm Bowel Dis* 2014;20:597–605.
22. Ruemmele FM, Turner D. Differences in the management of pediatric and adult onset ulcerative colitis–lessons from the joint ECCO and ESPGHAN consensus guidelines for the management of pediatric ulcerative colitis. *J Crohns Colitis* 2014;8:1–4.
23. Malaty HM, Abraham BP, Mehta S, et al. The natural history of ulcerative colitis in a pediatric population: a follow-up population-based cohort study. *Clin Exp Gastroenterol* 2013;6:77–83.
24. Turner D, Griffiths AM, Veereman G, et al. Endoscopic and clinical variables that predict sustained remission in children with ulcerative colitis treated with infliximab. *Clin Gastroenterol Hepatol* 2013;11: 1460–5.
25. Turner D, Mack D, Leleiko N, et al. Severe pediatric ulcerative colitis: a prospective multicenter study of outcomes and predictors of response. *Gastroenterology* 2010;138:2282–91.
26. Schechter A, Griffiths C, Gana JC, et al. Early endoscopic, laboratory and clinical predictors of poor disease course in paediatric ulcerative colitis. *Gut* 2015;64:580–8.
27. Abraham BP, Mehta S, El-Serag HB. Natural history of pediatric-onset inflammatory bowel disease: a systematic review. *J Clin Gastroenterol* 2012;46:581–9.
28. Dotson JL, Hyams JS, Markowitz J, et al. Extraintestinal manifestations of pediatric inflammatory bowel disease and their relation to disease type and severity. *J Pediatr Gastroenterol Nutr* 2010;51:140–5.
29. Rinawi F, Assa A, Hartman C, et al. Long-term extent change of pediatric-onset ulcerative colitis *J Clin Gastroenterol* 2017 [Epub ahead of print].
30. Aloi M, D'Arcangelo G, Pofi F, et al. Presenting features and disease course of pediatric ulcerative colitis. *J Crohns Colitis* 2013;7:e509–15.
31. Oliva-Hemker M, Hutfless S, Al Kazzi ES, et al. Clinical presentation and five-year therapeutic management of very early-onset inflammatory bowel disease in a large North American cohort. *J Pediatr* 2015;167:527.e1–3–32.e1–3.
32. Jakobsen C, Paerregaard A, Munkholm P, et al. Pediatric inflammatory bowel disease: increasing incidence, decreasing surgery rate, and compromised nutritional status: A prospective population-based cohort study 2007–2009. *Inflamm Bowel Dis* 2011;17:2541–50.
33. Adamiak T, Walkiewicz-Jedrzejczak D, Fish D, et al. Incidence, clinical characteristics, and natural history of pediatric IBD in Wisconsin: a population-based epidemiological study. *Inflamm Bowel Dis* 2013;19: 1218–23.
34. Malmborg P, Grahnquist L, Ideström M, et al. Presentation and progression of childhood-onset inflammatory bowel disease in Northern Stockholm County. *Inflamm Bowel Dis* 2015;21:1098–108.
35. Rinawi F, Assa A, Eliakim R, et al. Risk of colectomy in patients with pediatric-onset ulcerative colitis. *J Pediatr Gastroenterol Nutr* 2017;65: 410–5.

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RESEARCH Open Access

Oral administration of tannins and flavonoids in children with acute diarrhea: a pilot, randomized, control-case study

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Abstract

Background: AG is the most common cause of pediatric consultations among children between 2 and 5 years of age and it still leads to high mortality and morbidity. Its management is based on rehydration therapy, but this treatment is not effective in reducing duration of diarrhea. For this reason, other safer and less expensive interventions, which could be added to oral rehydration therapy, are of great interest.

Methods: A pilot, randomized, case-controlled trial was conducted in 60 children affected by AG (< 7 days) with mild-moderate dehydration, according to WHO recommendations, from1 year to 17 years old. Patients were divided into 2 Groups: Group 1 consisting of 30 children treated with Actitan F and standard oral rehydration (SOR); Group 2 consisting of 30 children who received only SOR. Both groups received treatment for seven days, respectively. Patients of Group 1 stopped for their own choice, SOR after the first 24 h and continued only with Actitan F.

Results: After 24 h of treatment, the median number of stools was 3.5 for Group 1, and 4 for Group 2. In Group 1 the difference between the number of stools at baseline ($n = 5$) and after 24 h of treatment ($n = 3.5$) was significant ($p < 0.0001$). At the end of treatment, the median duration of diarrhea in Group 1 was 5 days, compared with 4 days in the Group 2, this difference was not statically significant (p 0.48).

Conclusions: Oral administration of Actitan F associated with SOR seems safe and effective treatment in shortening the duration of AG in children. Further studies confirming these data are needed.

Trial registration: NCT03356327 (retrospectively registered).

Keywords: Acute gastroenteritis, Treatment, Tannins, Flavonoids

Background

Acute gastroenteritis (AG) is the most common cause of pediatric consultations among children between 2 and 5 years of age [1]. AG in children still leads to high mortality and morbidity [2].According to current European guidelines, the main treatment for AG is the oral rehydration with anhypo-osmolar solution and regular feeding without dietary changes, including milk consumption. However, these treatment, does not reduce severity and duration of symptoms. Considering the burden of AG to children and the high cost for healthcare system, effective and less expensive interventions, that could be added to the effect of oral rehydration therapy, are of great interest [3].

According to the European Society of Gastroenterology and Hepatology (ESPGHAN) guidelines for diarrhoea [4], probiotics, with evidence of efficacy such as *S. boulardii* and *L. rhamnosus GG*, can be considered as a supplement to hypo-osmolar solution. It is known that probiotics reduce the mean duration of diarrhoea by about 24 h, but their efficacy on stool amount is not described [4]. Although the grade of recommendation is very strong, quality of evidences is still low [5]. Other preparations used for treatment of diarrhoea include oral administration of immunoglobulins, antiperistaltic and antisecretory agents, such as various preparations of bismuth subsalicylate, cholestyramine and loperamide [6–10]. Use of

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antiperistaltic drugs is not effective and can cause serious side effects in children, including lethargy, seizures, Reye's syndrome, paralytic ileus and respiratory depression [11–13]. Use of loperamide, one of the most common antiperistaltic agents, is not recommended in young children and infants.

In conclusion, an ideal antidiarrheal agent should have a high index of safety even when used without close professional supervisions, and should be compatible with oral rehydration solutions, treating effectively diarrhea of any etiology with moderate cost. During last years, several randomized and controlled studies showed the efficacy of tannin-rich carob pod for the treatment of acute-onset diarrhea [10]. In 2003,Subbotina et al. demonstrated that tormentil root extract, which has high content of tannins, reduces the duration of rotavirus diarrhea compared with the control group in a pediatric population ($P < 0.0001$) [5].They also demonstrated that in the group treated with tormentil root extract, 8 of 20 children (40%) were diarrhea-free 48 h after admission to the hospital, compared with 1 of 20 in control group (5%, $P < 0.0001$) [5].

In this pilot study we aimed to evaluate the efficacy and the compliance of a treatment with Actitan-F, a natural molecular complex of tannins (from Agrimony and Tormentil) and flavonoids (Chamomile), associated with SOR, in a pediatric population of children affected by AG.

Methods

We included 60 children (mean age: 3.1 yrs., range 0.3-12 years) with a diagnosis of AG, referred between April and July 2017 to the Department of Translational Medicine, section of Pediatric, University of Naples Federico II.

Patients enrolled were children from 3 months to 12 years old, with a diagnosis of acute diarrhoea appeared less than 7 days before the admission, capability to oral rehydration, mild to moderate dehydration. Patients with diarrhoea over 7 days, serious somatic pathology and severe dehydration were excluded.

The study was approved by the Institutional Review Board of the University of Naples "Federico II" with the protocol number 25/17. At admission, written informed consent was obtained from participants' parents and from all patients older than 10 years. At first visit, a medical history was collected by one of the authors and all patients underwent clinical evaluation, including body weight and body temperature.

Frequency of bowel movements, stool consistency measured through the Bristol Stool Form Scale (BSFS) and other associated gastrointestinal symptoms, including nausea, vomiting, abdominal pain and rectal bleeding, were accurately recorded. The BSFS is the most commonly standardized instrument used to rate stool consistency in children [14]. On admission, the degree of dehydration was clinically determined for each patient, based on WHO recommendations and data were recorded on a scale from 1 to 3 (1 for mild or < 5%; 2 for moderate or 5 to 10%; 3 for severe or 10% and more) [15].

All enrolled children were randomly divided into two groups: Group 1 was treated with Actitan F and standard oral rehydration (SOR) and Group 2 was treated with SOR only ad libitum for 7 days (Fig. 1) SOR is a reduced osmolarity oral solution (50/60 mmol/L Na), which is the first line therapy recommended by ESPGHAN guideline for Acute Diarrhea [16]. Actitan F, instead, was orally administrated at dose of 1 sack every 4 h, maximum 4 sacks/day for 7 days. Caregivers were instructed to administer the daily dose after mixing the contents of the sachet with a small amount of water. The study products used in this trial were donated by Aboca® Società Agricola SpA., Località Aboca, 20, 52,037 Sansepolcro (AR) – Italy.

At home, all parents had to fulfil a daily diary to record number and consistency of stools, presence of fever, vomiting and children compliance with the therapy. During the final visit, scheduled after 7 days, the interim history was assessed, daily diaries were reviewed and discussed, and a physical evaluation was performed.

Outcomes

The primary outcome was to evaluate the efficacy of a treatment with Actitan-F, a natural molecular complex of tannins (from Agrimony and Tormentil) and flavonoids (Chamomile) added to SOR compared to SOR in a paediatric population of children affected by AG.

The secondary outcome was to assess safety, tolerability and compliance of the study products for short-term treatment.

Endpoints

The primary endpoint was the duration of diarrhoea, defined as the number of stools after 24 h of treatment or the time needed to normalize number and consistency of stools (compared with the period before the onset of diarrhoea). Secondary endpoint were the evaluation of vomiting, body weight, possible need of hospitalization, compliance to therapy.

Statistical analysis

Data were analysed using SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA) for descriptive and frequency results and presented as median and range for quantitative variables and percentiles for categorical variables. Significance was defined as P value < 0.05. Comparisons between the two groups used the Fisher's exact test for categorical variables and the Mann-Whitney U test for quantitative variables. $\chi 2$ test was used to

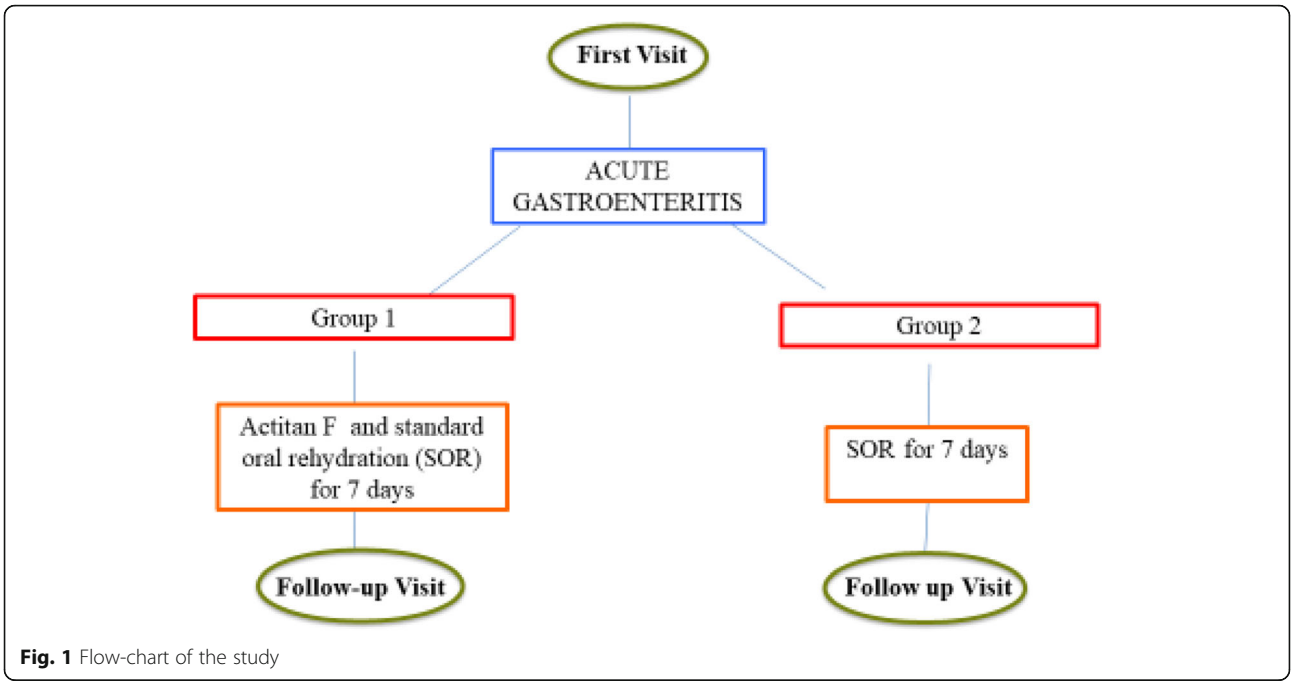

Fig. 1 Flow-chart of the study

determine patients' acceptance of the products (Actitan F and SOR) we tasted.

Results

All patients completed the study. Socio-demographic and clinical characteristics of the study population are showed in Table 1. At baseline, a mean of defecation/daily was 5 in both groups (ranged from 3 to 8 in Group 1 and from 2 to 10 in Group 2) ($p = 0.63$). The median number of stools after 24 h was 3.5 in Group 1 and 4 in Group 2. The difference in the number of stools the first day of treatment between the two groups was not statistically significant ($p = 0.4$); however, the difference between the number of stools at baseline and after 24 h of treatment was significant ($p = < 0.0001$) in the group treated with Actitan F. After 24 h of treatment, we found that the most of children (23/30, 76%) in Group 1, by their own choice, interrupted the assumption of SOR and continued to take only Actitan F. At the end of treatment, in Group 1 the primary endpoint was reached after 5 days while in Group 2 after 4 days, this difference was not statistically significant ($p = 0.48$) (Fig. 2). We also showed improved stool consistency from baseline to 24 h in the two groups, from liquid stools in 89.7 and 86.7% for Group 1 and Group 2, respectively, to 60 and 73.3% ($p = 0.34$). In particular, regarding stool consistency, we found that at day 6 in Group 1 76.9% of patients reported formed stools compared with 50% in Group 2 ($p = 0.028$). The presence of vomiting at baseline was 73.3% (22 children of 30) for Group 1 and 43.3% (13 children of 30) for the Group 2; at 24 h vomiting was present in 46.6% of the Group1 and 16% of the Group 2 with no statistical significant difference. Bloody diarrhoea was found in only 10 and 6% of patients at baseline for Group 1 and Group 2, respectively; at the end of treatment, no patient presented blood in the stool in both groups. As for weight and fever, we did not

Table 1 Demographics and Clinical characteristics at baseline

	Overall (*N*=60)	Group 1 (*N*=30)	Group 2 (*N*=30)	*p*-value
Median age (range), years	3.1 (0.3–12.7)	4.1 (0.5–12.1)	2.7 (0.3–12.7)	0.23
Starting date of diarrhea before consultation in days (range)	1 (1–4)	1 (1–4)	1 (1–3)	0.32
Median number of stools/day at baseline (range)	5 (2–10)	5 (3–8)	5 (2–10)	0.63
Consistency of stools at baseline (%)				0.35
Watery	81.4%	86.2%	76.7%	
Soft	18.6%	13.8%	23.3%	
Vomiting at baseline (median and range)	1.5 (0–23)	3 (0–23)	0.5 (0–7)	0.017
Fever (%)	50.0%	50.0%	50.0%	1.0

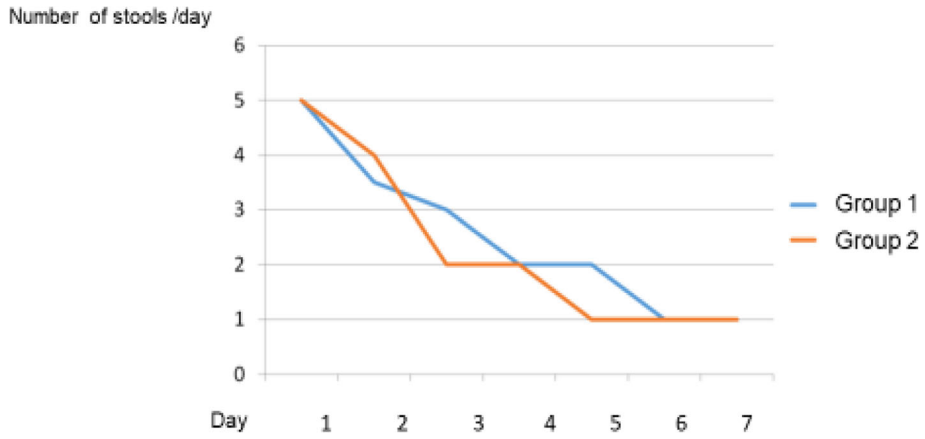

Fig. 2 Primary Endpoint: Bowel movements/ day

find any statistically significant difference between the groups. No one was hospitalized.

Compliance and patient acceptance of treatment

Concerning compliance with therapy, 8/30 children (26%) in Group 1 and 10/30 (30%) in Group 2 refused to take Actitan F and SOR, respectively ($p = 0.77$) (Fig. 3). Regarding the perception of the treatment efficacy, parents of children in Group 1 evaluated the Actitan F treatment very useful in 50% of cases, while parents of patients in Group 2 evaluated SOR quite effective in 68% of cases, this difference was statistically significant ($p < 0.0001$). Similar trend was present in the perception of improvement after 24 h, where we found a tendency to significance in Group 1 with respect to Group 2 ($p = 0.07$). No adverse events in both groups were reported during the study.

Discussion

In this pilot study, we investigated the therapeutic efficacy of a medical device made of natural complex substances in the treatment of children with AG. The positive clinical effect exerted by this new product on diarrhoea could be related to the tannins. Their action, indeed, is essentially focused on the intestinal mucosa, favouring its rapid recovery. More specifically, when tannins meet mucous membranes, they aspecifically interact with those membranes by making them more tight and less permeable; this process is called "astringency". This feature increases protection to the subadjacent layers of mucosa from the microorganisms and irritant chemicals [17].

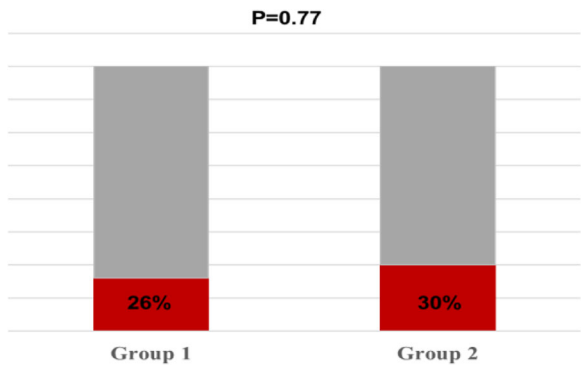

Fig. 3 Percentage of patients who complained with flavor of MDSM (Group1) and Oral solution (Group2) ($p = 0.77$)

The efficacy and safety of a similar product in treatment of acute diarrhoea in pediatrics have been already demonstrated during the last years. In 2009 Carretero et al. observed a significant decrease in the number of stools and an improvement in stool consistency in patients treated with oral rehydration solution (SOR) and gelatin tannate vs. patients treated only with SOR [18]. Loeb et al. showed that patients receiving a product containing tannate had a normalization of bowel movements, body temperature, weight, and ceased to vomit much faster than those receiving placebo [10].

Plein and colleagues studied the effect of tannate on diarrhoea in patients with Crohn's disease [19]. The results obtained demonstrated that there was a significant reduction in bowel movement frequency at the end of treatment. Another clinical experience was reported by Ziegenhagen and colleagues, who showed the better efficacy and safety profile of tannin salts versus activated charcoal. Moreover, in the group receiving tannin salts, the frequency of abdominal pain was lower than in the activated charcoal group (50 versus 82%) [20].

Although our study protocol included the administration of Actitan F plus SOR for a week in Group 1 and SOR alone in Group 2, most of the patients in the first Group interrupted the assumption of oral rehydration

after the first day of treatment. We can explain this choice considering that after 24 h of combined treatment the reduction in number of stools in Group 1 was significantly different than at baseline ($p < 0.0001$) (5 vs. 3.5 stools/day).

Due to the high perception of symptom improvement reported by parents of children in Group 1 after the first day of treatment, we can speculate that the rapid improvement of clinical condition may have induced parents to stop earlier the oral solution and continue only with Actitan F.

As a matter of fact, our results showing that the mean duration of diarrhea in both groups after 7 days of therapy was not statistically significant, should be interpreted in the light of what we have just described.

Regarding stool consistency, we showed no statistically significant differences between the two groups, however at day 6 of treatment we found that 76.9% of patients in Group 1 reported formed stools compared with 50% in Group 2, this data showed a tendency to significance with a $p = 0{,}028$, maybe do to the small number of patients enrolled. We can explain this better result on stool consistency comparing to SOR alone, knowing that it has already been demonstrated that tannins and flavonoids exerts their action by restoring the physiological function of the intestine barrier and have astringent properties [17].

Moreover, in our study any adverse event in children were observed in both groups, and this confirms the safety of tannins in the pediatric population. Compliance with administration of Actitan F was good; every patient received 75% of doses according to the patient's weight. This result confirms a good ease of administration.

In contrast to other studies, we did not perform stool cultures at baseline [21, 22]. Nevertheless, ESPGHAN does not recommend performing stool cultures for acute gastroenteritis in primary healthcare, and we did not aim to evaluate the impact of tannins based on the different aetiologies of diarrhoea.

Conclusion

Therefore, we can conclude that Actitan F in association with SOR, may offer a safe and cost-effective approach to AG in infants and children. However, further studies and large trials are required. Because this study showed promising results, it would be worthwhile to perform a large randomized control trial to unravel the efficacy of the Actitan F plus SOR in children with AG.

Abbreviations

AG: Acute gastroenteritis; SOR: standard oral rehydration

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Availability of data and materials

Data will not be shared in accordance to the Department's policy.

Authors' contributions

MR performed data acquisition, analysed the data, wrote the first draft of the manuscript, and approved the final version of the paper. VC performed data acquisition, critically revised the manuscript and approved the final version of the paper. VC, RB and AP performed data acquisition, critically revised the manuscript and approved the final version of the paper. EG designed the research study, analyzed the data, and approved the final version of the paper. AS designed the research study, critically revised the manuscript and approved the final version and the submission.

Ethics approval and consent to participate

The study was approved by the Independent Ethics Committee of the "Federico II" University of Naples (reference number: 25/17).

Competing interests

The authors declare that they have no competing interests.

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References

1. Van Damme P. Multicenter prospective study of the burden of rotavirus acute gastroenteritis in Europe, 2004–2005: the REVEAL study. J Infect Dis. 2007;195(Suppl 1):S4–16.
2. Ogilvie I. Burden of community-acquired and nosocomial rotavirus gastroenteritis in the pediatric population of Western Europe: a scoping review. BMC Infect Dis. 2012;12:62.
3. Szajewska H. Probiotics in gastrointestinal diseases in children: hard and not-so-hard evidence of efficacy. J Pediatr Gastroenterol Nutr. 2006;42:454–75.
4. Guarino A. Probiotics as prevention and treatment for diarrhea. Curr Opin Gastroenterol. 2009;25(1):18–23.
5. Subbotina Maria D. Effect of oral administration of tormentil root extract (Potentilla tormentilla) on rotavirus diarrhea in children: a randomized, double blind, controlled trial. Pediatr Infect Dis J. 2003;22:706–10.
6. Madkour AA. Cholestyramine in acute infantile diarrhea: a double-blind, placebo controlled clinical trial in hospitalized infants. Alexandria. J Pediatr. 1990;4:319–32.
7. Madkour AA. Smectite in acute diarrhea of children: a double-blind placebocontrolled clinical trial. Gastroenterology. 1991;100:A227.
8. Kaplan MA, Prior MJ, McKonly KI, et al. A multicenter randomized controlled trial of liquid loperamide product versus placebo in the treatment of acute diarrhea in children. Clin Pediatr. 1999;38:579–91.
9. Ebina T. Prevention of rotavirus infection with cow colostrum containing antibody against human rotavirus. Lancet. 1983;2:1029–30.
10. Loeb H. Tannin-rich carob pod for the treatment of acute-onset diarrhea. J Pediatr Gastroenterol Nutr. 1989;8:480–5.
11. Elaraby II. The use of chlorpromazine as an antisecretory agent in acute diarrhea of childhood. Alexandria J Pediatr. 1989;3:1–8.
12. Kassem AS. Loperamide in acute childhood diarrhoea: a double blind controlled trial. J Diarr Dis Res. 1983;1:10–6.
13. Kassem AS. Effect of antibiotics on the duration of diarrhea and speed of rehydration. Gazette Egyptian Paediatr Assoc 1983;31:117–118.
14. Lane MM. Reliability and validity of a modified Bristol stool form scale for children, J Pediatr. 2011;159:437–41.e1.
15. World Health Organization. The treatment of diarrhea: A manual for physicians and other senior health workers; 2005.
16. Guarino A. European Society for Pediatric Gastroenterology, hepatology, and nutrition/European Society for Pediatric Infectious Diseases Evidence-Based Guidelines for the Management of Acute Gastroenteritis in children in Europe: update 2014. JPGN. 2014;59(Issue 1):132–52.
17. Mills S, Bone K. The essential guide to herbal safety. UK: Churchil Livingstone; 2005.
18. Esteban Carretero J. A comparative analysis of ORS (oral rehydration solution) vs. ORS + gelatin tannin in two cohorts of pediatric patients with acute diarrhea. REV ESP ENFERM DIG (Madrid). 2009;101(1):41–8.

19. Plein K. Treatment of chronic diarrhea in Crohn disease. A pilot study of the clinical effect of tannin albuminate and ethacridine lactate. Fortschr Med. 1993;111:114–8.
20. Ziegenhagen DJ. Traveler's diarrhea in Turkey. Prospective randomized therapeutic comparison of charcoal versus tannin albuminate/ethacridine lactate. Med Klin. 1992;87:637–9.
21. Salazar-Lindo E. Racecadotril in the treatment of acute watery diarrhea in children. N Engl Med J. 2000;343:463–7.
22. Cezard J. Efficacy and tolerability of Racecadotril in acute diarrhea in children. Gastroenterology. 2001;120(799–805):13.

Probiotics in Pediatric Gastroenterology
Emerging Indications in Inflammatory Bowel Diseases

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Abstract: Etiology of inflammatory bowel disease (IBD) is not yet completely understood, but it is hypothesized that a disruption of the immune tolerance to gut microbiota, due to several potential factors like an abnormal gut microbiota composition and activity, may lead to IBD occurrence. Manipulation of the intestinal microbiota is an attractive target for the management of IBD, and probiotics could be useful to influence the disease's course. However, the existing literature on the usefulness of probiotics in IBD is relatively limited. At present, there is no evidence of efficacy for any bacterial strain in the induction or maintenance of remission in pediatric Crohn's disease, while there is limited evidence for the use of *VSL#3* and *Lactobacillus reuteri ATCC 55730*, in addition to standard therapy, for the induction of remission in pediatric ulcerative colitis. Moreover, current data assessing the therapeutic efficacy of probiotics in IBD do not fulfill evidence-based standards, with long-term maintenance studies and larger prospective randomized controlled trials still lacking.

Key Words: Crohn's disease, ulcerative colitis, bacterial strains, intestinal microbiota, children

(*J Clin Gastroenterol* 2018;52:S7–S9)

Inflammatory bowel disease (IBD) is a relapsing disorder of the gastrointestinal (GI) tract characterized by chronic inflammation secondary to a disregulation of the mucosal immune system. The most common forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC), which present with a combined prevalence of 450/100,000 in western populations. Etiology of IBD is not yet completely understood, but the most widely accepted theory involves genetic susceptibility (like NOD2 and ATG16L1 variants), alterations of the immune response, and environmental factors. It is hypothesized that a disruption of the immune tolerance to gut microbiota, due to several potential factors such as an abnormal gut microbiota composition and activity, may lead to IBD occurrence. The balance of beneficial versus aggressive intestinal microbes is responsible for either mucosal homeostasis or chronic inflammation.[1] In addition, environmental triggers are necessary to initiate or reactivate disease expression.

Intestinal microbiota composition is determined by genetic and environmental factors. Environmental contributions include colonization by maternal microbiota, with differences in case of vaginal delivery or cesarean section, breast or formula feeding, infections, and antibiotic use.[2] The different bacterial species and the host are mutually related, with bidirectional interactions such as modulation of metabolic activities and immune response by the intestinal microbiota, and intestinal microbiota composition linked to host's related factors as drugs, infections and, most of all, diet. The ability of the diet to influence intestinal microbiota has been the object of various clinical studies. Some evidence supports the hypothesis that western diet is able to select bacterial species with a proinflammatory effect that can contribute to the onset of numerous GI disorders related to the presence of dysbiosis, such as colon cancer and IBD.[3] CD represents a clear example of the relationship between diet and intestinal homeostasis, as its management, especially in children, is mainly based on nutritional therapy. Even if the mechanism by which nutritional therapy is able to induce remission in subjects with CD has not been clearly defined, it is currently known that the effects may be mediated by an interplay between intestinal microbiota and nutritional factors, as found by D'Argenio et al[4] that described a restoration of the intestinal microbiota composition in a patient with CD after induction therapy with exclusive enteral nutrition.

The aim of the present paper is to evaluate the interactions between the GI tract and the intestinal microbiota, to explore the theories behind the use of probiotics in IBD, and to summarize the evidence available on their efficacy in these disorders.

ROLE OF INTESTINAL MICROBIOTA IN IBD PATHOGENESIS

As already mentioned, there is strong evidence linking intestinal microbiota with IBD pathogenesis. Studies conducted in rodent models of intestinal inflammation showed that bacterial antigens are able to cause and support the appearance of chronic colitis and ileitis. Moreover, in different colitis-susceptible animal models it has been shown that normal luminal bacteria are necessary for the development of disease and for the activation of the immune system, while the germ-free state prevents the occurrence of inflammation.[2] In 2011, Steck et al[5] demonstrated that the metalloprotease gelatinase (GelE) produced by commensal strains of *Enterococcus faecalis* could aggravate murine intestinal inflammation damaging epithelial barrier integrity.

Intestinal microbiota includes also fungi and viruses, even if their impact on intestinal immune response is less known compared with bacteria. An association between

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virome and IBD pathogenesis has been suggested by the evidence of an increase in NOD2 signaling and of proinflammatory cytokines production during viral infections.[6] As for fungal microbiota, a role for its alterations in IBD pathogenesis has been hypothesized by Li et al[7] that found an increased fungal richness and diversity in the inflamed compared with the uninflamed mucosa of CD subjects, with expansion of *Candida* spp., *Cryptococcus neoformans*, *Alternaria brassicicola*, and *Gibberella moniliformis*, and a correlation between fungal microbiota and CD activity index. They also found significant differences in fecal fungal microbiota of subjects with CD compared with healthy subjects.

Even if a clear cause-effect relationship between microbiome and IBD occurrence has never been shown, several studies suggested a contribution of dysbiosis to disease symptoms appearance. In fact, in subjects with IBD intestinal inflammation is usually reported in mucosal areas with high bacterial abundance, and bacterial counts are higher compared with healthy subjects.[8] Moreover, antibiotic exposure has been associated with an 84% relative risk (RR) increase of IBD development in children and adults, and it is now known that specific antibiotics can induce remission in active IBD.[9]

MICROBIOTA ALTERATIONS AND ROLE OF PROBIOTICS IN IBD

Microbiota composition of healthy individuals is characterized by high diversity, with a predominance of Firmicutes and Bacteroidetes and low concentrations of Enterobacteriaceae. In recent years, the results of various microbiome studies have provided a lot of information regarding its perturbations in IBD. Patients with IBD present a different microbiota composition compared with healthy controls, with reduced bacterial diversity, decreased Bifidobacteria, expansion of Enterobacteriaceae, and parallel contraction of certain *Clostridium* subsets.[10] Many bacterial species that are decreased in IBD exhibit protective functions that mediate mucosal homeostasis (eg, Bifidobacteria are among the strains with probiotic activity). Typical microbiome changes in IBD include an augmentation of proinflammatory species, such as *Escherichia* and *Fusobacterium*, and a reduction in anti-inflammatory species such as *Faecalibacterium prausnitzii*.[11]

IBD represents a great burden for both the patients and the health care system. Medical therapies are associated with relevant side effects, including malignancies, whereas surgical therapies may have significant effects on the patient's quality of life. For this reason, "alternative" treatments are becoming very popular among IBD patients, especially in children. Considering all the above-mentioned evidence on a role of bacteria in IBD pathogenesis, manipulation of the intestinal microbiota appears as an attractive target for the therapeutic management of UC and CD, and probiotics seem a logical option to shift the microbial balance of the microbiota and influence the disease's course.[12]

There are several mechanisms by which probiotics can exert a positive effect in IBD. Some probiotic strains can avoid pathogen colonization by increasing the mucus release from goblet cells thus creating a mucus barrier, or providing a physical barrier, or increasing the integrity of the tight junctions. Other strains are able to release antimicrobial factors, while some others can modulate the host's immune response by signaling dendritic cells, or regulating cytokines production.[13]

Nevertheless, in spite of the validity of the conceptual basis for the use of probiotics, and of the experimental data available (mainly studies conducted in animal models) there are still doubts on their utility in IBD. In fact the existing literature on the usefulness of probiotics in IBD—especially randomized controlled trials (RCTs)—is relatively limited.

PROBIOTICS IN CD

A meta-analysis published in 2014 by Shen et al[14] evaluated the effects of probiotics on the induction and maintenance of remission in subjects with IBD. The authors identified 3 RCTs evaluating the induction of remission in CD patients, without finding a significant benefit of probiotics against placebo ($P = 0.35$, RR = 0.89), none of the studies involved pediatric patients. With regard to the maintenance of remission, they identified 7 RCTs conducted in CD subjects, with 3 trials enrolling children, and found no significant difference between the interventions ($P = 0.71$, RR = 1.09). The pediatric trials also reported the remission/response rates in active CD and none described any effect of probiotics. Focusing on the different bacterial strains, no effect of *Bifidobacteria*, *Escherichia coli*, and *Lactobacillus* was identified as a maintaining therapy in CD. The authors concluded that the evidence is insufficient to suggest a role of probiotics for CD management.

A RCT conducted in 2005 by Bousvaros et al[15] compared the duration of remission adding *Lactobacillus GG* (*LGG*) or placebo to standard therapy [including aminosalicylates (ASA), 6-mercaptopurine, azathioprine, and low-dose alternate day corticosteroids], in children with CD. The authors failed to demonstrate an effect of *LGG* against placebo in prolonging remission time, as the median time to relapse was 9.8 months in the *LGG* group versus 11 months in the placebo group ($P = 0.24$).

According to the consensus guidelines of the European Crohn's and Colitis Organisation (ECCO) and of the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) on the clinical management of pediatric CD, probiotics are not recommended for the maintenance of remission, and there is no benefit for reducing the risk of relapse.[16] This recommendation has been confirmed by the 2018 ESPGHAN Position Paper on nutrition in IBD, that does not recommend probiotics in the induction or maintenance of remission of pediatric CD.[17]

PROBIOTICS IN UC

The aforementioned meta-analysis by Shen et al[14] included 12 RCTs on UC and 4 RCTs on pouchitis. Only 1 study was conducted in children. Nine of the RCTs evaluated the effect of different bacterial strains (*Bifidobacteria*, *E. coli Nissle1917*, and *VSL#3*) for the induction of remission in UC, and suggested a beneficial effect of probiotics ($P = 0.01$, RR = 1.51). However, the subgroup analysis based on the probiotic strain highlighted a significantly increased remission/response rate only for *VSL#3* ($P = 0.004$, RR = 1.74). Considering the maintenance of remission, the analysis of the 5 RCTs performed in subjects with UC and of the 4 trials conducted in subjects with pouchitis did not find a beneficial effect of probiotics compared with controls ($P = 0.47$, RR = 0.89 and $P = 0.10$, RR = 0.28 in UC and pouchitis, respectively). The only pediatric trial included[18] found a significant effect of *VSL#3* as maintaining therapy in UC ($P = 0.02$, RR = 0.29), whereas 3 other trials performed in subjects with

pouchitis found that *VSL#3* was effective for preventing clinical relapse ($P < 0.00001$, RR = 0.20).

Another pediatric trial not included in the 2014 meta-analysis[19] evaluated the effect of an enema solution containing *Lactobacillus reuteri ATCC 55730* or placebo in addition to oral mesalazine, in children with active distal UC. The authors described a significant decrease of clinical, endoscopic, and histologic features in the *L. reuteri* group compared with the placebo group. Moreover, in the intervention group, an increase in the mucosal expression of interleukin (IL)-10 and a decrease in the mucosal expression of IL-1b, tumor necrosis factor-α, and IL-8 was also documented.

The ECCO/ESPGHAN consensus guidelines on the management of pediatric UC[20] assert that there is insufficient evidence to recommend the routine probiotic use for the induction or maintenance of remission. However, probiotics may be considered in case of intolerance to 5-ASA and mild UC, or as an adjuvant therapy in case of mild residual activity. As for pediatric pouchitis, there was a 100% consensus for the usefulness of probiotics in the maintenance of an antibiotic-induced remission in subjects with recurrent pouchitis.

The 2018 ESPGHAN Position Paper on nutrition in IBD[17] concludes that there is limited evidence in favor of the use of *VSL#3* or *L. reuteri ATCC 55730* in addition to standard therapy for the induction of remission in pediatric UC, and that there is evidence in favor of *VSL#3* or *E. coli Nissle* as an alternative to 5-ASA in the maintenance of remission in mild to moderate pediatric UC.

CONCLUSIONS

In conclusion, at present, there is no evidence of efficacy for any probiotic strain in the induction or maintenance of remission in pediatric CD, whereas there is limited evidence for the use of *VSL#3* and *L. reuteri ATCC 55730*, in addition to standard therapy, for the induction of remission of pediatric UC. However, as IBD is a heterogenous condition, presenting with various phenotypes, it is likely that the patient-probiotic interactions examined are not exhaustive. Moreover, current data assessing the therapeutic efficacy of probiotics in IBD do not fulfill evidence-based standards, with long-term maintenance studies and larger prospective RCTs still lacking.

REFERENCES

1. Larmonier CB, Shehab KW, Ghishan FK, et al. T lymphocyte dynamics in inflammatory bowel diseases: role of the microbiome. *Biomed Res Int*. 2015;2015:504638.
2. Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology*. 2008;134:577–594.
3. Albenberg LG, Wu G. Diet and the intestinal microbiome: associations, functions, and implications for health and disease. *Gastroenterology*. 2014;146:1564–1572.
4. D'Argenio V, Precone V, Casaburi G, et al. An altered gut microbiome profile in a child affected by Crohn's disease normalized after nutritional therapy. *Am J Gastroenterol*. 2013;108:851–852.
5. Steck N, Hoffmann M, Sava IG, et al. *Enterococcus faecalis* metalloprotease compromises epithelial barrier and contributes to intestinal inflammation. *Gastroenterology*. 2011;141:959–971.
6. Kim YG, Park JH, Reimer T, et al. Viral infection augments Nod1/2 signaling to potentiate lethality associated with secondary bacterial infections. *Cell Host Microbe*. 2011;9:496–507.
7. Li Q, Wang C, Tang C, et al. Dysbiosis of gut fungal microbiota is associated with mucosal inflammation in Crohn's disease. *J Clin Gastroenterol*. 2014;48:513–523.
8. Jonkers D, Penders J, Masclee A, et al. Probiotics in the management of inflammatory bowel disease: a systematic review of intervention studies in adult patients. *Drugs*. 2012;72:803–823.
9. Suskind DL, Cohen SA, Brittnacher MJ, et al. Clinical and fecal microbial changes with diet therapy in active inflammatory bowel disease. *J Clin Gastroenterol*. 2018;52:155–163.
10. Gosiewski T, Strus M, Fyderek K, et al. Horizontal distribution of the fecal microbiota in adolescents with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr*. 2012;54:20–27.
11. Gevers D, Kugathasan S, Denson LA, et al. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe*. 2014;15:382–392.
12. Guandalini S. Update on the role of probiotics in the therapy of pediatric inflammatory bowel disease. *Expert Rev Clin Immunol*. 2010;6:47–54.
13. Gareau MG, Sherman PM, Walker WA. Probiotics and the gut microbiota in intestinal health and disease. *Nat Rev Gastroenterol Hepatol*. 2010;7:503–514.
14. Shen J, Zuo ZX, Mao AP. Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials. *Inflamm Bowel Dis*. 2014;20:21–35.
15. Bousvaros A, Guandalini S, Baldassano RN, et al. A randomized, double-blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. *Inflamm Bowel Dis*. 2005;11:833–839.
16. Ruemmele FM, Veres G, Kolho KL, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. *J Crohns Colitis*. 2014;8:1179–1207.
17. Miele E, Shamir R, Aloi M, et al. Nutrition in pediatric inflammatory bowel disease: a position paper on behalf of the porto inflammatory bowel disease group of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr*. 2018;66:687–708.
18. Miele E, Pascarella F, Giannetti E, et al. Effect of a probiotic preparation (VSL#3) on induction and maintenance of remission in children with ulcerative colitis. *Am J Gastroenterol*. 2009;104:437–443.
19. Oliva S, Di Nardo G, Ferrari F, et al. Randomised clinical trial: the effectiveness of *Lactobacillus reuteri* ATCC 55730 rectal enema in children with active distal ulcerative colitis. *Aliment Pharmacol Ther*. 2012;35:327–334.
20. Turner D, Levine A, Escher JC, et al. Management of pediatric ulcerative colitis: joint ECCO and ESPGHAN evidence-based consensus guidelines. *J Pediatr Gastroenterol Nutr*. 2012;55:340–361.

Sporadic pediatric severe familial adenomatous polyposis: A case report

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Abstract. Familial adenomatous polyposis (FAP) is an autosomal dominant hereditary precancerous condition caused by germline pathogenetic variants in the tumor suppressor adenomatous polyposis coli (*APC*) gene. Patients with FAP develop multiple gastrointestinal adenomatous polyps usually at the age of ~20 years, which, if untreated, become cancerous in 100% of cases. Genotype-phenotype associations have been extensively described; however, inter- and intra-familial variability exists. It is crucial to characterize the causative pathogenetic variant in each pedigree in order to develop a cancer prevention program and follow-up strategy for at-risk families. The present report describes a severe case of sporadic FAP that was diagnosed when the patient was ~2 years old. The patient was a carrier of the *de novo* pathogenic c.4132 C>T (p.Gln1378X) variant. Additionally, the patient was a carrier of the homozygous c.5465 T>A (p.Asp1822Val) polymorphism, inherited from both parents. However, it remains unclear whether or not this polymorphism is involved in the phenotypic manifestation. This case highlights the need to extend molecular screening to very young children when they show iron-deficiency, anaemia and/or rectal bleeding, even in the absence of a familial history of disease.

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Abbreviations: CRC, colorectal cancer; FAP, familial adenomatous polyposis; APC, adenomatous poliposis coli; EGD, esophagogastroduodenoscopy; SNPs, single nucleotide polymorphisms

Key words: colorectal cancer, pediatric early onset familial adenomatous polyposis, *de novo* germline *APC* gene variants

Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the second in women, with 1.4 million estimated cases worldwide, and 700,000 estimated deaths (1,2). Familial adenomatous polyposis (FAP) is an autosomal dominant pre-cancerous syndrome characterized by the development of hundreds to thousands of colorectal adenomatous polyps that, if untreated, lead to CRC in the third to fourth decade of life (3-5). According to the European Medicines Agency, in 2009, there were 3-10/100,000 new cases in the European Union (6).

Germline pathogenic variants in the tumor suppressor adenomatous polyposis coli (*APC)* gene are responsible for FAP (7). *De novo* pathogenetic variants in *APC* are also found in the majority of sporadic cases of FAP (8,9). The *APC* gene is located on chromosome 5q and encodes a 312 kDa protein that is involved in several cellular processes, such as cell migration, adhesion and cell cycle regulation, as well as chromosome segregation, signal transduction and apoptosis (10,11). The tumour suppressing activity of APC depends on its capacity to regulate β-catenin levels in the nucleus. In fact, in the absence of Wnt signalling, APC induces β-catenin degradation. If the extracellular Wnt signal is absent, APC-induced β-catenin degradation is inhibited, β-catenin accumulates in the nucleus and modulates gene transcription. Pathogenic variants that disrupt APC interaction with β-catenin are oncogenic (6-11).

The classical symptoms of FAP, including bleeding, diarrhoea and abdominal pain, are generally diagnosed at around 20-25 years of age. Genetic testing is performed to verify the clinical diagnosis and to identify asymptomatic carriers in affected families. Early FAP detection by genetic screening in at-risk families is crucial in order to implement effective prophylaxis strategies. Moreover, the results of genotyping should be considered together with clinical data to decide the type and time of surgery (12).

Various symptomatic young patients with a family history of FAP have been reported, however we describe a case of severe paediatric FAP caused by a *de novo* pathogenic variant.

Case report

The patient described herein is a ten-year-old boy clinically diagnosed with FAP at the age of nine and recruited to the Ambulatorio di Pediatria of AOU Federico II of Naples in December 2016. The anamnesis revealed that symptoms, i.e. diarrhoea and rectal bleeding, first appeared at the age of two and became more frequent during late childhood. Colonoscopies and esophagogastroduodenoscopy performed in December 2015, December 2016, March 2018 and February 2019 showed hundreds of subcentimetre polyps throughout the colon (Fig. 1A) as well as polyps in the gastric fundus (>10 mm) and body (<5 mm) (Fig. 1B), respectively, thereby confirming the diagnosis of FAP. Histological analysis revealed a *Helicobacter pylori* infection-negative chronic gastritis-like inflammation of the stomach, inflammatory infiltrate in the ileum and low grade dysplasia of the glandular epithelium of the colon. Haematochemical analysis revealed microcytic anaemia with a haemoglobin level of 10.3 g/dl and a mean cellular volume of 66.5fl. The results of physical analysis were unremarkable. As shown in Fig. 2A, there was no family history of FAP, other polyposis syndromes or colon cancer. The patient's parents received clinical and genetic counselling and provided informed consent to molecular screening.

Genomic DNA was extracted from peripheral blood lymphocytes of the proband and his parents as previously described (13). Briefly, 2 ml of the patient's blood were incubated at 37˚ in a red blood lysis buffer (0.15 M NH_4Cl_2 and 0.17 M Tris-HCl, pH 7.65) for 15 min and centrifuged. The lymphocyte pellet was resuspended in a DNA extraction buffer (1 M Tris.HCl, 0.5 M EDTA and 5 M NaCl) and digested with Proteinase K and 10% SDS at 60˚C for ten minutes. After adding 6M NaCl, the sample was centrifuged, the DNA in the supernatant was precipitated with absolute ethanol and resuspended in an appropriate volume of deionized sterile water. The quality and the quantity of the DNA was spectrophotometrically assessed with the NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). An absorbance ratio at 260 and 280 nm in the range of 1.8-2.0 was considered good for further analysis.

APC exons 1 to 15 were amplified by polymerase chain reaction (PCR) using 100 ng of the proband genomic DNA and primer pairs described by Groden *et al* (7) in a 50 μl reaction mixture (Table I). The amplification protocol was as follows: 5 min at 95˚C, then 35 cycles of 20 sec at 95˚C, 30 sec at 60˚C and 45 sec at 72˚C, and a final extension of 5 min at 72˚C. All reactions were run in the MyCycler thermal cycler (Bio-Rad). Amplified fragments were run on a 1X agarose gel and visualized with ethidium bromide, then purified using the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's recommendations and subjected to automated Sanger sequencing (Fig. 2B and C).

The sequences analysis was performed by alignment with those present in the GenBank database using the BLASTn software (http://www.ncbi.nlm.nih.gov/blast/html). The accession number of the used reference sequence was NM_000038.4. The same procedure was followed for the genetic analysis of *APC* exon 15 (fragment H) on the DNA of both the patient's parents (Fig. 2B and C).

Genetic counselling excluded a family history of adenomatous polyposis syndrome. Indeed, the patient's parents reported that none of his first grade relatives, including themselves, had symptoms correlated to FAP or to those developed by the proband, such as chronic gastritis-like inflammation of the stomach in the absence of *Helicobacter pylori* infection. However, none of them underwent colonoscopy.

Sequence analysis of the *APC* gene revealed the causative pathogenetic variant in the proband: A heterozygous C to T transition in the exon 15H called c.4132 C>T (p.Gln1378X), which changes the glutamine at codon 1378 in a premature stop codon (Fig. 2C). The proband was also carrier of the rs459552, [c.5465 T>A (p.Asp1822Val)] polymorphism, in fragment L of exon 15 of the APC gene (data not shown). Such polymorphism causes the substitution of a valine with an aspartate at codon 1822 and is reported as benign in the ClinVar database (https://www.ncbi.nlm.nih.gov). Furthermore, the *APC* genetic carrier test for c.4132 C>T (p.Gln1378X) mutation was negative in the proband's parents (Fig. 2C), whereas both carried the rs459552 polymorphism.

Discussion

Approximately 70-80% of FAP cases are caused by inherited pathogenetic variants of the *APC* gene, whereas up to 25% of cases are attributed to *de novo* germline pathogenetic variants (3). Herein, we report the case of a ten-year old boy, clinically diagnosed with severe FAP, in the absence of a family history of polyposis. Genetic analysis of *APC* confirmed the clinical diagnosis. In fact, a stop codon variant, namely c.4132 C>T (p.Gln1378X) in fragment H of exon 15, was identified as the cause of FAP.

Although pathogenetic *APC* germline variants have a penetrance of ~100%, very close genotype-phenotype correlations and marked heterogeneity in the phenotypic expression of FAP are well known. Severe phenotypes, characterized by more than 5,000 polyps and early onset of the disease are associated with pathogenetic variants between codons 1250 and 1464, whereas patients with classical FAP develop hundreds to thousands of adenomatous polyps in the colorectum during the second and third decades of life (14,15). Germline pathogenetic variants between codons 168 and 1680 and deletion of entire *APC* locus are responsible for classical FAP (4,16). Attenuated phenotypes, characterized by a few polyps (~10-100) are associated with pathogenetic variants at the extreme 5' or 3' end of the *APC* gene or in alternatively spliced exon 9. Alterations in the region between codons 1286 and 1513, i.e., the mutation cluster region, probably provide a strong selective advantage to tumour cells. Indeed, the majority of somatic variants and germline *APC* variants are clustered in this region and cause aggressive phenotype (4,16). Thus, in accordance with previous studies, our patient, who carried a pathogenetic truncating variant at codon 1378, had a very early onset of the disease and severe FAP phenotype (17,18). The c.4132 C>T variant is reported as somatic by Liu *et al* (19) and in the *'Catalogue of Somatic Mutations in Cancer (COSMIC)'* (http://cancer.sanger.ac.uk/cosmic/mutation/overview?id=18862). However, Friedl and Aretz (20) found this variant as germlinal in a

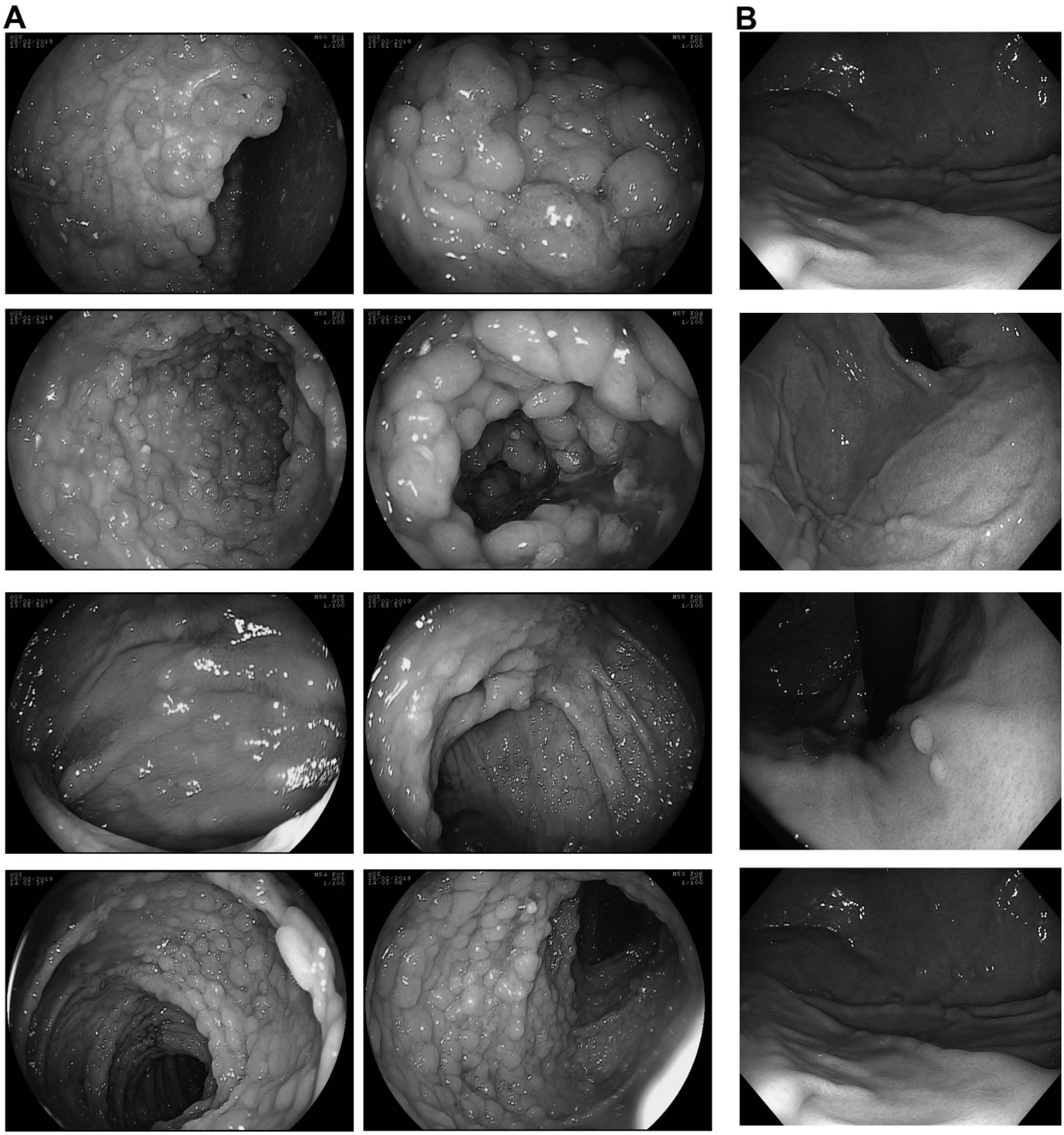

Figure 1. Clinical features of the proband. Colonoscopy and esophagogastroduodenoscopy performed during the last hospitalization in February 2019 revealed evidence of multiple (A) colorectal and (B) gastric adenomatous polyps.

FAP patient, but no information about the patient's history was provided (20). Moreover, to date, no genotype/phenotype correlations have been reported.

The patient also carried a second DNA variant, namely, c.5465 T>A (Asp1822Val). This polymorphism, which was also identified in the patient's parents, causes a valine/aspartate substitution at codon 1822. Several studies have investigated the role of polymorphisms in determining the risk of developing FAP or CRC, hypothesizing that the common variants in CRC genes now considered benign variants are, rather, low-risk alleles.

De Rosa *et al* (21) were the first to identify the Asp1822Val polymorphism. They analysed the genotype of two generations of individuals in ten families and concluded that it was not a disease-causing variant since it was found almost with the same frequency in normal and affected individuals, and it didn't modify the phenotype in any of the FAP patients. Furthermore, according to Fernández-Rozadilla *et al* (22), Asp1822Val is unrelated to CRC development and alleles T and A are equally distributed among cases and controls (P=0.2197) (22). In contrast, in a case-control study in which 1,785 CRC patients and 1,306 controls were analyzed, Picelli *et al* (23) found that the Asp1822Val polymorphism showed an odds ratio slightly lower in subjects carrying CRC than in controls (OR=0.75, CI=0.59-0.94), which suggested that the Asp1822Val substitution could protect from CRC (23). Finally, Wong *et al* (24) evaluated the association between eight *APC* single nucleotide polymorphisms and the risk of developing colorectal adenoma

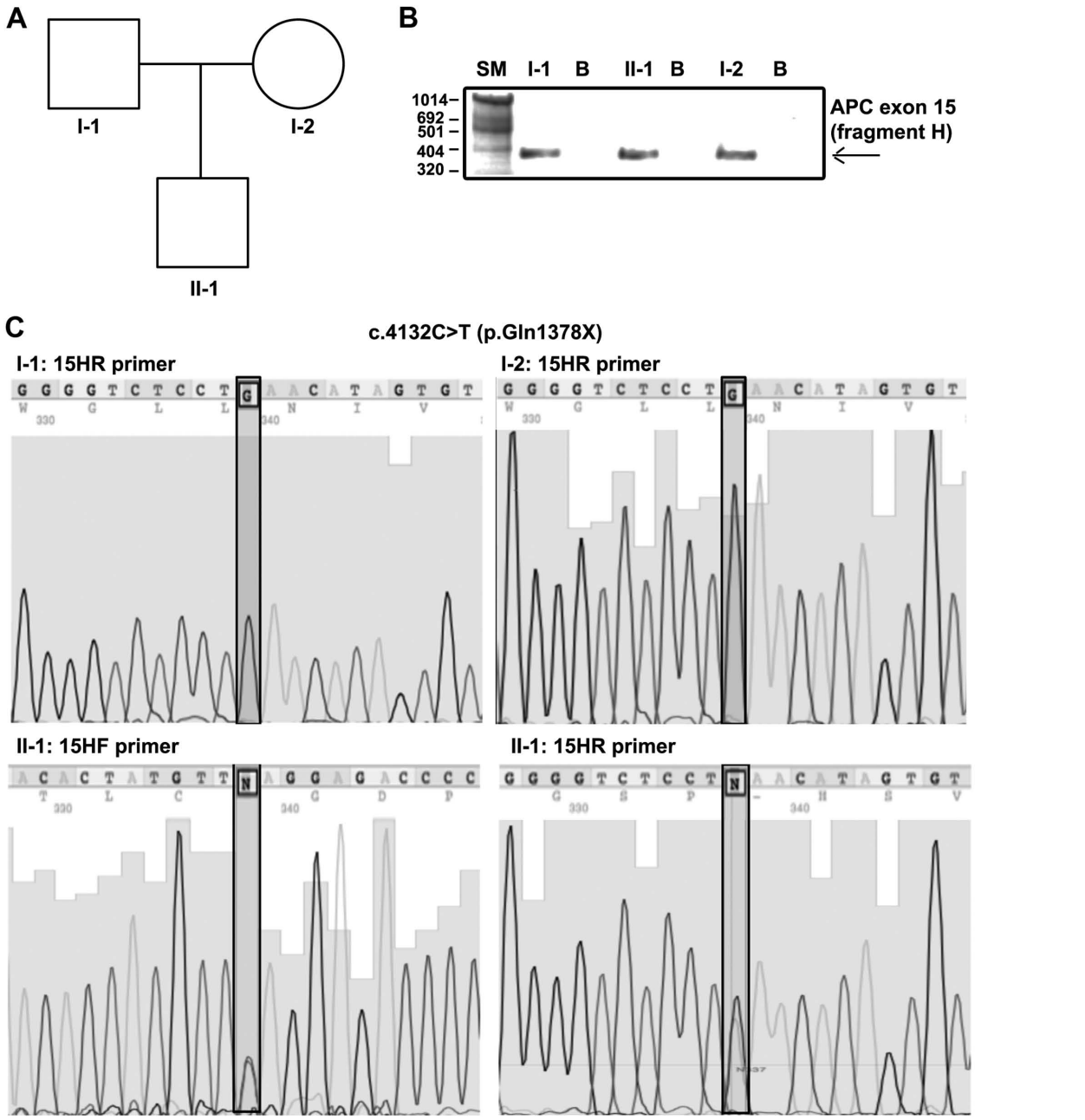

Figure 2. Molecular analysis of c.4132 C>T (p.Gln1378X) variant within the family. (A) Pedigree of the analyzed family. (B) PCR-electrophoresis gel image of exon 15 (fragment H) amplified from the proband's and the proband's parents' genomic DNA and (C) sequence analysis of APC exon 15 (fragment H) performed on fragments amplified from the genomic DNA of the proband (II.1) and his parents (subjects I-1 and I-2). Electropherograms showing the identified variant c.4132 C>T (p.Gln1378X) are reported. Specific nucleotides at position 4132 are shown in black boxes in each electropherogram. APC, adenomatous poliposis coli; SM, DNA size marker; I-1, II-1 and I-2, amplicons obtained by using DNA template from subjects I-1, II-1 and I-2, respectively; B, blank, PCR negative control performed without DNA template.

and concluded that none of the single nucleotide polymorphisms were associated with an increased risk. However, p. Asp1822Val was reported to influence the risk of colorectal adenoma in individuals with higher fat intake.

In conclusion, we found that the germline Gln1378X APC variant is the cause of a very severe sporadic FAP in the proband analyzed in the present study. Notably, the patient had the initial typical symptoms of the disease at the age of two years, although the clinical diagnosis was made when he was nine years old. Young symptomatic FAP patients have been described previously, but rarely in families with negative family history (25). We reported a severe case of FAP with onset in the toddler years, which represents a *de novo* pathogenetic variant (9). In both cases, the very young age at initial presentation (~2 years), as well as the absence of a family history of FAP, delayed the diagnosis for many years. Therefore, we reiterate the importance of endoscopic investigation in a child with iron-deficiency anaemia and a history of rectal bleeding should undergo endoscopic investigation notwithstanding no having a family history of FAP.

Further studies are required to determine whether Gln1378X and p.Val1822Asp play an additive role in his FAP phenotypic manifestations or not.

Table I. Primer pairs used for the genetic analysis of adenomatous poliposis coli exons 1 to 15.

Primers	Forward sequence (5'-3')	Reverse sequence (5'-3')
1 FP/RP	AGGTCCAAGGGTAGCCAAGG	TAAAAATGGATAAACTACAATTAAAAG
2 FP/RP	AAATACAGAATCATGTCTTGAAGT	ACACCTAAAGATGACAATTTGAG
3 FP/RP	TAACTTAGATAGCAGTAATTTCCC	ACAATAAACTGGAGTACACAAGG
4 FP/RP	ATAGGTCATTGCTTCTTGCTGAT	TGAATTTTAATGGATTACCTAGGT
5 FP/RP	CTTTTTTTGCTTTTACTATTAACG	TGTAATTCATTTTATTCCTAATAGCTC
6 FP/RP	GGTAGCCATAGTATGATTATTTCT	CTACCTATTTTTATACCCACAAAC
7 FP/RP	AAGAAAGCCTACACCATTTTTGC	GATCATTCTTAGAACCATCTTGC
8 FP/RP	ACCTATAGTCTAAATTATACCATC	GTCATGGCATTAGTGACCAG
9 FP/RP	AGTCGTAATTTTGTTTCTAAACTC	TGAAGGACTCGGATTTCACGC
9a FP/RP	TCATTCACTCACAGCCTGATGAC	GCTTTGAAACATGCACTACGAT
10 FP/RP	AAACATCATTGCTCTTCAAATAAC	TACCATGATTTAAAAATCCACCAG
10a FP/RP	AGACTAGGACTGAGACATTAATCATC	GGTGAGGAGTGAGAAGAAGGTAATC
11 FP/RP	GATGATTGTCTTTTTCCTCTTGC	CTGAGCTATCTTAAGAAATACATG
12 FP/RP	TTTTAAATGATCCTCTATTCTGTAT	ACAGAGTCAGACCCTGCCTCAAAG
13 FP/RP	TTTCTATTCTTACTGCCTAGCATT	ATACACAGGTAAGAAATTAGGA
14 FP/RP	TAGATGACCCATATTCTGTTTC	CAATTAGGTCTTTTTGAGAGTA
15A FP/RP	GTTACTGCATACACATTGTGAC	GCTTTTTGTTTCCTAACATGAAG
15B FP/RP	AGTACAAGGATGCCAATATTATG	ACTTCTATCTTTTTCAGAACGAG
15C FP/RP	ATTTGAATACTACAGTGTTACCC	CTTGTATTCTAATTTGGCATAAGG
15D FP/RP	CTGCCCATACACATTCAAACAC	TGTTTGGGTCTTGCCATCTT
15E FP/RP	AGTCTTAAATATTCAGATGAGCAG	GTTTCTCTTCATTATATTTTATGCTA
15F FP/RP	AAGCCTACCAATTATAGTGAACG	AGCTGATGACAAGATGATAATG
15G FP/RP	AAGAAACAATACAGACTTATTGTG	ATGAGTGGGGTCTCCTGAAT
15H FP/RP	ATCTCCCTCCAAAAGTGGTGC	TCCATCTGGAGTACTTTCTGTG
15I FP/RP	AGTAAATGCTGCAGTTCAGAGG	CCGTGGCATATCATCCCCC
15J FP/RP	CCCAGACTGCTTCAAAATTACC	GAGCCTCATCTGTACTTCTGA
15K FP/RP	CCCTCCAAATGAGTTACGTGA	TTGTGGTATAGGTTTTACTGGTG
15L FP/RP	ACCCAACAAAAATCAGTTAGATG	GTGGCTGGTAACTTTAGCCTC
15M FP/RP	ATGATGTTGACCTTTCCAGGG	ATTGTGTAACTTTTCATCAGTTGC
15N FP/RP	AAAGACATACCAGACAGAGGG	CTTTTTTGGCATTGCGGAGCT
15O FP/RP	AAGATGACCTGTTGCAGGAATG	GAATCAGACGAAGCTTGTCTAGAT
15P FP/RP	CCATAGTAAGTAGTTTACATCAAG	AAACAGGACTTGTACTGTAGGA
15Q FP/RP	CAGCCCCTTCAAGCAAACATG	GAGGACTTATTCCATTTCTACC
15R FP/RP	CAGTCTCCTGGCCGAAACTC	GTTGACTGGCGTACTAATACAG
15S FP/RP	TGGTAATGGAGCCAATAAAAAGG	TGGGAGTTTTCGCCATCCAC
15T FP/RP	TGTCTCTATCCACACATTCGTC	ATGTTTTTCATCCTCACTTTTTGC
15U FP/RP	GGAGAAGAACTGGAAGTTCATA	TTGAATCTTTAATGTTTGGATTTGC
15V FP/RP	TCTCCCACAGGTAATACTCCC	GCTAGAACTGAATGGGGTACG
15W FP/RP	CAGGACAAAATAATCCTGTCCC	ATTTTCTTAGTTTCATTCTTCCTC

FP, forward primer; RP, reverse primer.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

MDR and PI designed the study. MDR, AC, AA and FC performed genetic analysis. EM and MR provided sample

collection and clinical support. MDR and PI contributed to data interpretation. MDR and AC wrote the manuscript, and FD and RL critically revised the manuscript and participated in the analysis and interpretation of the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent has been provided by the proband's parents. All methods are part of the clinical practice necessary to carry out the molecular analysis of the APC gene, requested by the proband's parents. Furthermore, the proband's parents agree that the DNA sample no longer needed for the study is used for medical research purposes in an anonymous form and/or in epidemiological cases. The procedures reported in this study were performed in accordance with the rules of the Good Clinical Practice Guidelines (GCP) and the ethical principles set out in the Declaration of Helsinki. The study was also authorized by 'Comitato etico per le attività Biomediche-Carlo Romano of the University of Naples Federico II' (protocol no. 35/17).

Patient consent for publication

Not applicable. All identifying information, case details, personal information or images that may enable an individual to be identified, are not included in the text.

Competing interests

The authors declare that they have no competing interests.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136: E359-E386, 2015.
2. De Rosa M, Rega D, Costabile V, Duraturo F, Niglio A, Izzo P, Pace U and Delrio P: The biological complexity of colorectal cancer: Insights into biomarkers for early detection and personalized care. Therap Adv Gastroenterol 9: 861-886, 2016.
3. Ma H, Brosens LA, Offerhaus GJ, Giardiello FM, de Leng WW and Montgomery EA: Pathology and genetics of hereditary colorectal cancer. Pathology 50: 49-59, 2018.
4. Fearnhead NS, Britton MP and Bodmer WF: The ABC of APC. Hum Mol Genet 10: 721-733, 2001.
5. De Rosa M, Galatola M, Borriello S, Duraturo F, Masone S and Izzo P: Implication of adenomatous polyposis coli and MUTYH mutations in familial colorectal polyposis. Dis Colon Rectum 52: 268-274, 2009.
6. Half E, Bercovich D and Rozen P: Familial adenomatous polyposis. Orphanet J Rare Dis 4: 22, 2009.
7. Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, Albertsen H, Joslyn G, Stevens J, Spirio L, Robertson M, *et al*: Identification and characterization of the familial adenomatous polyposis coli gene. Cell 66: 589-600, 1991.
8. Leoz ML, Carballal S, Moreira L, Ocaña T and Balaguer F: The genetic basis of familial adenomatous polyposis and its implications for clinical practice and risk management. Appl Clin Genet 8: 95-107, 2015.
9. Auricchio R, De Rosa M, Quaglietta L, Miele E, Boccia G, Staiano A and Izzo P: A dramatic case of early-onset familial adenomatous polyposis. Clin Genet 67: 104-106, 2005.
10. Aoki K and Taketo MM: Adenomatous polyposis coli (APC): A multi-functional tumor suppressor gene. J Cell Sci 120: 3327-3335, 2007.
11. Polakis P: The adenomatous polyposis coli (APC. tumor suppressor. Biochim Biophys Acta 1332: F127-F147, 1997.
12. Dodaro C, Grifasi C, Florio J, Santangelo ML, Duraturo F, De Rosa M, Izzo P and Renda A: The role of mutation analysis of the APC gene in the management of FAP patients. A controversial issue. Ann Ital Chir 87: 321-325, 2016.
13. Galatola M, Paparo L, Duraturo F, Turano M, Rossi GB, Izzo P and De Rosa M: Beta catenin and cytokine pathway dysregulation in patients with manifestations of the 'PTEN hamartoma tumor syndrome'. BMC Med Genet 13: 28, 2012.
14. Nagase H and Nakamura Y: Mutations of the APC (adenomatous polyposis coli) gene. Hum Mutat 2: 425-434, 1993.
15. Gayther SA, Wells D, SenGupta SB, Chapman P, Neale K, Tsioupra K and Delhanty JD: Regionally clustered APC mutations are associated with a severe phenotype and occur at a high frequency in new mutation cases of adenomatous polyposis coli. Hum Mol Genet 3: 53-56, 1994.
16. De Rosa M, Scarano MI, Panariello L, Carlomagno N, Rossi GB, Tempesta A, Borgheresi P, Renda A and Izzo P: Three submicroscopic deletions at the APC locus and their rapid detection by quantitative-PCR analysis. Eur J Hum Genet 7: 695-703, 1999.
17. Newton KF, Mallinson EK, Bowen J, Lalloo F, Clancy T, Hill J and Evans DG: Genotype-phenotype correlation in colorectal polyposis. Clin Genet 81: 521-531, 2012.
18. Fostira F, Thodi G, Sandaltzopoulos R, Fountzilas G and Yannoukakos D: Mutational spectrum of APC and genotype-phenotype correlations in Greek FAP patients. BMC Cancer 10: 389, 2010.
19. Liu X, Shan X, Friedl W, Uhlhaas S, Propping P, Li J and Wang Y: May the APC gene somatic mutations in tumor tissues influence the clinical features of Chinese sporadic colorectal cancers? Acta Oncol 46: 757-762, 2007.
20. Friedl W and Aretz S: Familial adenomatous polyposis: Experience from a study of 1164 unrelated german polyposis patients. Hered Cancer Clin Pract 3: 95-114, 2005.
21. De Rosa M, Scarano MI, Panariello L, Salvatore F and Izzo P: A novel Mbo II polymorphism in exon 15 of the human adenomatous polyposis coli gene. Clin Genet 53: 315-316, 1998.
22. Fernández-Rozadilla C, de Castro L, Clofent J, Brea-Fernández A, Bessa X, Abulí A, Andreu M, Jover R, Xicola R, Llor X, *et al*: Single nucleotide polymorphisms in the Wnt and BMP pathways and colorectal cancer risk in a spanish cohort. PLoS One 5: e12673, 2010.
23. Picelli S, Zajac P, Zhou XL, Edler D, Lenander C, Dalén J, Hjern F, Lundqvist N, Lindforss U, Påhlman L, *et al*: Common variants in human CRC genes as low-risk alleles. Eur J Cancer 46: 1041-1048, 2010.
24. Wong HL, Peters U, Hayes RB, Huang WY, Schatzkin A, Bresalier RS, Velie EM and Brody LC: Polymorphisms in the adenomatous polyposis coli (APC) gene and advanced colorectal adenoma risk. Eur J Cancer 46: 2457-2466, 2010.
25. Septer S, Lawson CE, Anant S and Attard T: Familial adenomatous polyposis in pediatrics: Natural history, emerging surveillance and management protocols, chemopreventive strategies, and areas of ongoing debate. Fam Cancer 15: 477-485, 2016.

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ORIGINAL ARTICLE

Exclusive enteral nutrition effect on the clinical course of pediatric Crohn's disease: a single center experience

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Abstract

The aim of this study was to evaluate the short- and long-term outcomes of exclusive enteral nutrition (EEN) versus corticosteroids (CS) as induction therapy, in a cohort of pediatric patients with Crohn's disease (CD). A retrospective study of patients with CD has been conducted. Clinical characteristics, laboratory parameters, and pediatric Crohn's disease activity index (PCDAI) were evaluated at diagnosis and at different follow-up points. Subjects were divided in EEN-induction group, receiving EEN, and CS-induction group, treated with oral CS. We evaluated 47 patients in the EEN-induction group and 21 patients in the CS-induction group. After 8 weeks from diagnosis, we detected a significant improvement in CRP ($p = 0.001$) and albumin ($p = 0.05$), in EEN-induction group compared with the CS-induction group. PCDAI was significantly lower in the EEN-induction group versus the CS-induction group after 8 weeks ($p = 0.04$) and 1 year ($p = 0.03$) of follow-up. After 2 years from diagnosis, the number of subjects needing immunomodulators (IMM, azathioprine or methotrexate) was significantly higher in the CS-induction group compared with the EEN-induction group ($p = 0.02$).

Conclusion: EEN has the same effectiveness of CS therapy in induction of remission but seems to have a more pronounced effect on disease activity. In our cohort, the need to use IMM seems to be reduced in subjects initially treated with EEN.

What is Known:
- *Exclusive enteral nutrition (EEN) has the same effectiveness of corticosteroids (CS) in the induction of remission in pediatric Crohn's disease.*
- *EEN offers numerous advantages over CS, in terms of improved nutrition and mucosal healing.*

What is New:
- *Induction of remission with EEN seems to have a more pronounced effect on disease activity compared to induction with CS.*
- *In our cohort, induction of remission with EEN seems to reduce the need of therapy with immunomodulators at 2 years of follow-up.*

Elena Scarpato, Caterina Strisciuglio and Massimo Martinelli contributed equally to this work.

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Abbreviations

AZT	Azathioprine
BMI	Body mass index
CD	Crohn's disease
CS	Corticosteroids
ECCO	European Crohn's and Colitis Organization
EEN	Exclusive enteral nutrition
EIM	Extra-intestinal manifestations
ESPGHAN	European Society for Pediatric Gastroenterology, Hepatology and Nutrition
IFX	Infliximab
PCDAI	Pediatric Crohn's Disease Activity Index
IMM	Immunomodulators
MTX	Methotrexate

Introduction

Crohn's disease (CD) is a chronic inflammatory condition that may involve any part of the gastrointestinal tract, typically following a relapsing-remitting course. It may present at any age, but in up to 25% of patients, CD is diagnosed during childhood [1]. In this period of life, CD presents often with a more complicated disease course compared with adult patients. The cumulative risk of progression to complicated CD is similar to adults but, due to the early onset of disease, children are more likely to have undergone surgery by young adulthood [2]. CD has a heavy impact on the patient's nutritional status, with about 90% of patients showing weight loss at diagnosis. Although the etiology of nutritional problems and growth failure is multifactorial, malnutrition owing to inadequate nutrient intake is the primary cause [3–6]. Moreover, the chronic inflammatory state of CD has a remarkable effect on the patient's growth rate. The ultimate benefit of any treatment for CD is the ability to reduce frequency and severity of inflammatory relapses that contribute to long-term cumulative bowel damage, and these are the endpoints against which each treatment must be measured [7, 8]. The consensus guidelines of the European Crohn's and Colitis Organization (ECCO) and of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) suggest to use exclusive enteral nutrition (EEN) for children with inflammatory luminal diseases as a first-line therapy to induce remission, due to its excellent safety profile [7]. Comparable pediatric remission rates have been reported following treatment with either EEN or corticosteroids (CS) [9–11]. The potential benefits of EEN extend beyond nutrition alone and include improved mucosal healing, linear growth, and bone health [12–15]. Although CS are clinically efficacious and associated with improvements on endoscopic assessment, mucosal healing is significantly less frequent if CS are used, compared with EEN [16]. Their use is also associated with undesirable side effects including deleterious effects on growth and bone mineral density. Short-term EEN efficacy has been clearly demonstrated; however, the relative importance of initial choice for the induction therapy on medium- to long-term outcomes was not as well studied. The aims of our study were to evaluate the short- and long-term clinical effects of EEN versus CS in our cohort of children with CD.

Methods

Population

Our study population included all the subjects aged less than 18 years who received a diagnosis of CD between January 2003 and December 2013 and who were followed up at the Endoscopy and Motility Unit of the Department of Pediatrics, University of Naples "Federico II." Subjects with complex perianal fistulas were excluded from the analysis. The included patients were divided in 2 groups according to the different induction therapy received at CD diagnosis: EEN-induction group, consisting of patients who received EEN, and CS-induction group, consisting of patients treated with oral CS.

Data collection

The demographic and clinical data of each patient were retrospectively collected from medical records, at diagnosis and at different follow-up times (8 weeks, T1; 6 months, T2; 1 year, T3; 2 years, T4). Disease activity was scored using the Pediatric Crohn's Disease Activity Index (PCDAI) [17]. Disease activity was defined as "mild" or "moderate to severe" in the case of PCDAI scores < 30 or > 30, respectively. Remission was defined after the physician's global assessment as a PCDAI score ≤ 10, in the absence of clinical symptoms. Clinical relapse was defined as the occurrence or worsening of symptoms accompanied by a PCDAI score > 10 points, in a subject who had already reached clinical remission. CD was classified according to Paris classification [18]. Extra-intestinal manifestations (EIM) included eye, joint, skin, or liver involvement and persistent fever.

Weight, height, and body mass index (BMI) were collected at diagnosis and at the different time points. Growth velocity was calculated at each visit from two consecutive height measurements performed within an interval of at least 6 months. In order to compare parameters from subjects with different age

and gender, weight, height, BMI, and growth velocity z-scores were calculated considering the general Italian population as a reference. Growth failure was defined as a height for age z-score lower than − 1.64; obesity was defined as a BMI z-score higher than 2. The fasting laboratory parameters (including Hb, ESR, CRP, albumin, and fecal calprotectin) were collected at diagnosis and at the different time points.

Therapeutic approach

Therapeutic decisions, at baseline and follow-up, were made by two expert pediatric gastroenterologists (AS and EM), in line with the validated international guidelines [19]. We included in the analysis our cohort followed before the publication of the 2014 ECCO-ESPGHAN pediatric guidelines with CD risk stratification [7] and prior to biologics' optimization. Therefore, the first therapeutic choice was always represented by EEN (polymeric formula for 6–8 weeks, followed by a gradual introduction of foods during the subsequent 4 weeks). Partial EN was not continued at the end of the induction. CS therapy (oral methylprednisolone: 1 mg/kg/day, max 40 mg/day per 4 weeks, followed by gradual tapering off by week 11) was used in patients who refused EEN or in those patients where EEN was considered not sufficient to induce disease remission, according to the physician's discretion. Children who could not wean steroids after week 12 were defined as steroid-dependent. After the induction, all patients that reached clinical remission started a maintenance therapy with aminosalicylates (5-ASA; mesalazine 50 mg/kg/day, max 4 g/day) or IMM therapy at a standardized dose (azathioprine (AZA), 2–2.5 mg/kg/day; or methotrexate (MTX), 15 mg/m^2/week), according to the physician's discretion. Patients in whom induction therapy had failed, or patients with clinical relapse, were treated with a second cycle of CS or EEN as induction therapy and started IMM as a maintenance therapy. Early use of IMM was defined as use within the first 8 weeks of disease. Biologics were started as a second-line therapy after IMM failure.

Statistical analysis

Variables were screened for their distribution, and appropriate parametric or non-parametric tests were adopted as necessary. Continuous variables were expressed by mean and standard deviation. Qualitative variables were expressed by frequency and percentage. The Student's t test and the Mann-Whitney test for continuous variables and the χ^2 and Fisher's exact tests for categorical variables were used, where appropriate. Statistical significance was predetermined as $p < 0.05$. SPSS version 20 was used for all statistical analyses (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics

We included 68 children with CD who received either EEN (n = 47) or CS (n = 21) as the sole induction therapy at diagnosis. Clinical characteristics of the study population at baseline are summarized in Table 1. At diagnosis, there were no significant differences in age, gender, disease's location, and behavior between the two groups. The only exception was represented by EIMs that were significantly more frequent in subjects from the CS-induction group (10/47, 21%, in the EEN-induction group versus 11/21, 52%, in the CS-induction group; p = 0.02). In addition, no significant difference was found in disease activity according to PCDAI (median PCDAI 26.2, range 10–45, and median 32.5, range 10–60, in the EEN-induction group and CS-induction group, respectively; p = 0.13), with 31/47 (66%) in the EEN-induction group and 10/21 (48%) in the CS-induction group showing a "mild" disease and 16/47 (34%) in the EEN-induction group

Table 1 Baseline clinical characteristics of 68 CD pediatric patients, according to the Paris classification (17)

	EEN-induction (N = 47)	CS-induction (N = 21)	p*
Gender			
Male (%)	27 (57)	12 (54.5)	ns
EIM (%)	10 (21)	11 (52)	**0.02**
Disease activity			
PCDAI; median (range)	26.2 (10–45)	32.5 (10–60)	ns
Mild (PCDAI < 30)	31 (66)	10 (48)	ns
Moderate to severe (PCDAI > 30)	16 (34)	11 (52)	ns
Age at diagnosis			
Months; median (range)	129 (37–212)	158 (47–205)	ns
A1a	13 (28)	4 (19)	ns
A1b	34 (72)	17 (81)	ns
Disease location			
L1 (%)	9 (19)	2 (10)	ns
L2 (%)	10 (21)	8 (38)	ns
L3 (%)	28 (60)	11 (52)	ns
Disease behavior			
B1(%)	33 (70)	14 (67)	ns
B2 (%)	14 (30)	7 (33)	ns
P (%)	7 (15)	7 (33)	ns
Growth			
G0 (%)	44 (94)	17 (81)	ns
G1 (%)	3 (6)	4 (19)	ns

CD Crohn's disease, *EEN* exclusive enteral nutrition, *CS* corticosteroids, *EIM* extra-intestinal manifestations

*Fisher's exact test

and 11/21 (52%) in the CS-induction group showing a "moderate to severe" disease ($p = 0.18$). Finally, no statistically significant differences were found in anthropometric and laboratory parameters between the two groups (Tables 2 and 3).

T1 outcomes

At 8 weeks from diagnosis, 32/47 (68%) in the EEN-induction group and 10/21 (48%) in the CS-induction group achieved clinical remission ($p = 0.17$). Eight out of 47 (17%) from EEN-induction group versus 5/21 (24%) from CS-induction group did not respond to induction ($p = 1$). One out of 21 (5%) patients from the CS-induction group presented steroid dependence and could not stop steroid therapy at the end of the induction. In the EEN-induction group, 8/47 (17%) needed a course of CS therapy because of a failure of induction with EEN. The number of subjects who needed an early use of IMM was 6/47 (13%) in the EEN-induction group and 5/21 (24%) in the CS-induction group ($p = 0.29$). In the EEN-induction group compared with the CS-induction group, we detected a significant improvement in CRP values (median 0.3, range 0.3–4.6, and median 0.7, range 0.3–51, respectively; $p = 0.001$), albumin values (median 4.5, range 3.4–5.2, and median 4.3, range 3.6–4.9, respectively; $p = 0.05$), and PCDAI values (median 10, range 0–30, and median 15, range 0–45, respectively; $p = 0.04$). Moreover, we found a trend toward statistical significance for ERS values (median 9, range 1–57, and median 14.5, range 2–35, respectively, $p = 0.06$). The number of subjects with CRP values < 5 mg/dL was significantly lower in the EEN-induction group (47/47, 100%) compared with the CS-induction group (18/21, 86%; $p = 0.03$), while there were no significant differences in the number of subjects with normal albumin, normal calprotectin, and PCDAI score ≤ 10. In addition, we found that the difference in Hb values between T1 and T0 was significantly higher in the EEN-induction group compared with the CS-induction group (median 1.2, range − 2.9–3.5, and median − 0.2, range − 6.2–4.3, respectively; $p = 0.048$). All data on laboratory parameters are summarized in Table 2. No differences in anthropometric parameters were found (Table 3).

T2 outcomes

After 6 months from diagnosis, 39/47 (83%) in the EEN-induction group and 13/21 (62%) in the CS-induction group were in remission ($p = 0.07$). Twelve out of 47 (25.5%) subjects from the EEN-induction group and 7/21 (33%) subjects from the CS-induction group had at least 1 relapse ($p = 0.56$). Two out of 47 (4%) from the EEN-induction group and 1/21 (5%) from the CS-induction group had to perform a course of CS therapy ($p = 1$). Two out of 47 (4%) in the EEN-induction group and 1/21 (5%) in the CS-induction group introduced IMM therapy ($p = 1$). In addition, 1/47 (2%) from the EEN-induction group and 0/21 (0%) from the CS-induction group were on therapy with IFX ($p = 1$). The number of subjects needing the introduction of IMM therapy after 6 months from diagnosis was 8/47 (17%) in the EEN-induction group compared with 6/21 in the CS-induction group (29%), without significant differences between the two groups ($p = 0.33$). As described in Table 2, we found no differences in PCDAI median values and laboratory parameters, with the only exception of the difference in Hb values between T2 and T0, which was significantly higher in the EEN-induction group compared with the CS-induction group (median 1.7, range − 1.7–4.4, and median 0.4, range − 5.4–6.9, respectively; $p = 0.03$). No differences in anthropometric parameters were found (Table 3).

T3 outcomes

At 1 year of follow-up, the number of subjects included in the EEN-induction group was 46, since 1 patient was transferred to the adult care center. Similarly, the subjects evaluated in the CS-induction group were 20 because 1 patient was lost to follow-up (Fig. 1). Thirty-five out of 46 (76%) in the EEN-induction group versus 11/20 (55%) in the CS-induction group were in clinical remission ($p = 0.14$), with 19/46 (41%) subjects from the EEN-induction group and 13/20 (65%) subjects from the CS-induction group that had experienced at least 1 relapse ($p = 0.10$). Moreover, because of a clinical relapse, 3/46 (6.5%) from the EEN-induction group and 1/20 (5%) from the CS-induction group needed to perform a course of CS therapy ($p = 1$), 9/46 (19.5%) from the EEN-induction group and 6/20 (30%) from the CS-induction group started IMM therapy ($p = 0.35$), and 1/46 (2%) from the EEN-induction group versus 2/20 (10%) from the CS-induction group started IFX ($p = 0.21$). So, after 1 year of follow-up, 17/46 (37%) from the EEN-induction group and 12/20 (60%) from the CS-induction group had started a therapy with AZT or MTX ($p = 0.1$), and 2/46 (4%) in the EEN-induction group versus 2/20 (10%) in the CS-induction group had started IFX, with no significant differences between the groups ($p = 0.58$). Considering disease activity, PCDAI values were significantly lower in the EEN-induction group compared with the CS-induction group (median PCDAI 3.75, range 0–40, and median 10, range 0–40, respectively; $p = 0.03$). The difference in Hb values between T3 and T0 was confirmed to be significantly higher in the EEN-induction group compared with the CS-induction group (median 2, range − 0.7–5, and median 1.3, range − 5.2–5.3, respectively; $p = 0.03$). No differences in other laboratory parameters (Table 2) and in anthropometric parameters (Table 3) were found.

Table 2 Laboratory parameters and disease activity at all time points; median (range)

	T0			T1			T2			T3			T4		
	EEN-induction (47)	CS-induction (21)	*p**	EEN-induction (47)	CS-induction (21)	*p**	EEN-induction (47)	CS-induction (21)	*p**	EEN-induction (46)	CS-induction (20)	*p**	EEN-induction (37)	CS-induction (19)	*p**
PCDAI	26.2 (10–45)	32.5 (10–60)	ns	10 (0–30)	15 (0–45)	**0.04**	5 (0–35)	10 (0–30)	ns	3.75 (0–40)	10 (0–40)	**0.03**	5 (0–30)	7.5 (0–35)	ns
- PCDAI ≤ 10	2 (4)	1 (5)	ns	32 (68%)	10 (48%)	ns	39 (83%)	13 (62%)	ns	35 (76%)	11 (55%)	ns	31 (84%)	10 (53%)	**0.02**
- Δ PCDAI TX-T0 °	-	-	-	− 19 (− 42.5–2.5)	− 16.2 (− 45–15)	ns	20 (−5–45)	22.5 (2.5–42.5)	ns	20 (−10–42.5)	17.5 (−20–35)	ns	22.5 (− 5–38)	22.5 (− 25–40)	ns
Hb; g/dL	10.2 (8–14)	11.7 (7–18)	ns	11.5 (9.2–15.2)	11.3 (7.9–15.2)	ns	12.1 (9–15.9)	11.6 (7.6–14.1)	ns	12.5 (9.4–16.3)	12.7 (8.5–15.7)	ns	12.7 (7.3–17.2)	12.5 (9.8–15.8)	ns
- Δ Hb TX-T0 °	-	-	-	1.2 (− 2.9–3.5)	− 0.2 (− 6.2–4.3)	**0.048**	1.7 (−1.7–4.4)	0.4 (− 5.4–6.9)	**0.003**	2 (− 0.7–5)	1.3 (− 5.2–5.3)	**0.038**	2.5 (− 8.8–5)	1.3 (− 5.3–6.2)	**0.002**
ESR; mm	28.5 (2–111)	26 (3–77)	ns	9 (1–57)	14.5 (2–35)	ns	9 (2–70)	6 (2–38)	ns	10 (2–50)	10 (2–40)	ns	5 (2–30)	8 (2–29)	ns
- Δ ESR TX-T0 °	-	-	-	− 20 (− 96–14.6)	− 17.5 (− 69–18)	ns	19 (− 29.6–74)	12 (− 28–73)	ns	20 (−20–67)	8.38 (− 7–67)	ns	21 (−4–96)	7 (− 14–72)	ns
CRP; mg/dL	4.0 (0.3–146.2)	4.3 (0.3–115)	ns	0.3 (0.3–4.6)	0.7 (0.3–51)	**0.001**	0.3 (0.3–88.7)	0.5 (0.3–4.4)	ns	0.3 (0.2–34.2)	0.45 (0.3–41)	ns	0.3 (0.3–20.9)	0.7 (0.3–20.2)	ns
- CRP < 5	25 (53%)	11 (52%)	ns	47 (100%)	18 (86%)	**0.03**	43 (91%)	21 (100%)	ns	40 (87%)	18 (90%)	ns	34 (92%)	16 (84%)	ns
- Δ CRP TX-T0 °	-	-	ns	− 3.7 (− 145–0.58)	− 3.6 (− 113–47.9)	ns	− 2.9 (− 141–82.1)	− 3.9 (− 114.2–3.9)	ns	− 2.0 (− 94.5–20.5)	− 2.8 (− 114–33.4)	ns	− 2.4 (− 94–20.5)	− 3.0 (− 114.3–6.7)	ns
Calpr; mcg/gr	423 (30–1250)	471.5 (95–770)	ns	291.5 (15–1470)	435 (20–610)	ns	253 (15–680)	343 (15–498)	ns	138 (15–500)	181.5 (15–500)	ns	191.5 (15–500)	208 (15–613)	ns
-Calpr < 100	2/45 (4%)	1/18 (5%)	ns	6/44 (14%)	1/18 (5.5%)	ns	9/39 (23%)	4/17 (23.5%)	ns	16/41 (39%)	4/16 (25%)	ns	12/32 (37.5%)	3/15 (20%)	ns
- Δ Calpr TX-T0 °	-	-	ns	− 115 (− 970–1062)	− 36.5 (− 557–395)	ns	− 115 (− 1075–353)	− 117.5 (− 415–128)	ns	− 192.5 (− 1197–500)	− 145 (− 465–317)	ns	− 198 (−812–300)	− 87 (− 480–405)	ns
Alb; g/dL	3. 5 (2.5–5)	3.6 (2.5–4.4)	ns	4.5 (3.4–5.2)	4.3 (3.6–4.9)	**0.05**	4.5 (3.2–5.6)	4.4 (3.9–5)	ns	4.6 (3.6–5.5)	4.6 (3.6–5.2)	ns	4.6 (3.5–5.6)	4.6 (3.1–5.1)	ns
- Alb > 3.5	28 (59.5%)	13 (62%)	ns	44 (94%)	18 (86%)	**ns**	44 (94%)	21 (100%)	ns	46 (100%)	20 (100%)	ns	37 (100%)	16 (84%)	**0.03**
- Δ Alb TX-T0 °	-	-	ns	0.95 (− 3.5–1.9)	0.3 (− 3.7–2)	ns	0.95 (− 0.6–2.1)	0.95 (− 0.1–2.1)	ns	1.05 (− 0.8–2.3)	0.9 (−0.2–2.4)	ns	1 (− 3.7–2.1)	1.05 (− 0.8–2.5)	ns

*Mann-Whitney test; *SD* standard deviation, *EEN* exclusive enteral nutrition, *CS* corticosteroids, *PCDAI* pediatric Crohn's disease activity index, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *Calpr* calprotectin, *Alb* albumin

°Δ value TX-T0 defines the median (range) difference in PCDAI/Hb/ESR/CRP/calprotectin/albumin between each time point and T0 (Tx-T0)

Table 3 Anthropometric parameters at all time points

	T0			T1			T2			T3			T4		
	EEN (47)	CS (21)	p*	EEN (47)	CS (21)	p*	EEN (47)	CS (21)	p*	EEN (46)	CS (20)	p*	EEN (37)	CS (19)	p*
Height z-score Median (range)	−0.2 (−2.1–1.5)	−0.7 (−2.9–1.6)	ns	−0.2 (−2–2)	−0.7 (−3.1–1.7)	ns	−0.1 (−2.4–2.2)	−0.35 (−2.7–0.9)	ns	−0.2 (−2.3–2.1)	−0.3 (−2.6–2.7)	ns	−0.2 (−1.9–1.8)	−0.6 (−3–1.2)	ns
Weight z-score Median (range)	−0.9 (−3.3–3)	−1.2 (−4.2–1.4)	ns	−0.3 (−2.9–3)	−0.6 (−2.3–1.9)	ns	−0.3 (−2.2–3.1)	−0.5 (−2.6–1.7)	ns	−0.4 (−2.4–3)	−0.5 (−2.4–2.2)	ns	0.1 (−2.4–2)	−0.5 (−2–1.2)	ns
BMI z-score Median (range)	−1.2 (−4–2)	−0.8 (−5–1)	ns	−0.3 (−3.1–2.4)	−0.1 (−1.5–1.4)	ns	−0.4 (−2.6–2.3)	−0.33 (−1.9–1.5)	ns	−0.2 (−3.9–2.3)	−0.2 (−1.8–1.9)	ns	−0.02 (−2.8–1.9)	0.06 (−2–1.7)	ns
Growth failure n (%)	11 (23.4)	6 (27.3)	ns	3 (6.4)	0 (0)	ns	3 (6.4)	1 (4.5)	ns	4 (8.7)	1 (5)	ns	3 (8.1)	3 (15.8)	ns
Normal growth n (%)	35 (74.5)	16 (72.7)	ns	43 (91.5)	22 (100)	ns	43 (91.5)	21 (95.5)	ns	41 (89.1)	19 (95)	ns	34 (91.9)	16 (84.2)	ns
Normal GV n (%)	-	-	-	-	-	-	28 (59.6)	17 (77.3)	ns	29 (63)	15 (75)	ns	21 (56.7)	13 (68.4)	ns
Obese n (%)	1 (2.1)	0 (0)	ns	1 (2.1)	0 (0)	ns	1 (2.1)	0 (0)	ns	1 (2.2)	0 (0)	ns	0 (0)	0 (0)	ns

*Fisher's exact test or Mann-Whitney test were used for categorical and continues variables, respectively; *EEN* exclusive enteral nutrition, *CS* corticosteroids, *BMI* body mass index, *GV* growth velocity

T4 outcomes

At 2 years of follow-up, the number of subjects included in the EEN-induction group was 37, because 1 patient was transferred to the adult care center and 8 patients were lost to follow-up. The subjects evaluated in the CS-induction group were 19 since 1 patient was transferred to the adult care center. Patient flow through the study is described in Fig. 1. The number of subjects who experienced at least one relapse was not significantly different between the two groups, with 21/37 (57%) in the EEN-induction group versus 15/19 (79%) in the CS-induction group ($p = 0.14$). However, we found a significant difference in the number of subjects in clinical remission between the two groups, with 31/37 (84%) from the EEN-induction group compared with 10/19 (53%) from the CS-induction group that had a PCDAI score ≤ 10 in the absence of clinical symptoms ($p = 0.02$). One out of 37 (3%) in the EEN-induction group and 1/19 (5%) in the CS-induction group needed a course of CS therapy ($p = 1$), 2/37 (5%) from the EEN-induction group and 4/19 (21%) from the CS-induction group started a therapy with IMM ($p = 0.16$), and 4/37 (11%) in the EEN-induction group and 2/19 (10.5%) in the CS-induction group started a therapy with IFX ($p = 1$). Considering the global need to start IMM therapy, we detected a significant difference between the two groups: with 19/37 (51%) in the EEN-induction group versus 16/19 (84%) in the CS-induction group ($p = 0.02$).

Finally, there was no difference in the number of subjects who needed to start IFX (6/37, 16% and 4/19, 21% in the EEN-induction group and CS-induction group, respectively; $p = 0.71$). Clinical outcomes at all time points for both study groups are summarized in Table 4. Overall, 3/47 (6%) subjects from the EEN-induction group had to stop IMM because of side effects. Specifically 2 subjects had an adverse event to AZT (1 neutropenia and 1 pancreatitis), while 1 subject did not tolerate MTX due to GI symptoms. In the CS-induction group, 2/21 (9.5%) subjects had adverse events that required discontinuation of IMM therapy, with 1 subject showing intolerance only to AZT (pancreatitis), while the other being intolerant to both AZT and MTX (allergic reaction). No difference was found in the rate of adverse events between the groups ($p = 0.64$).

As for disease activity and laboratory parameters, no differences were found in median PCDAI scores and laboratory parameters, between the two groups. However, the number of subjects with PCDAI score ≤ 10 was significantly higher in the EEN-induction group (31/37, 84%) compared with the CS-induction group (10/19, 53%; $p = 0.02$), and significantly more subjects had albumin values > 3.5 g/dL in the EEN-induction group (37/37, 100%) versus CS-induction group (16/19, 84%; $p = 0.03$). The difference in Hb values between T4 and T0 was significantly higher in the EEN-induction group compared with the CS-induction group (median 2.5, range − 8.8–5, and median 1.3, range − 5.3–6.2, respectively; $p = 0.02$). Laboratory

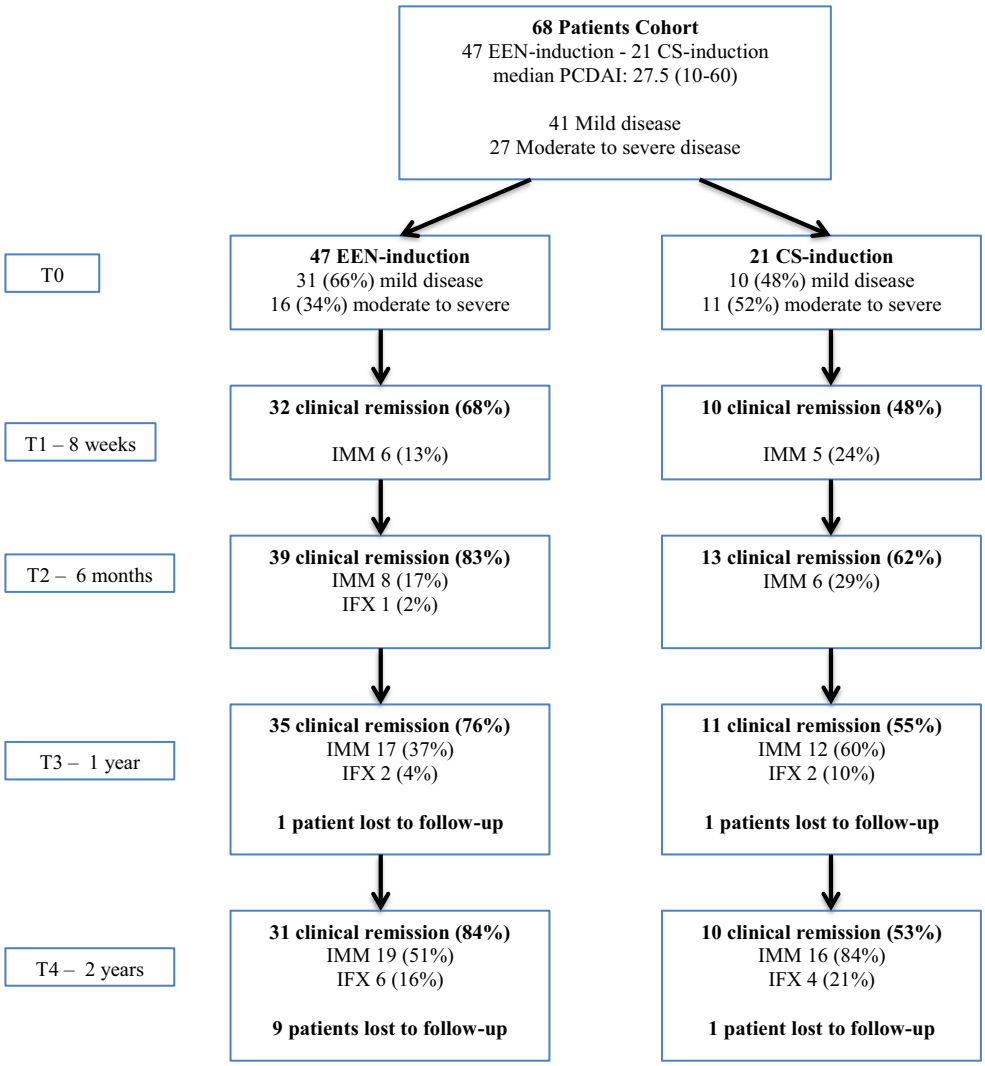

Fig. 1 Patient flow through the study

parameters at all time points are summarized in Table 2. Moreover, no statistically significant differences were found in the anthropometric parameters, between the two groups. However, after 2 years from diagnosis, median BMI z-scores improved from − 1.2 (range 4–2) to − 0.02 (range − 2.8–1.9) in the EEN-induction group ($p < 0.001$) and from − 0.8 (range – 5–1) to 0.06 (range – 2–1.7) in the CS-induction group ($p = 0.03$). Growth parameters at all time points are shown in Table 3.

Discussion

Our data confirm that EEN has the same effectiveness of CS therapy in the induction of clinical remission and suggest a more pronounced effect on disease activity, as demonstrated by the more significant improvement of PCDAI scores at the end of induction and still after 1 year of follow-up in the EEN-induction group compared with the CS-induction

Table 4 Clinical outcomes at all time points

	T1			T2			T3			T4		
	EEN (47)	CS (21)	*p**	EEN (47)	CS (21)	*p**	EEN (46)	CS (20)	*p**	EEN (37)	CS (19)	*p**
At least 1 relapse; n (%)	10 (21)	6 (27)	ns	12 (25.5)	7 (33)	ns	19 (41)	13 (65)	ns	21 (57)	15 (79)	**ns**
AZT or MTX; n (%)	6 (13)	5 (24)	**ns**	8 (17)	6 (29)	ns	17 (37)	12 (60)	ns	19 (51)	16 (84)	**0.02**
IFX n (%)	0 (0)	0 (0)	ns	1 (2)	0 (0)	ns	2 (4)	2 (10)	ns	6 (16)	4 (21)	ns

*Fisher's exact test; *EEN* exclusive enteral nutrition, *CS* corticosteroids, *AZT* azathioprine, *MTX* methotrexate, *IFX* infliximab

group and by the higher number of subjects in clinical remission after 2 years of follow-up, in the EEN-induction group compared with the CS-induction group. Moreover, according to the data from our cohort, induction with EEN seems to reduce the long-term need of IMM therapy. Although we are aware that the retrospective nature of the study may overestimate the benefits of EEN-induction on the need to start IMM, due to the risk of a selection bias, most of our results are in line with the previous published literature. The precise mechanism of action of EEN therapy has not been clearly elucidated. However, according to the available evidence, EEN is more than simple bowel rest, as confirmed by the direct anti-inflammatory effect with regulation of pro-inflammatory cytokine production [20], by the improvement in barrier function and enterocyte differentiation [21, 22], and by the modulation of intestinal microbiota [23]. Previous data showed that clinical response to polymeric diet is associated with a decrease in serum tumor necrosis factor-alpha levels, a downregulation of pro-inflammatory cytokines, and a significant healing of intestinal mucosa [24]. On the contrary, CS have poor ability to modify submucosal inflammatory process [25]. Moreover, it is estimated that nearly half of CD patients who initially respond to CS subsequently develop a dependency on them or have a relapse within 1 year [26]. It is evident that CS do not change the course of CD owing to their inability to affect mucosal lesions of the gut. Our data showed that, 8 weeks after diagnosis, there were no significant differences in the number of patients who achieved clinical remission with EEN compared with those who had used CS. These findings are in agreement with a Cochrane review published in 2018 by Narula et al. [15] that confirmed comparable remission rates between adult and pediatric patients treated with enteral nutrition versus steroids. In particular, the subgroup analysis by age showed that CS were superior to EEN in adults, while enteral nutrition was superior to CS in children. Another recent meta-analysis by Yu et al. [27], including pediatric studies comparing EEN versus CS for the treatment of pediatric CD, confirmed that EEN has the same effectiveness of CS in the achievement of remission. Indeed, numerous studies show that EEN is at least as efficacious as CS therapy in inducing remission and reducing disease activity over the short term for children with CD, but EEN offers numerous advantages in terms of improved nutrition and mucosal healing [28–31].

At the end of induction, we found a significant decrease in PCDAI scores, in patients from EEN group compared with those from the CS group. These findings are in agreement with the meta-analysis from Yu et al. [27] that described a distinct decline of PCDAI in patients who received EEN compared with those who received CS. Also Borrelli et al. [28] found that in children with newly diagnosed CD, a short course of nutritional therapy is as effective as a short course of oral CS in achieving clinical remission, measured with PCDAI. However, nutritional therapy was significantly more effective than CS in healing inflammatory lesions of the gut as documented by endoscopy and histology [12, 32].

Considering nutritional status, after 8 weeks of treatment, the improvement in albumin levels was significantly higher with EEN compared with CS treatment, suggesting a direct effect of EEN on nutritional status. Nevertheless, an improvement in weight for age, height for age, and BMI, both at short- and long-term follow-up, was found without significant difference in both groups, as described also by Yu et al. [27].

Finally, our study compared the long-term effects of the two therapies. At 1 year and 2 years of follow-up, we found no significant differences in the relapse rate between the two groups, in accordance with the meta-analysis from Yu et al. [27] and with data from the study by Cohen-Dolev et al. [29] that followed up newly diagnosed pediatric patients with mild to moderate CD for 2 years in the GROWTH CD study and found no differences in time to relapse or relapse rate in subjects initially treated with EEN compared with those treated with CS.

Furthermore, in our cohort, the use of IMM is less frequent in subjects initially treated with EEN compared with those treated with CS. This result could be related to a protective role of EEN induction therapy in the first years after diagnosis, despite the many variables influencing disease course. Our results are in accordance with the study from Lambert et al. [16], which was the first pediatric study to compare the 2-year outcomes of children treated with EEN to children treated with CS. The authors found that the use of EEN as initial induction therapy determined higher rates of remission, improved growth patterns, lower rates of relapse, and less exposure to CS. Also Berni Canani et al. [33] described positive effects at 12 months in subjects initially treated with EEN compared with subjects who received CS, despite the use of the same maintenance therapy, assuming a role of the more pronounced effect of EEN on mucosal healing.

This study has several limitations including its retrospective nature. Therefore, the two comparative groups were not allocated randomly and we were not able to specifically explore the physician and patient's factors regarding treatment choices and preferences, leading to a potential selection bias. In addition, considering that EIM were more frequent in the CS group compared with the EEN group, it is possible that more severe cases were preferentially treated with CS. This could also partially explain the worst 2-year outcome of subjects initially treated with CS.

Similarly, also the need to start IMM may represent the consequence of a more severe phenotype, rather than a direct benefit of EEN, and therefore we cannot exclude that a selection bias occurred from the starting allocation to the CS-induction group. A larger sample size and longer follow-up

time may have allowed detection of significance in a number of observed trends, especially anthropometric data.

Conclusion

In conclusion, accepting the limitations of our study design, our data confirm the importance of the standard use of EEN as primary therapy in children with newly diagnosed CD, due to the significant amelioration of disease activity and considering the possible reduction in the need of IMM. As well underlined in the most recent meta-analysis, further randomized, controlled studies on defining EEN regimens, influence of dietetic support and protocols on treatment success, and longer-term outcomes are required.

Authors' contributions E.S: Dr. Scarpato conceptualized the study, acquired the data, performed the data analysis, drafted the initial manuscript, and approved the final manuscript as submitted. Dr. Scarpato agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

C.S.: Dr. Strisciuglio conceptualized the study, acquired the data, performed the data analysis, drafted the initial manuscript, and approved the final manuscript as submitted. Dr. Strisciuglio agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

M. M.: Dr. Martinelli conceptualized the study, performed the data analysis, drafted the initial manuscript, and approved the final manuscript as submitted. Dr. Martinelli agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

M. R.: Dr. Russo acquired the data, performed the data analysis, drafted the initial manuscript, and approved the final manuscript as submitted. Dr. Russo agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

S. C.: Dr. Cenni acquired the data, drafted the initial manuscript, and approved the final manuscript as submitted. Dr. Cenni agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

M. C.: Dr. Casertano acquired the data, drafted the initial manuscript, and approved the final manuscript as submitted. Dr. Casertano agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

M. R. S.: Dr. Serra acquired the data, performed the data analysis, drafted the initial manuscript, and approved the final manuscript as submitted. Dr. Serra agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

A. S.: Dr. Staiano contributed to conception of the study, revised the article critically for important intellectual content, and approved the final manuscript as submitted. Dr. Staiano agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

E. M.: Dr. Miele contributed to conception of the study, revised the article critically for important intellectual content, and approved the final manuscript as submitted. Dr. Miele agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Compliance with ethical statements

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. The Institutional Review Board of the University of Naples "Federico II" approved the study protocol and questionnaire with the registration number 128/18. The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

References

1. Shen YM, Wu JF, Chen LH et al (2011) Characteristics and incidences of pediatric Crohn's disease in decades before and after 2000. Pediatr Neonatol 52:317–320
2. Gasparetto M, Guariso G, Pozza LV et al (2016) Clinical course and outcomes of diagnosing inflammatory bowel disease in children 10 years and under: retrospective cohort study from two tertiary centres in the United Kingdom and in Italy. BMC Gastroenterol 16:35
3. Shamir R, Phillip M, Levine A (2007) Growth retardation in pediatric Crohn's disease: pathogenesis and interventions. Inflamm Bowel Dis 13(5):620–628
4. Walters TD, Griffiths AM (2009) Mechanisms of growth impairment in pediatric Crohn's disease. Nat Rev Gastroenterol Hepatol 6(9):513–523
5. Griffiths AM, Nguyen P, Smith C et al (1993) Growth and clinical course of children with Crohn's disease. Gut 34(7):939–943
6. Ley D, Duhamel A, Behal H, Vasseur F, Sarter H, Michaud L, Gower-Rousseau C, Turck D (2016) Growth pattern in pediatric Crohn's disease is related to inflammatory status. J Pediatr Gastroenterol Nutr 63(6):637–643
7. Ruemmele FM, Veres G, Kolho KL, Griffiths A, Levine A, Escher JC, Amil Dias J, Barabino A, Braegger CP, Bronsky J, Buderus S, Martín-de-Carpi J, de Ridder L, Fagerberg UL, Hugot JP, Kierkus J, Kolacek S, Koletzko S, Lionetti P, Miele E, Navas López VM, Paerregaard A, Russell RK, Serban DE, Shaoul R, van Rheenen P, Veereman G, Weiss B, Wilson D, Dignass A, Eliakim A, Winter H, Turner D, European Crohn's and Colitis Organisation, European Society of Pediatric Gastroenterology, Hepatology and Nutrition (2014) Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. J Crohns Colitis 8(10):1179–1207
8. Perrin JM, Kuhlthau K, Chughtai A et al (2008) Measuring quality of life in pediatric patients with inflammatory bowel disease: psychometric and clinical characteristics. J Pediatr Gastroenterol Nutr 46(2):164–171
9. Whitten KE, Rogers P, Ooi CK, Day AS (2012) International survey of enteral nutrition protocols used in children with Crohn's disease. J Dig Dis 13(2):107–112
10. Heuschkel RB, Menache CC, Megerian JT, Baird AE (2000) Enteral nutrition and corticosteroids in the treatment of acute

Crohn's disease in children. J Pediatr Gastroenterol Nutr 31(1):8–15
11. de Bie C, Kindermann A, Escher J (2013) Use of exclusive enteral nutrition in paediatric Crohn's disease in the Netherlands. J Crohns Colitis 7(4):263–270
12. Grover Z, Burgess C, Muir R, Reilly C, Lewindon PJ (2016) Early mucosal healing with exclusive enteral nutrition is associated with improvement outcomes in newly diagnosed children with luminal Crohn's disease. J Crohns Colitis 10(10):1159–1164
13. Dziechciarz P, Horvath A, Shamir R et al (2007) Meta-analysis: enteral nutrition in active Crohn's disease in children. Aliment Pharmacol Ther 26(6):795–806
14. Day AS, Lopez RN (2015) Exclusive enteral nutrition in children with Crohn's disease. World J Gastroenterol 21(22):6809–6816
15. Narula N, Dhillon A, Zhang D et al (2018) Enteral nutritional therapy for induction of remission in Crohn's disease. Cochrane Database Syst Rev 4:CD000542
16. Lambert B, Lemberg DA, Leach ST, Day AS (2012) Longer-term outcomes of nutritional management of Crohn's disease in children. Dig Dis Sci 57(8):2171–2177
17. Hyams JS, Ferry GD, Mandel FS, Gryboski JD, Kibort PM, Kirschner BS, Griffiths AM, Katz AJ, Grand RJ, Boyle JT, Michener WM, Levy JS, Lesser ML (1991) Development and validation of a pediatric Crohn's disease activity index. J Pediatr Gastroenterol Nutr 12(4):439–447
18. Levine A, Griffiths A, Markowitz J, Wilson DC, Turner D, Russell RK, Fell J, Ruemmele FM, Walters T, Sherlock M, Dubinsky M, Hyams JS (2011) Pediatric modification of the Montreal classification for inflammatory bowel disease: The Paris Classification. Inflamm Bowel Dis 17:1314–1321
19. Van Assche G, Dignass A, Reinisch W, van der Woude J et al (2010) The second European evidence-based consensus on the diagnosis and management of Crohn's disease: special situations. J Crohns Colitis 4(1):63–101
20. Greenberg GR, Fleming CR, Jeejeebhoy KN et al (1998) Controlled trial of bowel rest and nutritional support in the management of Crohn's disease. Gut 29(10):1309–1315
21. Budd GR, Aitchison A, Day AS et al (2017) The effects of polymeric formula on enterocyte differentiation. Innate Immun 23(3): 240–248
22. De Jong NS, Leach ST, Day AS (2007) Polymeric formula has direct anti-inflammatory effects on enterocytes in an in vitro model of intestinal inflammation. Dig Dis Sci 52(9):2029–2036
23. Lionetti P, Callegari ML, Ferrari S et al (2005) Enteral nutrition and microflora in pediatric Crohn's disease. J Parenter Enter Nutr 29(4 Suppl):S173–S175 discussion S175-8, S184-8
24. Bannerjee K, Camacho-Hubner C, Babinska K (2004) Anti-inflammatory and growth-stimulating effects precede nutritional restitution during enteral feeding in Crohn disease. J Pediatr Gastroenterol Nutr 38(3):270–275
25. Swaminath A, Feathers A, Ananthakrishnan AN (2017) Systematic review with meta-analysis: enteral nutrition therapy for the induction of remission in paediatric Crohn's disease. Alimen Pharmacol Ther 46(7):645–656
26. Tung J, Loftus EV Jr, Freese DK et al (2006) A population-based study of the frequency of corticosteroid resistance and dependence in pediatric patients with Crohn's disease and ulcerative colitis. Inflamm Bowel Dis 12(12):1093–1100
27. Yu Y, Chen KC, Chen J (2019) Exclusive enteral nutrition versus corticosteroids for treatment of pediatric Crohn's disease: a meta-analysis. World J Pediatr 15(1):26–36
28. Borrelli O, Cordischi L, Cirulli M, Paganelli M, Labalestra V, Uccini S, Russo PM, Cucchiara S (2006) Polymeric diet alone versus corticosteroids in the treatment of active pediatric Crohn's disease: a randomized controlled open-label trial. Clin Gastroenterol Hepatol 4(6):744–753
29. Cohen-Dolev N, Sladek M, Hussey M et al (2018) Differences in outcomes over time with exclusive enteral nutrition compared with steroids in children with mild to moderate Crohn's disease: results from the GROWTH CD study. J Crohn's Colitis 12(3):306–312
30. Fell JM, Paintin M, Arnaud-Battandier F et al (2000) Mucosal healing and a fall in mucosal pro-inflammatory cytokine m-RNA induced by a specific oral polymeric diet in pediatric Crohn's disease. Aliment Pharmacol Ther 14(3):281–289
31. Newby EA, Sawczenko A, Thomas AG et al (2005) Interventions for growth failure in childhood Crohn's disease. Cochrane Database Syst Rev 3:CD003873
32. Grover Z, Muir R, Lewindon P (2014) Exclusive enteral nutrition induces early clinical, mucosal and transmural remission in pediatric Crohn's disease. J Gastroenterol 49(4):638–645
33. Berni Canani R, Terrin G, Borrelli O, Romano MT, Manguso F, Coruzzo A, D'Armiento F, Romeo EF, Cucchiara S (2006) Short- and long-term therapeutic efficacy of nutritional therapy and corticosteroids in paediatric Crohn's disease. Dig Liver Dis 38:381–387

Chapter 8

- Curriculum Vitae –

Main research fields:

- Pediatric gastroenterology (main fields : functional gastrointestinal disorders, inflammatory bowel disease)
- Gastrointestinal Motility
- Intestinal Microbiota

List of publications in the years 2017-2020

1 **Marina Russo**, Francesca Paola Giugliano, Paolo Quitadamo, Valeria Mancusi, Erasmo Miele and Annamaria Staiano Efficacy of a Mixture of Probiotic Agents as Complementary Therapy for Chronic Functional Constipation in Childhood. Ital J Pediatr. 2017 Mar 7;43(1):24.

2 Martinelli M, Giugliano FP, **Russo M,** Giannetti E, Andreozzi M, Bruzzese D, Perrone L, Staiano A, Miraglia Del Giudice E, Miele E, Marzuillo P, Strisciuglio C. The Changing Face of Pediatric Ulcerative Colitis: A Population-based Cohort Study. J Pediatr Gastroenterol Nutr 2018 Jun;66(6):903-908

3 **Russo M,** Coppola V, Giannetti E, Buonavolontà R, Piscitelli A, Staiano A. Oral administration of tannins and flavonoids in children with acute diarrhea: a pilot, randomized, control-case study. Ital J Pediatr. 2018 Jun 4;44(1):64.

4 Scarpato E, **Russo M,** Staiano A. Probiotics in Pediatric Gastroenterology: Emerging Indications: Inflammatory Bowel Diseases. J Clin Gastroenterol Nov/Dec 2018;52 Suppl 1, Proceedings from the 9th Probiotics, Prebiotics and New Foods, Nutraceuticals and Botanicals for Nutrition & Human and Microbiota Health Meeting, held in Rome, Italy from September 10 to 12, 2017:S7-S9.

5 **Russo M,** Strisciuglio C, Scarpato E, Bruzzese D, Casertano M, Staiano

A.Functional Chronic Constipation: Rome III Criteria Versus Rome IV Criteria. J Neurogastroenterol Motil. 2019 Jan 31;25(1):123-128.

6 Alessandrella A , Turco R , **Russo M** , Poziello A , Miele E , Staiano A High-resolution anorectal manometry in children with functional constipation with or without fecal incontinence. Neurogastroenterol Motil. 2020 Sep;32(9):e13882.

7 Turco R, **Russo M**, Bruzzese D, Staiano A. Efficacy of a partially hydrolysed formula, with reduced lactose content and with Lactobacillus reuteri DSM 17938 in infant colic: A double blind, randomised clinical trial. Clin Nutr. 2020 Jun 12:S0261-5614(20)30285-5.

8 Cerasuolo A, Miele E, **Russo M,** Aversano A, Cammarota F, Duraturo F, Liccardo R, Izzo P and De Rosa M, Sporadic pediatric severe familial adenomatous polyposis: a case report. Mol Clin Oncol 2020 Sep;13(3):20.9.

9 **Marina Russo,** Caterina Strisciuglio, Elena Scarpato, Dario Bruzzese, Marianna Casertano, and Annamaria Staiano. Functional Chronic Constipation: Rome III Criteria Versus Rome IV Criteria. J Neurogastroenterol Motil. 2019 Jan 31;25(1):123-128.

Abstracts and Communications in the years 2017-2020

1. **Russo M**, Strisciuglio C, Scarpato E, Bruzzese D, Casertano M, Staiano A.Functional Chronic Constipation: Rome III Criteria Versus Rome IV Criteria. XXIV Congresso Nazionale SIGENP 2017 , Roma 5-7 Ottobre 2017
2. Turco R, **Russo M**, Bruzzese D, Staiano A. Efficacy of a partially hydrolysed formula, with reduced lactose content and with Lactobacillus reuteri DSM 17938 in infant colic: A double blind, randomised clinical trial. Giornata di Ricerca Pediatrica del DISMET, Dipartimento di Scienze Mediche Traslazionali Università Federico II di Napoli, 21st June 2018.
3. Turco R, **Russo M**, Bruzzese D, Staiano A. Efficacy of a partially hydrolysed formula, with reduced lactose content and with Lactobacillus reuteri DSM 17938 in infant colic: A double blind, randomised clinical trial. FNM 2018,

3rd Meeting of the Federation of Neurogastroenterology and Motility, Amsterdam 28 August - 1st september 2018

4. Renata Auricchio, Roberta Mandile , Maria Immacolata Spagnuolo, **Marina Russo**, Deianira Pedoto, Nicoletta Pellino, Maria Antonia Maglio, Riccardo Troncone. Non-coeliac villous atrophy in children: clinical and immunohistochemical features. ESPGHAN 52 th, Glasgow 3-8 June 2019
5. A. Alessandrella, **M. Russo**, R. Turco, E. Miele, A. Staiano High resolution anorectal manometry in children with functional constipation with or without fecal incontinence. XXVI Congresso Nazionale SIGENP 2019 , Verona 16-19 Ottobre 2019

Invited as a Speaker years 2017-2020

- NFM 2018, 3rd Meeting of the Federation of Neurogastroenterology and Motility, Amsterdam August 28- September 1st 2018.

Theaching Activities

- Professor of the Postgraduate Course in Paediatric Gastroenterology, Hepatology and Nutrition held at the Department of Paediatrics of the University of Naples “Federico II”, Edition 2017 and 2018.