Trends in clinical practice in common pediatric gastro-intestinal disorders

van Kalleveen, M.W.

Publication date
2023
Document Version
Final published version

Trends in clinical practice in common pediatric gastro-intestinal disorders

Michael W. van Kalleveen

Trends in clinical practice in common pediatric gastro-intestinal disorders

Michael W. van Kalleveen

Printing: Gildeprint Enschede, gildeprint.nl
Layout and design: Eduard Boxem, persoonlijkproefschrift.nl

Trends in clinical practice in common pediatric gastro-intestinal disorders

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. ir. P.P.C.C. Verbeek
ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
op vrijdag 27 januari 2023, te 13.00 uur
door Michael William van Kalleveen
geboren te UTRECHT

Promotiecommissie

Promotores:	prof. dr. M.A. Benninga	AMC-UvA
	prof. dr. P.H.M. Savelkoul	Vrije Universiteit Amsterdam
Copromotores:	dr. T.G.J. de Meij	VU Medisch Centrum
	prof. dr. F.B. Plötz	AMC-UvA
Overige leden:	prof. dr. M.L. Mearin Manrique	Universiteit Leiden
	prof. dr. H.S.A. Heymans	AMC-UvA
	prof. dr. F.A. Wijburg	AMC-UvA
	prof. dr. J. Frenkel	Universiteit Utrecht
	prof. dr. D. Pajkrt	AMC-UvA
	dr. B.G.P. Koot	AMC-UvA

Faculteit der Geneeskunde

TABLE OF CONTENT

‘‘By three methods we may learn wisdom: First, by reflection, which is noblest; Second, by imitation, which is easiest; and third by experience, which is the bitterest.’’

Confucius (551 BC to 479 BC)

1

General introduction and thesis outline

A girl with abdominal pain

Rosie is a 9-year old girl without a relevant medical history. During the last four months, she experienced abdominal pain, crampy and located throughout the entire abdomen. Most of the time the abdominal pain increased during defecation. Her stool consistency varies, but mostly consists of diarrhea, without blood, up to five times a day. The abdominal pain is present more on week days than on weekend days. She did not have complaints of nausea. There were no alarm symptoms, such as jaundice, bilious vomiting, unexplained fever, joint pains or weight loss. Rosie has a negative family history for inflammatory bowel disease. Rosie was reported sick at primary school at least once a week, for several months and therefore was referred to her general practitioner. One of Rosie's aunts was recently diagnosed with celiac disease (CD), this worried Rosie's mother because she also had abdominal pain and variable consistency of her stools. After multiple appointments at the general practitioner, Rosie was referred to a pediatrician in order to find a cause for her complaints. After a thorough medical history and physical evaluation, the pediatrician suspected Rosie to suffer from a functional abdominal pain disorder (FAPD). Consequently, the pediatrician performed additional diagnostic blood tests, including a complete blood count and CD serology, and fecal examination for *Giardia lamblia* (*G. lamblia*), according to the Dutch national guidelines on chronic abdominal pain. Blood results, including celiac disease serology, were normal. Rosie's stools turned out to be negative for *G. lamblia,* but as a unrequested result her dual-feces-test, applied for ruling out *G. lamblia* was positive for *Dientamoeba fragilis* (*D. fragilis*). After reviewing Rosie's test results, the pediatrician realized that the pathogenicity of *D. fragilis* is questionable. Despite his uncertainty the pediatrician decided to treat Rosie with a ten day course of Metronidazole. Six weeks after Rosie's first outpatient visit Rosie comes back for routine control at the pediatricians office. Rosie experienced a lot of side effects of Metronidazole, but despite the side effects finished her course of antibiotics. Rosie's symptoms were still present during two days a week, but control of eradication of *D. fragilis* was not performed by the pediatrician. The course of the case made the pediatrician start to think…

Reflection on Rosie's case

The diagnostic work-up and determination of therapeutic strategies in children with chronic abdominal pain (CAP) is commonly challenging for clinicians since abdominal pain can be caused by a wide variety of organic abdominal and extra-abdominal diseases. Furthermore, young children can be limited in their ability to provide accurate history and parents can have difficulties interpreting the presenting symptoms. These factors can cause uncertainty for clinicians which could lead to the execution of diagnostic tests, such as blood tests, fecal tests and even radiological imaging. Another complicating factor is the absence of a clear-cut guideline for diagnostic- and therapeutic management of children with CAP. When treating children with abdominal pain differentiating between acute- and chronic abdominal pain is puzzling, but essential. Acute abdominal pain usually lasts for hours to days, is frequently described

as sharp or stabbing and is usually limited to one episode whereas CAP usually lasts weeks to months and is usually diffuse, dull and poorly localized. It is important to assess the symptoms in accordance with the age of the child since the age of the child affects some of the likely diagnoses. Also, clinicians should assess the presence of alarm symptoms such as unexplained fever, bloody diarrhea, jaundice, bilious vomiting, weight loss, joint pain and lethargy, which could imply more serious causes of abdominal pain. However, in up to 90% of cases, CAP in children is mostly without any organic cause and is classified as functional. In Rosie's case, there were no alarm symptoms present. There was a positive family history for celiac disease, but her aunt only is a second degree relative which makes it less likely that Rosie had celiac disease. Every other piece of information in Rosie's case points towards a functional origin. The pediatrician in Rosie's case was aware of the current national guidelines for chronic abdominal pain in children, however, as an unrequested result of the dual-feces-test to rule out *G. lamblia* he found Rosie to test positive for *D. fragilis*. Clinical insecurity in pediatricians, or insecurities in parents, may lead to the execution of additional tests which sometimes lead to the generation of 'unwanted' results such as the positive fecal test for *D. fragilis* in Rosie's case [1–3]. These 'unwanted' results could generate further insecurities, especially when there is an ongoing debate regarding the pathogenicity of these results such as the positive dual-feces-test for *D. fragilis* in Rosie's case.

Identifying children with an organic cause of CAP can be puzzling and doubt in parents or treating clinicians can lead to the execution of unnecessary diagnostic tests because they do not want to miss organic disease. This execution of unnecessary diagnostic tests could lead to false- positive or –negative results, financial costs and unnecessary anxiety in children and their parents. This thesis focused on the daily clinical practice in children referred with CAP. Aim was to provide insight in the diagnostic work-up of children referred with CAP in the presence of available guidelines, with a main focus on two organic causes of CAP, namely, celiac disease and *D. fragilis*. Causes with an increasing prevalence, with shifting paradigms in diagnostic- and therapeutic management and with increasing health care costs.

Chronic abdominal pain

Chronic abdominal pain (CAP) is one of the most common clinical conditions amongst children and adolescents and can be very disabling. Global prevalences of abdominal pain in children vary widely, but a meta-analysis of epidemiologic studies on the prevalence of abdominal pain, including 196,472 children, reported a pooled global prevalence of 13.5% [4]. The prevalence of pediatric CAP in Western countries is reported even higher, with prevalence rates up to 19% amongst school-going children in Europe and the United States [5]. CAP has a significant social burden during childhood, whereas children with CAP experience a significantly lower quality of life compared to their healthy peers and abdominal pain is ranked second as a cause of school absence in Western countries [6,7]. Despite a variety of treatments and frequent medical attention,

a significant proportion of children with abdominal pain will experience persisting symptoms with up to 30% of children experiencing symptoms for more than five years, continuing into adulthood [8,9]. Furthermore, adolescents with abdominal pain have an increased risk for developing anxiety disorders or depression at the adult age [7,10].

CAP often is a reason for referral to a pediatrician and it is estimated that 2-4% of referrals to a pediatrician are caused by CAP [11]. CAP in children and adolescents can be caused by a variety of organic disorders, such as celiac disease, inflammatory bowel disease, intestinal parasites, constipation, (recurrent) urinary tract infections, gastro-esophageal disorders and in rare occasions by neoplasms [12–14]. However, in a vast majority of approximately 90% of children who seek medical attention for CAP, no explanatory organic cause can be identified and these children are often diagnosed with one of the functional abdominal pain disorders (FAPD)[15]. One way for pediatricians to provide themselves with more diagnostic certainty is to conduct additional diagnostic tests to differentiate between functional and organic causes of CAP. In children with CAP, most pediatricians perform a series of blood, stool, and urine tests in order to differentiate between functional and organic disease, even in the absence of so called 'red flags' [3]. Consequently, the health care costs for children with chronic abdominal pain in Europe and the United States are rising with health care expenses of the diagnostic work-up and consultation by clinicians up to 2,500 euro per child in the Netherlands and up to 6,000 dollar per child in the United States [16–18]. A clear clinical compass for pediatricians in treating children with CAP is warranted. In **Chapter 2** we present a single center study amongst general pediatricians in the Netherlands investigating the amount-, and variation of additional diagnostic tests performed in children with CAP in the presence of current national guidelines We aimed to assess current clinical practice in diagnosing children with CAP and aimed to investigate the heterogeneity in clinical practice caused by the current national guidelines.

Functional abdominal pain disorders

Functional abdominal pain is abdominal pain without any structural or biochemical cause such as an anatomical, metabolic, infectious, inflammatory or neoplastic cause. The first description of functional abdominal pain disorders goes back to 1958, when Apley and Naish first referred to this condition as 'recurrent abdominal pain' [19]. Apley and Naish defined it as "at least three episodes of abdominal pain, severe enough to affect the activities of the child over a period longer than three months" [19]. Since 1999, with the introduction of the pediatric Rome-II criteria, RAP was referred to as 'abdominal pain-related functional gastrointestinal disorders', but nowadays, under the current Rome-IV criteria, it is referred to as 'functional abdominal pain disorders' (FAPD) [20]. FAPD consists of four main diagnostic entities: irritable bowel syndrome (IBS), functional dyspepsia (FD), abdominal migraine (AM) and functional abdominal pain-not otherwise specified (FAP-NOS) [20]. Diagnostic criteria for FAPD include: the presence of symptoms during at least four days per month (or once a week) and the persistence

of symptoms during two subsequent months [20]. The pathophysiological mechanisms underlying functional abdominal pain disorders are not yet clearly understood but two decades of research suggest that interactions in the "gut-brain-axis" are key in the development of FAPD [21]. Various factors have been suggested to cause these interactions such as: low grade intestinal inflammation, post-infectious changes or chronic infections, genetic factors, disturbances in gut microbiota, immune activation and altered intestinal permeability, disordered bile salt metabolism, abnormalities in serotonin metabolism and, finally, by alterations in brain function [21]. However, this has mostly been investigated in adults and precise underlying mechanisms remain largely undetermined in children.

Celiac disease

Celiac disease (CD) is a chronic immune-mediated enteropathy of the small intestine which is precipitated by exposure to dietary gluten in genetically susceptible individuals [22,23]. Approximately 95% of children with CD is positive for the human leukocyte antigen (HLA) DQ2.5 and most of the remaining 5% is either carrier of HLA-DQ8 or HLA-DQ2.2 [22,23]. The pathophysiology of CD is characterized by adaptive and innate immune responses towards gliadin peptides, which derive from incomplete digestion of gluten in the gut lumen. Gliadin peptides are formed by the trans-tissue glutaminase enzyme and the presentation of these gliadin peptides to HLA-DQ2 or DQ8-carrying antigen presenting cells in the plaques of Peyer in the intestinal mucosa leads to the activation of $CD4^+$ T-cell lymphocytes [23]. The cytokines produced by these T-cell lymphocytes lead towards an influx of cytotoxic T-cell lymphocytes which causes mucosal damage and, ultimately, villous atrophy and subsequent reduced resorption of minerals and micronutrients [22,23].

The prevalence of pediatric CD in the Western population, including the Netherlands, has been estimated at 0.5–1% [24–n27]. Historically, an esophagogastroduodenoscopy with histological examination of duodenal biopsies, classified according to the Marsh criteria, was necessary to establish the diagnosis CD [28,29]. But, through the past decades, highly sensitive and specific serological tests for the detection of CD have been developed. The sensitivity and specificity for the anti-tissue transglutaminase type 2 (TG2A) test are as high as 96 and 99% and for the endomysial antibody (EMA) test as high as 95 and 100%, respectively [30–32]. As a result of this, nowadays, in accordance with the current 2019 European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines for diagnosing CD, in children with typical symptoms, the combination of high TG2A antibody levels (at least 10 times the upper limit of normal) with anti-EMA and human leukocyte antigen (HLA), type DQ2 or DQ8, is enough for the diagnosis CD, thus circumventing the need for endoscopy [33].

It is evident that CD can affect individuals of any age and that patients may present with various atypical symptoms [34–38]. The clinical spectrum of CD was historically

characterized as a pediatric illness with the typical malabsorption syndrome presented by failure to thrive, distended abdomen and chronic diarrhea [39]. However, over the past two decades, also in the Netherlands, the traditional clinical picture has shifted towards display of more atypical, often extra-intestinal symptoms, like iron deficiency anemia, altered bone metabolism, short stature and elevation of liver serum transaminases [35–38]. However, the studies were not recently conducted and there was a lack of information on the current clinical spectrum of pediatric CD in the Netherlands. In **Chapter 3** we present a single center study amongst Dutch children with CD where we investigate the current clinical spectrum of CD in the Netherlands.

Due to the nature of CD, children with CD are susceptible to various vitamin and mineral deficiencies during diagnosis. However, the latest, updated versions of international guidelines such as the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) and North American Society for Pediatric Gastroenterology Hepatology and Nutrition (NASPGHAN) provide no clear statement on biochemical measurements of vitamin and minerals during diagnosis and follow-up of children with CD [29,40]. In the Netherlands, dated national guidelines do provide statements for biochemical follow-up in children with CD [41]. The study in **Chapter 4** explores the amount-, variation and diagnostic yield of performed additional diagnostic tests by pediatricians during follow-up of CD, in regard of the current national guidelines for CD. We aimed to assess guideline adherence amongst pediatricians and to investigate the clinical relevance of the laboratory tests suggested by the Dutch national guidelines for follow-up of pediatric CD.

Dientamoeba fragilis

Dientamoeba fragilis is a flagellate anaerobic parasite that inhabits the human gastrointestinal tract. The first description of *D. fragilis* was already 100 years ago by Jepps and Dobell, but there is still a lack of consensus on the potential pathogenicity of this protozoa [42,43]. It remains unclear why some patients harboring *D. fragilis* manifest clinical symptoms while others are only asymptomatic carriers. Possible interactions with the gut microbiota by the parasite or the possibility of different virulent strains remain unidentified. The protozoan appears to be particularly prevalent amongst children, yet it appears that the view on its pathogenicity in adults is more widespread accepted than in children [44]. A large series of scientific reports (primarily case reports or prospective or retrospective studies), from the time of its discovery until now, have provided support *D. fragilis* to be a potential pathogen [45–47]. However, this is opposed by the only conducted randomized controlled trial regarding the effect of metronidazole on gastrointestinal symptoms in children which showed no beneficial effect over placebo [48]. These conflicting results lead to the performance of the survey in **Chapter 5** where we explore the clinical attitude of Dutch general practitioners and pediatricians regarding potential pathogenicity, and diagnostic/ and therapeutic considerations towards *D. fragilis* in children and to the study described in **Chapter 6**

in which we explore if children with symptomatic *D. fragilis* infections might have a different gut microbiota in terms of composition and diversity, possibly leading to the displayed gastrointestinal symptoms.

The clinical presentation of *D. fragilis* varies, from asymptomatic carriers to a wide spectrum of gastrointestinal complaints, of which the most frequently reported symptoms are abdominal pain, and diarrhea [45,47,49–52]. These symptoms, however, are very common in the pediatric population. The diagnosis of *D. fragilis* is made by examination of stools and can be performed by using light microscopy (LM) after permanent staining of fixed stool samples, culture, or molecular techniques [53]. Until recently, microscopy was the most often used diagnostic tool worldwide for the diagnosis of *D. fragilis*. However, more recently, molecular diagnosis with real-time polymerase chain reaction (RT-PCR) was introduced for the diagnosis of *D. fragilis* [53]. This method proved to have a strongly increased sensitivity as compared to microscopy with a sensitivity of 100% [45,54]. Worldwide prevalence rates of *D. fragilis* vary between 0.3% and 52%, depending on the study cohort and used diagnostic modality [45,46,53] The reported prevalence rates may have increased due to the routine introduction of the more sensitive RT-PCR techniques [45]. *D. fragilis* infections can be treated by a single course of antibiotics, but available current literature suggest various kinds of possibly effective antibiotics in variable dosages and duration of treatment [53,55]. The most effective antibiotic regiment remains to be elucidated. In **Chapter 7** we present a systematic review which reviews the diagnostic considerations and efficacy of antibiotic treatment in children with *D. fragilis* infections.

REFERENCES

1. Wright N, Hammond P, Curry J. Chronic abdominal pain in children : help in spotting the organic diagnosis. Arch Dis Child Educ Pract. 2013;98:32–9.
2. Yacob D, Di Lorenzo C. How to Deal with Pediatric Functional Gastrointestinal Disorders. Curr Pediatr Rep. 2013;1:198–205.
3. Rajindrajith S, Zeevenhooven J, Devanarayana NM, Jayasiri B, Perera C, Benninga MA. Functional abdominal pain disorders in children. Expert Rev Gastroenterol Hepatol. 2018;12(4):369–90.
4. Korterink JJ, Diederen K, Benninga MA, Tabbers MM. Epidemiology of pediatric functional abdominal pain disorders: A meta-analysis. PLoS One. 2015;10(5):1–17.
5. Chitkara DK, Rawat DJ, Talley NJ. The epidemiology of childhood recurrent abdominal pain in western countries: A systematic review. Am J Gastroenterol. 2005;100(8):1868–75.
6. Drossman DA, Li Z, Andruzzi E, Temple RD, Talley NJ, Grant Thompson W, et al. U. S. Householder survey of functional gastrointestinal disorders - Prevalence, sociodemography, and health impact. Dig Dis Sci. 1993;38(9):1569–80.
7. Youssef NN, Atienza K, Langseder AL, Strauss RS. Chronic Abdominal Pain and Depressive Symptoms: Analysis of the National Longitudinal Study of Adolescent Health. Clin Gastroenterol Hepatol. 2008;6(3):329–32.
8. Campo J V., Di Lorenzo C, Chiappetta L, Bridge J, Colborn DK, Gartner JC, et al. Adult outcomes of pediatric recurrent abdominal pain: do they just grow out of it? Pediatrics. 2001;108(1).
9. Gieteling MJ, Bierma-Zeinstra SM, Van Leeuwen Y, Passchier J, Berger MY. Prognostic factors for persistence of chronic abdominal pain in children. J Pediatr Gastroenterol Nutr. 2011;52(2):154–61.
10. Shelby GD, Shirkey KC, Sherman AL, Beck JE, Haman K, Shears AR, et al. Functional abdominal pain in childhood and long-term vulnerability to anxiety disorders. Pediatrics. 2013;132(3):475–82.
11. Starfield B, Gross E, Wood M. Psychosocial and psychosomatic diagnoses in primary care of children. Pediatrics. 1980;(66):159–67.
12. Boyle J. Pediatric Gastrointestinal Disease: Pathophysiology, Diagnosis, Management. 4th ed. Walker W, Goulet O, Kleinman R, editors. Hamilton, ON: BC Dekker Inc; 2004. 232 p.
13. Lake A. Chronic Abdominal Pain in Childhood: Diagnosis and Management. Am Fam Physician. 1999;59(7):1823–30.
14. Noe J, Li B. Navigating recurrent abdominal pain through clinical clues, red flags, and initial testing. Pediatr Ann. 2009;38(5):259–66.
15. Spee LAA, Lisman-Van Leeuwen Y, Benninga MA, Bierma-Zeinstra SMA, Berger MY. Prevalence, characteristics, and management of childhood functional abdominal pain in general practice. Scand J Prim Health Care. 2013;31(4):197–202.
16. Dhroove G, Chogle A, Saps M. A million-dollar work-up for abdominal pain: Is it worth it? J Pediatr Gastroenterol Nutr. 2010;51(5):579–83.

17. Hoekman DR, Rutten JMTM, Vlieger AM, Benninga MA, Dijkgraaf MGW. Annual Costs of Care for Pediatric Irritable Bowel Syndrome, Functional Abdominal Pain, and Functional Abdominal Pain Syndrome. J Pediatr. 2015;167(5):1103-1108.e2.

18. Park R, Mikami S, Leclair J, Bollom A, Lembo C, Sethi S, et al. Inpatient burden of childhood functional GI disorders in the USA: An analysis of national trends in the USA from 1997 to 2009. Neurogastroenterol Motil. 2015;27(5):684–92.

19. Apley J, Naish N. Recurrent abdominal pains: A field survey of 1,000 school children. Arch Dis Child. 1958;33(168):165–70.

20. 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.

21. Holtmann GJ, Ford AC, Talley NJ. Pathophysiology of irritable bowel syndrome. Lancet Gastroenterol Hepatol. 2016;1(2):133–46.

22. Green P, Lebwohl B, Greywoode R. Celiac disease. J Allergy Clin Immunol. 2016;135(5):1099–106.

23. Lindfors K, Ciacci C, Kurppa K, Lundin KEA, Makharia GK, Mearin ML, et al. Coeliac disease. Nat Rev Dis Prim. 2019;5(1).

24. Ludvigsson JF, Leffler DA, Bai JC, Biagi F, Fasano A, Green PHR, et al. The Oslo definitions for coeliac disease and related terms. Gut. 2013;62(1):43–52.

25. Mustalahti K, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S, et al. The prevalence of celiac disease in Europe: Results of a centralized, international mass screening project. Ann Med. 2010;42(8):587–95.

26. Steens RFR, Csizmadia CGDS, George EK, Ninaber MK, Hira Sing RA, Mearin ML. A National Prospective Study on Childhood Celiac Disease in the Netherlands 1993–2000: An Increasing Recognition and a Changing Clinical Picture. J Pediatr. 2005;147(2):239–43.

27. Rubio-Tapia A, Ludvigsson JF, Brantner TL, Murray JA, Everhart JE. The Prevalence of Celiac Disease in the United States. Am J Gastroenterol. 2012;107(10):1538–44.

28. Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). Gastroenterology. 1992 Jan;102(1):330–54.

29. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease. J Pediatr Gastroenterol Nutr. 2012;54(1):136–60.

30. Fabiani E, Peruzzi E, Mandolesi A, Garbuglia G, Fanciulli G, D'Appello AR, et al. Anti-human versus anti-guinea pig tissue transglutaminase antibodies as the first-level serological screening test for coeliac disease in the general population. Dig Liver Dis. 2004;36(10):671–6.

31. Kolho KL, Savilahti E. IgA endomysium antibodies on human umbilical cord: an excellent diagnostic tool for celiac disease in childhood. J Pediatr Gastroenterol Nutr. 1997;24(5):563–7.

32. Lewis NR, Scott BB. Systematic review: the use of serology to exclude or diagnose coeliac disease (a comparison of the endomysial and tissue transglutaminase antibody tests). Aliment Pharmacol Ther. 2006;24(1):47–54.

33. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin M, Ribes-Koninckx C, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition guidelines for diagnosing coeliac disease 2019. J Pediatr Gastroenterol Nutr. 2019;70(1):141–56.

34. Almallouhi E, King KS, Patel B, Wi C, Juhn YJ, Murray JA, et al. Increasing Incidence and Altered Presentation in a Population-based study of Pediatric Celiac Disease in North-America. J Pediatr Gastroenterol Nutr. 2018;65(4):432–7.

35. Catassi C, Gatti S, Fasano A. The New Epidemiology of Celiac Disease. J Pediatr Gastroenterol Nutr. 2014;59(July):S7–9.

36. Khatib M, Baker RD, Ly EK, Kozielski R, Baker SS. Presenting Pattern of Pediatric Celiac Disease. J Pediatr Gastroenterol Nutr. 2016;62(1):60–3.

37. Roma E, Panayiotou J, Karantana H, Constantinidou C, Siakavellas SI, Krini M, et al. Changing pattern in the clinical presentation of pediatric celiac disease: a 30-year study. Digestion. 2009;80(3):185–91.

38. Krauthammer A, Guz-mark A, Zevit N, Marderfeld L, Waisbourd-Zinman O, Silbermintz A, et al. Two decades of pediatric celiac disease in a tertiary referral center: What has changed? Dig Liver Dis. 2020;0(0):1–5.

39. Losowsky MS. A History of Coeliac Disease. Dig Dis. 2008;26:112–20.

40. Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S, et al. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr. 2005;40(1):1–19.

41. CBO. Richtlijn Coeliakie en Dermatitis Herpetiformis. Haarlem: Nederlandse Vereniging van Maag-Darm-Leverartsen; 2008. Available from: https://www.mdl.nl/files/richlijnen/richtlijn_Coeliakie_definitief.pdf

42. Jepps MW, Dobell C. Dientamoeba fragilis n.g., n.sp., new intestinal amoeba from man. Parasitology. 1918;10:352–67.

43. Wong ZW, Faulder K, Robinson JL. Does Dientamoeba fragilis cause diarrhea? A systematic review. Parasitol Res. 2018;117(4):971–80.

44. Elbakri A, Al-qahtani A, Samie A. Advances on Dientamoeba fragilis Infections. In: Samie A, editor. An Overview of Tropical Diseases. IntechOpen; 2015. p. 61–81.

45. Stark D, Barratt J, Chan D, Ellis T. Dientamoeba fragilis , the Neglected Trichomonad of the Human Bowel. Clin Microbiol Rev. 2016;29(3):553–80.

46. Barratt JLN, Harkness J, Marriott D, Ellis JT, Stark D. A review of Dientamoeba fragilis carriage in humans: Several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. Gut Microbes. 2011;2(1):3–12.

47. Garcia L. Dientamoeba fragilis, One of the Neglected Intestinal Protozoa. J Clin Microbiol. 2016;54:2243–50.

48. Röser D, Simonsen J, Stensvold CR un., Olsen KEP, Bytzer P, Nielsen H V., et al. Metronidazole therapy for treating dientamoebiasis in children is not associated with better clinical outcomes: a randomized, double-blinded and placebo-controlled clinical trial. Clin Infect Dis. 2014;58(12):1692–9.

49. Stark D, Barratt J, Roberts T, Marriott D, Harkness J, Ellis J. A review of the clinical presentation of dientamoebiasis. Am J Trop Med Hyg. 2010;82(4):614–9.

50. Johnson EH, Windsor JJ, Clark CG. Emerging from obscurity: Biological, clinical, and diagnostic aspects of Dientamoeba fragilis. Clin Microbiol Rev. 2004;17(3):553–70.

51. Grendon J, DiGiacomo R, Frost F. Descriptive features of Dientamoeba fragilis infections. J Trop Med Hyg. 1995;(98):309–15.

52. Norberg A, Nord C, Evengard B. Dientamoeba fragilis - A protozoal infection which may cause severe bowel distress. Clin Microbiol Infect. 2003;9(1):65–8.

53. Van Gestel RSFE, Kusters JG, Monkelbaan JF. A clinical guideline on Dientamoeba fragilis infections. Parasitology. 2018;146(9):1131–9.

54. Stark D, Barratt J, Roberts T, Marriott D, Harkness J, Ellis J. Comparison of microscopy, two xenic culture techniques, conventional and real-time PCR for the detection of Dientamoeba fragilis in clinical stool samples. Eur J Clin Microbiol Infect Dis. 2010;29(4):411–6.

55. Nagata N, Marriott D, Harkness J, Ellis JT, Stark D. Current treatment options for Dientamoeba fragilis infections. International Journal for Parasitology: Drugs and Drug Resistance. 2012.

2

Large variation in clinical practice amongst pediatricians in treating children with recurrent abdominal pain

Michael W. van Kalleveen

Elise Noordhuis

Carole Lasham

Frans B. Plötz

Pediatric Gastroenterology, Hepatology and Nutriton *2019;22(3):225-232*

ABSTRACT

Purpose: To evaluate intra- and inter-observer variability and guideline adherence amongst pediatricians in treating children aged between 4 and 18 years referred with recurrent abdominal pain (RAP) without red flags.

Methods: The first part of the study is a retrospective single-center cohort study. The diagnostic work-ups of eight pediatricians were compared to the national guidelines. Intra- and inter-observer variability were examined by Cramer's V test. Intra-observer variability was defined as the amount of variation within a pediatrician and inter-observer variability as the amount of variation between pediatricians in the application of diagnostic work-up in children with RAP. Prospectively, the same pediatricians were requested to provide a report on their management strategy with a fictitious case to prove similarities in retrospective diagnostic work-up.

Results: A total of 10 patients per pediatrician were analyzed. Retrospectively, a (very) weak association between pediatricians' diagnostic work-ups was found (0.22), which implies high inter-observer variability. The association between intra-observer diagnostic was moderate (range, 0.35–0.46). The Cramer's V of 0.60 in diagnostic work-up between pediatricians in the fictitious case implied the presence of a moderately strong association and lower inter-observer variability than in the retrospective study. Adherence to the guideline was 66.8%.

Conclusion: We found a high intra- and inter-observer variability and moderate guideline adherence in daily clinical practice amongst pediatricians in treating children with RAP in a teaching hospital.

INTRODUCTION

Recurrent abdominal pain (RAP) is common amongst children and adolescents and often a reason for referral to a paediatrician. RAP is classified according to the paediatric Rome criteria as an abdominal pain related-functional gastrointestinal disorder (AP-FGID) [1,2]. In approximately 10% an identifiable somatic cause for RAP is identified [1,2]. Clinical decision rules and guidelines have been developed to guide clinicians in the decision to perform or to omit additional laboratory testing in children with RAP.

The Dutch evidence based guidelines advise detailed history and physical examination to detect alarm symptoms, the so-called "red flags", in children with RAP. In the absence of these "red flags" clarification and reassurance is justified. According to the guidelines, extensive diagnostic tests are not recommended in view of a low pre-test probability of finding a somatic cause [3]. In addition, this policy may serve to reduce financial costs, minimize nonspecific findings, and remove fear for painful diagnostic testing [4-6]. Despite well-defined guidelines, it is unknown whether paediatricians adhere to the guidelines during daily clinical practice.

The present study was undertaken to evaluate current clinical practice in children referred with RAP without alarm symptoms amongst paediatricians in a large teaching hospital. We retrospectively studied adherence to the Dutch guidelines and prospectively studied adherence to a synthetic case, and were especially interested in guideline adherence and intra- and interobserver variability.

METHODS

Study design

This single center study was conducted at the Tergooi Hospital in Blaricum, the Netherlands between August 2016 and December 2016. Tergooi Hospital is a 496-bed teaching hospital and serving a population of approximately 250.000 habitants.

The first part of the study was a retrospective single-center cohort study. The second part was a prospective survey study amongst paediatricians working at the Tergooi Hospital. Both parts were not subject to the Dutch Medical Research in Human Subjects Act.

Retrospective cohort study

Participants

Pediatricians were included for intra- and interobserver variability analysis if they worked in Tergooi Hospital since January 2013 and if they had at least 10 patients with RAP between 2013 and 2015 in their care. The number of included paediatricians was

based on the maximum attainable number of patients with a minimum of 10 patients per paediatrician. Intra-observer variability was defined as the amount of variation within a paediatrician and inter-observer variability as the amount of variation between paediatricians in application of diagnostic work-up in children with RAP.

Study protocol

Patients were eligible for inclusion if they were between 4 and 18 years old and attended the outpatient department of Tergooi Hospital between January 1st 2013 and December 31st 2015 with RAP. Included patients were referred to a paediatrician by a general practitioner (GP). Diagnostic work-up and follow-up was performed by the same paediatrician (with exception of medical students and paediatric trainees under supervision). RAP was the major symptom and at least present during three episodes in three months (severe enough to affect daily activities). Patients were excluded from this study if "red flags" in medical history were present, which were defined as unintentional weight loss, gastrointestinal blood loss, vomiting (prolonged, bilious or projectile), chronic diarrhoea (≥ 3 watery stools per day, longer than two weeks), arthralgia, unexplained fever and/or positive family history for inflammatory bowel disease (IBD), celiac disease or familial Mediterranean fever. Patients were also excluded if abnormalities during physical examination were found (i.e. abnormal growth curve, fever, uveitis, mouth ulcers, erythema nodosum, arthritis, icterus, suspected anaemia, persistent abdominal pain localized in the right upper or lower quadrant and/or perianal abnormalities). Finally, patients under 4 years old were excluded because of a higher pre-test probability of underlying somatic causes [4,5].

Data extraction

The patient care administration department at the Tergooi Hospital provided a list of children classified with 'recurrent abdominal pain' and their paediatrician during the study period. The medical records of included patients were reviewed in reverse chronicle order to represent the most recent population. The following data were obtained from the medical records: demographic characteristics**,** diagnosis according to Rome III criteria [1], characteristics of outpatient visits and performed diagnostic work-up by paediatrician.

Guideline adherence was studied by comparing diagnostic work-ups with the national guidelines. The guidelines recommended to order a complete blood count (CBC), CRP and celiac serology. In patients who suffer from diarrhea additional feces for Giardia Lamblia was advised and in patients who were suspected for inflammatory bowel disease a fecal calprotectin was advised. Guideline adherence was defined as: 0%: no adherence, 1-49%: very weak to weak adherence, 50-80%: moderate adherence, >80%: moderate strong to very strong adherence.

Prospective survey
Paediatricians who were included in the first part of the study were invited to complete a questionnaire. The survey concerned questions about diagnostic work-up in a synthetic case and guideline adherence. Briefly, the questionnaire consisted of several items, demographic characteristics, diagnostic work-up in a fictitious case of a child with RAP without red flags, reasons and considerations to perform diagnostic tests in children with RAP and questions and reasons about the use of guidelines (awareness, application, individual preferences and reasons to deviate).

Statistical analysis
For the statistical analysis the SPSS (SPSS version 22.0, SPSS Inc., Chicago) software and Microsoft Excel (2013) were used. Demographic and clinical characteristics were presented by descriptive statistics. A Kruskal-Wallis test was performed to analyse differences between paediatricians on several domains (i.e. patients' characteristics and clinical work-up). An independent Student's T-test was performed to analyse differences in duration of symptoms in months and time of follow-up between functional and organic RAP. For all comparisons an α-value of <0.05 was considered significant. Cramer's V test was used to study retrospective intra- and interobserver variability and prospective interobserver variability of our nominal variables. Intra- and interobserver variability was studied by means of a Cramer's V-test. The result of a Cramer's V-test lies between 0 and 1 and is interpreted as followed: 0 = no association, 0.01-0.3 = very weak to weak association, 0.3-0.5 = moderate association and >0.5 = a moderate strong to very strong association.

RESULTS

Retrospective cohort study

Participants and patients
During the study period 587 children visited the outpatient department of Tergooi Hospital with RAP (Figure 1). After first reviewing these 587 children, 189 children were excluded and 398 records remained for analysis in reverse chronicle order. After reviewing the 398 files, 8 of 10 paediatricians met the inclusion criteria (≥10 patient with RAP). Included paediatricians were anonymously categorized (A till H). Per included paediatrician the 10 most recently diagnosed patients with RAP were selected.

We found that 70% (n=56) of patients with RAP were classified as AP-FGID according to Rome III criteria. In 26% (n=21) an organic cause was found, and in 4% (n=3) a combination of an organic and functional cause was found, respectively.

Diagnostic work-up

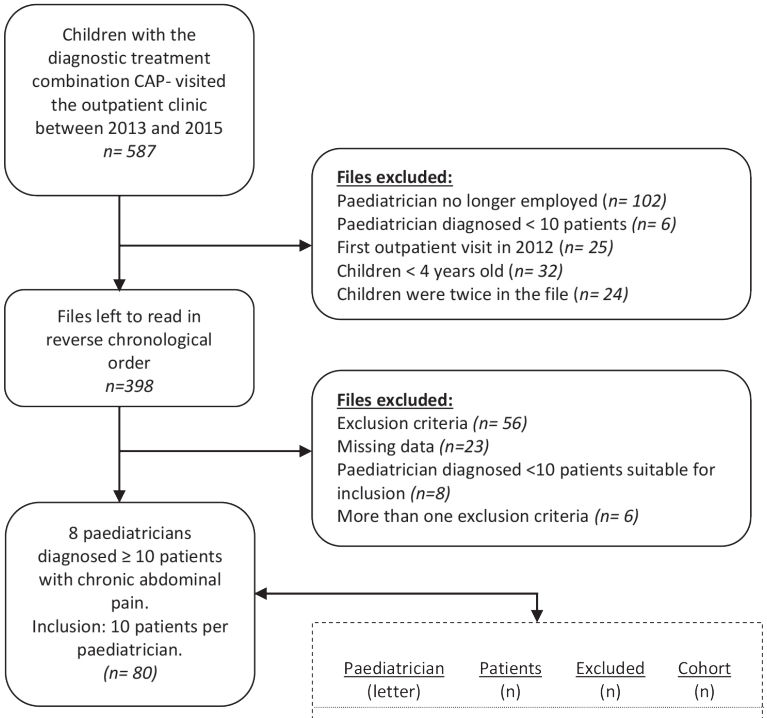
The clinical work-up per pediatrician in patients with RAP are shown in Table 1. There were no statistically significant differences between the included paediatricians except for the total number of outpatient visits (p= 0.045). Paediatricians performed an average of 5.3 (range 2.8-8.7) tests per patient.

Intra- and interobserver variability

A very weak association (Cramer's V value 0.22) between paediatricians' diagnostic work-ups was found, which implies a high interobserver variability. In terms of intra-observer variability, a moderate association (mean Cramer's V value 0.40, range: 0.35-0.46) was found for all paediatricians.

Guideline adherence

The adherence to the guidelines was moderate strong for performing a CBC (83%, range: 50-100%), moderate for performing celiac serology (76%, range: 50-100%) and weak for performing CRP (41%, range: 0-80%). None of the paediatricians strictly performed the defined combination of CBC, CRP and celiac serology. A sensitivity test was performed to determine the degree of national guideline adherence by the paediatricians (Table 1). This sensitivity test measured the proportion of tests that was recommended by the guidelines (CRP, CBC and celiac serology). The mean sensitivity was 66.8% (range: 47-80%), which represents a moderate adherence.



Paediatrician (letter)	Patients (n)	Excluded (n)	Cohort (n)
A	16	6	10
B	37	5	10
C	49	10	10
D	24	3	10
E	55	7	10
F	24	11	10
G	67	7	10
H	90	8	10
I	6	n/a	n/a
J	33	25	**0**

Figure 1. Study flow chart.

Prospective survey

The survey was completed by the eight paediatricians who participated in the retrospective study. Diagnostic tests performed by paediatricians in retrospect (R, n=10 patients) and in the prospective synthetic case (P, n=1 patient) are shown in Table 2. Both in prospect and in retrospect the CBC and celiac serology were the most performed tests, followed by stool examination for parasites. The Cramer's V of 0.60 in diagnostic work-up between paediatricians in the synthetic case implies a moderate strong association and lower interobserver variability than in the retrospective study. Reasons to deviate from the guidelines included feelings of being insufficiently informed about the guidelines, disagreement with the guidelines and not being convinced of the added value.

Table 1. Clinical characteristics of patients per paediatrician.

	Total (n=80)	A (n= 10)	B (n= 10)	C (n= 10)	D (n= 10)	E (n= 10)	F (n=10)	G (n=10)	H (n=10)	P value
Age (y)	9.7 (4-17)	10.2 (7-17)	7.1 (4-14)	11.2 (4-17)	8.1 (4-16)	10.1 (8-13)	10.1 (4-16)	10.3 (5-15)	10.4 (7-15)	0.173
Sex (male)	53.75%	60%	60%	30%	60%	50%	60%	50%	60%	0.873
Outpatients visits	2.1 (1-7)	1.6 (1-2)	2.2 (1-4)	3.3 (2-7)	2.5 (1-4)	2 (1-4)	1.4 (1-2)	1.8 (1-3)	1.9 (1-3)	0.045
Telephone consultations	1.1 (0-4)	1.2 (0-3)	1.1 (0-3)	1.1 (0-3)	1.1 (0-4)	1 (0-2)	1 (0-2)	1.3 (0-3)	0.8 (0-3)	0.961
Duration of symptoms (mo)	9.3 (0.5-72)	10.1 (1-24)	11.4 (2-48)	31 (4.5-72)	22.2 (1.5-42)	10.6 (3-24)	9.7 (1-18)	7 (0.5-12)	11.3 (0.75-36)	0.155
Follow-up (wk)	8.8 (0-74)	8.3 (0-20)	8 (0-27)	20.6 (2-74)	10.5 (2-42)	7 (2-13)	5 (1-9)	6.1 (1-21)	5 (0-12)	0.356
Time to diagnosis (wk)	5.6 (0-74)	4.7 (0-20)	5.8 (0-27)	15.1 (0-74)	5.8 (0-39)	4.8 (2-13)	2.8 (1-5)	3.6 (0-8)	2.8 (0-11)	0.645

Data are presented as median (interquartile range) or number (%)

Table 2. Percentage diagnostic tests performed in retrospective (n=10) and in a fictitious case (n=1).

	Total retro	Total pros	AR	AP	BR	BP	CR	CP	DR	DP	ER	EP	FR	FP	GR	GP	HR	HP
Hematology panel, Celiac serology, CRP	67%	13%	70%		47%		77%		67%		53%		67%	X	80%		73%	
Hematology panel	83%	63%	80%		50%		100%		90%	X	80%	X	90%	X	80%	X	90%	X
Celiac serology	77%	63%	50%		60%		100%		90%	X	80%	X	70%	X	100%	X	60%	X
CRP	41%	13%	80%		30%		30%		20%		-		40%	X	60%		70%	
ESR	69%	13%	80%		40%		50%		90%		60%		90%		60%		80%	X
LBP panel	40%	13%	-		30%		70%		20%		70%		60%		30%		40%	X
Kidney panel	41%	13%	-		30%		80%		30%		80%		60%		30%		20%	X
Electrolytes	6%	-	-		-		20%		-		10%		20%		-		-	
Thyroid panel	25%	-	-		-		60%		50%		50%		40%		-		-	
Iron levels	11%	13%	-		-		10%		10%		50%	X	-		-		20%	
Allergy panel	5%	-	20%		-		10%		10%		-		-		-		-	
Urine	16%	-	20%		20%		20%		-		40%		-		10%		20%	
Parasites	74%	50%	90%	X	90%	X	90%		90%		70%	X	70%		10%		80%	X
Calprotectin	46%	25%	70%	X	30%		80%	X	30%		30%		50%		30%		50%	
SSYC	13%	-	30%		-		20%		20%		-		-		-		30%	
H. pylori screening	21%	13%	40%	X	30%		30%		-		30%		20%		20%		-	
Abdominal US	19%	-	-		-		70%		-		30%		30%		-		20%	
Immuno-globulins	5%	-	-		-		-		30%		-		10%		-		-	
No tests performed	10%	-	30%		30%		-		-		10%		-		-		10%	

A till H are the anonymized pediatricians. Every first column represents the retrospective part of the study, every second column represents the result from the prospective survey. CRP: C-reactive protein. ESR: Erythrocyte Sedimentation Rate. LBP: Liver, Biliary, Pancreatic. SSYC: Shigella, Salmonella, Yersinia, Campylobacter. H. Pylori: Helicobacter Pylori. Abdominal US: Abdominal Ultrasound.

DISCUSSION

The aim of this study was to objectify intra- and interobserver variability and the degree of guidelines adherence in diagnostic work-up in children with RAP without red flags. We observed that guidelines adherence was moderate and inconsistent and the inter- and intra-variability in diagnostic work-up in children with RAP was large. To our knowledge, simultaneously reporting intra- and interobserver variability in diagnostic work-up has not been reported before. These results demonstrate that paediatricians rather prefer their clinical experience above practising evidence-based guidelines. The consequences of such an approach on patient related outcomes were not examined.

The results of our study are in line with various other paediatric studies that evaluated diagnostic work-up in common paediatric disorders [7-10]. For example, in a multi-centre retrospective cohort study in 30 large paediatric centres in the United States a variation of 38-89% in performing chest X-rays in hospitalized infants <1 year old with bronchiolitis was reported [7]. Also, the performance of diagnostic tests, for example, CBC's, blood cultures, blood chemistries, viral studies, inflammatory markers and chest radiographs in children with community-acquired pneumonia across emergency departments in the United States showed a large variation [8]. Low adherence has also been reported in the presence of well-defined evidence based guidelines [9,10]. Niele et al. reported that almost 50% of clinicians in the Netherlands managing children with minor traumatic brain injury often deviate from the evidence based guidelines [10]. Urkin et al. reported non-adherence in 50% of paediatricians managing children with acute pharyngitis [9].

The results of our study indicate that clinical decision making is based on the combination of evidence based guidelines and clinical experience. The main reason of guideline deviation in our study was disagreement with the guidelines. Reasons for guideline deviation amongst Dutch GP's included lack of agreement with the recommendations by the guidelines, lack of knowledge regarding the guidelines, and unclear recommendations by the guidelines as main reasons to deviate [11]. The degree of adherence to evidence based guidelines is also partly influenced by the clinicians number of years in practice [9,10,12]. Experienced clinicians are more likely to deviate from guidelines [10,12]. This may also be true for the paediatricians involved in our study, all were experienced paediatricians with at least 10 years of clinical practise. Adherence is also higher if guidelines recommendations are based on stronger evidence than those based on lower evidence [13]. Finally, Urkin et al. reported various cultural, psychological and local factors that influenced the behaviour of clinicians treating children with acute pharyngitis, which cannot be easily incorporated into guidelines [9].

Although non-adherence to evidence based guidelines may have clinical consequences, the opposite is also true in case of strict adherence to guidelines. Both approaches may

result in performing unnecessary diagnostic tests leading to possible false- positive or false-negative results and generates financial costs. For example, strict adherence to mild traumatic head injury increases CT scan use, which may increases the risk of non-specific findings [14].

Based on our results we think it is very important that guidelines are evaluated frequently, in particular whether the guidelines perform well diagnostically and how applicable the guidelines are to clinical practice. This also increases the awareness of differences in diagnostic approach within a team of paediatricians as well as in individuals. Furthermore, patient related outcome measures should also be take into account when evaluating diagnostic work-up or treatment in common paediatric disorders. Finally, it is important to explore influencing factors for not adhering to evidence based guidelines.

Various studies have been conducted to identify possible factors for improving paediatricians adherence to guidelines [15-17]. Paul et al. reported improved adherence to paediatric septic shock guidelines by monthly educational meetings, hospital-wide internet based learning modules, creating pocket cards and by a survey regarding barriers to adherence in order to make local modifications for the guidelines [15]. Also, e-learning significantly improved guideline adherence in paediatricians treating children with acute gastro-enteritis [16]. Finally, Redaèlli et al. reported that a physician's free choice between additional e-learning, training of practical nurses or e-learning and training of practical nurses improved asthma guidelines adherence with 10% [17]. However, a more individualized approach was considered necessary [17].

This study has some limitations. First, the use of a synthetic case has limitations. A paper case does not offer the opportunity to detect subtle signs during clinical presentation and therefore does not completely represent reality. Second, although we have found lower percentages of children diagnosed with functional RAP (70%) than reported literature (90%), it remains very questionable whether a positive stool on parasites fully explains symptoms of organic RAP [18]. Therefore, our percentage of children with functional RAP might be an underestimate. Our sample of patients represents, however, a general paediatric population and the patient characteristics per paediatrician were comparable. The major strength of our study is that we examined both retrospective and prospective intra- and interobserver variability. We were able to illustrate differences and similarities in retrospective clinical work-up and in a synthetic case in children with RAP. To our knowledge, very little is known regarding factors determining intra-observer variability.

In conclusion, we found a high intra- and interobserver variability and moderate guideline adherence in children with RAP in daily clinical practice amongst paediatricians.

We advocate to evaluate whether the guidelines perform well diagnostically and how applicable the guidelines are to clinical practice

Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Baber KF, Anderson J, Puzanovova M, Walker, LS, Rome II versus Rome III classification of functional gastrointestinal disorders in pediatric chronic abdominal pain. *Journal of pediatric gastroenterology and nutrition. 2008;47(3):299–302.*
2. Zeevenhooven J, Koppen IJ, Benninga MA, The New Rome IV Criteria for Functional Gastrointestinal Disorders in Infants and Toddlers. *Paediatr. Gastroenterol Hepatol Nutr. 2017 Mar;(20):1-13; DOI:*10.5223/pghn.2017.20.1.1
3. Di Lorenzo C, Colletti RB, Lehmann HP, Boyle JT, Gerson WT, Hyams JS et al., Chronic Abdominal Pain In Children: A Technical Report of the American Academy of Pediatrics and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition: AAP Subcommittee and NASPGHAN Committee on Chronic Abdominal Pain. *Journal of Pediatric Gastroenterology and Nutrition. 2005;40(3):249-261*
4. Wright NJ, Hammond PJ, Curry JI, Chronic abdominal pain in children: help in spotting the organic diagnosis. *Archives of Disease in Childhood – Education and Practice. 2013;98:32-39; DOI*:10.1136/archdischild-2012-302273
5. Zorgpad buikpijn (2013), opgesteld door kinderartsen van het Tergooi Ziekenhuis
6. Yacob D, Di Lorenzo C, How to Deal with Pediatric Functional Gastrointestinal Disorders. *Current Pediatrics Reports. 2013;1(3):198-205*
7. Christakis DA, Cowan CA, Garrison MM, Molteni, R, Marcuse E, Zerr DM, Variation in Inpatient Diagnostic Testing and Management of Bronchiolitis. *Pediatrics. 2005;115(4)879-884; DOI: 10.1542/peds.2004-1299*
8. Florin TA, French B, Zorc JJ, Alphern ER, Shah SS, Variation in emergency department diagnostic testing and disposition outcomes in pneumonia. *Pediatrics. 2013;132(2):237-44; DOI:10.1542/peds.2013-0179*
9. Urkin J, Allenbogen M, Friger M, Vinker S, Reuveni H, Elahayani A, Acute Pharyngitis: Low Adherence to Guidelines Highlights Need for Greater Flexibility in Managing Paediatric Cases. *Acta Paediatrica. 2013;102(11):1075-1080.*
10. Niele N, Willemars L, van Houten, M, Plötz FB, National Survey on Managing Minor Childhood Traumatic Brain Injuries in the Netherlands Shows Low Guideline Adherence and Large Interhospital Variations. Acta Paediatrica 2017 Sep 16. doi: 10.1111/apa.14076. [Epub ahead of print]
11. Lugtenberg B, Zegers van Schaick JM, Westert GP, Burgers JS, Why don't physicians adhere to guideline recommendations in practice? An analysis of barriers among Dutch general practitioners. *Implementation Science.* 2009;12(4):54. *DOI: 10.1186/1748-5908-4-54.*
12. Halm EA, Atlas SJ, Borowsky LH, Benzer TI, Metlay JP, Chang Y, et al., Understanding physician adherence with a pneumonia practice guideline: effects of patient, system, and physician factors. *Arch Intern Med.* 2000;160(1):98-104. DOI: 10.1001/archinte.160.1.98
13. Cnossen MC, Scholten AC, Lingsma HF, Synnot A, Tavender E, Gantner D, et al., Adherence to Guidelines in Adult Patients with Traumatic Brain Injury: A Living Systematic Review. *J Neurotrauma. 2016*; doi: 10.1089/neu.2015.4121. [Epub ahead of print]

14. Jansen PR, Dremmen M, van den Berg A, Dekkers, IA, Blanken LME, Muetzel RL et al. Incidental Findings on Brain Imaging in the General Pediatric Population. N Engl J Med 2017; 377(16):1953-1595 doi: 10.1056/NEJMc1710724

15. Paul R, Melendez E, Stack A, Caparo A, Monuteaux M, Neuman MI, Improving Adherence to PALS Septic Shock Guidelines. Pediatrics 2014;133(5):e1358-66

16. Nicastro E, Lo Vecchio A, Liguoro I, Chmielewska A, De Bruyn C, Dolinsek J, et al., The Impact of E-learning on Adherence to Guidelines for Acute Gastroenteritis: a Single-Arm Intervention Study. PLoS One 2015;10(7):e0132213

17. Redaèli M, Vollmar HC, Simic D, Maly-Schürer C, Löscher S, Koneczny N, Guideline Implementation Study on Asthma: Results of a pragmatic implementation approach. Z Evid Fortbild Qual Gesundhwes 2015;109(2):124-31.

18. Maas L, Dorigo-Zetsma JW, de Groot CJ, Bouter S, Plötz FB, van Ewijk BE, Detection of intestinal protozoa in paediatric patients with gastrointestinal symptoms by multiplex real-time PCR. *Clin. Microbiol. Infect 2014;20(6):545-50. DOI: 10.1111/1469-0691.12386*

3

Clinical spectrum of paediatric coeliac disease: a 10-year single-centre experience

Michael W. van Kalleveen

Tim de Meij

Frans B. Plötz

European Journal of Pediatrics 2018;177(4):593-602

ABSTRACT

This study was undertaken to gain insight in the clinical spectrum of paediatric coeliac disease (CD) in a Dutch teaching hospital. We retrospectively compared the frequency of CD in children with a wide spectrum of complaints with and without CD antibodies in serum and were interested if certain complaints are more pathognomonic for CD. Furthermore, we expected that over a period of 10-year incidence rates of CD would have increased and shifted towards an atypical presentation with more non-gastrointestinal symptoms with increasing age. A retrospective, single-centre, case-control study was performed. All patients who presented at the Department of Paediatrics, Tergooi Hospital, with symptoms suspected for CD were eligible for inclusion during the study period from 1 January 2007 till 31 December 2016. Children were diagnosed with CD according to the 2005 and 2012 ESPGHAN guideline between 2007 and 2016, respectively. Demographic data, presenting symptoms, prevalence of associated conditions and serology results were examined. A total of 105 new cases of paediatric CD were observed, with an average of 10 new cases each year. The calculated incidence was 21.09 (CI 17.49–25.22)/100,000 under 18 years of age. About 40% were infants and toddlers, predominantly presenting with gastrointestinal symptoms. Primary and high school children had more display of atypical symptoms ($p = 0.001$, $p = 0.017$) and non-gastrointestinal symptoms ($p = 0.009$, $p = 0.009$) than infants and toddlers. In 8.6% of the CD patients, mostly primary school aged female patients, the serology was repeated at least once in time to become positive. The median time for serology to become positive was 609 days (range 140–1054).

Conclusion: As it is well known, our study supports the increasing notion of a shift in the clinical spectrum of presenting symptoms in paediatric CD towards an atypical presentation, with more non-gastrointestinal symptoms and a diagnosis at a later age in a Dutch population, whereas the number of new cases did not increase over the years.

INTRODUCTION

Coeliac disease (CD) is a chronic immune-mediated enteropathy of the small intestine which is precipitated by exposure to dietary gluten in genetically susceptible individuals [3, 22]. The prevalence of paediatric CD in the Western population, including the Netherlands, has been estimated at 0.5–1% [9, 22, 26, 31, 34]. Until 2012, esophagogastroduodenoscopy with histological examination of duodenal biopsies, classified according to the Marsh criteria, was necessary to establish the diagnosis according to European Society for Paediatric Gastroenterology and Nutrition (ESPGHAN) guidelines [16, 23]. Current ESPGHAN and Dutch guidelines state that in children with typical symptoms, the combination of high anti-transglutaminase type 2 (anti-tTG) antibody levels (at least 10 times the upper limit of normal) with anti-endomysial antibodies (anti-EMA) and human leukocyte antigen (HLA), type DQ2 or DQ8, is enough for the diagnosis, thus circumventing the need for endoscopy [17]. Sensitivity and specificity for the anti-tTG2 test are as high as 96 and 99% and for the endomysial antibody (EMA) test as high as 95 and 100%, respectively [11, 19, 30].

By application of these serological tests as a screening tool in asymptomatic individuals, genetic predisposition or positive family history of CD, many asymptomatic cases became evident, contributing to the idea of CD as an iceberg conception [9, 12, 27, 29]. It is evident that CD can affect individuals of any age and that patients may present with various atypical symptoms [1, 8, 12, 18, 27, 29, 32, 34]. The clinical spectrum of CD was historically characterized as a paediatric illness with the typical malabsorption syndrome presented by failure to thrive, distended abdomen and chronic diarrhoea [21]. Over the past years, also in the Netherlands, the traditional clinical picture has shifted towards display of more atypical, often extra-intestinal symptoms, like iron deficiency anaemia, altered bone metabolism, short stature and elevation of liver serum transaminases [1, 5, 8, 12, 18, 27, 29, 32, 34]. However, it is not known if increased awareness of CD also results in increased incidence rates, therefore increasing the visible part of the coeliac iceberg. Since 2000, this has not been documented in the Netherlands [34].

This study was undertaken to gain insight in the clinical spectrum of paediatric CD in a Dutch teaching hospital. We therefore retrospectively compared the frequency of CD in children with a wide spectrum of complaints with and without CD antibodies in serum at our hospital and were interested if certain complaints were more pathognomonic for CD. Furthermore, we expected that over a period of 10-year incidence rates of CD increased and shifted towards an atypical presentation with more non-gastrointestinal symptoms with increasing age.

METHODS

Study design

This study was a retrospective single-centre, case-control study at the Tergooi Hospital in Blaricum, the Netherlands. Approval for the study was obtained from the Scientific Review Committee of Tergooi Hospital.

Participants

All patients age ≤ 18 years, who presented at the Department of Paediatrics of the Tergooi Hospital with symptoms suspected for CD were eligible for inclusion during the study period from 1 January 2007 till 31 December 2016. In these children, IgA levels of tTG and EMA and serum IgA levels were measured. If IgA deficiency was present, IgG levels of tTG and EMA were measured. Serum IgA deficiency was defined as < 0.20 g/L. A tTG-level of > 8 U/mL was considered positive.

Children were classified as positive CD cases if they were diagnosed with CD during 2007–2011 based on the 2005 ESPGHAN guidelines or diagnosed with CD during 2012–2016 based on the 2012 ESPGHAN guidelines for the diagnosis of CD in children and adolescents [16, 17]. Briefly, the old 2005 guideline stated that every CD serology (tTG/EMA) positive patient needed to be confirmed histologically by a esophagogastroduodenoscopy with duodenal biopsy. This guideline was used for patients who presented between 2007 and 2011. In these children, a Marsh score of ≥2 (crypt hyperplasia of the duodenum and an increased number of intraepithelial lymphocytes) was confirmative for CD. The new 2012 guideline was used for patients who presented between 2012 and 2016. The 2012 guideline stated that children with a tTG > 10× upper limit of normal (ULN) and a positive EMA and positive HLA haplotype (DQ2 or DQ8) are conclusive for the diagnosis CD; so, an esophagogastroduodenoscopy with duodenal biopsy can be omitted in that specific case. In every other case, a confirmative esophagogastroduodenoscopy with duodenal biopsy was necessary. In these children, a Marsh score of ≥2 was also confirmative for CD.

If serology was negative at first but became above the cut of value (> 10× ULN) after repeated testing, the amount of requested serological tests and the time interval to develop positive CD serology were extracted. The time interval to develop positive CD serology was defined as the date of positive CD serology minus the date of first requested CD serology and was expressed in days. Patients were excluded from this study if a confirmative esophagogastroduodenoscopy showed a Marsh score ≤1, revision of paper charts revealed a different diagnosis, the diagnosis was made in another hospital or if the diagnosis was established before 2007. The patient care administration provided a list of patient with the diagnosis CD during the study period.

To investigate if certain complaints are more pathognomonic for CD, we included a matched control group for age at diagnosis (up to 6 months younger or older), sex and period of presentation (up to 6 months earlier or later), in whom CD serology was negative during the study period. Control patients were selected based on a list with all the requested CD serology in patients aged 0–18 years during the study period that was provided by the Department of Microbiology of the Tergooi Hospital. If there was more than one suitable control patient available, the closest match (based on age and time of presentation) was selected. Patients referred for screening because of positive family history or CD-associated diseases were excluded as possible control patients. However, if a patient with a positive family history or CD-associated disease was referred because of complaints, they were included as a possible control patient.

The study population was divided into three separate age groups: under 4 years old (infants and toddlers), 4–12 years old (primary school) and over 12 years old (high school).

PROCEDURE AND MATERIALS

Data extraction

The following demographic characteristics were extracted from the paper charts: age, gender, ethnicity, age at time of first presentation of symptoms, year of presentation, height and weight at first presentation, body mass index, date of first visit, date of diagnosis of CD and family history for CD. The date of diagnosis was defined as the date the blood tests were performed or as the date on which the esophagogastroduodenoscopy was performed. The results of the different serological tests for CD (tTG/EMA) and HLA haplotypes were extracted from the electronic patient file using the ChipSoft HiX EPD software (ChipSoft HiX, version 6.1, Intermax, Rotterdam), which contained all results of laboratory tests performed during the study period.

Symptoms

Based on previous studies, presenting symptoms were categorized into “classical” and “atypical” symptoms and extracted from the paper charts [1, 5, 8, 12, 18, 27, 29, 32]. Classical symptoms included chronic diarrhoea, failure to thrive, distended abdomen, irritability, anorexia and coeliac crisis (defined as a life-threatening syndrome in which patients with CD have profuse diarrhoea and severe metabolic disturbances). Atypical symptoms were defined as recurrent abdominal pain, constipation, short stature, vomiting, iron deficiency anaemia, arthritis, aphthous stomatitis, dermatitis herpetiformis-like rash, pubertal delay, elevated liver enzymes, dental enamel defects and fatigue.

Since a number of these presenting symptoms were subjective symptoms, we used demarcated definitions beforehand. Chronic diarrhoea was defined as having defaecation ≥3 times a day during at least 14 days [36]. Failure to thrive was defined as a deflective weight-to-height curve or as being ≤−2SDs off on the weight-to-length curve. Recurrent abdominal pain was defined as having intermitting abdominal pain for at least 2 months [2]. Constipation was defined as having defaecation three times or less a week or as having pain during defaecation with the production of hard stools [6]. Short stature was defined as being two or more SDs smaller than children with the same age, or as being two or more SDs under their target height [28]. The presence of an iron deficiency anaemia was based on haematological references for children [4]. If there was no definition possible, the symptom was needed to be described by a paediatrician in the paper chart. Patients were described having a classical presentation if they presented with at least one classical symptom. If both classical and atypical symptoms were present, the patient was classified into classical or atypical based on the largest amount of classical or atypical symptoms.

Statistical analysis

For the statistical analysis, the SPSS (SPSS version 22.0, SPSS Inc., Chicago) software was used. Categorical variables between groups were studied by means of a chi-squared test. For small groups, the Fisher's exact test was used. Normally distributed continuous variables between two groups were studied by means of an independent samples t test. Continuous variables between the three groups were studied by means of one-way analysis of variance. For all comparisons, an α value of < 0.05 was considered significant.

In order to calculate incidence rates, demographic data about children aged 0–18 years old of the adherence area of the Tergooi Hospital (Gooi and Vechtstreek area) were provided by the Central Bureau of Statistics (CBS, The Hague, the Netherlands). The numbers of new outpatients per year was provided by the administration office.

RESULTS

Study population

During the study period, 147 patients met the inclusion criteria, of whom42 patients were excluded from participation for various reasons (Fig. 1). Of the 105 included CD patients, 52 (49.5%) were diagnosed according to the old 2005 ESPGHAN guideline (median age 4.3, range 1.0–17.6) and 53 patients (50,5%) were diagnosed according to the new 2012 ESPGHAN guideline (median age 5.2, range 1.0–17.3) ($p = 0.388$, not shown) (Table 1).

The majority of the CD patients was female (67%, ratio 2:1) (Table 1). The median age at time of first presentation was 5.8 years (range 1.0–17.6). We observed that 43 patients (41%) were diagnosed at the toddler age (0–3 years), 50 patients (48%) at the

primary school age (4–12 years) and 12 patients (11%) at the high school age (> 12 years), respectively. Almost 25% of the patients (all diagnosed by symptoms, not by screening) had a positive family history for CD. There were no auto-immune hepatitis, auto-immune thyroid disease, IgA nephropathy, Turner syndrome or Williams syndrome observed in the studied population.

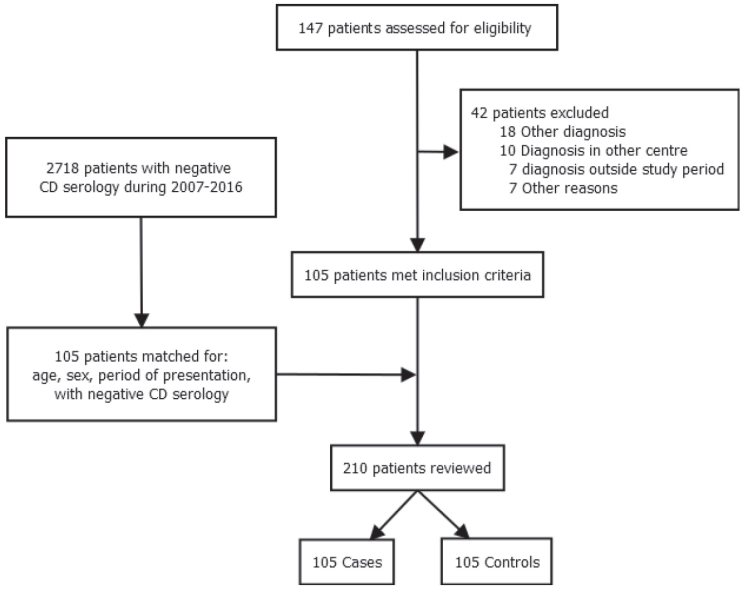


Figure 1. Flowchart of the studied population with coeliac disease.

A total of 2718 patients were screened as control patients and manually matched for age (up to 6 months younger or older), sex and period of presentation (up to 6 months earlier or later). In 96 of 105 patients, only 1 suitable control patient (based on matching criteria) was available. In the other nine patients, the closest match (based on age and time of presentation) was selected.

Table 1. Demographic data of the studied population.

Characteristics	Total (n=210)		P value	Infants & Toddlers (0-3yrs) (n=86)		P value	Pre-school (4-12yrs) (n=100)		P value	High-school (>12yrs) (n=24)		P value
	Cases	Controls		Cases	Controls		Cases	Controls		Cases	Controls	
Sex	105	105	N.S.	43	43	N.S.	50	50	N.S.	12	12	N.S.
Male	35 (33.3)	35 (33.3)		14 (32.6)	14 (32.6)		16 (32.0)	16 (32.0)		5 (41.7)	5 (41.7)	
Female	70 (66.7)	70 (66.7)		29 (67.4)	29 (67.4)		34 (68.0)	34 (68.0)		7 (58.3)	7 (58.3)	
Length (cm)	111.3 ± 26.8	111.0 ± 27.7	N.S.	87.8 ± 10.1	86.3 ± 10.0	0.494	118.9 ± 14.0	120.0 ± 16.0	0.725	163.3 ± 11.8	162.2 ± 10.1	0.815
Weight (kg)	21.9 ± 14.1	21.7 ± 14.2	N.S.	12.1 ± 2.9	11.6 ± 2.8	0.422	22.6 ± 6.6	23.0 ± 7.8	0.748	53.9 ± 12.2	52.6 ± 13.0	0.795
BMI (kg/m^2)	16.1 ± 2.2	16.0 ± 2.6	N.S.	15.5 ± 1.3	15.5 ± 1.4	N.S.	15.6 ± 1.5	15.6 ± 1.9	N.S.	20.0 ± 3.1	20.0 ± 4.8	N.S.
Age of presentation (years)	5.8 ± 4.2	5.7 ± 4.3	N.S.	2.3 ± 1.0	2.1 ± 1.0	0.303	6.6 ± 2.1	6.6 ± 2.1	N.S.	14.7 ± 2.1	14.9 ± 1.9	N.S.
Time to diagnosis (days)	25.7 ± 74.3	20.1 ± 51.2	0.525	42.5 ± 108.0	14.3 ± 41.3	0.113	16.8 ± 35.2	27.5 ± 62.0	0.291	2.58 ± 6.6	10.1 ± 25.4	0.332
Positive family member for CD	25 (23.8)	5 (4.8)	0.000	7 (16.3)	3 (7.0)	0.178	16 (32.0)	2 (4.0)	0.000	2 (16.7)	0 (0.0)	0.478
1st degree	19 (18.1)	3 (2.9)	0.000	4 (9.3)	3 (7.0)	N.S.	14 (28.0)	0 (0.0)	0.000	1 (8.3)	0 (0.0)	N.S.
2nd degree	6 (5.7)	2 (1.9)	0.149	2 (4.7)	0 (0.0)	0.494	3 (6.0)	2 (4.0)	N.S.	1 (8.3)	0 (0.0)	N.S.
3rd/4th degree	2 (1.9)	0 (0.0)	0.249	2 (4.7)	0 (0.0)	0.494	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	-
Ethnicity	97 (92.4)	97 (92.4)	0.506	37 (86.0)	40 (93.0)	0.359	47 (94.0)	45 (90.0)	0.701	12 (100)	12 (100)	N.S.
Caucasian	2 (1.9)	0 (0.0)	-	2 (4.7)	0 (0.0)	-	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	-
African-american	1 (1.0)	1 (1.0)	-	0 (0.0)	0 (0.0)	-	1 (2.0)	1 (2.0)	-	0 (0.0)	0 (0.0)	-
Asian	5 (4.8)	7 (6.7)	-	4 (0.0)	3 (7.0)	-	2 (4.0)	4 (8.0)	-	0 (0.0)	0 (0.0)	-
Not-reported			-			-			-			-
Presence of CD associated disease	8 (7.6)	6 (5.7)	0.580	4 (9.3)	2 (4.7	0.676	3 (6.0)	2 (4.0)	N.S.	1 (8.3)	2 (16.7)	N.S.
Type 1 DM	4 (3.8)	5 (4.8)	N.S.	0 (0.0)	1 (2.3)	N.S.	3 (6.0)	2 (4.0)	-	1 (8.3)	2 (16.7)	N.S.
IgA-deficiency	4 (3.8)	0 (0.0)	0.121	4 (9.3)	0 (0.0)	0.116	0 (0.0)	0 (0.0)	N.S.	0 (0.0)	0 (0.0)	-
Down syndrome	0 (0.0)	1 (1.0)	N.S.	0 (0.0)	1 (2.3)	N.S.	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	-

Nominal variables expressed in means ± SD. Figures in parentheses are percentages of the total study population. N.S. means not significant.

Table 2. Presenting symptoms during first outpatient visit.

Symptom	Total (n= 210)		P value	Infants & Toddlers (0-3yrs) (n=86)		P value	Pre-school (4-12yrs) (n=100)		P value	High-school (>12yrs) (n=24)		P value
	Cases	Controls		Cases	Controls		Cases	Controls		Cases	Controls	
Chronic diarrhoea	30 (28.6)	18 (17.1)	0.049	21 (48.8)	14 (32.6)	0.124	7 (14.0)	3 (6.0)	0.182	2 (16.7)	1 (8.3)	0.537
Failure to Thrive (FTT)	39 (37.1)	14 (13.3)	<0.001	26 (60.5)	11 (25.6)	0.001	11 (22.0)	3 (6.0)	0.021	2 (16.7)	0 (0.0)	0.140
Abdominal distention	45 (42.9)	4 (3.8)	<0.001	31 (72.1)	3 (7.0)	<0.001	13 (26.0)	1 (2.0)	0.001	1 (8.3)	0 (0.0)	0.307
Irritability	17 (16.2)	11 (10.5)	0.223	8 (18.6)	10 (23.3)	0.596	8 (16.0)	1 (2.0)	0.014	1 (8.3)	0 (0.0)	0.307
Anorexia	25 (23.8)	23 (21.9)	0.742	16 (37.2)	8 (18.6)	0.054	8 (16.0)	12 (24.0)	0.317	1 (8.3)	3 (25.0)	0.273
Recurrent abdominal pain	57 (54.3)	50 (47.6)	0.334	14 (32.6)	16 (37.2)	0.651	33 (66.0)	28 (56)	0.305	10 (83.3)	6 (50.0)	0.083
Constipation	22 (21.0)	17 (16.2)	0.375	11 (25.6)	10 (23.3)	0.802	9 (18.0)	6 (12.0)	0.401	2 (16.7)	1 (8.3)	0.537
Short stature	15 (14.3)	8 (7.6)	0.055	3 (7.0)	1 (2.3)	0.090	11 (22.0)	7 (14.0)	0.298	1 (8.3)	0 (0.0)	0.307
Vomiting	19 (18.1)	17 (16.2)	0.714	16 (37.2)	9 (20.9)	0.096	3 (6.0)	6 (12.0)	0.295	0 (0.0)	2 (16.7)	0.140
Bloating	9 (8.6)	6 (5.7)	0.421	3 (7.0)	4 (9.3)	0.693	4 (8.0)	2 (4.0)	0.400	2 (16.7)	0 (0.0)	0.140
Iron deficiency anaemia	24 (22.9)	10 (9.5)	0.009	14 (32.6)	5 (11.6)	0.019	10 (20.0)	4 (8.0)	0.084	0 (0.0)	1 (8.3)	0.307
Missing values	5 (4.8)	6 (5.7)	-	1 (2.3)	5 (11.6)	-	3 (6.0)	0 (0.0)	-	1 (8.3)	1 (8.3)	-
Aphtous stomatitis	0 (0.0)	1 (1.0)	1.0	0 (0.0)	0 (0.0)	-	0 (0.0)	1 (2.0)	1.0	0 (0.0)	0 (0.0)	-
Elevated liver enzymes	28 (26.7)	18 (17.1)	0.095	19 (44.2)	6 (14.0)	0.002	9 (18.0)	12 (24.0)	0.461	0 (0.0)	0 (0.0)	-
Missing values	51 (48.6)	66 (62.9)	-	14 (32.6)	29 (67.4)	-	31 (62.0)	31 (62.0)	-	6 (50.0)	6 (50.0)	-
Fatigue	34 (32.4)	22 (21)	0.061	13 (30.2)	8 (18.6)	0.209	18 (36.0)	9 (18.0)	0.043	3 (25.0)	5 (41.7)	0.386

Figures in parentheses are percentages of subgroup columns. Cases and controls comprise half amount of (sub)total patient numbers. There were no celiac crises, arthritis, dermatitis herpetiformis, pubertal delays and dental enamel defects observed.

Diagnostics

CD serology was performed in 100% of all CD subjects (median serum tTG-IgA total CD population 128 U/L, range 0–1300, median serum tTG-IgA matched controls 0.1 U/L, range 0–1.4). In 63 of 105 patient s (60%), an Esophagogastroduodenoscopy (EGD) with duodenal biopsies was necessary to establish the diagnosis, in the other 42 patients, CD serology alone was conclusive.CD serology was not conclusive (< 10× ULN of tTG-IgA/IgG) in 19.0% (n = 20) of the CD population (18 in tTG-IgA group, 2 in tTG-IgG group). In 100% of these CD patients, a confirmative esophagogastroduodenoscopy with duodenal biopsies was performed. In the 2005 ESPGHAN group, 51 patients (98%) had a Marsh score of 3. In the 2012 ESPGHAN group, 12 patients (22,6%) underwent an esophagogastroduodenoscopy with duodenal biopsies and 12 patients (100%) had a Marsh score of 3.

We observed that in 8.6% (n = 9) of the CD patients, CD serology had to be repeated at least once in time to become positive. The majority of these patients was female (67%, n=6) and of primary school age (78%, n=7). The median time for serology to become positive after repeated testing (n=2) was 609 days (range 140–1054). The EMA test was initially negative in these patients but became positive after repeated testing. Diagnostic features of these patients can be found in Supplementary Table 1. None of these patients was on a gluten restricted diet, and only one of these nine patients used immunosuppressive drugs (inhalation corticosteroids) and had a CD-associated IgA deficiency.

Incidence

Demographic data regarding children aged 0–18 years provided by the Central Bureau of Statistics revealed that a total of 545,216 children, 278,458 males and 266,758 females, respectively, lived in the adherence area of the Tergooi Hospital. Patients that were excluded because of the diagnosis made in another hospital were included in the incidence calculation. The calculated incidence rate was 21.09 (CI 17.49–25.22)/100,000 inhabitants ≤18 years and for males of 14.36 (CI 10.40–19.37)/100,000 inhabitants and for females 28.12 (CI 22.27–35.04)/100,000 inhabitants, respectively.

Between 1 January 2012 and 31 December 2016, a total of 32.286 new outpatients visited our outpatient clinic, of which 46 patients were diagnosed with CD resulting in a crude incidence rate of 1.43 (CI 1.055–1.884)/1000 new outpatients visiting our outpatient clinic.

Clinical presentation of CD patients and matched controls

The most common presenting symptoms of CD in our population were recurrent abdominal pain (54.3%) and distended abdomen (Table 2). The classical triad of CD symptoms (failure to thrive, distended abdomen and chronic diarrhoea) occurred in 32.6% of the cases with a mean age of 1.8 ± 0.9 years. Infants and toddlers presented

with more gastrointestinal symptoms (diarrhoea, distended abdomen, recurrent abdominal pain, constipation, vomiting, elevated liver enzymes) than primary and high school children (p = 0.009 and p = 0.009) (Fig. 2). The clinical spectrum shifted from a classical presentation in almost 90% of the toddlers to an atypical presentation in primary school children (42%, p = 0.001) and high school children (41.7%, p = 0.017) (Fig. 2). Subgroup analysis showed that this effect could be attributed to female gender (p = 0.002 vs. p = 0.190 for male gender).

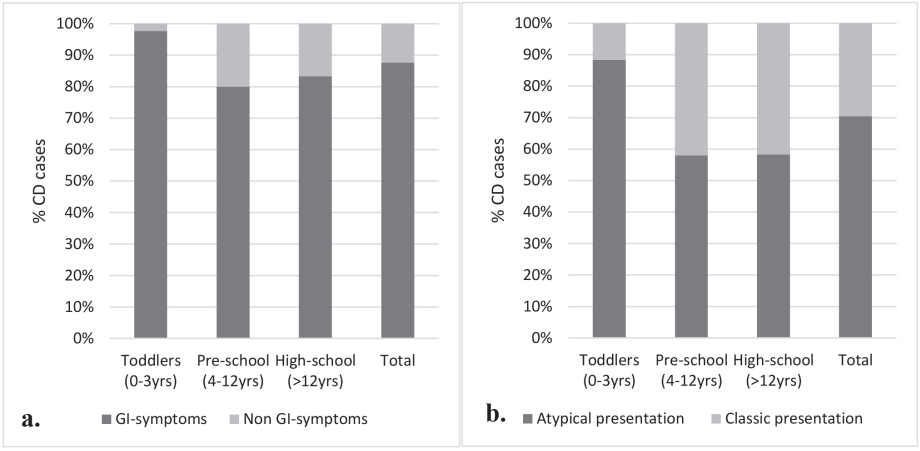


Figure 2. Distribution of new CD cases according to symptoms present at diagnosis. **a** Classification according to the presence of GI-symptoms vs. non-GI symptoms. **b** Classification according to atypical presentation vs. classical presentation.

Differences in presenting symptoms, according to the different age groups, between CD patients and control patients are depicted in Table 2. There were no statistically significant differences in presenting symptoms during first outpatient visits between high school children with CD and their matched control patients (Table 2).

DISCUSSION

The aim of our study was to describe the incidence and current clinical spectrum of paediatric CD in our hospital over the past 10 years. We observed 105 new cases of paediatric CD, of which 40% were infants and toddlers, predominantly presenting with gastrointestinal symptoms and 60% were primary or high school children presenting with an atypical presentation in 42 and 41.7%, respectively. We did not observe a difference in presentation between patients with CD and controls with increasing age. In almost 25% of our CD population, the family history for CD was positive. In almost

10% of the CD population, mostly females of primary school age, CD serology had to be repeated at least once in time to become positive.

We found that 41%of the patients with CD were diagnosed at the infant and toddler age. The mean age at time of diagnosis was 5.8 years. Previously, van Gils et al. found that 52% of the children with CD were diagnosed before the age of 4 years and the median age at time of diagnosis for the total CD population was 3 years [15]. These differences may be explained by the fact that the study ran between 1980 and 2015. In particular during the early stages of that study, probably most infants presented at the "classical way" and the invisible part of the coeliac iceberg was missed because of the absence of serological testing.

The shift in the clinical spectrum of paediatric CD has also been described in other studies [1, 8, 12, 18, 27, 29, 32, 34]. In the Netherlands, a decrease in rates of classical symptoms and low rates for atypical symptoms as presenting symptoms during first presentation were observed [34]. Our study found lower rates for classical symptoms and higher rates for atypical symptoms like recurrent abdominal pain (54.3 vs. 16%) and fatigue (32.4 vs. 12%), suggesting an ongoing shift in clinical spectrum. However, since no Dutch studies were conducted in the last 17 years, it is difficult to confirm this observation. Also, international studies in children with CD found atypical symptoms as the most common presenting symptoms suppand an atypical presentation varying from 36 to 41% [1, 8, 18, 27, 29, 32]. A recent American study even showed that nonspecific or extra-gastrointestinal symptoms were the most common presenting symptoms in 43% of the patients [1].

Our study showed that the classic CD triad of symptoms (chronic diarrhoea, failure to thrive and distended abdomen) are still pathognomic for paediatric CD, mostly for the infants and toddlers group, but with increasing age, atypical symptoms become more relevant. In our study population, iron deficiency anaemia is frequently seen in CD patients. Iron deficiency anaemia on itself can be caused by multiple diseases; however, in combination with for instance recurrent abdominal pain or chronic diarrhoea, it is very well justified to test for CD in paediatric patients. Recurrent abdominal pain is the most frequent presenting symptom of our studied population; however, the a priori chance of recurrent abdominal pain being a predominant presenting symptom of paediatric CD is very low. In primary and high school children, fatigue is more frequently seen but it is a too atypical symptom to be pathognomic for paediatric CD.

In our study in about 10% of the CD patients, CD serology had to be repeated at least once before becoming positive. The majority of these patients were primary school aged females with numerous subjective complaints. It is known that CD antibodies are not detectable in the blood of all patients with CD [20]. Also, it recently became clear that tTG levels are not sufficient to diagnose CD in North-American practices

without intestinal biopsies [10]. This could also be possible for European or Dutch practices. From a clinical point of view, this remains a challenge. According to the ESPGHAN recommendations, these children with (a specific) symptoms compatible with CD but with negative serology, CD serology should be repeated and eventually diagnostic duodenal biopsies should be performed [17]. However, in general hospitals, a large number of children are seen with various complains, whereas the a priori chance that isolated recurrent abdominal pain or fatigue in this age group is caused by CD is very low. We only found an average of 10 new cases per year, whereas the number of children with subjective complaints is much higher. Despite this, we underscore the important role for CD screening or case finding to prevent under diagnosis of CD because of the significant clinical consequences of missing CD in children [14, 25].

Our calculated (gender-specific) incidence rates were higher than the incidence rates found by Burger et al. in 2010 (21.09 vs. 12.29/100,000 inhabitants ≤18 years) [7]. We cannot compare our crude calculated incidence rate of 1.43/1000 new outpatients to the incidence rates of 0.81/1000 live births during 1993–2000 and 1.1/1000 live births in 2000 [9, 34].We observed a male to female ratio of 1.0:2.0 comparable to other Dutch studies [7, 9, 20, 24, 33, 34]. These results may indeed suggest that the visible part of the coeliac iceberg is increasing. However, we cannot draw definite conclusions, in particular, since data on older cohorts within our hospital are lacking. Also, our calculated incidence rate could still be an underestimate of the true incidence rate for paediatric CD because of the possibility of patients being referred directly towards an academic hospital for esophagogastroduodenoscopy with duodenal biopsies instead of being referred to a general hospital first.

In our study, about 25% of the children with CD had a family history positive for CD, which is a higher percentage than 14% reported in a previous Dutch study and 18% in an Australian study [15, 35]. A large American multi-centre epidemiologic study conducted in 32 states between 1996 and 2001 already showed that the prevalence of CD in first and second degree relatives is high, even in asymptomatic relatives [13]. In our study, every patient was diagnosed by complaints, not by screening. We therefore underscore the ESPGHAN 2012 guideline that justifies the use of HLA diagnostics as a screening tool for CD in family members of patients with CD [17].

Our study has several limitations. First, it is a single-centre, retrospective study. Nevertheless, we believe that our study is representative for clinical and epidemiological trends for paediatric CD in the Netherlands, because our hospital covers a large residential area which is both rural and urban, and because previous studies found relatively small differences in paediatric CD incidence between the different provinces in the Netherlands [7, 15]. Second, it is not feasible to measure the “true” incidence of CD due to the nature of the disease and its symptomatology and the lack of a nationwide screening programme. Patients are detected only by symptomatic disease or by

screening of asymptomatic high-risk individuals and the remainder are missed. Third, some of the presenting symptoms we have investigated like, for instance irritability, anorexia and fatigue are subjective symptoms. We aimed to overcome this by defining most of these presenting symptoms using demarcated definitions, although a certain degree of subjectivity could not be avoided. Fourth, the number of high school patients was relatively small, making the group underpowered for detection of differences between high school children with CD and their matched controls.

CONCLUSION

Our study supports the increasing notion of a shift in the clinical spectrum of presenting symptoms in paediatric CD towards an atypical presentation, with more non-gastrointestinal symptoms and a diagnosis at a later age in a Dutch population, whereas the number of new cases did not increase over the years. We advise a larger multi-centre or nationwide study to be conducted to confirm these results.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Almallouhi E, King KS, Patel B, Wi C, Juhn YJ, Murray JA, Absah I (2017) Increasing incidence and altered presentation in a population-based study of pediatric celiac disease in North America. J Pediatr Gastroenterol Nutr 65(4):432–437. https://doi.org/10.1097/MPG.0000000000001532
2. American Academy of Pediatrics subcommittee on chronic abdominal pain (2005) Chronic abdominal pain in children. Pediatrics 115(3):812–815. https://doi.org/10.1542/peds.2004-2497
3. American Gastroenterological Association (2001) American Gastroenterological Association medical position statement: celiac sprue. Gastroenterology 120(6):1522–1525. https://doi.org/10.1053/gast.2001.24055
4. Baker RD, Greer FR (2010) Committee on nutrition American Academy of Pediatrics. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0–3 years of age). Pediatrics 126(5):1040–1050. https://doi.org/10.1542/peds.2010-2576.
5. Barker JM, Liu E (2008) Celiac disease: pathophysiology, clinical manifestations, and associated autoimmune conditions. Adv Pediatr Infect Dis 55(1):349–365. https://doi.org/10.1016/j.yapd.2008.07.001
6. Bharucha AE, Dorn SD, Lembo A, Pressman A (2013) American Gastroenterological Association Medical position statement on constipation. Gastroenterology 144(1):211–217. https://doi.org/10.1053/j.gastro.2012.10.029
7. Burger JPW, Roovers EA, Drenth JPH, Meijer JWR, Wahab PJ (2014) Rising incidence of celiac disease in the Netherlands; an analysis of temporal trends from 1995 to 2010. Scand J Gastroenterol 49(8):933–941. https://doi.org/10.3109/00365521.2014.915054
8. Catassi C, Gatti S, Fasano A (2014) The new epidemiology of celiac disease. J Pediatr Gastroenterol Nutr 59(July):S7–S9. https://doi.org/10.1097/01.mpg.0000450393.23156.59.
9. Csizmadia CG, Mearin ML, von Blomberg BME, Brand R, Verloove-Vanhorick SP (1999) An iceberg of childhood coeliac disease in the Netherlands. Lancet 353(9155):813–814. https://doi.org/10.1016/S0140-6736(99)00243-3
10. Elitsur Y, Sigman T, Watkins R, Porto AF, Leonard Puppa EL, Foglio EJ, Preston DL (2017) Tissue transglutaminase levels are not sufficient to diagnose celiac disease in North American practices without intestinal biopsies. Dig Dis Sci 62(1):175–179. https://doi.org/10.1007/s10620-016-4354-4
11. Fabiani E, Peruzzi E, Mandolesi A, Garbugli G, Fanciulli G, D'Appello AR et al (2004) Anti-human versus anti-guinea pig tissue transglutaminase antibodies as the first-level serological screening test for coeliac disease in the general population. Dig Liver Dis 36(10):671–676. https://doi.org/10.1016/j.dld.2004.05.008
12. Fasano A (2005) Clinical presentation of celiac disease in the pediatric population. Gastroenterology 128(4 Suppl 1):S68–S73. https://doi.org/10.1053/j.gastro.2005.02.015

13. Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, Elitsur Y, Green PHR, Guandalini S, Hill ID, Pietzak M, Ventura A, Thorpe M, Kryszak D, Fornaroli F, Wasserman SS, Murray JA, Horvath K (2003) Prevalence of celiac disease in at-risk and notat-risk groups in the United States: a large multicenter study. Arch Intern Med 163(3):286–292. https://doi.org/10.1001/archinte.163.3.286

14. van Gils T, Nijeboer P, van Wanrooij RL, Bouma G, Mulder CJJ (2015) Mechanisms and management of refractory coeliac disease. Nat Rev Gastroenterol Hepatol 12(10):572–579. https://doi.org/10.1038/nrgastro.2015.155.

15. van Gils T, Rootsaert B, Bouma G, Mulder CJ (2016) Celiac disease in The Netherlands: demographic data of members of the Dutch Celiac Society. J Gastrointestin Liver Dis 25(4):441–445. https://doi.org/10.15403/jgld.2014.1121.254.gil

16. Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandaline S et al (2005) Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 40(1):1–19. https://doi.org/10.1097/00005176-200501000-00001

17. Husby S, Koletzko S, Korponay-Szabó IR,Mearin ML, Phillips A, Shamir R, Troncone R, Giersiepen K, Branski D, Catassi C, Lelgeman M, Mäki M, Ribes-Koninckx C, Ventura A, Zimmer KP, ESPGHAN Working Group on Coeliac Disease Diagnosis, ESPGHAN Gastroenterology Committee, European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (2012) European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 54(1):136–160. https://doi.org/10.1097/MPG.0b013e31821a23d0

18. Khatib M, Baker RD, Ly EK, Kozielski R, Baker SS (2016) Presenting pattern of pediatric celiac disease. J Pediatr Gastroenterol Nutr 62(1):60–63. https://doi.org/10.1097/MPG.0000000000000887

19. Kolho KL, Savilahti E (1997) IgA endomysium antibodies on human umbilical cord: an excellent diagnostic tool for celiac disease in childhood. J Pediatr Gastroenterol Nutr 24(5):563–567. https://doi.org/10.1097/00005176-199705000-00014

20. Lewis NR, Scott BB (2006) Systematic review: the use of serology to exclude or diagnose coeliac disease (a comparison of the endomysial and tissue transglutaminase antibodies tests). Aliment Pharmacol Ther 24(1):47–54. https://doi.org/10.1111/j.1365-2036.2006.02967.x

21. Losowsky MS (2008) A history of coeliac disease. Dig Dis 26(2):112–120. https://doi.org/10.1159/000116768

22. Ludvigsson JF, Leffler DA, Bai JC, Biagi F, Fasano A, Green PH et al (2013) The Oslo definitions for coeliac disease and related terms. Gut 62(1):43–52. https://doi.org/10.1136/gutjnl-2011-301346

23. Marsh MN (1992) Gluten, major histocompatibility complex, and the small intestine: a molecular and immunobiologic approach to the spectrum of gluten sensitivity (‘celiac sprue’). Gastroenterology 102(1):330–354. https://doi.org/10.1016/0016-5085(92)91819-P

24. Mearin ML (2007) Celiac disease among children and adolescents. Curr Probl Pediatr Adolesc Health Care 37(3):86–105. https://doi.org/10.1016/j.cppeds.2007.01.001

25. Mulder CJ,Wierdsma NJ, BerkenpasM, Jacobs MAJM, Bouma G (2015) Preventing complications in celiac disease: our experience with managing adult celiac disease. Best Pract Res Clin Gastroenterol 29(3):459–468. https://doi.org/10.1016/j.bpg.2015.05.006

26. Mustahlahti K, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S et al (2010) The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. Ann Med 42(8):587–595. https://doi.org/10.3109/07853890.2010.505931

27. Nenna R, Tiberti C, Petrarca L, Lucantoni F, Mennini M, Luparia RP et al (2013) The celiac iceberg. J Pediatr Gastroenterol Nutr 56(4):416–421. https://doi.org/10.1097/MPG.0b013e31827b7f64

28. Pedicelli S, Peschiaroli E, Violi E, Cianfarani S (2011) Controversies in the definition and treatment of idiopathic short stature (ISS). J Clin Res Pediatr Endocrinol 1(3):105–115. https://doi.org/10.4008/jcrpe.v1i3.53

29. Roma E, Panayiotou J, Karantana H, Constantinidou C, Siakavellas SI, KriniM, Syriopoulou VP, Bamias G (2009) Changing pattern in the clinical presentation of pediatric celiac disease: a 30-year study. Digestion 80(3):185–191. https://doi.org/10.1159/000227275

30. Rostom A, Dubé C, Cranney A, Saloojee N, Sy R, Garritty C, Sampson M, Zhang L, Yazdi F, Mamaladze V, Pan I, MacNeil J, Mack D, Patel D, Moher D (2005) The diagnostic accuracy of serologic tests for celiac disease: a systematic review. Gastroenterology 128(4 Suppl 1):S38–S46. https://doi.org/10.1053/j.gastro.2005.02.028

31. Rubio-Tapia A, Ludvigsson JF, Brantner TL, Murray JA, Everhart JE (2012) The prevalence of celiac disease in the United States. Am J Gastroenterol 107(10):1538–1544. https://doi.org/10.1038/ajg.2012.219

32. Shahraki T (2016) Clinical spectrum of celiac disease in children in Sistan and Baluchestan Province. Arch Iran Med19(11). http://www.ams.ac.ir/AIM/NEWPUB/16/19/11/004.pdf. Accessed May 4, 2017.

33. Spijkerman M, Tan IL, Kolkman JJ, Withoff S, Wijmenga C, Visschedijk MC, Weersma RK (2016) A large variety of clinical features and concomitant disorders in celiac disease: a cohort study in the Netherlands. Dig Liver Dis 48(5):499–505. https://doi.org/10.1016/j.dld.2016.01.006

34. Steens RFR, Csizmadia CGDS, George EK, Ninaber MK, Hira Sing RA,Mearin ML(2005) A national prospective study on childhood celiac disease in the Netherlands 1993–2000: an increasing recognition and a changing clinical picture. J Pediatr 147(2):239–243. https://doi.org/10.1016/j.jpeds.2005.04.013

35. Stone ML, Bohane TD, Whitten KE, Tobias VH, Day AS (2005) Age related clinical features of childhood coeliac disease in Australia. BMC Pediatr 5(1):11. https://doi.org/10.1186/1471-2431-5-11

36. Vanderhoof JA (1998) Chronic diarrhea. Pediatr Rev 19(12):418–422. https://doi.org/10.1542/pir.19-12-418.

4

Guideline adherence and clinical relevance of laboratory investigations during follow-up of paediatric coeliac disease: A Dutch single-centre cohort study

Michael W. van Kalleveen

Tim C. Dykstra

Tim G.J. de Meij

Frans B. Plötz

Acta Paediatrica *2021;110(9):2641-2647*

ABSTRACT

Aim: Dutch national guidelines on follow-up of paediatric celiac disease (CD) are available. The primary aim was to evaluate guideline adherence by paediatricians during follow-up. The secondary aim was to determine the clinical relevance and diagnostic yield of routine laboratory tests suggested by these guidelines.

Methods: A retrospective, single-centre, cohort study was performed in paediatric CD patients who visited Tergooi Hospital, the Netherlands, between January 2017 and December 2019, with follow-up of at least twelve months after diagnosis. We analysed guideline adherence, number of outpatient visits and all laboratory data.

Results: We included 91 CD children with a median follow-up of 4.0 years (range 1-16 years) and 162 follow-up visits. Strict adherence amongst paediatricians during follow-up was 8.0% (13/162 cases). A total of 1570 laboratory tests were performed of which 45.4% (713/1570) was in strict compliance with the Dutch national guidelines. Clinically relevant deviations were observed in 5.3% of requested laboratory tests.

Conclusion: Strict guidelines adherence amongst paediatricians in follow-up of paediatric CD was low and the clinical relevance of the suggested routine laboratory tests is limited. This underlines the increasing notion that evidence-based guidelines on follow-up of CD are warranted.

INTRODUCTION

The prevalence of paediatric coeliac disease (CD) in the Western population is estimated at 1%, [1–4]. CD is usually diagnosed in childhood, with an increasing age at diagnosis over the past decades [1,2,5]. Patients may present with a wide spectrum of classical and atypical (extra)intestinal symptoms [1,4,5]. The only effective treatment for CD is a lifelong gluten free diet (GFD) [6]. Possible concomitant vitamin and mineral deficiencies can be corrected by supplements [7]. Reported GFD adherence or incidental gluten intake varies between 23-98%, with higher reported adherence in younger children [8,9]. GFD leads to restoration of gut function, normalization of laboratory results and deficiencies, and recovery from other complications. Anti-transglutaminase type 2 antibodies (TG2A) return to normal values in 20-72% of children after one year and 58-95% of children after two years [10–13].

International guidelines regarding the follow up of paediatric CD are lacking. The European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) and North American Society for Paediatric Gastroenterology Hepatology and Nutrition (NASPGHAN) provide no evidence-based protocol regarding follow-up in terms of frequency and duration of visits and biochemical and serological measurements in their latest updated guidelines [15,16]. Available, but dated, Dutch national guidelines for diagnosing and follow-up of paediatric CD recommend yearly follow-up visits with length and weight measurements, monitoring GFD adherence and laboratory testing (during diagnosis and follow-up) for CD serology, haemoglobin, haematocrit, mean corpuscular volume, folic acid, vitamin B12, calcium, alkaline phosphatase, iron levels (serum iron or serum ferritin) and, when indicated, for thyroid function [17]. Both adherence to existing national guidelines and clinical relevance of laboratory tests during follow-up of paediatric patients with CD remain largely unknown [14]. In the Netherlands, children with CD are not exclusively seen by paediatric gastroenterologists for diagnosis and during follow-up. Routine care is also, and in fact in the majority of cases, provided by general paediatricians and general practitioners and children are also seen by dieticians.

The primary aim of this study was to evaluate adherence to Dutch national guidelines amongst paediatricians in children with CD during follow-up. Secondary aim was to determine the clinical relevance of all laboratory tests during follow-up of paediatric CD.

PATIENTS AND METHODS

Study design and patients

This study was a retrospective, single centre, cohort study at Tergooi hospital in Blaricum, the Netherlands. Tergooi hospital is a 496-bed teaching hospital and serves a population of approximately 250,000 habitants, with approximately 5,000 paediatric

out-patient visits yearly. All patients under 18 years of age with a diagnosis of CD, who underwent follow-up of at least 12 months after the initial diagnosis at the department of Paediatrics of the Tergooi hospital, were included during the study period from 1st January 2017 until 31st December 2019. Children diagnosed between 2004 and 2011 were diagnosed based on 2005 ESPGHAN guidelines for CD and children diagnosed between 2012 and 2019 were diagnosed based on 2012 ESPGHAN guidelines [15].

Study protocol

The 2008 Dutch national guidelines, as stated by the Dutch Society for Gastroenterology, recommends yearly monitoring of length and weight measurements, anamnestic GFD adherence, haemoglobin, haematocrit, mean corpuscular volume, folic acid, vitamin B12, calcium, alkaline phosphatase, iron levels (serum iron or serum ferritin) and TG2A in GFD adherent paediatric patients [17]. In GFD non-adherent children, these measurements are suggested to be monitored more frequently [17]. Thyroid stimulating hormone (TSH) and free T4 should be tested yearly, upon clinical indication as judged by the clinician [17].

Data collection

The following demographic and patient characteristics were extracted from the electronic patient file system ChipSoft HiX EPD software (ChipSoft HiX, version 6.1, Intermax, Rotterdam) and stored coded using Castor EDC (Castor Electronic Data Capture, 2019): age during follow-up, gender, year of diagnosis, year of follow-up, height and weight, body mass index, primary responsible physician, family history for CD, GFD compliance, comorbidities, CD complications, use of medication and every performed laboratory test during follow-up. We numbered total outpatient visits and follow-up visits in which laboratory tests were performed. The laboratory tests were compared to reference range values, specified for age and sex [18,19]. Growth charts of all patients were analyzed individually. Growth delay was defined as a deviation of ≥ -1SD in 1 year in length, and/or weight to length charts during the study period. If follow-up ended during puberty and no length gain was obtained thereafter, this was not considered pathological.

Data analysis

Since we aimed to evaluate adherence to the Dutch national CD guidelines by paediatricians and the clinical relevance and diagnostic yield of laboratory tests suggested by these guidelines, length and weight measurements and GFD adherence were not included in the analysis on guideline adherence. Guideline adherence was therefore defined as yearly measurement of the nine laboratory parameters recommended by the Dutch national guidelines [17]. In case laboratory tests were performed in addition to these recommended parameters, we considered this as adherent to the guidelines. If the timeframe between outpatient visits for CD follow-up was longer than 12 months or if at least one laboratory parameter was not measured,

this was considered non-adherent. Since monitoring of TSH and free T4 in children is only suggested on indication, these parameters were not included in the analysis of guidelines adherence, but only used for analysis of the diagnostic yield of routine follow-up. If a patient was seen multiple times during the study period of three years, guideline adherence was analysed every single year. If one patient had more than one follow-up visit per year and the required nine parameters were evaluated in these follow-up visits combined, this was also considered adherent. Haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), erythrocytes and relative distribution width (RDW) were clustered into two single elements: haemoglobin/haematocrit (Hb/Ht) and "red blood cell indicators" (MCV/MCH/RDW/erythrocytes). Guideline adherence was determined by comparison of performed laboratory tests to those suggested by the Dutch national guidelines, by a research fellow (MK), independent from the treating physicians.

As secondary aim, we determined the clinical relevance and diagnostic yield of all performed laboratory tests during follow-up of CD. Every measured laboratory test in patients where paediatricians were adherent or non-adherent to the guidelines were analysed individually, analysed for deviations and determined for its clinical significance. Deviations in laboratory tests were defined as a laboratory test value outside the reference range, corrected for age and sex. Deviations were determined clinically relevant if: laboratory test values were outside the reference range in combination with clinical complaints and/or requiring supplementation or if deviations could result in earlier follow-up (for instance anaemia without the presence of clinical complaints). If these criteria were not fulfilled than it was considered not relevant. The clinical relevance of deviations in laboratory tests were scored by two clinical researchers (MK/FP). If there was no consensus, a third researcher was consulted (TM).

Reference values

During the study period TG2A IgA and anti-deaminated gliadin peptide reference values used in Tergooi hospital were adjusted on July 1st 2018 from negative <5 U/ml to <20 U/ml, weakly positive from 5-10 U/ml to 20-30 U/ml and positive from >10 U/ml to >30 U/ml, therefore a differentiation in time was made before and after July 1st 2018 for final statistical analysis. Endomysial antibodies (EMA) were categorised in negative, weakly positive and positive. All haematology, chemistry, endocrinal and immunological tests were collected and compared to reference values. Tergooi hospital changed its measuring equipment (besides CD immunology) in some laboratory tests at February 12th 2019. In case reference values had been adjusted during the study period because of a change in test material, interpretation of data was done accordingly.

Statistical analysis

For the statistical analysis, the SPSS software (SPSS, version 26, SPSS Inc., Chicago) was used. Descriptive statistics and 95% confidence interval were calculated for laboratory

results for absolute deviations. Categorical variables between the study groups were analysed using Pearson's chi-square test or Fisher's exact test when the expected frequencies were low. For all comparisons, a p-value of <0.05 was considered significant.

Ethical standards

The study was approved by the Medical Ethics Review Committee of Tergooi hospitals (Reference number 19.59). The study was not subject to the Medical Research Involving Human Subjects Act, because no intervention was performed and only clinical data were collected.

Table 1. Clinical characteristics and anthropometric data.

Characteristic	Guideline adherent (n=13)	Guideline non-adherent (n=149)	P-value
Age (years)	8.0 [3 – 16]	9.0 [2 – 17]	0.40
Gender	6	106	-
Male	7	43	-
Female			
Length (cm)	135.5 [91.8 – 174.4]	139.8 [90.2 – 190.9]	0.30
SD length	-0.86 [-2.19 - 2.09]	-0.27 [-2.96 – 224]	0.44
Weight (kg)	32.3 [16.3 – 74.5]	31.8 [13.0 – 84.2]	0.88
SD weight	0.52 [-2.23 – 2.35]	-0.01 [-2.32 – 2.60]	0.08
BMI (kg/m^2)	17.43 [14.86 – 24.49]	16.42 [13.20 – 24.47]	0.10
SD BMI	0.96 [-1.96 – 2.69]	0.11 [-2.11 – 2.38]	0.01
Years of follow-up	4.0 [1 – 8]	4.0 [1 -16]	0.49
Comorbidities	0	1	-
Alport syndrome	0	5	-
ADHD/ADD	1	3	-
Asthma	0	1	-
Crohn's disease	1	1	-
IgA-deficiency			

Nominal variables expressed in median + range

RESULTS

Study population

We included 91 CD children with 176 follow-up visits (cases) in which additional blood tests were performed. Of these visits, 14 were excluded since some patients were seen multiple times per year. Therefore 162 cases were included for statistical analysis. There were 52 follow-up visits in 2017, 49 in 2018 and 61 in 2019, respectively.

Patient characteristics

The majority of children with CD were female (62/91, 68.1%). Median age at follow-up of our studied population was 9.0 years (range 2.0 – 17.6). Clinical characteristics and anthropometric data is presented in table 1. During the last follow-up visit six children were of toddler age (6.5%), 53 children of pre-school age (57.6%) and 32 children of high-

school age (35.2%). A total of 39 children had one follow-up visit, 28 children had two follow-up visits, 19 children had three follow-up visits, three children had four follow-up visits and two children had five follow-up visits within the studied period of three years (mean 1.9 ± 1.0). Mean follow-up since diagnosis was 4.0 years (range 1-16). Analysis of all individual growth charts did not show growth delay in our studied population.

Guidelines adherence

Overall, paediatricians adhered strictly to the Dutch national guidelines in 8.0% (13/162) of cases (Table 2). In children were paediatricians were non-adherent to the guidelines, haemoglobin/haematocrit 78.5% (117/149), red cell indicators 78.5% (117/149) and TG2A serology 92.6% (138/149) were the most requested laboratory tests, whereas folic acid 6.7% (10/149) and vitamin B12 8.7% (13/149) were the least requested laboratory tests. Screening for thyroid disease was performed in 27.8% (45/162) of total cases.

Laboratory investigations

A total of 1570 laboratory tests (CD serology included) were requested in 162 cases, of which 45.4% (713/1570) was in compliance with the Dutch national guidelines (Table 2). We observed deviations in 12.9% (203/1570) of all requested laboratory tests, of which only 2.5% (40/1570) was considered clinically relevant. Of these clinically relevant deviations in laboratory tests 95.6% (36/40) were deviations in laboratory tests recommended by the Dutch national guidelines. These 40 deviations included low haemoglobin (n=3), decreased levels of serum ferritin (n=4), deviations in thyroid function (n=4), elevations in liver enzymes (elevation <2x ULN (n=4)) and elevations in TG2A (n=25)(table 1). Overall, clinically relevant deviations in laboratory tests were observed in 5.0% (36/713) of laboratory tests recommended by the Dutch national guidelines (CD serology included). Data on laboratory tests and deviations can be found in the supplemental tables (Table S1-S4).

DISCUSSION

This study was undertaken to evaluate adherence to Dutch national guidelines during follow-up of paediatric CD patients and to evaluate the clinical relevance of laboratory tests recommended by the Dutch national guidelines. Strict adherence to the Dutch national guidelines was observed in only 8.0% of paediatric CD patients in a general teaching hospital in the Netherlands. Despite low adherence to the guidelines, 95.6% of observed clinically relevant deviations of laboratory tests were deviations in laboratory tests recommended by the Dutch national guidelines. However, the overall clinical relevance of routine laboratory tests seems limited since we observed clinically relevant deviations in only 5.0% of requested laboratory tests recommended by the Dutch national guidelines.

Our study shows that non-adherence to guidelines during the follow up of paediatric CD led to the execution of unnecessary tests with only 4.4% clinically relevant deviations in laboratory tests. Non-adherence to guidelines amongst paediatricians, despite the presence of well-defined evidence based guidelines, has been shown by previous research in CD, where for instance biopsy guidelines are not strictly followed by paediatric gastroenterologists and in several other paediatric conditions [21–24]. Previous studies provided several reasons why clinicians would deviate from available guidelines of which some are difficult to address: availability of too many guidelines, decreased sense of autonomy, guidelines are not specific enough, oversimplification of medicine, uncertainty regarding the evidence on which guidelines are based and the way clinicians have treated patients throughout their career without problems [25–27]. Furthermore, variation in clinical practice can also be caused by various cultural, psychological and local factors which influences the behavior of clinicians which cannot be incorporated into guidelines. It is known that publication of guidelines do not guarantee the clinical implementation and adherence by clinicians [21,23,24]. Although non-adherence to guidelines may consequently lead to the execution of unnecessary tests leading to false-positive or false-negative results, unnecessary anxiety in children and their parents, and the generation of financial costs, the reverse is also true in case of strict adherence. Whether or not strict guideline adherence during follow-up of paediatric CD leads to a reduction in the execution of unnecessary tests, anxiety, financial costs and improvement of care cannot be concluded based upon this study.

The clinical relevance of the laboratory tests suggested by the Dutch national CD guidelines seems limited since we observed clinically relevant deviations in only 5.0% (36/713) of requested laboratory tests recommended by the Dutch national guidelines. Our results are in line with previous studies conducted in relatively small paediatric CD populations, whereas iron deficiency (5-20.5%), hypocalcaemia (0%), folic acid deficiency (0-1.4%) and vitamin B12 deficiency (0-1%) were reported in only a small proportion of children [14,25]. Notably, normalisation of observed deficiencies occurred in practically all patients after a year of compliance to a GFD without the use of supplements [14]. We observed no growth delay or deviations in length- or weight-length curves, which is an interesting finding since growth delay is a common finding in children with CD [29]. However, growth delay is mostly present during diagnosis and more frequent at particular young age and in children with severe clinical and histological presentation of CD [29].

Table 2. Laboratory investigations requested during follow-up of paediatric CD patients.

	Guideline adherent (n=13)	Deviations	Guideline non-adherent (n=149)	Deviations	Total cases (n=162)	Deviations
Dutch national guidelines						
Haemoblobin/haematocrit	13 (100)	1 (7.7)	117 (78.5)	11 (9.4)	130 (80.2)	12 (9.2)
Red cell indicators	13 (100)	1 (7.7)	117 (78.5)	4 (3.4)	130 (80.2)	5 (3.8)
Calcium	13 (100)	0 (0)	38 (25.5)	0 (0)	51 (35.5)	0 (0)
Alkaline phosphatase	13 (100)	1 (7.7)	22 (14.8)	14 (63.3)	35 (21.6)	15 (42.8)⊥
Folic acid	13 (100)	0 (0)	10 (6.7)	0 (0)	23 (14.2)	0 (0)
Vitamin B12	13 (100)	3 (23.1)	13 (8.7)	0 (0)	26 (16.0)	3 (11.5)
Ferritin	13 (100)	1 (7.7)	38 (25.5)	3 (7.9)	51 (31.5)	4 (7.8)
Iron	13 (100)	5 (41.7)	30 (20.1)	13 (43.3)	42 (25.9)	18 (42.9)
TG2A serology	13 (100)	4 (30.8)	138 (92.6)	21 (15.2)	151 (93.2)	25 (16.6)
TSH	4 (30.8)	0 (0)	41 (27.5)	3 (7.3)	45 (27.8)	3 (6.7)
Free T4	4 (30.8)	0 (0)	25 (16.8)	1 (4.0)	29 (17.8)	1 (3.4)
Outside of guidelines						
ESR	0 (0)	0 (0)	13 (8.7)	1 (7.7)	13 (8.0)	1 (7.7)
Leukocyte count	13 (100)	0 (0)	71 (47.7)	6 (8.5)	84 (51.9)	6 (7.1)
Leukocyte differentiation	13 (100)	0 (0)	59 (39.6)	18 (30.5)	72 (44.4)	18 (25.0)
Reticulocytes	0 (0)	0 (0)	1 (0.7)	0 (0)	1 (0.6)	0 (0)
Trombocyte count	13 (100)	0 (0)	71 (47.7)	1 (1.4)	84 (51.9)	1 (1.2)
CRP	2 (15.4)	0 (0)	13 (8.7)	1 (7.8)	15 (9.3)	1 (6.7)
Sodium	0 (0)	0 (0)	14 (9.4)	1 (7.1)	14 (8.6)	1 (7.1)
Potassium	0 (0)	0 (0)	14 (9.4)	0 (0)	14 (8.6)	0 (0)
Magnesium	2 (15.4)	0 (0)	6 (4.0)	1 (16.7)	8 (4.9)	1 (12.5)
Phosphate	0 (0)	0 (0)	30 (20.1)	4 (13.3)	30 (18.5)	4 (13.3)
Urea	0 (0)	0 (0)	13 (8.7)	1 (7.8)	13 (8.0)	1 (7.7)
Creatinine	0 (0)	0 (0)	17 (11.4)	13 (76.5)	17 (10.5)	13 (76.5)†
ASAT	2 (15.4)	0 (0)	17 (11.4)	4 (23.5)	19 (11.7)	4 (21.1)
ALAT	3 (23.1)	0 (0)	18 (12.1)	0 (0)	21 (13.0)	0 (0)
GGT	0 (0)	0 (0)	12 (8.1)	0 (0)	12 (7.4)	0 (0)
LD	1 (7.7)	0 (0)	7 (4.7)	1 (14.3)	8 (4.9)	1 (12.5)
Glucose	0 (0)	0 (0)	11 (7.4)	0 (0)	11 (6.8)	0 (0)
Albumin	13 (100)	0 (0)	47 (31.5)	4 (8.5)	60 (37.0)	4 (6.7)
Total protein	0 (0)	0 (0)	2 (1.3)	0 (0)	2 (1.2)	0 (0)
Vitamin D	10 (76.9)	0 (0)	30 (20.1)	6 (20.0)	40 (24.7)	6 (15.0)
Transferrin	12 (92.3)	0 (0)	30 (20.1)	1 (3.3)	42 (25.9)	1 (2.4)
Iron saturation	12 (92.3)	4 (33.3)	30 (20.1)	11 (36.7)	42 (25.9)	15 (35.8)
Total IgA	13 (100)	2 (15.4)	142 (95.3)	5 (3.5)	155 (95.7)	7 (4.5)
Total IgG	2	0 (0)	1 (0)	1 (0)	3 (1.9)	0 (0)
Endocrinology						
LH	0 (0)	0 (0)	2 (1.2)	1 (50.0)	2 (1.2)	1 (50.0)
FSH	0 (0)	0 (0)	2 (1.2)	0 (0)	2 (1.2)	0 (0)
Testosteron	0 (0)	0 (0)	2 (1.2)	1 (50.0)	2 (1.2)	1 (50.0)
IGF-1	0 (0)	0 (0)	5 (3.4)	0 (0)	5 (3.1)	0 (0)
Anti-TPO	0 (0)	0 (0)	1 (1.2)	0 (0)	1 (0.6)	0 (0)
Celiac serology						
Endomysial antibodies	5 (38.5)	3 (60.0)	44 (29.5)	24 (54.5)	49 (30.2)	27 (55.1)
DGP-IgG	2 (15.4)	0 (0)	14 (9.4)	2 (14.3	16 (9.9)	2 (12.5)

Values in parentheses represent percentages of vertical column.
*Laboratory values as suggested by the national guidelines. †Deviations in shown creatinine values are deviations based upon reference values for adults. ⊥Most deviations in shown ALP values occurred since ALP values were not corrected for age anymore after change of measuring equipment. Anti-TPO: anti-thyroid peroxidase; ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase; CRP: C-reactive protein; DGP: deamidated gliadin peptide; ESR: erythrocyte sedimentation rate; FSH: follicle stimulating hormone; G-GT: gamma-glutamyl transferase; IGF-1: insulin-like growth factor-1; LD: lactate dehydrogenase; LH: luteinizing hormone; TG2A: anti-tissue transglutaminase type 2 anti-bodies; TSH: thyroid stimulating hormone;

It is remarkable that even in the latest international guidelines the follow-up in paediatric CD patients is not addressed. These guidelines provide no recommendations on how often outpatients should be seen during follow-up and which additional laboratory tests should be performed. Our results suggest that routine control of vitamin B12 and folic acid seems unnecessary since deficiencies seem to occur only rarely in the paediatric population. However, since these parameters were only requested in 16% and 14% of cases, firm recommendations cannot be made. Since iron deficiency anaemia is commonly seen as an adjuvant intestinal symptom in the paediatric CD population we suggest serum ferritin and haemoglobin as routine biochemical measurements during follow-up [1,5,13,26]. Despite hypocalcaemia only seems to occur rarely, it seems rather important to keep this parameter inclined in routine biochemical follow-up since its potential effects on bone metabolism and growth and its importance in prevention of osteoporosis in the paediatric CD population. We would suggest to add vitamin D to the suggested routine biochemical measurements of children with CD, despite that vitamin D deficiency is frequently found in a general paediatric population and supplementation of vitamin D in GFD-adherent children not always leads to normalisation of vitamin D levels [18,30]. However, routine assessment of vitamin D levels and subsequent supplementation of deficiencies can be considered as good clinical practice in children because of its potential beneficial effects on bone metabolism and future prevention of osteoporosis. Furthermore, in GFD-adherent children TG2A serology remained negative over time after becoming negative [10–13]. This could implicate that routine assessment of TG2A serology in GFD-adherent children, and children with accidental gluten intake, may not be necessary after becoming negative [7].

In summary, when future guidelines regarding follow-up of paediatric CD are constructed we advocate that certain elements need to be taken into account: assessment of growth, GFD adherence and laboratory testing for vitamin and mineral deficiencies. The assessment of vitamin and mineral deficiencies during follow-up need to be based upon the presence of deficiencies during diagnosis. Routine assessment of haemoglobin, serum ferritin, calcium (+albumin) and vitamin D seems indicated on annual base. If deficiencies are present at diagnosis routine assessment and supplementation are indicated. Routine assessment of TG2A serology depends on GFD adherence and needs to be performed until normalization of TG2A levels in GFD-adherent children and thereafter should be performed every two years to monitor GFD adherence. In GFD non-adherent children routine assessment of TG2A serology needs to be performed more frequently until normalization, whereas monitoring every three to six months would seem sufficient. Screening of thyroid function needs to be performed on indication.

Our study comes with one main limitation. Our study is a retrospective study and retrospective studies are always dependent on accurate record keeping. However, our study mostly covers requested laboratory parameters which were recorded digitally in electronic patient files therefore no requested laboratory parameters could be missed.

CONCLUSION

Strict adherence to Dutch national guidelines amongst paediatricians during the follow-up of paediatric CD was observed in only 8% of patients. The clinical relevance of recommended laboratory tests by the Dutch national CD guidelines seems limited. In order to prevent unnecessary additional diagnostic tests, we advocate that future (inter) national guidelines also address evidence-based recommendations on biochemical follow-up in paediatric CD.

Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

1. van Kalleveen M, de Meij T, Plötz F. Clinical spectrum of paediatric coeliac disease: a 10-year single-centre experience. Eur J Pediatr. 2018;177(4):593–602.
2. Almallouhi E, King KS, Patel B, Wi C, Juhn YJ, Murray JA, et al. Increasing Incidence and Altered Presentation in a Population-based study of Pediatric Celiac Disease in North-America. J Pediatr Gastroenterol Nutr. 2018;65(4):432–7.
3. Ludvigsson J, Murray J. Epidemiology of Celiac Disease. Gastroenterol Clin NA. 2018;1–18.
4. Lindfors K, Ciacci C, Kurppa K, Lundin KEA, Makharia GK, Mearin ML, et al. Coeliac disease. Nat Rev Dis Prim. 2019;5(1).
5. Roma E, Panayiotou J, Karantana H, Constantinidou C, Siakavellas SI, Krini M, et al. Changing pattern in the clinical presentation of pediatric celiac disease: a 30-year study. Digestion. 2009;80(3):185–91.
6. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin M, Ribes-Koninckx C, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition guidelines for diagnosing coeliac disease 2019. J Pediatr Gastroenterol Nutr. 2019;70(1):141–56.
7. Bascuñán K, Vespa M, Magdalena A. Celiac disease: understanding the gluten-free diet. Eur J Nutr. 2016;56(2):449–59.
8. Husby S, Bai JC. Follow-up of Celiac Disease. Gastroenterol Clin NA. 2019;48(1):127–36.
9. Myléus A, Reilly N, Green P. Rate, Risk Factors, and Outcomes of Nonadherence in Pediatric Patients with Celiac Disease: A Systematic Review. Clin Gastroenterol Hepatol. 2019;18(3):562–73.
10. Czaja-Bulsa G, Bulsa M. Adherence to Gluten-Free Diet in Children with Celiac Disease. Nutrients. 2018;10(10):pii: E1424.
11. Isaac D, Rajani S, Yaskina M, Huyhn H, Turner J. Anti-tissue transglutaminase normalization post diagnosis in children with celiac disease. J Pediatr Gastroenterol Nutr. 2017;65(2):196–9.
12. Gidrewicz D, Trevenen C, Lyon M, Decker Butzner Ã. Normalization Time of Celiac Serology in Children on a Gluten-free Diet. J Pediatr. 2017;64(3):362–7.
13. Hogen Esch C, Wolters V, Gerritsen S, H P, von Blomberg B, van Hoogstraten I, et al. Specific Celiac Disease Antibodies in Children on a Gluten-Free Diet. Pediatrics. 2011;128(3):547–52.
14. Krauthammer A, Guz-mark A, Zevit N, Marderfeld L, Waisbourd-Zinman O, Silbermintz A, et al. Two decades of pediatric celiac disease in a tertiary referral center: What has changed? Dig Liver Dis. 2020;0(0):1–5.
15. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease. J Pediatr Gastroenterol Nutr. 2012;54(1):136–60.
16. Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S, et al. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr. 2005;40(1):1–19.

17. CBO. Richtlijn Coeliakie en Dermatitis Herpetiformis [Internet]. Haarlem: Nederlandse Vereniging van Maag-Darm-Leverartsen; 2008. Available from: https://www.mdl.nl/files/richlijnen/richtlijn_Coeliakie_definitief.pdf

18. Wessels M, van Veen I, Vriezinga S, Putter H, Henri E, Maria H, et al. Complementary Serologic Investigations in Children with Celiac Disease Is Unnecessary during Follow-Up. J Pediatr. 2015;169:1–6.

19. NVKC. Algemeen overzicht referentiewaarden Nederlandse Vereniging voor Klinische Chemie en Laboratoriumgeneeskunde. Available from: https://www.nvkc.nl/algemeen-overzicht-referentiewaarden

20. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011 (WHO/NMH/NHD/MNM/11.1). 2011. Available from: https://www.who.int/vmnis/indicators/haemoglobin.pdf

21. Wallach T, Genta RM, Lebwohl B, Green PHR, Reilly NR. Adherence to Celiac Disease and Eosinophilic Esophagitis Biopsy Guidelines Is Poor in Children. J Pediatr Gastroenterol Nutr. 2017;65(1):64–8.

22. Niele N, Willemars L, van Houten M, Plötz F. National survey on managing minor childhood traumatic head injuries in the Netherlands shows low guideline adherence and large interhospital variations. Acta Paediatr. 2017;107(1):168–9.

23. van Kalleveen M, Noordhuis E, Lasham C, Plötz F. Large variation in clinical practice amongst pediatricians in treating children with recurrent abdominal pain. Pediatr Gastroenterol Hepatol Nutr. 2019;22(3):225–32.

24. van der Weijden BM, Achten NB, Bekhof J, Evers EE, Berk M, Kamps AWA, et al. Multicentre study found that adherence to national antibiotica recommendations for neonatal early-onset sepsis was low. Acta Paediatr. 2021;110(3):791–8.

25. Baron D, Metnitz P, Rhodes A, Kozek-Langenecker S. Clinical guidelines: How can we improve adherence and implementation. Eur J Anaesthesiol. 2017;34(6):329–31.

26. Gagliardi A, Brouwers M, Palda V, Lemieux-Charles L, Grimshaw J. How can we improve guideline use? A conceptual framework of implementability. Implement Sci. 2011;6(1):26.

27. Cook D, Pencille L, Dupras D, Linderbaum J, Pankratz VS, Wilkinson JM. Practice variation and practice guidelines: Attitudes of generalist and specialist physicians, nurse practitioners, and physician assistants. PLoS One. 2018;13(1):1–12.

28. Deora V, Aylward N, Sokoro A, El-matary W. Serum Vitamins and Minerals at Diagnosis and Follow-up in Children With Celiac Disease. J Pediatr Gastroenterol Nutr. 2017;65(2):185–9.

29. Nurminen S, Kivelä L, Taavela J, Huhtala H, Mäki M, Kaukinen K, et al. Factors associated with growth disturbance at celiac disease diagnosis in children: A retrospective cohort study. BMC Gastroenterol. 2015;15(1):1–8.

30. Kreutz JM, Adriaanse MPM, van der Ploeg EMC, Vreugdenhil ACE. Narrative review: Nutrient deficiencies in adults and children with treated and untreated celiac disease. Nutrients. 2020;12(2).

5

Diagnostic and therapeutic considerations towards *Dientamoeba fragilis* in children: a survey amongst general practitioners and pediatricians in the Netherlands

Michael W. van Kalleveen

M. van Bergen

Marc A. Benninga

Paul H.M. Savelkoul

Frans B. Plötz

Tim G.J. de Meij

Journal of Pediatric Gastroenterology, Hepatology and Nutrition 2021;73(6):e121-e125

ABSTRACT

This survey was undertaken to obtain insight in the attitude of Dutch physicians towards pathogenicity, diagnostic- and therapeutic approach towards *Dientamoeba fragilis* (*D. fragilis)* in children. Physicians were invited by e-mail for a questionnaire. A total of 211/450 physicians (46.9%) completed the questionnaire, including 67 general practitioners (GPs) and 144 pediatricians. Of all respondents, 175/211 (82.9%) considered *D. fragilis* a "potential pathogen", when other causes of gastro-intestinal complaints are ruled out. Only 16/211 (7.6%) performed diagnostic tests regularly. Diagnostic tests were performed by 162/211 (77%) of respondents in children with diarrhea and abdominal pain in consideration of duration of symptoms. Fecal polymerase chain reaction (PCR) was diagnostic modality of preference. 89/142 (62.7%) prescribed metronidazole as antibiotic of first choice. This study shows heterogeneity in clinical practice amongst Dutch physicians regarding diagnostic- and therapeutic approach of *D. fragilis* in children. Different attitude towards pathogenicity and inconsistent guidelines could be causative factors.

INTRODUCTION

Dientamoeba fragilis (*D. fragilis*) is a single-celled protozoan parasite, which can inhabit the human bowel and was first described over 100 years ago [1]. *D. fragilis* can be detected in children with abdominal pain and diarrhea, but also in asymptomatic children [2–4]. Some studies even report *D. fragilis* to be more prevalent amongst healthy controls than in children with gastro-intestinal complaints [5–7]. Consequently, the potential pathogenicity of *D. fragilis* is still under debate. During the past decades replacement of light microscopy by highly sensitive real-time polymerase chain reaction (RT-PCR) techniques for the diagnosis of *D. fragilis* led to an worldwide increase in detection rate, with incidence rates varying between 0.3% - 52% depending on the studied cohort and used diagnostic modality [3,8]. Treatment guidelines of *D. fragilis* mainly consist of a single set of antibiotics with paromomycin having the best eradication rates followed by clioquinol and metronidazole [8–10].

The debatable pathogenicity and increased incidence of *D. fragilis* may challenge physicians responsible for the care of children how to manage *D. fragilis*. Therefore the aim of this survey was to obtain insight in clinical practice amongst physicians responsible for the care of children, hence general practitioners (GPs) and pediatricians, regarding pathogenicity, diagnostic approach and therapeutic strategies of *D. fragilis* in children in the Netherlands.

METHODS

Participants

Physicians eligible for inclusion were Dutch GPs and pediatricians. Both residents and interns were invited for participation and were required to be currently practicing in the Netherlands. Respondents who provided incomplete questionnaires were excluded from the study.

The scientific review committee of the Amsterdam University Medical Center reviewed and approved the application for this study and concluded that it was not subject to the Medical Research Involving Human Subjects Act (WMO, METc#: 2020.0640).

Procedure and materials

Participating GPs were randomly selected through a list of Dutch GPs in the surrounding area of Amsterdam, provided by the department of family medicine of the Amsterdam UMC. Participants were contacted and invited for participation by e-mail. Questionnaires were send by e-mail containing an internet link to the online questionnaire. Participating pediatricians were selected by contacting departments of Pediatrics of hospitals in the surrounding area of Amsterdam but also three hospitals outside this region in order to obtain a comparable, more rural, insight in clinical practice: Amsterdam UMC

(location VUmc and AMC), OLVG (location east and west), Spaarne Gasthuis Haarlem, Amstelland Hospital Amstelveen, Noordwest Hospital (Alkmaar and Den Helder) Dijklander Hospital (Hoorn and Purmerend), St. Anthonius Hospital Nieuwegein, Rode Kruis Hospital Beverwijk. Gelre Hospital (Zutphen and Apeldoorn), Rijnstate Hospital Arnhem and Isala Hospital Zwolle. Included hospitals were both secondary and tertiary care centers. After contacting the secretaries of these departments, they administered the questionnaires towards participating clinicians through e-mail.

Questionnaire

The questionnaire was developed using the Survalyzer software (Survalyzer BV) for online questionnaires. Before entering the questionnaire, participants received information on the study by e-mail, including aim of the study, procedure, possible (dis-)advantages and a privacy statement (Supplementary data 1). Completion took approximately 2-10 minutes. All questions included an open text box in case the multiple-choice options were not conclusive. Open text answers were categorized and encoded into subgroups. A full version of the questionnaire is provided in the supplementary data (supplementary data 2). Briefly, the questionnaire consisted of three parts with a total of 25 questions. The first part provided questions regarding demographic data of the participants. The second part provided questions regarding pathogenicity and diagnostic considerations regarding *D. fragilis* in children. The third part provided questions regarding treatment and follow-up. Finally, an open text box was provided for responders to note questions or provide comments on the questionnaire.

Encoding data and statistics

All data were imported from Survalyzer (Survalyzer BV) into SPSS (SPSS version 26.0, SPSS Inc., Chicago). Data was analyzed by means of descriptive statistics. Results are shown in percentages of the total amount of the responders per question since the amount of provided questions by the questionnaire varied per care giver based upon the answers. In case multiple answers on a question were possible, responses are presented in percentages of the amount of care givers per given option. Categorical variables between groups were studied by means of a chi-square test. For small groups the Fisher's exact test was used. For all comparisons, an p-value of <0.05 was considered significant.

RESULTS

Demographic data of participants

A total of 211 out of 450 invited physicians (46.9%) completed the questionnaire. Of the 211 included respondents 67/211 (31%) were GPs and 144/211 (69%) pediatricians (Table 1). Of the 144 pediatricians, 95/144 (66.4%) were general pediatricians, 12/144 (8.4%) were pediatric gastroenterologists and 32/144 (22.4%) were fellows in pediatrics.

All respondents

A total of 175/211 (82.9%) respondents considered *D. fragilis* to be a potential pathogen, but only 16/211 (7.6%) performed diagnostic tests regularly (Table 1). Of all respondents, 125/162 (77%) performed diagnostic tests for *D. fragilis* when diarrhea and/or abdominal pain were presenting symptoms and 140/162 (86.4%) of the respondents handled in consideration of duration of these symptoms, whereas the majority of respondents (102/140, 72.9%) performed diagnostic tests <2 months after onset of symptoms (Table 1). PCR was the test of preference for 63/162 (38.9%). When a positive test result was found, 78/162 (48.1%) ruled out first other causes when initiating treatment. Some responded to take the Cq-value of the PCR test into account when performing treatment (open text answers). When performing treatment, 89/142 (62.7%) prescribed metronidazole as preferred antibiotic, followed by clioquinol (28.2%)(Table 2). A vast majority of respondents 96/130 (73.8%) do not control effect of eradication of *D. fragilis*, but when follow-up is performed, 26/35 (74.2%) of respondents, perform follow-up within 6 weeks after treatment (Table 2).

Differences between pediatricians and pediatric gastroenterologists

Pediatric gastroenterologists consider *D. fragilis* more often "strictly commensal" than pediatricians (25% vs. 19%). Diagnostic tests are performed by 7/12 (58.3%) of pediatric gastroenterologists, but pediatricians perform diagnostic tests earlier (54% vs. 43% <2 months). Pediatric gastroenterologists prescribe clioquinol (37.5% vs. 33.3%) and metronidazole (50% vs. 61.9%) in a similar amount as pediatricians. Control of eradication is performed more frequent by pediatric gastroenterologists than by pediatricians (62.5% vs. 28.4%).

Differences between GP's and pediatricians

Pediatricians consider *D. fragilis* more often "strictly commensal" than GPs, 19% vs. 7%, p=0.03. GPs responded to perform diagnostic tests for *D. fragilis* more often than pediatricians (combined response "sometimes/regularly/rarely" 76.2% vs. 55.4%, p=<0.01)(Table 1). When diagnostic tests are performed, GPs perform diagnostic tests earlier than pediatricians (88.2% vs. 54% <2 months, p=0.04). When performing treatment pediatricians prescribe clioquinol more often as preferred antibiotic than GPs (33.3% vs. 20.7%, p=0.10). Control of eradication of *D. fragilis* is not performed frequently but GPs perform control of eradication earlier than pediatricians (83.4% vs. 66.7% <6 weeks, p=0.37)(Table 2).

Table 1. Pathogenicity and diagnostic considerations.

	General practitioners (n=67)	Pediatricians (n=144)	p-value	Total (n=211)
Pathogenicity *"I consider D. fragilis:"*	***Respondents (n=67)***	***Respondents (n=144)***		***Respondents (n=211)***
Strictly pathogenic	1 (1.5)	0 (0.0)	N.S.	1 (0.5)
Mostly commensal, but able to cause symptoms	59 (88.0)	116 (80.6)	0.18	175 (82.9)
Strictly commensal	5 (7.5)	28 (19.4)	0.03	33 (15.6)
No opinion	2 (3.0)	0 (0.0)	N.S.	2 (1.0)
Diagnostic considerations	***Respondents (n=67)***	***Respondents (n=144)***		***Respondents (n=211)***
"Do you perform diagnostics for D. fragilis?"				
Regularly	4 (6.0)	12 (8.3)	0.56	16 (7.6)
Sometimes	42 (62.7)	66 (45.7)	0.03	108 (51.2)
Rarely	5 (7.5)	2 (1.4)	0.02	7 (3.3)
Never	8 (11.9)	40 (27.9)	0.01	48 (22.7)
Not specific, unrequested result	8 (11.9)	24 (16.7)	0.37	32 (15.2)
"Presenting symptoms when performing diagnostics?" (multiple responses possible)	***Respondents (n=58)***	***Respondents (n=104)***		***Respondents (n=162)***
Diarrhea	51 (87.9)	74 (71.2)	0.03	125 (77.2)
Abdominal pain	42 (72.4)	82 (78.8)	0.24	126 (77.8)
Bloating	15 (25.9)	27 (26.0)	0.99	42 (25.9)
Weight loss	8 (13.8)	16 (15.4)	0.79	24 (14.8)
Bloody stools	6 (10.3)	22 (21.2)	0.08	28 (17.3)
Anorexia	5 (8.6)	12 (11.5)	0.56	17 (10.5)
Flatulence	5 (8.6)	13 (12.5)	0.45	18 (11.1)
Nausea	4 (6.9)	16 (15.4)	0.12	20 (12.3)
Vomiting	0 (0.0)	8 (7.7)	0.03	8 (4.9)
Other	9 (15.5)	26 (25.0)	0.26	35 (21.6)
"Duration of symptoms in consideration when testing for D. fragilis?"	***Respondents (n=58)***	***Respondents (n=104)***	0.01	***Respondents (n=162)***
Yes	56 (96.5)	84 (80.8)	-	140 (86.4)
No	2 (3.5)	20 (19.2)	-	22 (13.6)
"Duration of symptoms when you perform tests for D. fragilis?"	***Respondents (n=56)***	***Respondents (n=84)***		***Respondents (n=140)***
<2 week	0 (0.0)	1 (1.2)	N.S.	1 (0.7)
2-4 weeks	16 (28.6)	13 (15.5)	0.06	29 (20.7)
1-2 months	30 (53.6)	42 (50.0)	0.68	72 (51.4)
3-4 months	4 (7.1)	21 (25.0)	0.01	25 (17.9)
>5 months	4 (7.1)	4 (4.8)	0.55	8 (5.7)
Other: variable by patient	2 (3.6)	3 (3.5)	N.S.	5 (3.6)
"What kind of test do you perform?"	***Respondents (n=58)***	***Respondents (n=104)***		***Respondents (n=162)***
Fecal PCR	18 (31.0)	45 (43.3)	0.13	63 (38.9)
DFT	7 (12.1)	33 (31.7)	0.01	40 (24.7)
TFT	13 (22.4)	21 (20.2)	0.74	34 (21.0)
Other: decided by laboratory	20 (34.5)	5 (4.8)	<0.01	25 (15.4)

Numbers in parentheses are percentages of vertical column; N.S. means not significant; DFT: dual-feces-test; PCR: polymerase chain reaction; TFT: triple-feces-test;

Table 2. Therapeutic considerations.

	GPs (n=67)	Pediatricians (n=144)	p-value	Total (n=211)
"Do you perform treatment for a positive D. fragilis test?"	***Respondents (n=58)***	***Respondents (n=104)***		***Respondents (n=162)***
Yes	3 (5.2)	12 (11.5)	0.11	15 (9.3)
Yes, but only when other causes are ruled out	29 (50.0)	49 (47.1)	0.62	78 (48.1)
No, I perform expectative management	9 (15.5)	23 (22.1)	0.15	32 (19.8)
Other: incidental or shared decision making	17 (29.3)	20 (19.2)	0.14	37 (22.8)
"Do you perform treatment when D. fragilis is a unrequested result"	***Respondents (n=7)***	***Respondents (n=24)***		***Respondents (n=31)***
Yes, but only when other causes are ruled out	2 (28.6)	5 (20.8)	0.67	7 (22.6)
No, I perform expectative management	2 (28.6)	14 (58.4)	0.17	16 (51.6)
Other: incidental or shared decision making	3 (42.8)	5 (20.8)	0.24	8 (25.8)
"First choice of antibiotics?"	***Respondents (n=58)***	***Respondents (n=84)***		***Respondents (n=142)***
Metronidazole	37 (63.8)	52 (61.9)	0.82	89 (62.7)
Paromomycin	0 (0.0)	1 (1.2)	-	1 (0.7)
Clioquinol	12 (20.7)	28 (33.3)	0.10	40 (28.2)
Doxycycline	0 (0.0)	1 (1.2)	-	1 (0.7)
Other: Probiotics, diet, advice microbiologist	9 (15.5)	2 (2.4)	<0.01	11 (7.7)
"Do you perform control of eradication?"	***Respondents (n=49)***	***Respondents (n=81)***		***Respondents (n=130)***
Yes	4 (8.2)	19 (23.5)	0.03	23 (17.7)
Yes, but only if symptoms persist	3 (6.1)	4 (4.9)	0.77	7 (5.4)
No, I do not perform control of eradication	38 (77.5)	58 (71.6)	0.45	96 (73.8)
Inconclusive	4 (8.2)	0 (0.0)	-	4 (3.1)
"When do you perform control of eradication?"	***Respondents (n=12)***	***Respondents (n=23)***	-	***Respondents (n=35)***
<2 weeks after treatment	0 (0.0)	4 (17.4)	0.06	4 (11.4)
<4 weeks after treatment	7 (58.4)	6 (26.1)	0.94	13 (37.2)
<6 weeks after treatment	3 (25.0)	6 (26.1)	0.47	9 (25.7)
>6 weeks after treatment	1 (8.3)	4 (17.4)	0.68	5 (14.3)
Inconclusive	1 (8.3)	3 (13.0)		4 (11.4)
"What would you do when eradication is not achieved?"	***Respondents (n=12)***	***Respondents (n=23)***		***Respondents (n=35)***
I prescribe a second (different) course of antibiotics	3 (25.0)	10 (43.5)	0.28	13 (37.1)
I perform expectative management	1 (8.3)	2 (8.7)	0.97	3 (8.6)
Other: Shared decision making or dependent on symptoms	7 (58.4)	10 (43.5)	0.40	17 (48.6)
Other: probiotics/diet	1 (8.3)	1 (4.3)	0.63	2 (5.7)

Numbers in parentheses are percentages of vertical column;

DISCUSSION

This survey amongst Dutch GPs and pediatricians showed that 83.4% of participants consider *D. fragilis* a potential pathogen when other causes of gastrointestinal complaints are ruled out. Diarrhea and abdominal pain are presenting symptoms when physicians perform diagnostic tests the most, mainly in consideration of duration of symptoms. PCR is the diagnostic modality of preference and when treatment is performed, metronidazole is the antibiotic of preference for both GP's and pediatricians. Follow-up after treatment or control of eradication of *D. fragilis* is not routinely performed by the majority of physicians.

The majority of respondents considered *D. fragilis* to be a potential pathogen in children when other causes of gastrointestinal complaints are ruled out. An interesting finding since strong evidence regarding pathogenicity of *D. fragilis* is lacking [11–13]. In several reports over the past decades, it has been described that, at least in a subgroup of patients, treatment of *D. fragilis* resulted in clinical improvement of symptoms [9,10]. Furthermore, two of three Koch´s postulates were fulfilled in rodent models and even different subtypes of *D. fragilis* with unique virulent factors could be identified [14–17]. However, there is also some evidence that supports *D. fragilis* to be non-pathogenic. A recent case-control study found no alterations in gut microbiota in children with symptomatic and asymptomatic *D. fragilis* as cause for the gastrointestinal symptoms. Another case-control study found less gastrointestinal symptoms in *D. fragilis* positive controls than in *D. fragilis* negative cases [12,13].

GPs more often perform diagnostic tests for *D. fragilis* than pediatricians. This could possibly be explained by the differences in attitude regarding potential pathogenicity and by different present guidelines for GPs and pediatricians regarding diagnosis and treatment of *D. fragilis* [8,18,19]. Guidelines for GPs suggest to test for *D. fragilis* if abdominal pain and/or diarrhea is present ≥10 days and persistent and suggest treatment with metronidazole, whereas guidelines for pediatricians advice not to test and not to treat *D. fragilis* [24,25]. Diagnostic tests for *D. fragilis* were most commonly performed in children with diarrhea and abdominal pain, however, symptoms as abdominal pain and/or diarrhea are frequently present in the pediatric population which makes it challenging for clinicians to select children who may benefit from diagnostic work-up and targeted therapy [9]. PCR is the diagnostic modality of preference for both GPs and pediatricians for diagnosing *D. fragilis*. However, the use of only PCR, without performing light microscopy, comes with certain challenges and clinicians should be aware that a combination of the two is recommended to ensure the reliability of the test [8,9].

The majority of clinicians only performs treatment for *D. fragilis* when all other somatic causes are excluded, which is in line with current GP and microbiology guidelines [8,18].

Interestingly, two thirds of both GPs and pediatricians prescribe metronidazole as first-line therapy. A remarkable finding since the only conducted randomized controlled trial regarding treatment of *D. fragilis* showed no beneficial effect of metronidazole over placebo in relief of clinical symptoms [20]. Furthermore, clioquinol and paromomycin appear to have superior eradication rates and resolution of clinical symptoms compared to metronidazole [9,21]. Treatment of *D. fragilis* should therefore be performed with clioquinol or paromomycin [8]. Follow-up is not performed by the majority of clinicians, which could be explained by the fact that follow-up is not addressed in most guidelines. However, international microbiology guidelines recommend to perform control of eradication of *D. fragilis* 3 to 4 weeks after treatment, particularly in case of persisting symptoms [8,9].

Strength of this study is that we were able to study a representative population of Dutch GPs and pediatricians in both urban and rural parts of the Netherlands and that a response rate of almost 50% was obtained. This study also has some limitations. First, a substantial number of participants responded that they receive a positive test result for *D. fragilis* as unrequested test result when testing for other infectious causes. This could have led to an overestimation of performed diagnostic tests and treatment approach. Secondly, we did not use a validated questionnaire since these were not available for this topic. However, we did provide open text boxes for respondents to provide alternative answers when the provide answers were not sufficient.

CONCLUSION

Our study shows heterogeneity in clinical practice amongst Dutch GPs and pediatricians regarding pathogenicity, diagnostic approach and therapeutic management of *D. fragilis* in the pediatric population. Different attitude towards potential pathogenicity between GPs and pediatricians and different available guidelines for both groups could be causative factors. Future studies regarding potential pathogenicity of *D. fragilis* and randomized controlled trials in children with well-defined clinical symptoms, appropriate diagnostic tests, follow-up and comparison of different treatment regiments with placebo are warranted. Future microbiological and clinical guidelines should state similar information to enhance unambiguity in clinical practice.

RECOMMENDATIONS

Based upon previously published literature we endorse the following recommendations on the testing and management of *D. fragilis* in the pediatric population [8–10]. Testing for *D. fragilis* should be reserved for children with (sub)acute gastrointestinal symptoms in whom other causes are excluded [8,9]. Diagnostic modality of preference is a fecal PCR, if possible combined with light microscopy [8,9]. Initiating treatment should be performed by means of shared decision making whereas parents should be informed

about the debated pathogenicity of the parasite and potential failure of treatment. Treatment should be performed by a single course of paromomycin or clioquinol [8–10,21]. Control of eradication should be performed 3-4 weeks post-treatment. If *D. fragilis* persists, consider testing all members of the same household.

REFERENCES

1. Jepps MW, Dobell C. Dientamoeba fragilis n.g., n.sp., new intestinal amoeba from man. Parasitology. 1918;10:352–67.
2. Barratt JLN, Harkness J, Marriott D, Ellis JT, Stark D. A review of Dientamoeba fragilis carriage in humans: Several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. Gut Microbes. 2011;2(1):3–12.
3. Stark D, Barratt J, Chan D, Ellis T. Dientamoeba fragilis , the Neglected Trichomonad of the Human Bowel. Clin Microbiol Rev. 2016;29(3):553–80.
4. Stark D, Barratt J, Roberts T, Marriott D, Harkness J, Ellis J. A review of the clinical presentation of dientamoebiasis. Am J Trop Med Hyg. 2010;82(4):614–9.
5. De Wit MAS, Koopmans MPG, Kortbeek LM, Van Leeuwen NJ, Bartelds AIM, Van Duynhoven YTHP. Gastroenteritis in sentinel general practices, the Netherlands. Emerg Infect Dis. 2001;7(1):82–91.
6. De Jong MJ, Korterink JJ, Benninga MA, Hilbink M, Widdershoven J, Deckers-Kocken JM. Dientamoeba fragilis and chronic abdominal pain in children: A case-control study. Arch Dis Child Educ Pract Ed. 2014;99(12):1109–13.
7. de Boer MD, Schuurs TA, Vermeer M, Ruijs GJHM, van der Zanden AGM, Weel JF, et al. Distribution and relevance of Dientamoeba fragilis and Blastocystis species in gastroenteritis: results from a case-control study. Eur J Clin Microbiol Infect Dis. 2020 Jan 1;39(1):197–203.
8. Van Gestel RSFE, Kusters JG, Monkelbaan JF. A clinical guideline on Dientamoeba fragilis infections. Parasitology. 2018;146(9):1131–9.
9. van Kalleveen M, van Gool T, Klarenbeek N, Benninga M, Savelkoul P, de Meij T, et al. Dientamoeba fragilis in children: a systematic review on diagnostic considerations and efficacy of treatment. Expert Rev Gastroenterol Hepatol. 2020;14(4):231–42.
10. Nagata N, Marriott D, Harkness J, Ellis JT, Stark D. Current treatment options for Dientamoeba fragilis infections. International Journal for Parasitology: Drugs and Drug Resistance. 2012.
11. Wong ZW, Faulder K, Robinson JL. Does Dientamoeba fragilis cause diarrhea? A systematic review. Parasitol Res. 2018;117(4):971–80.
12. van Kalleveen MW, Budding AE, Benninga MA, Savelkoul PHM, van Gool T, van Maldeghem I, et al. Intestinal Microbiota in Children With Symptomatic Dientamoeba fragilis Infection: A Case-control Study. Pediatr Infect Dis J. 2021 Apr;40(4):279–83.
13. Dullaert-de Boer M, Schuurs T, Vermeer M, Ruijs G, van der Zanden A. Distribution and relevance of Dientamoeba fragilis and Blastocystis species in gastroenteritis: results from a case-control study. Eur J Clin Microbiol Infect Dis. 2020;39(1):197–203.
14. Munasinghe V, Vella N, Ellis J, Windsor P, Stark D. Cyst formation and faecal – oral transmission of Dientamoeba fragilis – the missing link in the life cycle of an emerging pathogen. Int J Parasitol. 2013;43(11):879–83.
15. El-gayar E, Mokhtar A, Hassan W. Study of the pathogenic potential of Dientamoeba fragilis in experimentally infected mice. Parasite Epidemiol Control. 2016;1:136–43.
16. Cacciò S. Acta Tropica Molecular epidemiology of Dientamoeba fragilis. Acta Trop. 2018;184:73–7.

17. Johnson J, Clark C. Cryptic Genetic Diversity in Dientamoeba fragilis. J Clin Microbiol. 2000;38:4653–4.

18. Belo J, Bos M, Brühl P, Lemmen W, Pijpers M, van den Donk M, et al. NHG-Standaard Acute diarree (derde herziening). Huisarts Wet. 2014;57(9):462–71.

19. Rutten JMTM, Korterink JJ, Venmans LMAJ, Benninga MA, Tabbers MM. Guideline on functional abdominal pain in children. Ned Tijdschr Geneeskd. 2017;161:D781.

20. Röser D, Simonsen J, Stensvold CR un., Olsen KEP, Bytzer P, Nielsen H V., et al. Metronidazole therapy for treating dientamoebiasis in children is not associated with better clinical outcomes: a randomized, double-blinded and placebo-controlled clinical trial. Clin Infect Dis. 2014;58(12):1692–9.

21. Burgaña A, Abellana R, Yordanov S, Kazan R, Pérez A, Castillo C, et al. Paromomycin is superior to metronidazole in Dientamoeba fragilis treatment. IJP Drugs Drug Resist. 2019;11:95–100.

6

Intestinal Microbiota in Children With Symptomatic *Dientamoeba fragilis* Infection: A Case-Control Study

Michael W. van Kalleveen
Andries E. Budding
Marc A. Benninga
Paul H.M. Savelkoul
Tom van Gool
Iris van Maldeghem
Wendelien Dorigo-Zetsma
Aldert Bart
Frans B. Plötz
Tim G.J. de Meij

The Pediatric Infectious Diseases Journal *2021;40(4):279-283*

ABSTRACT

Background: *Dientamoeba fragilis* in children has been associated with gastrointestinal symptoms, like abdominal pain and diarrhea. The mechanism underlying these symptoms in children with *D. fragilis* remains unclear. We hypothesized that concomitant microbial alterations, which have been described in other parasitic infections, may be associated with gastrointestonal symptoms in *D. fragilis*.

Methods: In this case-control study performed in two centers, 19 children referred to a pediatrician because of gastrointestinal symptoms and with a positive fecal PCR for *D. fragilis* were included as cases. We included 19 healthy children as controls, and matched for age and gender, selected from an existing cohort of 63 children. A PCR for *D. fragilis* was performed on fecal samples of the 19 controls to assess *D. fragilis* carriership in this asymptomatic group. Microbiota was analyzed with the IS-pro technique and the intestinal microbiota composition and diversity was compared between the two groups.

Results: Microbiota of children with *D. fragilis* and gastrointestinal symptoms did not significantly differ in terms of composition and diversity compared to controls, both on phylum and species level. In the asymptomatic controls, a positive fecal PCR for *D. fragilis* was found in 16 of 19 (84.2%).

Conclusion: Intestinal microbiota do not seem to play a key role in the presence of clinical symptoms in children with *D. fragilis.* The pathogenicity of *D. fragilis* and pathophsiological pathways underlying development of gastrointestinal symptoms remains yet to be clarified.

INTRODUCTION

Dientamoeba fragilis is a flagellate anaerobic parasite inhabiting the human gastrointestinal tract. *D. fragilis* can be diagnosed using light microscopy on fresh or permanently stained stool samples, or by real time polymerase chain reaction (PCR) techniques on the stool [1]. Worldwide prevalence rates of *D. fragilis* vary between 0.3% and 62%, depending on the studied cohort and used diagnostic modality [1]. Reported rates may have increased over the past years due to the routine introduction of real-time PCR techniques, which is characterized by a higher sensitivity compared to previous methods [1,2]. There is still a lack of consensus on the potential pathogenicity of this parasite, despite its discovery around 100 years ago [3]. Data derived from large case series suggest that *D. fragilis* is associated with clinical symptoms, whereas other studies and the single performed randomized controlled trial in children do not support this concept of pathogenicity [1,4–10].

The clinical presentation of children with *D. fragilis* varies from asymptomatic carriership to a wide spectrum of gastrointestinal complaints, of which abdominal pain and diarrhea are the most frequently reported symptoms [1,5]. It remains unknown why some patients harboring *D. fragilis* manifest clinical symptoms while others are asymptomatic. A possible, yet unexplored, hypothesis for development of clinical symptoms in a selection of children with *D. fragilis* might be the presence of microbial alterations (or dysbiosis) related to the presence of *D. fragilis.* Intestinal dysbiosis is commonly defined as a change in composition of resident commensal bacterial communities relative to bacterial communities present in healthy subjects. The supposed relation between dysbiosis and development of gastrointestinal symptoms has been related to exposure of the intestinal mucosa to a variety of factors such as bacterial products, bacterial endotoxins, ammonia, indoles, phenols and hydrogen sulphide, which all have substantial effects on mucosal and intestinal health [11]. The presence of these toxic metabolites is dependent on types of fermentation in the bowel, which on its turn is dependent on the composition of the intestinal microbiota, as well as on the substrates for fermentation [11]. The relationship between microbial dysbiosis and gastrointestinal symptoms has been described in several parasitic infections, such as *Blastocystis hominis, Entamoeba histolyica and Giardia lamblia,* and also in irritable bowel syndrome (IBS) [12–15]. So far, however, no studies have described the microbiota in children with *D. fragilis* and searched for a relation between dysbiosis and symptoms in this population.

This study was undertaken to explore the hypothesis that gastrointestinal symptoms in children with *D. fragilis* infection are associated with bacterial dysbiosis. Aim of this study was to compare the microbiota of children with *D. fragilis* presenting with gastrointestinal symptoms with an asymptomatic control population and to search for a microbial signature associated with symptoms. Detection of such a microbial signature could possibly lead to the development of novel microbiota-based therapeutic strategies in the treatment of symptomatic *D. fragilis* subjects.

MATERIAL AND METHODS

Study design

This was a case-control study, conducted between January 2014 and June 2016, in an academic hospital (Amsterdam UMC, location VUmc) and a teaching hospital (Tergooi hospital, Blaricum) in the Netherlands. The study population was divided into two subgroups; (1) children positive for *D. fragilis* presenting with gastrointestinal complaints and (2) a matched pediatric control population without gastrointestinal complaints. Approval for the study was obtained from the Scientific Review Committee of both hospitals.

Participants

All children aged ≤ 18 years referred to the department of pediatrics of one of the participating centers with gastrointestinal symptoms lasting longer than two weeks and clinically suspected of having a parasitic gastrointestinal illness, based on the judgement of the treating pediatrician, and with a positive fecal PCR for *D. fragilis* were eligible to participate. The applied fecal PCR test for *D. fragilis* has been described previously and is standardized and validated for application in clinical practice [16]. Exclusion criteria included an underlying diagnosis of a chronic gastrointestinal disease, like celiac disease, functional constipation and inflammatory bowel disease (IBD), a culture-proven infectious gastroenteritis in the last 6 months prior to inclusion, history of surgery of the gastrointestinal tract (except appendectomy), use of antibiotics, immune modulating agents or probiotics within six months prior to inclusion and a co-infection with another parasite than *D. fragilis*. Written informed consent for participation in this study was given by the parents and by the child when older than 12 years of age.

Pediatric controls were selected from a cohort consisting of 63 healthy children aged between three and eighteen years [17]. None of the control children had gastrointestinal symptoms as reported by a detailed questionnaire. Fecal samples of these children were collected during the same study period (January 2014 – June 2016) and in the same geographical region, using a similar protocol for sampling, collection, storage and microbial analysis of the fecal samples [17]. Controls were 1:1 matched based on age and gender with the symptomatic *D. fragilis* cases. When more than one suitable control was available, the closest match (based on age) was selected. We applied the same exclusion criteria for the symptomatic and healthy pediatric control population. A real-time PCR for detection of *D. fragilis* was applied on the fecal samples of these controls to determine the prevalence of *D. fragilis* in this subgroup [18].

Procedure

Every participant was instructed to collect a fecal sample (see following paragraph fecal sampling for further details) and to complete a short questionnaire, including items regarding standard demographics, health status, use of antibiotics and other medication

and present (gastrointestinal) symptoms. Data on applied detection test for *D. fragilis* was collected from the patients file.

Fecal sampling

Sterile plastic containers and an information letter were provided to parents and children, with instructions on procedure of collection and storage of the fecal samples. Fecal samples were collected in this sterile plastic container at home before initiation of therapy and stored within one hour after defecation at -20°C before further handling. The same fecal sample was used for diagnosing *D. fragilis* as for microbiota profiling. The intestinal microbiota was analyzed on fecal samples of both groups by IS-pro, a PCR-based microbiota profiling technique [19].

Microbiota analysis by IS-pro

Fecal samples were analyzed by the standard IS-pro procedure as described earlier [17,19]. Isolated Bacterial DNA was amplified in a standardized multiplex PCR-amplification with the IS-pro assay (InBiome BV, Amsterdam, The Netherlands) as provided by the manufacturer. Briefly, IS-pro differentiates bacterial species by the length of the 16S-23S rDNA IS region with taxonomic classification by phylum-specific fluorescently labelled PCR primers. Two labelled forward primers and three universal unlabelled reverse primers are used for this amplification. The labelled primers are specific for the phyla *Firmicutes*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia* (FAFV) and *Bacteriodetes* respectively. For the *Proteobacteria* a separate PCR-amplification was performed with a labelled forward primer and unlabelled reverse primers. Amplified DNA fragments were separated for analysis on an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, Carlsbad, California, USA). In order to control for potential contamination, all negative control samples were taken along with each DNA isolation run. Negative control samples were taken through the entire identical IS-pro process as patient samples.

Data analysis

IS-Pro

Preprocessing of microbial data was performed by the IS-Pro proprietary software suite (InBiome BV, Amsterdam, The Netherlands), which led to distinctive microbial profiles. Different types of information were obtained: the main phylae present in the human gastrointestinal tract (*Bacteroidetes*, *FAFV*, and *Proteobacteria*) were automatically sorted by color of peaks; Species were identified by the length of the 16S-23S rDNA IS region, displayed by number of nucleotides; And relative quantity of the PCR product (ie peak height), which is measured in relative fluorescence units. Specific peaks in the microbial profile (and its corresponding intensity as its abundance) were considered an operational taxonomic unit. Peak determination (ie species determination) was done by matching sample profiles to a known database (consisting of more than 1500 species)

of known bacterial species and their corresponding IS-lengths. Sampling effect on IS profiles was assessed in previous analyses, in which a correlation was found of 96% for *Bacteroidetes* and 90% for *FAFV* for samples of the same excrement [17].

Diversity

Diversity analysis was performed on all IS-pro data. The Shannon diversity index was calculated to define microbial diversity based on the resulting profiles by conventional statistics. Diversity was calculated for total microbial composition (by pooling all three main phylae *Bacteroidetes*, *FAFV* and *Proteobacteria*) and per phylum. The R 2.15.2 software package was used to perform diversity analysis. Spotfire software package (Tibco, Palo Alto, CA, USA) was used for fitted curves and data visualization.

Statistical analysis

For the statistical analysis, the SPSS (SPSS version 25.0, SPSS Inc., Chicago) software was used. Categorical variables between groups were studied by means of a chi-squared test. Normally distributed continuous variables between two groups were studied by means of an independent samples t-test. For all comparisons, an α value of < 0.05 was considered significant.

RESULTS

Participants

A total of 19 children with gastrointestinal symptoms with a positive fecal PCR for *D. fragilis* were included. Fecal samples were collected between 14-42 days following the onset of gastrointestinal symptoms and while these symptoms were still present. The mean age was 7.8 ± 3.8 years and 10 were male (52.6%)(Table 1). The most common presenting gastrointestinal symptoms of the children with *D. fragilis* were abdominal pain (100%) and diarrhea (78.9%).

We matched 19 controls to these 19 patients with positive *D. fragilis* based on age and gender. The mean age of the healthy control population was 7.2 ± 4.0 years (Table 1). We performed a fecal PCR for *D. fragilis* on the fecal samples of these asymptomatic children to assess *D. fragilis* carrierschip and found a positive result in 16 children (84.2%)

Table 1. Demographic data of the studied population.

Characteristics	Symptomatic patients	Healthy controls
N	19	19
Gender (%) Male Female	10 (52.6) 9 (47.4)	10 (52.6) 9 (47.4)
Age	7.8 ± 3.8	7.2 ± 4.0
D. fragilis positive PCR	19 (100)	16 (84.2)
Presenting symptom (%)		
Abdominal pain	19 (100)	-
Diarrhea	15 (78.9)	-
Nausea	6 (31.6)	-
Vomiting	5 (26.3)	-
Fatigue	3 (15.8)	-
Failure to thrive	2 (10.5)	-
Bloating	1 (5.3)	-
Constipation	1 (5.3)	-
Irritablility	1 (5.3)	-

Nominal variables expressed in means ± SD

Microbiota analysis

The most abundant species in the IS-profiles of both children with *D. fragilis* and the controls were observed within the phylum *Bacteroidetes*, presented by the species *Alistipes finegoldii* (231 and 396 nt length position), *Alistipes putrenidis* (235 nt) and *Bacteroides vulgatus* (479 nt). A clustered heatmap did not reveal specific clustering between the two subgroups, neither at phylum level nor at species level (figure 1). The most dominant species within the *FAFV* phyla was *Faecalibacterium prausnitzii* (318 nt) and in both groups *Sutterella Wadsworthensis* (663 nt) was the most common species in the *Proteobacteria* phylum. Principle coordinate analysis (PCoA) of microbial profiles of both groups revealed no segregation between children with *D. fragilis* and symptoms and asymptomatic controls, neither for all phyla together, nor per phylum. Shannon diversity indices were not statistically different between the two studied groups for all phylae together (Cases: 3.15 ± 0.27; IQR: 3.01 – 3.25; Controls: 3.10 ± 0.38; IQR: 2.94 - 3.30, p=0.85) nor per phylum: *Bacteroidetes* (Cases: 2.48 ± 0.25; IQR: 2.37 – 2.61; Controls: 2.46 ± 0.34; IQR: 2.45 - 2.59; p= 0.77), *FAFV* (Cases: 1.80 ± 0.53; IQR: 1.55 – 2.19; Controls: 1.77 ± 0.49; IQR: 1.39 – 2.15; p= 0.89), *Proteobacteria* (Cases: 1.87 ± 0.56; IQR: 1.77 – 2.19; Controls: 1.71 ± 0.62; IQR: 1.39 – 2.10; p= 0.41)(figure 2).

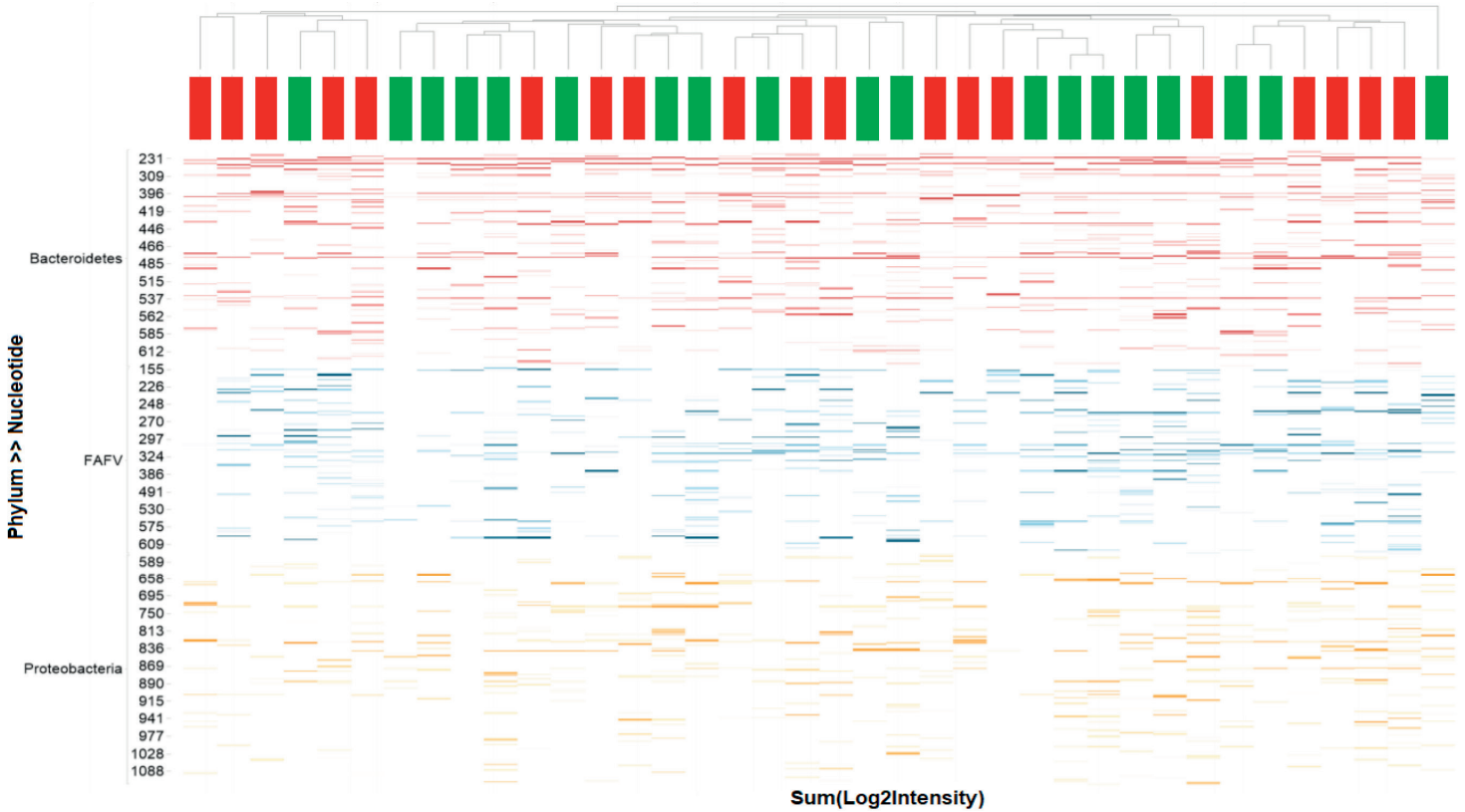


Figure 1. Clustered heatmap displaying IS-profiles of childrenden with *D. fragilis* and gastrointestinal symptoms.
Clustered heatmap displaying IS-profiles of 19 children with *D. fragilis* and gastrointestinal symptoms (red) and 19 asymptomatic controls (green). Individual subjects are shown on the X-axis; On the Y-axis, IS-fragment lenghts are expressed (in number of nucleotides), corresponding with bacterial strain type (OTU). Brown peaks represent *Bacteroidetes*, blue peaks represent *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia* (FAFV), yellow peaks represent *Proteobacteria*. Intensity of colors reflect relative dominance of each indicted bacterial strain, white signals represent less prevalent IS-fragment lengths. No clustering between the two groups was observed, indicating that the groups could not be distinguished based on IS-profiles using this unsupervised method. The most abudant OTUs in both study groups were observed within the phylum *Bacteroidetes*, corresponding to *Alistipes finegoldii* (231 and 396 nt length position), *Alistipes putrenidis* (235 nt) and *Bacteroides vulgatus* (479 nt)

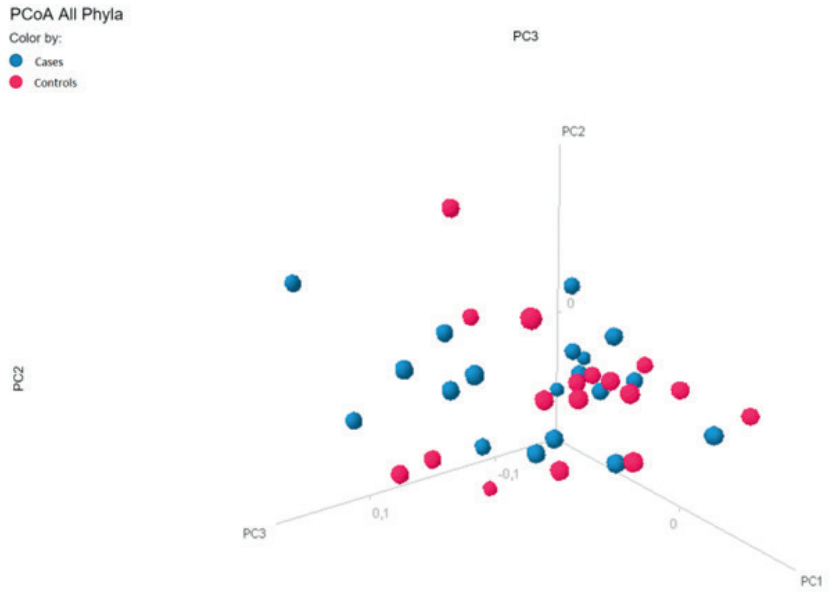


Figure 2. Principle coordinate analysis of microbial profiles of children with D. fragilis and gastrointestinal symptoms and asymptomatic controls.
Principle coordinate analysis scatterplot displaying overall bacterial community composition, showing no separate clustering of microbial profiles of children with D. fragilis and gastrointestinal symptoms (blue) and asymptomatic controls (red).

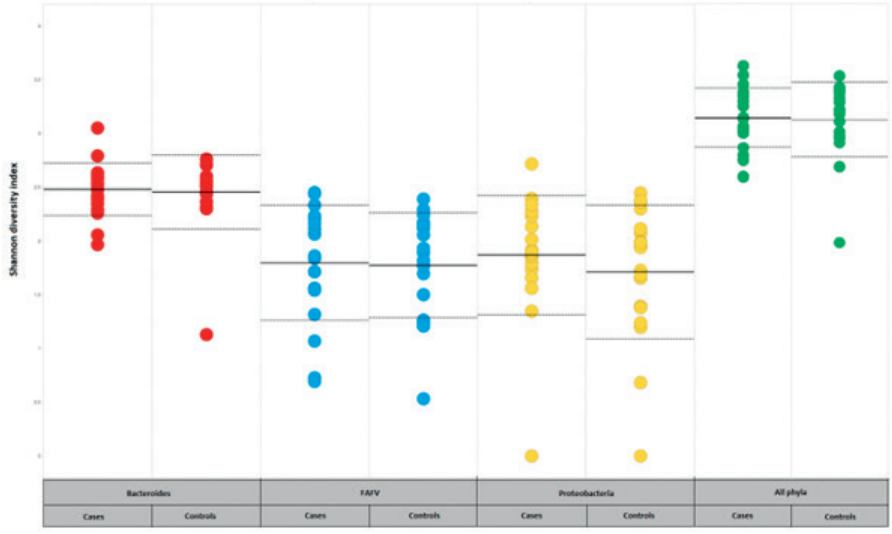


Figure 3. Diversity indices of children with D. fragilis and gastrointestinal symptoms and asymptomatic controls.
Shannon diversity index of 19 children with D. fragilis and gastrointestinal symptoms (cases) and 19 asymptomatic controls. Showing similar indices on phylum level for both groups. Red: Bacteroidetes. Blue: *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia* (FAFV), Yellow: *Proteobacteria*. Green: All phyla.

DISCUSSION

This case-control study was undertaken to explore the hypothesis that gastrointestinal symptoms in children with *D. fragilis* are associated with dysbiosis. We did not observe statistically significant microbial differences between children with *D. fragilis* and gastrointestinal symptoms and an asymptomatic control population in terms of composition and diversity.

We observed a high prevalence of *D. fragilis* carriership in our matched, asymptomatic control population of 84.2%. Such a high prevalence of *D. fragilis* in an asymptomatic pediatric population could be interpreted as illustration of the non-pathogenic character of this parasite in the majority of cases. The stool of these patients should preferebaly be examined by microscopy for proof of true replication of *D. fragilis* by detection of actively dividing trophozoites, however there was no sufficient fecal material left for this additional microscopic analysis. The prevalence of *D. fragilis* in our control population could therefore be an overestimation of the real prevalence in this cohort. Using real-time PCR, prevalence rates of *D. fragilis* of up to 50% in asymptomatic Dutch children, were described by previous studies [8,16]. Furthermore, in other western European countries (Germany and Denmark) corresponding prevalence rates were found [7,20]. A future study could be conducted using real-time PCR combined with microscopic examination of permanently stained slides of stools (in which actively dividing parasites (trophozoites) can be demonstrated.

Mounting evidence suggests that certain intestinal parasites are linked to maintain intestinal homeostasis [21]. One of the suggested mechanisms for microbial alterations in symptomatic *Blastocystis spp.* infections is the production of polyketide synthase and two non-ribosomal peptide synthases by *Blastocystis spp.* which are known to produce antibiotic effects and therefore influence intestinal microbiota composition [22,23]. For instance, an increased relative abundance of *Bacteroides spp.* was found in patients with *Blastocystis spp.* positive individuals compared to controls, although the study design did not allow conclusions whether this dysbiosis was associated with the presences of clinical symptoms [12–14]. Our study suggests that alterations in intestinal microbiota are not asscociated with development of gastrointestinal symptoms in children with a positive *D. fragilis* fecal PCR. Other suggested mechanisms for symptomatic disease in *Blastocystis spp.* are increased intestinal permeability due to tight junction proteins degradation and apoptosis and upregulation of pro-inflammatory cytokines [23–26]. Whether this is also valid for symptomatic *D. fragilis* infections remains to be elucidated.

One could argue that the negative results found in our study are due to selection bias by only including children referred to a pediatrician because of gastrointestinal complaints. These symptoms might hypothetically have been of functional origin, with the presence of *D. fragilis* as an innocent bystander. Treatment and follow-up

in the cases was not performed in a standardized matter. Therefore, retrospective assessment of the effect of prescribed antibiotics on symptoms and eradication of *D. fragilis* would not have realiably clarified whether symptoms were indeed caused by *D. fragilis* and was therefore not performed. Although some studies reported a weak association between *D. fragilis* and IBS, a systematic review on the role of *D. fragilis* in IBS showed no association (OR: 1.13, 95%CI: 0.22-5.72)[12,27–29]. Notably, some studies even reported a higher prevalence of *D. fragilis* in control patients (18%) than in IBS patients (11%) [27,30,31].

The choice of microbiota analytical techniques may also influence outcome. Differences in applied techniques and protocols may lead to variation in outcome that even outweighs biological differences, illustrating the need for standardization and to use similar techniques and protocols when comparing microbial data of different populations [32]. In this study, we used of IS-pro as microbiota profiling technique instead of more regular 16S rRNA based sequencing techniques. We selected IS-pro mainly for two reasons: the microbiota of children selected from the control population originally were analyzed by IS-pro [17]. In that study, 454-pyrosequencing was also applied for validation, showing comparable outcomes with IS-pro. Furthermore, an advantage of IS-pro over most sequencing techniques is its capacity to generate results within hours following sampling, illustrating its potential to be applied in daily clinical practice.

Strength of this study is that we were able to study a representative population for several reasons. First, we have studied patients with a positive *D. fragilis* test and with a combination of gastrointestinal symptoms. A previous study conducted in children showed that the majority of children with symptomatic *D. fragilis* also reported the presence of a combination of gastrointestinal symptoms rather than a single complaint [16]. Second, other causes for gastrointestinal symptoms in cases, like celiac disease, IBD, or other infectious disorders, were excluded. Third, the inclusion of patients with gastrointestinal symptoms lasting longer than two weeks further reduces the possibility of short lasting other infectious causes (ie viral infections that were not tested for in our fecal samples). Our study comes with several limitations. First, our studied population was relatively small, any subtle microbial differences, possibly related to specific clinical symptoms could therefore have been missed. Second, our study had a retrospective design and symptoms and response to treatment were therefore not reported in a standardized way. Third, since the majority of asymptomatic controls turned out to be carrier of *D. fragilis*, any effect of *D. fragilis* on microbiota composition, irrespective of the presence of clinical symptoms, could not be assessed. Fourth, the results found in our study only excluded compositional differences of the intestinal microbiota between the subgroups. Functional differences of the intestinal microbiota, by metabolomics and metagenomics, have not been studied and should be considered

in future studies. The results of this study suggest that dysbiosis is not the primary pathofysiological mechanism underlying gastrointestinal symptoms in children with *D. fragilis.* Our study results support the necessity of future randomized studies to elucidate the pathogenicity of *D. fragilis* and to unravel the underlying mechanisms leading to clinical symptoms.

CONCLUSION

The intestinal microbiota of children with *D. fragilis* presenting with gastrointestinal symptoms and asymptomatic children did not differ in composition and diversity, suggesting that the intestinal microbiota does not play a key role in the presence of gastrointestinal symptoms in children with *D. fragilis*. The pathogenicity of *D. fragilis* and pathophsiological pathways underlying development of gastrointestinal symptoms remains yet to be clarified.

Conflict of interest

Andries E. Budding (AEB) is CEO of InBiome and has the proprietary rights on the IS-pro platform technology. Marc A. Benninga (MAB) is a scientific consultant for Shire, Sucampo, Astrazeneca, Norgine, Zeria, Coloplast, Danone, Friesland Campina, Sensus and Novalac. Paul H.M. Savelkoul (PHS) is scientific advisor of InBiome and has to proprietary rights ont the IS-pro platform technology. The other authors have no relevant affliations or financial involvement with any organisation or entitiy with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Acknowledgements

The authors thank Malieka van der Lugt-Degen and Linda Poort, research technicians, for their support in the laboratory and Anat Eck-Hauer for helping construct the analytical models.

REFERENCES

1. Stark D, Barratt J, Chan D, Ellis T. Dientamoeba fragilis , the Neglected Trichomonad of the Human Bowel. Clin Microbiol Rev. 2016;29(3):553–80.
2. Rijsman LH, Monkelbaan JF, Kusters JG. Clinical consequences of polymerase chain reaction-based diagnosis of intestinal parasitic infections. J Gastroenterol Hepatol. 2016;31(11):1808–15.
3. Wong ZW, Faulder K, Robinson JL. Does Dientamoeba fragilis cause diarrhea? A systematic review. Parasitol Res. 2018;117(4):971–80.
4. Barratt JLN, Harkness J, Marriott D, Ellis JT, Stark D. A review of Dientamoeba fragilis carriage in humans: Several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. Gut Microbes. 2011;2(1):3–12.
5. Garcia L. Dientamoeba fragilis, One of the Neglected Intestinal Protozoa. J Clin Microbiol. 2016;54:2243–50.
6. Brands M, van de Vijver E, Haisma S, Heida A, van Rheenen P. No association between abdominal pain and Dientamoeba in Dutch and Belgian children. Arch Dis Child. 2019;104:1–4.
7. Jokelainen P, Hebbelstrup Jensen B, Andreassen B, Petersen A, Röser D, Krogfelt K, et al. Dientamoeba fragilis, a Commensal in Children in Danish Day Care Centers. J Clin Microbiol. 2017;55(6):1707–13.
8. De Jong MJ, Korterink JJ, Benninga MA, Hilbink M, Widdershoven J, Deckers-Kocken JM. Dientamoeba fragilis and chronic abdominal pain in children: A case-control study. Arch Dis Child Educ Pract Ed. 2014;99(12):1109–13.
9. Röser D, Simonsen J, Stensvold CR un., Olsen KEP, Bytzer P, Nielsen H V., et al. Metronidazole therapy for treating dientamoebiasis in children is not associated with better clinical outcomes: a randomized, double-blinded and placebo-controlled clinical trial. Clin Infect Dis. 2014;58(12):1692–9.
10. van Kalleveen M, van Gool T, Klarenbeek N, Benninga M, Savelkoul P, de Meij T, et al. Dientamoeba fragilis in children: a systematic review on diagnostic considerations and efficacy of treatment. Expert Rev Gastroenterol Hepatol. 2020;14(4):231–42.
11. Hawrelak J, Myers S. The Cause of Intestinal Dysbiosis: A Review. Altern Med Rev. 2004;99(22):180–97.
12. Nourrisson C, Scanzi J, Pereira B, Nkoudmongo C, Wawrzyniak I, Dapoigny M, et al. Blastocystis Is Associated with Decrease of Fecal Microbiota Protective Bacteria: Comparative Analysis between Patients with Irritable Bowel Syndrome and Control Subjects. PLoS One. 2014;9:e111868.
13. O'brien-Andersen L, Karim AB, Roager HM, Vigsnæs LK, Krogfelt KA. Associations between common intestinal parasites and bacteria in humans as revealed by qPCR. Eur J Clin Microbiol Infect Dis. 2016;35:1427–31.
14. Iebba V, Santangelo F, Totino V, Pantanella F, Monsia A, Di Cristanziano V, et al. Gut microbiota related to Giardia duodenalis, Entamoeba spp. and Blastocystis hominis infections in humans from Côte d' Ivoire. J Infect Dev Ctries. 2016;10:1035–41.

15. Pittayanon R, Lau J, Yuan Y, Leontiadis G, Tse F, Surette M, et al. Gut Microbiota in Patients With Irritable Bowel Syndrome: A Systematic Review. Gastroenterology. 2019;157(1):97–108.

16. Maas L, Dorigo-Zetsma JW, de Groot CJ, Bouter S, Plötz FB, Van Ewijk BE. Detection of intestinal protozoa in paediatric patients with gastrointestinal symptoms by multiplex real-time PCR. Clin Microbiol Infect. 2014;20(6):545–50.

17. de Meij T, Budding A, de Groot E, Jansen F, Kneepkens C, Benninga M, et al. Composition and stability of intestinal microbiota of healthy children within a Dutch population. FASEB J. 2015;30(4):1512–22.

18. van Hattem J, Arcilla M, Grobusch M, Bart A, Bootsma M, van Genderen P, et al. Travel-related acquisition of diarrhoeagenic bacteria, enteral viruses and parasites in a prospective cohort of 98 Dutch travellers. Travel Med Infect Dis. 2017;Sep(19):33–6.

19. Budding A, Grasman M, Lin F, Bogaards J, van Bodegraven A, Savelkoul P. IS-pro: high-throughput molecular fingerprinting of the intestinal microbiota. FASEB J. 2010;24(11):4556–64.

20. Preiß U, Ockert G, Brömme S, Otto A. Dientamoeba fragilis infection, a cause of gastrointestinal symptoms in childhood. Klin Pädiatrie. 1990;202(02):120–3.

21. Stensvold C, van der Giezen M. Associations between Gut Microbiota and Common Luminal Intestinal Parasites. Trends Parasitol. 2018;34(5):369–77.

22. Denoeud F, Roussel M, Noel B, Wawrzyniak I, da Silva C, Diogon M, et al. Genome sequence of the stramenopile Blastocystis, a human anaerobic parasite. Genome Biol. 2011;12:29.

23. Poirier P, Wawrzyniak I, Vivarès C, Delbac F, el Alaoui H. New Insights into Blastocystis spp .: A Potential Link with Irritable Bowel Syndrome. PLoS Pathog. 2012;8(3):1–4.

24. Puthia M, Sio S, Lu J, Tan K. Blastocystis ratti Induces Contact-Independent Apoptosis , F-Actin Rearrangement , and Barrier Function Disruption in IEC-6 Cells. Infect Immun. 2006;74(7):4114–23.

25. Mirza H, Wu Z, Teo J, Tan K. Statin pleiotropy prevents rho kinase-mediated intestinal epithelial barrier compromise induced by Blastocystis cysteine proteases. Cell Microbiol. 2012;14(9):1474–84.

26. Puthia M, Lu J, Tan K. Blastocystis ratti Contains Cysteine Proteases That Mediate Interleukin-8 Response from Human Intestinal Epithelial Cells in an Nf-kB-dependent manner. Eukaryot Cell. 2008;7(3):435–43.

27. Rostami A, Riahi S, Haghighi A, Saber V. The role of Blastocystis sp . and Dientamoeba fragilis in irritable bowel syndrome : a systematic review and meta-analysis. Parasitol Res. 2017;(116):2361–71.

28. Yakoob J, Jafri W, Beg M. Blastocystis hominis and Dientamoeba fragilis in patients fulfilling irritable bowel syndrome criteria. Parasitol Res. 2010;(107):679–84.

29. Borody T, Warren E, Wettstein A. Eradication of Dientamoeba fragilis can resolve IBS-like symptoms. J Gastroenterol Hepatol. 2002;17:A103.

30. Krogsgaard L, Engsbro A, Stensvold C, Nielsen H, Bytzer P. The Prevalence of Intestinal Parasites Is Not Greater Among Individuals With Irritable Bowel Syndrome : a Population-based case control study. Clin Gastroenterol Hepatol. 2014;13(3):507–13.

31. Jimenez-gonzalez D, Martinez-flores W, Romero-valdovinos M, Stark D, Souza-saldivar V. Blastocystis infection is associated with irritable bowel syndrome in a Mexican patient population. Parasitol Res. 2012;110:1269–75.

32. Lozupone CA, Stombaugh J, Gonzalez A, Ackermann G, Wendel D, Vázquez-Baeza Y, et al. Meta-analyses of studies of the human microbiota. Genome Res. 2013;23(10):1704–14.

7

Dientamoeba fragilis in children: A systematic review on diagnostic considerations and efficacy of treatment

Michael W. van Kalleveen, MD
Tom van Gool, MD, PhD
Nikki Klarenbeek, MD
Marc A. Benninga MD, PhD
Paul H.M. Savelkoul, PhD
Tim de Meij MD, PhD
Frans B. Plötz, MD, PhD

Expert Review of Gastroenterology & Hepatology *2020;14(4):231-242*

ABSTRACT

Introduction: The presence of *D. fragilis* in feces is characterized by asymptomatic carrier ship to a spectrum of gastrointestinal symptoms. However, a causal relationship remains to be elucidated. In this systematic review we aimed to evaluate the relationship between eradication of *D. fragilis* and symptoms to establish the strength of evidence that *D. fragilis* in symptomatic children warrants antibiotic treatment.

Areas covered: This systematic review covers a challenge in daily clinical practice. Is it necessary to test for *D. fragilis* in children with gastrointestinal symptoms and does a positive fecal PCR test warrant treatment?

Expert opinion: Testing for *D. fragilis* seems justified in a selection of children with persistent unexplained chronic abdominal pain and diarrhea. Treatment of *D. fragilis* should be withhold until other causes like celiac disease have been excluded. Both microscopic and Real Time-PCR methods (or a combination of the two) can be used for diagnosis. Paromomycin or clioquinol are antibiotics of choice based on their small spectrum of activity, fewer side effects and better eradication rates than metronidazole. Future randomized studies, with strict inclusion criteria, appropriate diagnostic testing and doses of antibiotics based on bodyweight are warranted.

INTRODUCTION

Dientamoeba fragilis is a flagellate anaerobic parasite that inhabits the human gastrointestinal tract. The first description of *D. fragilis* was already 100 years ago by Jepps and Dobell, but there is still a lack of consensus on the potential pathogenicity of this protozoa [1,2]. The protozoan appears to be particularly prevalent amongst children, yet it appears that the view on its pathogenicity in adults is more widespread accepted than in children [3]. However, a large series of scientific reports, from the time of its discovery until now, has provided support *D. fragilis* to be a potential pathogen [4–6]. The clinical presentation of *D. fragilis* varies, from asymptomatic carriership to a wide spectrum of gastrointestinal complaints, of which the most frequently reported symptoms are abdominal pain, altered bowel movements and diarrhea [4,6–14]. These symptoms, however, are very common in the pediatric population. For example, the prevalence of chronic abdominal pain varies between 0.3 to 19% and has a significant impact on clinical practice with 2-4% of all general pediatric visits [15–17]. Despite 90% of these gastrointestinal symptoms have a functional origin, most clinicians are tempted to perform an extensive diagnostic work-up, to exclude somatic disorders, including microscopic and molecular diagnosis of intestinal parasites, including *D. fragilis*, regardless of guidelines [18,19]. As such, *D. fragilis* is the most commonly detected protozoa in the stools of children [5].

Diagnosis of *D. fragilis* in stools can be performed by using light microscopy (LM) after permanent staining of fixed stool samples, culture, or molecular techniques [20]. Until recently, microscopy was the most often used diagnostic tool worldwide for the diagnosis of *D. fragilis*. However, more recently, molecular diagnosis with real time polymerase chain reaction (RT-PCR) was introduced for the diagnosis of *D. fragilis* [20]. This method proved to have a strongly increased sensitivity as compared to microscopy with a sensitivity of 100% [4,21]. Worldwide prevalence rates of *D. fragilis* vary between 0.3% and 52%, depending on the study cohort and used diagnostic modality [4,20]. The reported prevalence rates may have increased due to the routine introduction of the more sensitive RT-PCR techniques [4]. Despite its technical superiority, the increased use of fecal RT-PCR diagnostics comes with certain challenges that clinicians should be aware of [22].

The purpose of the current systematic review was to identify, critically appraise, and synthesize evidence from studies including children with gastrointestinal complaints treated for *D. fragilis* with antibiotics. We aimed to evaluate the relationship between eradication of *D. fragilis* and clinical symptoms and to establish the strength of evidence that *D. fragilis* in symptomatic children warrants antibiotic treatment.

METHODS

We used a PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) review protocol for data collection, analysis and reporting (eAppendix 1 in Supplement, contains full methodological details).

Study eligibility criteria

All studies including children (0-18 years) with gastrointestinal complaints treated for a *D. fragilis* infection with antibiotics were included. We pre-specified eligibility criteria as followed: any study design with original data, comparing one antibiotic treatment to another, or compared to no medical treatment, and reporting the gastrointestinal complaints and eradication of *D. fragilis* as an outcome. We excluded studies when participants were older than 18 years old or certain publication types as case reports, posters or guidelines.

Information sources and search strategy

The Cochrane Library and Medline databases were searched for systematic reviews and randomized controlled trials (RCTs) from inception to May 2019. We searched in all search fields for 'Dientamoebiasis'. In title/abstract fields we used 'dientamoebiasis', combined with 'child' or 'infant' or 'adolescent', and 'treatment, 'metronidazole' or 'clioquinol' or 'paromomycin' or 'secnidazole'. Exact search engine strings are detailed in the review protocol (eAppendix 1 in Supplement**)**. No exclusion criteria were applied. We examined reference lists of included studies and relevant reviews to identify additional eligible studies. We also reviewed all titles and abstracts of all papers citing *D. fragilis* infection in children identified through Google Scholar and/or Scopus/Web of Science search engines. All citations were combined and duplicates were manually excluded.

Study Selection and Data Extraction

Search results were independently screened by two reviewers (Michael van Kalleveen (M.K.) and Nikki Klarenbeek (N.K.)) who assessed each potentially eligible full-text paper according to predetermined inclusion criteria. In case of disagreement, a third researcher (Tim de Meij (T.M.)) had the decisive vote. Two authors (M.K., N.K.) extracted relevant data from papers as well as any available supplements. Two other authors (M.K., Frans Plötz (F.P.)) verified data-extraction for completeness and accuracy. The following general data were extracted; author, year and country; study design, populations and inclusion criteria. We extracted data on gastrointestinal complaints, *D. fragilis* eradication, diagnostic tests to detect *D. fragilis*, type and duration of antibiotic therapy, and duration of follow-up. If multiple papers reported data from the same source study, results were combined to avoid overlap among results. For studies eligible for meta-analysis, we retrieved supplementary data from original authors if exact data was not present in the original publication.

Assessment of Methodological Quality

We graded the quality of evidence of each finding based on the criteria established by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) working group [23–29]. The quality of the study methodology was independently classified by the two investigators as high, intermediate, low, or very low, based on study design, risk of bias, inconsistency, indirectness, imprecision, publication bias, large effect, dose response and residual confounding.

Synthesis of Results and Analysis

Our primary outcome was to investigate the effect of antibiotics on resolving gastrointestinal complaints. Our secondary outcome was efficacy of antibiotic treatment for *D. fragilis*. We also analyzed if eradication of *D. fragilis* was associated with a disappearance of the gastrointestinal symptoms.

Descriptive statistics were performed on all included studies. Data on study characteristics, interventions, outcomes, and important covariates were summarized using frequency and percentage for dichotomous outcomes, and means and standard deviation or median and inter-quartile range for continuous outcomes. For binary outcomes relative risk and number needed to treat with 95% confidence interval were used as an effect measure. For continuous outcomes, we used mean difference or standardized mean difference (if units differ). Statistical significance were determined at a level $\alpha \leq 0.05$. All analysis were performed using the R statistical software.

RESULTS

Characteristics and participants of included studies

After reviewing 102 identified publications for study eligibility, we carefully selected and evaluated 69 full-text articles (Figure 1). Eleven studies were included (Table 1) [13,14,30–38]. The included studies involved a total of 945 children. We found one randomized double blind placebo controlled trial, three prospective observational cohort studies, five retrospective cohort studies, and two case-control studies. *D. fragilis* was detected by LM in the older reported studies, whereas more recent studies used RT-PCR techniques. We observed a large variety in applied therapeutic strategies, including six different antibiotic therapies, different dosages, and different duration of therapy. Follow-up between the studies varied from unknown to eight weeks.

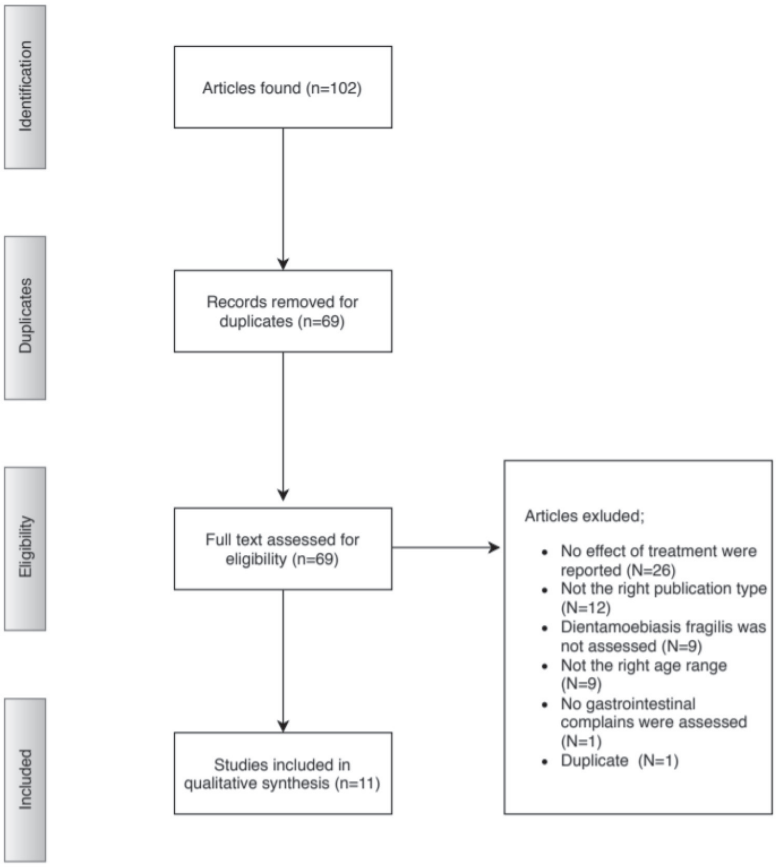


Figure 1. Flowchart of the selection procedure.

Risk of Bias and Quality of Evidence

The overall risk of bias was judged as high for eight studies and low for three studies (Table 2). We graded the overall quality of evidence for the primary outcome of reduction of gastrointestinal complaints as low, due to inclusion of small number of observational studies that had small effect sizes. We graded the quality of evidence regarding eradication of *D. fragilis* as very low, mainly due to small number of events across all studies.

Table 1. Quality assessment of included studies.

Study	Sort	Initial quality	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Large effect	Dose response	Residual confounding	QoE
Röser et al [37]	RCT	High	No	No	Yes (-1)	Yes (-1)	No	No	No	No	Low
Vandenberg et al [32]	PCS	Low	No	No	Yes (-1)	No	No	No	No	No	Very low
Gijsbers et al [33]	PCS	Low	Yes (-1)	No	No	No	No	No	No	No	Very low
Maas et al [34]	PCS	Low	Yes (-1)	No	No	No	No	No	No	No	Very low
Spencer et al [35]	RCS	Low	Yes (-1)	No	No	No	No	No	No	No	Very low
Preiss et al [14]	RCS	Low	Yes (-1)	No	No	No	No	No	No	No	Very low
Cuffari et al [13]	RCS	Low	Yes (-1)	No	No	No	No	No	No	No	Very low
Bosman et al [36]	RCS	Low	Yes (-1)	No	No	No	No	No	No	No	Very low
Ter Schure et al [30]	RCS	Low	No	No	No	No	No	No	No	No	Low
Banik et al [38]	RC-CS	Low	Yes (-1)	No	No	No	No	No	No	No	Very low
de Jong et al [31]	RC-CS	Low	Yes (-1)	No	No	No	No	No	No	No	Very low

QoE = Quality of evidence; PCS = Prospective cohort study; RC-CS = Retrospective case-control study; RCT = Randomized controlled trial

Gastrointestinal symptoms in the studied population

The children included in our reviewed studies had a wide variety of gastrointestinal symptoms (Table 2 and 3). The used definitions of gastrointestinal symptoms and the used inclusion criteria varied widely between studies. Therefore, the studied population consisted of a heterogeneous group of children. Many individuals with *D. fragilis* were asymptomatic carriers, but when symptomatic, the most common reported symptoms included abdominal pain (frequently lasting longer than two weeks), and diarrhea. Less frequently reported symptoms included nausea, vomiting, anal itching, constipation and anorexia. The duration of these gastrointestinal symptoms in our studied population was commonly insufficiently reported and varied between two weeks and at least three months.

Antibiotic therapy and gastrointestinal complaints

All eleven included studies evaluated the effect of antibiotic treatment on gastrointestinal complaints with eradication of *D fragilis* as main outcome. The gastrointestinal symptoms per study are presented in Table 3. We found that the only double blind, placebo controlled randomized trial in children reported no significant differences in visual analogue scale scores for abdominal pain before and after treatment (Table 1)[37]. The three prospective observational cohort studies reported a reduction in complaints varying from 36,8% to 86,7%, for the five retrospective cohort studies this varied between 57,1% and 90%, respectively [13,14,30,32–36]. Finally, the two case-control studies, in which the controls had no *D. fragilis* and no symptoms, reported resolution of gastrointestinal symptoms in 50,6% and 85,0% of cases [31,38]. Resolution of symptoms following metronidazole occurred in 142 of 230 patients (62.7%), for clioquinol in 89 of 141 patients (63.1%) and for paromomycin in 27 of 53 patients (50.9%).

Antibiotic therapy and eradication *D. fragilis*

A total of eight studies examined the effect of antibiotic treatment on *D. fragilis* eradication rate (Table 1). In all these studies side effects of treatment was not an outcome measure and therefore not reported. Duration of antibiotic treatment in the included studies varied between 3 to 10 days. Most patients (32.6%) were treated with metronidazole monotherapy. Also, the dosage of the prescribed antibiotics varied between the different studies (Table 1). These studies found an eradication of *D. fragilis* in 43.9-100% of patients for all types of antibiotic treatment. Efficacy of metronidazole varied from 62.5% (fourteen days post-treatment) in the only double blinded, placebo controlled randomized controlled trial to 100% in a small retrospective cohort study including four patients [30,31,34,36–38]. The average eradication rate for metronidazole was 69%. Eradication rates for paromomycin in two small prospective cohort studies varied from 80.0-86.8% [32,33]. The average eradication rate for paromomycin was 84.9%. For clioquinol eradication rates varied from 46.7% in a medium sized n=112 retrospective cohort study to 81% in a small sized retrospective cohort study [30,31,36]. The average eradication rate for clioquinol was 64.6%. In a subgroup of 2 patients in a retrospective cohort study an eradication rate of 100% for Iodoquinol was reported [13]. No eradication rates were reported for the total of 24 patients treated with (Oxy)tetracycline, doxycycline, erythromycin or hydroxyquinoline [14].

Table 2. Included studies.

Author	Study period	Study population	N	D	Treatment	Results primary outcome (n/N)	Results secondary outcome (n/N)	Follow-up
Randomized controlled trials								
Röser et al [37]	2011-2013	3-12 years Chronic GI-complaints >4 weeks	96	PCR	Metronidazole 3dd 40mg/kg/d 10d vs. Placebo	Abdominal pain VAS: -1.76 (CI: -2.47, -1.05) vs. Abdominal pain VAS: -1.57 (CI: -2.28, -0.85)	Eradication 1m: 30/44 (68.2%) 2m: 18/41 (43.9%) vs. 1m: 5/44 (11.4%) 2m: 8/42 (19%)	8 weeks
Prospective cohort studies								
Vandenberg et al [32]	unknown	1-15 years Suspected GI-infection caused by parasites	15	TFT	Paromomycin 25-35mg 3dd 7d	Clinical reduction: 13/15 (86.7%)	Eradication: 12/15 (80%)	4 weeks
Gijsbers et al [33]	2002-2004	4-16 years Children with chronic abdominal pain during at least 3 months	38	TFT	Paromomycin 25-35mg/kg/d 7d	Clinical reduction 14/38 (36.8%)	Eradication: 33/38 (86.8%)	6 months
Maas et al [34]	2010-2011	0-18 years 144 cases vs. 44 controls Any presentation of GI-symptoms >2 weeks or suspected parasite infection	148	PCR	Clioquinol 15mg/kg/d 10d or Metronidazole 30mg/kg/d 10d or Paromomycin (no dose specification)	***Cases (positive parasite PCR)*** Abdominal pain VAS: -1.1 (no separate outcome measures) ***Controls (negative parasite PCR)*** Abdominal pain VAS: -0.9 (no separate outcome measures)	***Cases (positive parasite PCR)*** Significant reduction in abdominal pain, nausea, chronic diarrhea, anal itching, vomiting, acute diarrhea ***Controls (negative parasite PCR)*** Significant reduction in abdominal pain, nausea, altered defecation pattern, chronic diarrhea	6 weeks

Table 2. Continued.

Author	Study period	Study population	N	D	Treatment	Results primary outcome (n/N)	Results secondary outcome (n/N)	Follow-up
					Retrospective cohort studies			
Spencer et al [35]	1976-1978	1-12 years Acute and chronic GI-complaints	35	LM	Iodoquinol 30-40mg/kg 21d (12/18) or Metronidazole 250mg 3dd 7d (5/18) or Tetracycline 250mg 2dd 5d (1/18)	Clinical reduction: 12/18 (67%)*	-	unknown
Preiss et al [14]	1985-1987	1-16 years Proven *D. fragilis* infection in patients with GI-complaints	115	TFT or LM	Metronidazole 30mg/kg/d 3dd 10d or Oxytetracycline 30-40mg/kg/d 4dd 10d or Doxycycline 2mg/kg/d 1dd 10d or Erythromycin 50mg/kg/d 4dd 10d or Hydroxyquinoline 20mg/kg/d 3dd 10d	Clinical reduction: 64/91 (70%) 8/9 (90%) 3/4 (75%) 3/6 (50%) 1/5 (20%)	-	unknown
Cuffari et al [13]	1990-1995	4-10 years Proven *D. fragilis* infection	7	LM	Metronidazole (no dose specification) or Iodoquinol (no dose specification) or No treatment	Clinical reduction: 4/4 (57.1%) 2/2 (100%) 1/1 (100%)	Eradication: 4/4 (100%) 2/2 (100%) 1/1 (100%)	unknown
Bosman et al [36]	unknown	3-10 years Proven *D. fragilis* infection	43	TFT	Clioquinol 15mg/kg/d 5-7d (27/43) or Metronidazole 15-30mg/kg/d 7d (10/43) or Tinidazole 50mg/kg/d 3d (6/43)	Eradication: 22/27 (81%) 11/16 (69%) (metronidazole/ tinidazole combined)	Clinical reduction: 19/22 (86.4%) 8/11 (72.7%) 2/10 (20%) persistent *D. fragilis* infection had clinical reduction	4 weeks

Table 2. Continued.

Author	Study period	Study population	N	D	Treatment	Results primary outcome (n/N)	Results secondary outcome (n/N)	Follow-up
Ter Schure et al [30]	2008-2010	0-18 years Children with positive *D. fragilis* PCR	151	PCR	Clioquinol 15mg/kg/d 3dd 5-10d or Metronidazole 30mg/kg/d 3dd 3-10d	Clinical reduction: 65/112 (58%) **Missing data on 25 patients** 17/39 (43.6%) **Missing data on 8 patients**	Eradication: 14/30 (46.7%) **Missing data on 25 patients** 11/21 (52.4%) **Missing data on 8 patients**	4-22 weeks
Retrospective case-control studies								
Banik et al [38]	2004-2010	0-15 years Children with positive *D. fragilis* PCR	82	PCR	Metronidazole (no dose specification/ duration)	***Cases (positive D. fragilis PCR)*** Clinical reduction: 35/41 (85%) 29/41 (71%) diarrhea ***Controls (negative D fragilis PCR)*** 14/41 (34%) diarrhea	***Cases (positive D. fragilis PCR)*** Eradication: 35/41 (85%) ***Controls (negative D fragilis PCR)*** Eradication: -	4 weeks
de Jong et al [31]	2011-2013	8-18 years	215	PCR	Metronidazole (no dose specification/ duration) or Clioquinol (no dose specification/ duration)	***Cases (patients with CAP >2m):*** 57/132 (43%) *D. fragilis* positive Clinical reduction: 14/39 (35.9%) 5/8 (62.5%) ***Controls (healthy patients):*** 39/77 (51%) *D. fragilis* positive	***Cases (patients with CAP >2m):*** Eradication: 23/39 (59%) 6/8 (75%) ***Controls (healthy patients):*** -	8 weeks

CAP: Chronic abdominal pain; D = Diagnosis; GI = Gastro-intestinal; LM = Light microscopy; m = months; N = number of studied patients; PCR = Polymerase chain reaction; TFT = Triple feces test; VAS: Visual analogue scale;
* 6/12 did not complete antibiotic treatment

Only one study reported the recurrence of *D. fragilis* after treatment with metronidazole and reported an increasing recurrence two months after treatment [37]. This study found that 14/48 children (29.2%) were still positive for *D. fragilis* 14 days after treatment with metronidazole, which increased to 23/41 (56.1%) after 56 days [37]. However, they did not reassess clinical symptoms at 2 months.

Table 3. Studied gastro-intestinal symptoms per included study.

Author	Symptoms observed	Definitions
Randomized controlled trials		
Röser et al [37]	Abdominal pain, diarrhea, loose stools, fever, headache, urticaria, nausea, vomiting, reflux, loss of appetite, FTT, anal itching, bloody stool	-
Prospective cohort studies		
Vandenberg et al [32]	Diarrhea, abdominal pain, fever, nausea, vomiting, anorexia, weight loss	***Diarrhea:*** at least 3 unformed or liquid stools per day for at least 3 days (acute <30d, persistent: >30d); ***Chronic abdominal pain:*** >3 months
Gijsbers et al [33]	Abdominal pain, constipation, diarrhea, bloody stools, flatulence, bloating, anorexia, early satiety, nausea, vomiting, awake at night	***Constipation:*** ≤2/week and/or hard stools; ***Diarrhea:*** unformed stools;
Maas et al [34]	Abdominal pain, nausea, acute diarrhea, chronic diarrhea, altered bowel habits, weight loss, vomiting, anal itching	***Diarrhea:*** acute: >3 loose stools a day <14 days, chronic: >14 days; ***Altered bowel habits:*** change in stool pattern other than diarrhea;
Retrospective cohort studies		
Spencer et al [35]	Abdominal pain, diarrhea, anorexia, weight loss, fever, irritability, vomiting, constipation, fatigue	-
Preiss et al [14]	Acute diarrhea, chronic diarrhea, bloody stools, abdominal pain, eosinophilia, urticaria	***Diarrhea:*** ≥3 loose stools during 24 hours;
Cuffari et al [13]	Diarrhea, abdominal pain, vomiting, bloody stools, fatigue, anorexia, weight loss	-
Bosman et al [36]	Abdominal pain, altered defecation pattern, fatigue, flatulence, nausea	-
Ter Schure et al [30]	Abdominal pain, loose or hard stools, nausea, flatulence, bloating, fatigue, anorexia, weight loss, vomiting, bloody stools, diarrhea, sleeplessness, fever	***Diarrhea:*** ≥3 stools a day
Retrospective case-control studies		
Banik et al [38]	Acute diarrhea, chronic diarrhea, abdominal pain, loose stools, vomiting, constipation	***Diarrhea:*** acute: <2 week, chronic: >2 weeks;
de Jong et al [31]	Abdominal pain, loose stools, constipation, nausea, vomiting, ructus, flatulence, bloating, abdominal cramps, bloody stools, altered defecation pattern, fatigue, weight loss, anorexia, fever, sleeplessness, headache, back pain, neck pain	-

Table 3, list of observed clinical symptoms per included study

DISCUSSION

This systematic review showed that resolution of gastrointestinal symptoms was reported in 36.8-90% of children treated with antibiotics for *D. fragilis* infection. Reported eradication rates for the different antibiotics varied from 43.6% to 100%. A higher eradication rate did not lead to a higher rate of symptom resolution.

Most studies included in our review reported resolution of symptoms, mainly abdominal pain and diarrhea, varying between 35.9-100% after treatment with antibiotics [14,31–35]. This response to antibiotics suggests that treatment of *D. fragilis* could be beneficial in a subset of patients. However, most studies had an observational design in which a placebo effect possibly played a significant role. The only conducted RCT included in our study did not show any effect of antibiotics on clinical symptoms, which profoundly limits the strength of evidence based on this systematic review that *D. fragilis* warrants treatment [37]. A large multicenter double-blind, placebo randomized controlled trial is required to give a definitive answer.

The only conducted RCT so far by Röser et al has some methodological flaws which need to be addressed. First, the null hypothesis of the study was no clinical- or microbiological measurable effect of metronidazole compared to placebo in treatment of children with *D. fragilis*. However, the observed absence of any difference did not prove that the null hypothesis was true. Second, the study was conducted in a small population of 48 children per group in the per protocol analysis and their most critical analysis (i.e. gastrointestinal symptoms among children with successful eradication versus placebo) only included 14 versus 44 subjects, even less than the original groups. This result was marked as a tertiary outcome by describing: “Furthermore, tertiary analyses did not show greater effect in eradicated vs non-eradicated patients”, suggesting that even in this small group there was a difference, yet not a “greater” one. Furthermore, this tertiary analysis was designed after unblinding of the data and the actual numbers related to this comparison where not shown, which is unfortunate since it reflects important data. Third, one of the confounding factors is that *D. fragilis* was also spontaneously eradicated from a part (4/48) of the placebo group, which may have impact on the primary outcome in a study with such small subject groups.

Reported eradication rates for the different antibiotics varied from 43.6-100%. Importantly, a higher eradication rate was not associated with a higher rate of symptom resolution. This suggests that, at least in a part of the studied population, *D. fragilis* may not be the causative factor for the different gastrointestinal symptoms. The success rates of the different antibiotics varied throughout the studies. Our review showed that clioquinol and metronidazole have a comparable and higher average success rate in resolution of gastrointestinal symptoms (63.1% vs. 62.7%) than paromomycin (50.9%). There was a lot of missing data regarding the resolution of symptoms in the

group treated with metronidazole. Therefore, this success rate could be an over- or an underestimation of the true success rate. The average eradication rate was also comparable between clioquinol and metronidazole (64.6% vs. 69%), but significantly higher for paromomycin (84.9%). The spectrum of activity for metronidazole and paromomycin is significantly wider than clioquinol, whereas clioquinol has limited antibacterial and antimycotic activity. It therefore seems that paromomycin is the most effective antibiotic in treating *D. fragilis*. A recent Spanish study compared paromomycin and metronidazole for treatment of *D. fragilis* and reported significant better eradication rates for paromomycin (81.8% vs. 65.4%) [39]. However, since 2011 paromomycin was removed from the community register of active orphan medicinal products in Europe and cannot easily be prescribed anymore. Considered its smaller spectrum of activity and its comparable efficacy to metronidazole, clioquinol is a good alternative antibiotic when paromomycin is not available [20,40]. Also, metronidazole has more side effects than paromomycin and clioquinol [40]. The design of the reviewed studies, and the limited number of inclusions per study prevented to draw conclusions regarding causality between eradication of *D. fragilis* and resolution of symptoms.

Over time, TFT and RT-PCR methods have been developed with significantly higher sensitivity than light microscopy [21,34,41–44]. The higher accuracy has led to a significantly higher detection rate of the *D. fragilis* protozoa in stools of patients [45]. For instance, a study by Calderaro et al reported that *D. fragilis* was detected in 69/959 (7%) samples by conventional methods, whereas PCR detected *D. fragilis* in the same samples in 186/959 (19%) samples [43]. Since RT-PCR techniques with superior detection rates detect even a small fraction of *D. fragilis* DNA, this might have led to an overestimation of the true *D. fragilis* incidence of the studies, which used PCR as diagnostic tool [30,31,34,37,38]. It is also complicated to compare incidences between studies which used different diagnostic modalities. To overcome this, semi-quantitative PCR techniques have been developed, but these were not used in the included studies. It is unknown what concentration (or load) of the protozoa, measured during quantitative fecal PCR, causes symptoms. The concentration (or load) can be expressed in a number of duplication cycles (C_q) needed to detect it during PCR. C_q values are inverse to the amount of target nucleic acid of a sample. A lower C_q value corresponds to a higher load of the parasite. However a threshold C_q value for a clinical significant concentration of *D. fragilis* (i.e. when it causes symptoms) is not known. Earlier studies report on genetic evidence of two variants of *D. fragilis* (genotypes 1 and 2) with a strong predominance of genotype 1 in humans [46,47]. It is unknown if these two variants differ in their pathogenicity and none of the included studies evaluated the presence of the different *D. fragilis* stains.

Strengths of our systematic review include a detailed search strategy, systematic data extraction and analysis. It provides a synthesis of a clinical frequent observed therapeutic dilemma and concern. However, our review also carries limitations. Careful

interpretation of the results from this systematic review and in particular consideration to local circumstances is warranted. Except for one, all included studies were non-randomized observational studies inducing high risk of bias, particularly regarding placebo effect, and limiting the quality of the evidence. Studies were conducted over a long time span in which adjustments to laboratory techniques to detect *D. fragilis* were made. Furthermore, studies included children from all ages in which it is a particular challenge to judge the reliability of the gastrointestinal complaints. Also, the duration of treatment and the dose regimen of the various antibiotics differed between studies, whereas some studies did not even describe a treatment dose and duration. Follow-up varied between studies and since no control group was included in most studies the spontaneous resolution of gastrointestinal complaints remains to be elucidated. Nevertheless, a significant placebo effect in observational studies needs to be taken into account. Finally, results of the included studies do not show a clear causal relation between eradication rate and symptom resolution, but this was only studied in small groups of patients and no study used quantitative fecal PCR. This does not provide evidence for a causal relationship and therefore limits the generalizability of our findings. However, there seems to be a moderate to good reported effect of antibiotic therapy on resolution of gastrointestinal complaints associated with *D. fragilis*.

EXPERT OPINION

Results of this study indicate that more extended data on relationship between *D. fragilis* and gastrointestinal symptoms and the indication for targeted treatment of *D. fragilis* in children is needed.

Potential pathogenicity

The pathogenicity of *D. fragilis* is still under debate despite the vast evidence that emerged from clinical studies (primarily case reports or prospective or retrospective studies). Over time only one RCT has been conducted regarding the effect of treatment with metronidazole on gastrointestinal symptoms in children with *D. fragilis* which showed no beneficial effect over placebo [37]. However, numerous reports published over the past decades showed that, at least in a subgroup of patients (in whom other causes like celiac disease, IBD and Giardia lamblia infection were excluded) treatment of *D. fragilis* frequently resulted in clinical improvement of gastrointestinal complaints [13,14,30,35,36,38]. Also, two promising rodent models have been developed in which three criteria of Koch's postulates were fulfilled [48,49]. All mice inoculated with *D. fragilis* became infected, in contrast to the negative controls, which remained uninfected. In these experiments, infected rodent groups produced unformed stools and experienced statistically significant weight loss. Histopathology of infected tissue demonstrated a mild inflammatory response compared to that of the control group. Fecal calprotectin levels were also more than two times higher in the infected group than in uninfected controls. Additionally, polymorphonuclear white blood cells were

microscopically detected in the feces of infected rodents. Finally, cysts recovered from the feces of infected mice could establish a new infection in naive mice and rats when administered orally [48,49]. In order to conduct future randomized studies, belief in the potential pathogenicity of *D. fragilis* is essential. We therefore believe *D. fragilis* to be a potential enteropathogen associated with gastrointestinal complaints.

Aims of future studies

Future studies should focus on finding practical criteria to identify children with *D. fragilis* infection who are most likely to benefit from specific treatment, the best diagnostic procedure, and most effective drug treatment with respect to parasitological clearance and resolution of symptomatology.

Identification of children in need of treatment

For clinicians it is most difficult to select the children who would truly benefit from treatment amongst the many children infected with *D. fragilis*. This finds its origin in three very different aspects; there is frequently occurring asymptomatic carriage of the parasite, especially when PCR was the method of diagnosis (in up to 50% of children in developed Western-European countries [31,50], a large non-specific symptomatology, and more recently, a strong increase of positive cases diagnosed with ultra-sensitive molecular tests (RT-PCR's).

Limited guidance by symptoms

Reported symptomatology most frequently associated with D. fragilis infection includes diarrhea and abdominal pain, which can be of an acute or chronic nature [4,6–14]. Unfortunately, in its nature, this symptomatology is non-specific as it is observed in many other intestinal infections and non-infectious disorders (see also Table 3). A more peculiar finding in *D. fragilis* infections is eosinophilia, which is reported in up to 50% of infected patients [4,13]. In comparison with infection with Giardia lamblia, also known as an important cause of abdominal pain and diarrhea in children, children with *D. fragilis* do suffer significantly less from nausea and/or vomiting, anorexia and weight loss [51]. On the other hand, symptomatology associated with *D. fragilis* infection has a strong similarity with symptomatology of functional gastrointestinal disorders in infants, toddlers and children. Ninety percent of children who present with chronic abdominal pain without diarrhea are classified as functional according to the Dutch evidence based guidelines [52].

Need to exclude other causes

In the work-up of children with abdominal pain and diarrhea of unknown origin, full parasitological, bacterial and (when symptoms are or of shorter duration) virologic examination, are indispensable to identify or rule out infectious agents as a potential cause of symptomatology. The prevalence of *D. fragilis* in developed countries, such as Denmark (43-68.3%), the Netherlands (51.1%), Sweden (73%) Spain (17.7%) or Italy

(21.4%) is high, but the percentage of children with associated symptoms remains unclear [53]. Therefore, it seems reasonable to consider a parasitological study in children with gastrointestinal symptoms and to treat *D. fragilis* when other prevalent causes, like celiac disease, are ruled out [20,52,54]. The underlying reason is that many humans, symptomatic and non-symptomatic, do harbor *D. fragilis* as a commensal parasite which does not inflict any disease and possibly may have yet unidentified beneficial effects to the host. As such, the finding of *D. fragilis* could be coincidental and unrelated to the true underlying cause of the disease. Diseases which first need to be excluded include celiac disease, inflammatory bowel disease, *Giardia lamblia* infection, lactose intolerance (or other possible food allergies) and thyroid disease [20,52].

Specific problems and possibilities with parasitological diagnosis

Parasitological diagnosis should be at very high standards in future studies. Evidence of clinical importance of *D. fragilis* is essentially based on a large series of reports in children and adults, indicating that treatment and elimination of *D. fragilis* frequently results in significant clinical improvement of patients experiencing gastrointestinal illness [4,6,40]. Many of these studies were performed decades ago in a time where parasitological diagnosis of *D. fragilis* infection solely relied on microscopic examinations of permanently stained slides of stools. Using this method, especially with repeated examinations, *D. fragilis* can reliably be demonstrated in stools. Microscopy has a specific advantage, namely that actively dividing parasites (trophozoites) can be demonstrated, which provides proof of true replication of the parasite in the intestine. In addition, the parasitic load (i.e. the number of parasites per microscopic field) can well be determined. The disadvantage of diagnosing *D. fragilis* by microscopy is the need for highly skilled laboratory personnel and extended study time to, especially, demonstrate low numbers of the parasite and exclusion of the infection. Therefore, for non-reference laboratories especially, the introduction of molecular diagnosis, especially RT- PCR for *D. fragilis*, was much welcomed because of its high sensitivity and, possibly, also, specificity of the test [4,44]. In addition, these tests are less time consuming and ask for less specific skills of laboratory personal [4]. However, large scale introduction of the ultrasensitive diagnostic PCR method in populations were *D. fragilis* infection is highly endemic (i.e. The Netherlands and Denmark), proved to have unforeseen large and negative impact on, especially, routine clinical practice. In comparison with earlier prevalences of *D. fragilis*, observed with the use of light microscopic diagnosis, prevalences with RT- PCR's in the same population often strongly increased [4]. These, sometimes perplexing newer data, did draw attention in international literature and led to questioning the specificity of the PCR's in use and clinical significance of the data [4]. Fueled by a strong increase of positive cases with D. fragilis with PCR, clinicians got worried about the large number of patients which would potentially need, and also requested, treatment for *D. fragilis* infection. This confusion finally resulted into excluding diagnosis of *D. fragilis* from routine clinical parasitological examinations in laboratories were PCR was the only method of diagnosis (Tom van Gool (TVG) personal communication).

Future studies of *D. fragilis* should be performed with diagnosis based on light microscopy or, when PCR is also available, the combination of PCR and light microscopy. In the Netherlands the combined use of both techniques is practiced nowadays in laboratories with the Dual-Feces-Test (DFT). In this test fresh and SAF fixed stool are collected by the patient on one day. Laboratory analysis for *D. fragilis* and other intestinal parasites subsequently is performed with a combination of (multiple) RT-PCRs and light microscopy. This methodology combines the advantages of fast and highly sensitive screening of the RT-PCR's, with reliable information as to the presence of active dividing parasites and the intestinal load of these, by counts of parasites per microscopic field. As an alternative for estimates of the parasitic load with microscopy, quantification of PCR results could be used. A classification into low, moderate and high DNA loads, preferentially related to results obtained with microscopy, should be provided by the laboratory. We suggest that very low DNA loads of *D. fragilis* without - or with only spare parasites visible with light microscopy -, are unlikely to be associated with symptomatology. In contrast, higher parasitic loads, both with DNA and light microscopy, can be associated with symptomatology as these – microscopic-findings are similar to the ones observed in previous studies in which patients did show significant clinical improvement after successful eradication of *D. fragilis* infection. However, this needs to be elucidated in future clinical studies. Another interesting development is the recently presented new method to detect *D. fragilis* in trichrome-stained, formalin fixed samples [45]. Introduction of this microscopy based method alone led to an 20-fold increase of new *D. fragilis* findings in Finland between 2007 and 2017 [45]. This approach may prove valuable, especially, in low-income countries, where the only tool available to diagnose *D. fragilis* is microscopy.

Treatment options for children

Best treatment for *D. fragilis* at present most likely is paromomycin (25-35 mg/kg/day in 3 doses for 7 days). Alternatives are treatment with iodoquinol (30-40mg/kg/d (max. 2g) in 3 doses for 20 days) or the related compound clioquinol (15 mg/kg/day for 7 days). Our systematic review showed that the highest average eradication rate was achieved by paromomycin (84.9%), followed by a comparable rates for metronidazole (69%) and clioquinol (64.6%). This is in line with a recent Spanish study which reported a significant higher eradication rate for paromomycin (81.8%) than metronidazole (65.6%) in treatment of *D. fragilis* [39]. Also, our systematic review showed that clioquinol (63.1%) and metronidazole (62.7%) had higher success rates in resolution of clinical symptoms than paromomycin (50.9%). However, studies conducted in various pediatric and adult populations with *D. fragilis* showed treatment efficacy for paromomycin varying between 80-100%, clioquinol 81.5-83% and metronidazole 12.5-83.3% [40]. However, most of these studies were case studies, included only a small amount of patients and failed to utilize adequate control groups [4,40]. Metronidazole has more side effects than both paromomycin and clioquinol. Also, metronidazole has a wider spectrum of antibiotic activity than both paromomycin and clioquinol, which could

potentially lead to large scale alterations of the intestinal microbiota. Randomized, double-blind, controlled trials using different registered antibiotics used for treating *D. fragilis* are warranted to address which antibiotic is the most effective. Until such time, it is rather difficult to state which antibiotic is the most effective in treating *D. fragilis*. However, taking everything mentioned above into account, paromomycin, iodoquinol or clioquinol seem to be antibiotics of choice.

Parasitological and clinical evaluation after treatment

Good judgement regarding clinical effectiveness of specific treatment for *D. fragilis* can only be obtained with reliable parasitological proof of eradication. Parasitological examinations direct after treatment i.e. at day 8 after start of treatment with paromomycin or clioquinol, and day 21 after start of iodoquinol. When a combination of PCR and microscopy are used for diagnosis one day sampling is sufficient. When only microscopy is used, stools collected over a period of two or three consecutive days should be collected to reach sufficient high sensitivity of diagnosis and, as a result, certainty of eradication. Repeated testing can be considered 1 to 2 weeks after end of treatment. Collecting data about clinical improvement should be obtained direct after treatment and two - four weeks thereafter.

Clarifying the mechanism between *D. fragilis* infection and symptomatology

It remains unclear why some patients harboring *D. fragilis* manifest clinical symptoms and others are only asymptomatic carriers. A possible, yet unexplored, hypothesis is imbalance in the intestinal microbiota (dysbiosis). The pathogenesis of gastrointestinal disorders with comparable clinical symptoms as reported in *D. fragilis*, such as irritable bowel syndrome or *B. hominis* infections (some in combination with *D. fragilis*), has also been associated with dysbiosis or alterations in the intestinal microbiota [55,56]. It could be hypothesized that *D. fragilis* provokes dysbiosis, comparable to observed microbial alterations in Blastocystis, which on its turn could provoke gastrointestinal symptoms, mimicking irritable bowel syndrome [55,56]. Future studies should explore the possible association between *D. fragilis* microbiota composition and clinical symptoms. Involvement of the microbiota in *D. fragilis* related symptoms may lead to development of novel microbiota-based diagnostic strategies, like stratification of patients who may benefit from treatment, and even therapeutic options. Another explanation could be that different subtypes of *D. fragilis* have different virulent factors and as such may be responsible for the large differences in clinical presentation [46,47].

Future studies

A double-blind randomized controlled trial in children with well-defined clinical symptoms, appropriate diagnostic testing and follow-up, comparing paromomycin or clioquinol with placebo is mandatory. Paromomycin (25 to 35mg/kg per day in three daily doses for 7 days) or clioquinol (when paromomycin is not available, in 15 mg/kg in three daily doses for 7 days), are the first antibiotics of choice because

treatment outcomes are better with paromomycin and clioquinol than metronidazole and its relative fewer side-effects. The effect of antibiotic treatment needs to be investigated by two ways, eradication of the parasite immediately after the antibiotic therapy and resolving of gastro-intestinal complaints approximately 6-8 weeks after antibiotic treatment. In addition to a randomized controlled trial, studies are needed to elucidate the mechanism of *D. fragilis* intestinal infection. For example, a clinical trial in children with a confirmed *D. fragilis* in feces, comparing the intestinal microbiome between asymptomatic children and children with gastrointestinal complaints. This information may answer the question if symptoms in patients with *D. fragilis* are caused by alterations in the intestinal microbiome, like has been supposed in IBS.

Declaration of interest

M.A. Benninga is a scientific consultant for Shire, Sucampo, Astrazeneca, Norgine, Zeria, Coloplast, Danone, Friesland Campina, Sensus, Novalac. The other authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed."

REFERENCES

1. Jepps MW, Dobell C. Dientamoeba fragilis n.g., n.sp., new intestinal amoeba from man. Parasitology. 1918;10:352–67.
2. Wong ZW, Faulder K, Robinson JL. Does Dientamoeba fragilis cause diarrhea? A systematic review. Parasitol Res. 2018;117(4):971–80.
3. Elbakri A, Al-qahtani A, Samie A. Advances on Dientamoeba fragilis Infections. In: An Overview of Tropical Diseases. IntechOpen; 2015. p. 61–81.
4. Stark D, Barratt J, Chan D, et al. Dientamoeba fragilis , the Neglected Trichomonad of the Human Bowel. Clin Microbiol Rev. 2016;29(3):553–80.
5. Barratt JLN, Harkness J, Marriott D, et al. A review of Dientamoeba fragilis carriage in humans: Several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. Gut Microbes. 2011;2(1):3–12.
6. Garcia L. Dientamoeba fragilis , One of the Neglected Intestinal Protozoa. J Clin Microbiol. 2016;54:2243–50.
7. Stark D, Barratt J, Roberts T, et al. A review of the clinical presentation of dientamoebiasis. Am J Trop Med Hyg. 2010;82(4):614–9.
8. Johnson EH, Windsor JJ, Clark CG. Emerging from obscurity: Biological, clinical, and diagnostic aspects of Dientamoeba fragilis. Clin Microbiol Rev. 2004;17(3):553–70.
9. Stark D, Beebe N, Marriott D, et al. Prospective study of the prevalence, genotyping, and clinical relevance of Dientamoeba fragilis infections in an Australian population. J Clin Microbiol. 2005;43(6):2718–23.
10. Girginkardesler N, Coskun S, Cüneyt Balcioglu I, et al. Dientamoeba fragilis , a neglected cause of diarrhea , successfully treated with secnidazole. Clin Microbiol Infect. 2003;(9):110–3.
11. Grendon J, DiGiacomo R, Frost F. Descriptive features of Dientamoeba fragilis infections. J Trop Med Hyg. 1995;(98):309–15.
12. Norberg A, Nord C, Evengard B. Dientamoeba fragilis - A protozoal infection which may cause severe bowel distress. Clin Microbiol Infect. 2003;9(1):65–8.
13. Cuffari C, Oligny L, Seidman E. Dientamoeba fragilis masquerading as an allergic colitis. J Pediatr Gastroenterol Nutr. 1998;26(1):16–20.
14. Preiß U, Ockert G, Brömme S, et al. Dientamoeba fragilis infection, a cause of gastrointestinal symptoms in childhood. Klin Pädiatrie. 1990;202(02):120–3.
15. Chitkara DK, Rawat DJ, Talley NJ. The epidemiology of childhood recurrent abdominal pain in western countries: A systematic review. Am J Gastroenterol. 2005;100(8):1868–75.
16. Starfield B, Gross E, Wood M. Psychosocial and psychosomatic diagnoses in primary care of children. Pediatrics. 1980;(66):159–67.
17. Youssef N, Murphy T, Langseder A, et al. Quality of Life for Children With Functional Abdominal Pain : A Comparison Study of Patients' and Parents ' Perceptions. Pediatrics. 2006;(117):54–9.

18. van Kalleveen MW, Noordhuis EJ, Lasham C, et al. Large variation in clinical practice amongst pediatricians in treating children with recurrent abdominal pain. Pediatr Gastroenterol Hepatol Nutr. 2019;22(3):225–32.

19. Baber KF, Anderson J, Puzanovova M, et al. Rome II versus Rome III classification of functional gastrointestinal disorders in pediatric chronic abdominal pain. J Pediatr Gastroenterol Nutr. 2008;47(3):299–302.

20. Van Gestel RSFE, Kusters JG, Monkelbaan JF. A clinical guideline on Dientamoeba fragilis infections. Parasitology. 2018;146(9):1131–9.

21. Stark D, Barratt J, Roberts T, et al. Comparison of microscopy, two xenic culture techniques, conventional and real-time PCR for the detection of Dientamoeba fragilis in clinical stool samples. Eur J Clin Microbiol Infect Dis. 2010;29(4):411–6.

22. Rijsman LH, Monkelbaan JF, Kusters JG. Clinical consequences of polymerase chain reaction-based diagnosis of intestinal parasitic infections. J Gastroenterol Hepatol. 2016;31(11):1808–15.

23. Balshem H, Helfand M, Schünemann HJ, et al. GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol. 2011;64(4):401–6.

24. Guyatt G, Oxman A, Vist G, et al. GRADE guidelines: 4. Rating the quality of evidence - Study limitations (risk of bias). J Clin Epidemiol. 2011;64(4):407–15.

25. Guyatt G, Oxman A, Montori V, et al. GRADE guidelines: 5. Rating the quality of evidence - Publication bias. J Clin Epidemiol. 2011;64(12):1277–82.

26. Guyatt G, Oxman A, Kunz R, et al. GRADE guidelines: 6. Rating the quality of evidence - Imprecision. J Clin Epidemiol. 2011;64(12):1283–93.

27. Guyatt G, Oxman A, Kunz R, et al. GRADE guidelines: 7. Rating the quality of evidence - Inconsistency. J Clin Epidemiol. 2011;64(12):1294–302.

28. Guyatt G, Oxman A, Kunz R, et al. GRADE guidelines: 8. Rating the quality of evidence - Indirectness. J Clin Epidemiol. 2011;64(12):1303–10.

29. Guyatt G, Oxman A, Sultan S, et al. GRADE guidelines: 9. Rating up the quality of evidence. J Clin Epidemiol. 2011;64(12):1311–6.

30. Ter Schure JMA, De Vries M, Weel JFL, et al. Symptoms and treatment of dientamoeba fragilis infection in children, a retrospective study. Pediatr Infect Dis J. 2013;32(4):148–50.

31. De Jong MJ, Korterink JJ, Benninga MA, et al. Dientamoeba fragilis and chronic abdominal pain in children: A case-control study. Arch Dis Child Educ Pract Ed. 2014;99(12):1109–13.

32. Vandenberg O, Souayah H, Mouchet F, et al. Treatment of Dientamoeba fragilis infection with paromomycin. Pediatr Infect Dis J. 2007;26(1):88–90.

33. Gijsbers CFM, Schweizer JJ, Büller HA. Protozoa as a cause of recurrent abdominal pain in children. J Pediatr Gastroenterol Nutr. 2013;57(5):603–6.

34. Maas L, Dorigo-Zetsma JW, de Groot CJ, et al. Detection of intestinal protozoa in paediatric patients with gastrointestinal symptoms by multiplex real-time PCR. Clin Microbiol Infect. 2014;20(6):545–50.

35. Spencer MJ, Garcia LS, Chapin MR. Dientamoeba fragilis: An intestinal pathogen in children. Am J Dis Child. 1979;133:390–3.

36. Bosman DK, Benninga MA, van de Berg P, et al. [Dientamoeba fragilis: possibly an important cause of persistent abdominal pain in children]. Ned Tijdschr Geneeskd. 2004;148(12):575–9.

37. Röser D, Simonsen J, Stensvold CR et al. Metronidazole therapy for treating dientamoebiasis in children is not associated with better clinical outcomes: a randomized, double-blinded and placebo-controlled clinical trial. Clin Infect Dis. 2014;58(12):1692–9.

38. Banik GR, Barratt JLN, Marriott D, et al. A case-controlled study of Dientamoeba fragilis infections in children. Parasitology. 2011;138(7):819–23.

39. Burgaña A, Abellana R, Yordanov S, et al. Paromomycin is superior to metronidazole in Dientamoeba fragilis treatment. IJP Drugs Drug Resist. 2019;11:95–100.

40. Nagata N, Marriott D, Harkness J, et al. Current treatment options for Dientamoeba fragilis infections. Int J Parasitol Drugs Drug Resist. 2012;2:204–15.

41. Incani R, Ferrer E, Hoek D, et al. Diagnosis of intestinal parasites in a rural community of Venezuela : Advantages and disadvantages of using microscopy or RT-PCR. Acta Trop. 2017;167:64–70.

42. Stark DJ, Beebe N, Marriott D, et al. Dientamoebiasis: Clinical importance and recent advances. Trends Parasitol. 2006;22(2):92–6.

43. Calderaro A, Gorrini C, Montecchini S, et al. Evaluation of a real-time polymerase chain reaction assay for the detection of Dientamoeba fragilis. Diagn Microbiol Infect Dis. 2010;(3):239–45.

44. Friesen J, Fuhrmann J, Kietzmann H, et al. Evaluation of the Roche LightMix Gastro parasites multiplex PCR assay detecting Giardia duodenalis, Entamoeba histolytica, cryptosporidia, Dientamoeba fragilis, and Blastocystis hominis. Clin Microbiol Infect. 2018;(24):1333–7.

45. Pietilä J, Meri T, Siikamäki H, et al. Dientamoeba fragilis – the most common intestinal protozoan in the Helsinki Metropolitan Area , Finland , 2007 to 2017. Euro Surveill. 2019;24:pii=1800546.

46. Cacciò S. Acta Tropica Molecular epidemiology of Dientamoeba fragilis. Acta Trop. 2018;184:73–7.

47. Johnson J, Clark C. Cryptic Genetic Diversity in Dientamoeba fragilis. J Clin Microbiol. 2000;38:4653–4.

48. Munasinghe V, Vella N, Ellis J, et al. Cyst formation and faecal – oral transmission of Dientamoeba fragilis – the missing link in the life cycle of an emerging pathogen. Int J Parasitol. 2013;43(11):879–83.

49. El-gayar E, Mokhtar A, Hassan W. Study of the pathogenic potential of Dientamoeba fragilis in experimentally infected mice. Parasite Epidemiol Control. 2016;1:136–43.

50. Jokelainen P, Hebbelstrup Jensen B, Andreassen B, et al. Dientamoeba fragilis, a Commensal in Children in Danish Day Care Centers. J Clin Microbiol. 2017;55(6):1707–13.

51. Vandenberg O, Peek R, Souayah H, et al. Clinical and microbiological features of dientamoebiasis in patients suspected of suffering from a parasitic gastrointestinal illness : A comparison of Dientamoeba fragilis and Giardia lamblia infections. Int J Infect Dis. 2006;10:255–61.

52. Tabbers M, Benninga M, Venmans L, et al. NVK Richtlijn functionele buikpijn bij kinderen (2015). 2015.

53. Menéndez C, Fernandéz-Suarez J, Boga Ribeiro J, et al. Epidemiological and clinical characteristics of Dientamoeba fragilis infection. Enferm Infecc Microbiol Clin. 2018;37(5):290–5.

54. van Kalleveen MW, de Meij T, Plötz FB. Clinical spectrum of paediatric coeliac disease: a 10-year single-centre experience. Eur J Pediatr. 2018;177(4):593–602.

55. O'brien-Andersen L, Karim AB, Roager HM, et al. Associations between common intestinal parasites and bacteria in humans as revealed by qPCR. Eur J Clin Microbiol Infect Dis. 2016;35:1427–31.

56. Nourrisson C, Scanzi J, Pereira B, et al. Blastocystis Is Associated with Decrease of Fecal Microbiota Protective Bacteria : Comparative Analysis between Patients with Irritable Bowel Syndrome and Control Subjects. PLoS One. 2014;9:e111868.

SUPPLEMENTAL MATERIAL

Supplemental Table 1. Search strategy in Pubmed May 27, 2019.

Set	Search terms	Result
#1	"Dientamoebiasis"[Mesh] OR Dientamoeb*[tiab]	343
#2	("Child"[Mesh] OR "Infant"[Mesh] OR "Adolescent"[Mesh] OR infan*[tw] OR child*[tw] OR adolescen*[tw] OR pediatric*[tw] OR paediatric*[tw] OR pube*[tw] OR juvenil*[tw] OR school*[tw] OR newborn*[tiab] OR new-born*[tiab] OR neonat*[tiab] OR neonat*[tiab] OR premature*[tiab] OR postmature*[tiab] OR premature*[tiab] OR post-mature*[tiab] OR preterm*[tiab] OR pre-term*[tiab] OR baby[tiab] OR babies[tiab] OR toddler*[tiab] OR youngster*[tiab] OR preschool*[tiab] OR kindergart*[tiab] OR kid[tiab] OR kids[tiab] OR playgroup*[tiab] OR playgroup*[tiab] OR playschool*[tiab] OR prepube*[tiab] OR preadolescen*[tiab] OR junior high*[tiab] OR highschool*[tiab] OR senior high[tiab] OR young people*[tiab] OR minors[tiab]) NOT (animals[mh] NOT (humans[mh] AND animals[mh]))	4150213
#3	"Therapeutics"[Mesh] OR "therapy" [Subheading] OR Therapeutic*[tiab] OR Therapy[tiab] OR Therapies[tiab] OR Treatment*[tiab] OR "Clioquinol"[Mesh] OR clioquinol[tiab] OR "Metronidazole"[Mesh] OR metronidazole[tiab] OR "Paromomycin"[Mesh] OR paromomycin[tiab] OR "Secnidazole"[Mesh] OR secnidazole[tiab]	10786782
#4	#1 AND #2 AND #3	48

Supplemental Table 2. Search strategy in Embase May 27, 2019.

Set	Search terms	Result
#1	'dientamoebiasis'/exp OR Dientamoeb*:ti,ab,kw AND ('juvenile'/exp OR infan*:ti,ab,kw OR child*:ti,ab,kw OR adolescen*:ti,ab,kw OR pediatric*:ti,ab,kw OR paediatric*:ti,ab,kw OR pube*:ti,ab,kw OR juvenil*:ti,ab,kw OR school*:ti,ab,kw OR newborn*:ti,ab,kw OR 'new born*':ti,ab,kw OR 'neo nat*':ti,ab,kw OR neonat*:ti,ab,kw OR premature*:ti,ab,kw OR postmature*:ti,ab,kw OR 'pre mature*':ti,ab,kw OR 'post mature*':ti,ab,kw OR preterm*:ti,ab,kw OR 'pre term*':ti,ab,kw OR baby:ti,ab,kw OR babies:ti,ab,kw OR toddler*:ti,ab,kw OR youngster*:ti,ab,kw OR preschool*:ti,ab,kw OR kindergart*:ti,ab,kw OR kid:ti,ab,kw OR kids:ti,ab,kw OR playgroup*:ti,ab,kw OR 'play group*':ti,ab,kw OR playschool*:ti,ab,kw OR prepube*:ti,ab,kw OR preadolescen*:ti,ab,kw OR 'junior high*':ti,ab,kw OR highschool*:ti,ab,kw OR 'senior high':ti,ab,kw OR 'young people*':ti,ab,kw OR minors:ti,ab,kw)	153
#2	"'therapy'/exp OR Therapeutic*:ti,ab,kw OR Therapy:ti,ab,kw OR Therapies:ti,ab,kw OR Treatment*:ti,ab,kw OR 'clioquinol'/exp OR clioquinol:ti,ab,kw OR 'metronidazole'/exp OR metronidazole:ti,ab,kw OR 'paromomycin'/exp OR paromomycine:ti,ab,kw OR 'secnidazole'/exp OR secnidazole:ti,ab,kw	12238474
#3	#1 AND #2	54

8

English summary

This thesis discusses diagnostic- and therapeutic dilemma's in daily clinical practice in children with chronic abdominal pain (CAP), with a main focus on celiac disease (CD) and *Dientamoeba fragilis* (*D. fragilis*) as potential causes of CAP. CAP is one of the most common clinical conditions amongst children and adolescents globally with a high prevalence in the Western pediatric population. Despite its high prevalence, only a small proportion of CAP is caused by organic disease and approximately 90% of children with CAP fulfill the diagnostic criteria of the functional abdominal pain disorders (FAPD). Identifying this small proportion of children with an organic cause of CAP is of great importance because of the significant implications on both growth and development of these organic causes of CAP. However, it can be puzzling for clinicians and most often leads to the execution of additional diagnostic tests to differentiate between functional and organic causes of CAP. The absence of clear-cut clinical guidelines, and the necessity to exclude organic causes to diagnose FAPD which is incorporated in its diagnostic criteria, potentially often leads to the execution of unnecessary diagnostic tests, potential false- positive or false-negative results, generation of extra financial costs and generation of results of which the clinical relevance can be unclear. Such as, for instance, the role of the flagellate anaerobic parasite *D. fragilis* in causing gastro-intestinal symptoms in children. What is clear, however, is, that testing for CD in children with CAP is essential because of the relatively high prevalence of the disease in the Western population and its significant consequences regarding treatment. However, over the past decades incidence rates and the clinical spectrum of presenting symptoms of children with CD changed significantly. This has led to shifting paradigms in diagnostic- and therapeutic management of children with CD and *D. fragilis*.

The aims of this thesis were to evaluate guideline adherence amongst clinicians treating children with CAP and CD, to investigate the current clinical spectrum of pediatric CD, to investigate the clinical relevance of the suggested routine biochemical tests during follow-up suggested by the guidelines, and to evaluate the role of *D. fragilis* in causing CAP in children.

Diagnostic work-up in children with CAP

The first part of this thesis focuses on the diagnostic work-up of children with CAP. CAP is common amongst children and adolescents and often reason for referral to a pediatrician. Diagnosing the underlying cause of CAP in children can be challenging for clinicians for several reasons: CAP can be caused by a wide variety of organic abdominal and extra-intestinal diseases, younger children can be limited in their ability to provide accurate history, parents can have difficulties interpreting the presenting symptoms and both parents and clinicians do not want to miss organic diseases. These factors combined can cause uncertainty amongst clinicians which could generate the execution of excessive, often unnecessary diagnostic tests such as blood tests, fecal tests and even radiological imaging. These factors lead to heterogeneity in clinical practice and to the generation of even more unnecessary diagnostic tests and insecurities when

"unwanted" results are found. To provide clinicians a clinical compass, guidelines are constructed, which aim to minimize heterogeneity in clinical practice and aim to provide only the essential number of diagnostic tests needed for the diagnosis. However, it has proven rather difficult to incorporate these factors into clear-cut clinical guidelines and adherence to clinical guidelines amongst clinicians is often considered poor due to a large variety of factors, both guidelines-related and clinician-related. For instance, clinicians believe that guidelines could be too unspecific, for others there are too many guidelines, or they feel that the process of clinical decision making cannot be standardized for the individual patient or that this process is simply too complex to incorporate into guidelines and finally, experienced clinicians have treated children the same way throughout their entire career without any problems. Therefore, the periodic evaluation of implemented clinical guidelines is essential in order to improve adherence and to optimize clinical care for children with CAP by means of implementation of up-to-date evidence.

In **Chapter 2** we retrospectively investigated intra- and inter-observer variability and guideline adherence amongst pediatricians in treating children with CAP in a large teaching hospital in the Netherlands. These children, aged 4-18 years, were referred with CAP without the presence of 'red flags'. We compared the diagnostic work-up of pediatricians with respect to the work-up suggested by the national guidelines (1). An organic cause for CAP was found in 26% of patients. None of the pediatricians strictly performed the work-up suggested by the guidelines (complete blood count, CRP and celiac serology) without performing additional tests, but 67% of pediatricians performed a diagnostic work-up which included these suggested tests. Both high intra-observer variability and inter-observer variability was observed. Furthermore, in a prospective survey with a fictitious case, reasons to deviate from the guidelines were studied. Reasons to deviate from the guidelines were: insufficiently informed about the content of the guidelines, (partly) disagreement with the content of the guidelines and not being convinced about the added value of the guidelines. These results suggest that pediatricians seem to rely more on their clinical expertise in diagnosing children with CAP, rather than relying on existing evidence-based guidelines.

Celiac disease

The second part of this thesis focuses on two aspects of celiac disease (CD) as one of the possible causes of CAP in children, since excluding CD as a cause of CAP in children is a key element during the diagnostic traject. Through the past decades, also in the Netherlands, awareness for CD has grown and the application of highly sensitive and specific serological tests for CD in (often) asymptomatic- or genetically predisposed individuals has led to an increased number of detected cases with an altered spectrum of clinical symptoms during clinical presentation of CD. The clinical spectrum seems to have shifted from the traditional picture of failure to thrive, distended abdomen and chronic diarrhea towards display of more atypical, often extra-intestinal symptoms.

Whether or not this alteration in clinical spectrum is also present amongst the Dutch pediatric population has not been examined in over more than a decade. It could indicate that clinicians should be aware to test for CD more often in children not only with intestinal symptoms but also in children with extra-intestinal symptoms. The other important aspect in treating children with CD lies within the follow-up. Due to the nature of CD, children with CD are susceptible to various vitamin and mineral and deficiencies, not only at diagnosis, but also if they are not fully compliant to a gluten free diet (GFD), during the years after diagnosis. Therefore, one would expect guidelines regarding follow-up of vitamin and mineral deficiencies. It seems rather peculiar that international guidelines regarding follow-up of pediatric CD are lacking and provide no further statement regarding follow-up in terms of frequency and duration of outpatient visits and biochemical and serological measurements in the latest updated guidelines. However, there are dated Dutch guidelines available for follow-up of pediatric CD which do provide some statements regarding outpatient visits, and periodic biochemical- and serological measurements. So far, guideline adherence and clinical relevance of recommended laboratory tests during follow-up of these dated guideline has not been examined.

First, in **Chapter 3** we performed a study investigating the current clinical spectrum of pediatric CD. We retrospectively analyzed children diagnosed with CD between 2007 and 2017, based upon the 2005 (2) or 2012 (3) European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) guidelines, for presenting clinical symptoms and added a matched control group for age at diagnosis, sex and period of presentation with negative CD serology to investigate if certain complaints are more pathognomonic for CD. We found 105 new cases of CD with a calculated incidence of 21.09/100.000 inhabitants under 18 years. About 40% were infants and toddlers, predominantly presenting with gastrointestinal symptoms. Primary school children and high-school children had more often atypical symptoms and non-gastrointestinal symptoms than infants and toddlers. The most common presenting symptoms of CD were recurrent abdominal pain and distended abdomen, but the classical triad of CD symptoms occurred in only 32.6% of patients, predominantly in infants and toddlers. The clinical spectrum shifted from a classical presentation in almost 90% of toddlers to an atypical presentation in 40-50% of primary school- and high-school children.

Second, in **Chapter 4** we performed a study which investigated guideline adherence amongst pediatricians treating children with CD and the clinical relevance and yield of the laboratory parameters suggested for routine biochemical follow-up. We found that strict guidelines adherence amongst pediatricians in follow-up of pediatric CD is low and that the clinical relevance of the suggested routine laboratory tests is limited. This underlines the increasing notion that the Dutch guidelines on follow-up of CD should be refined and evidence-based guidelines are warranted.

Dientamoeba fragilis

The third part of this thesis focuses on *Dientamoeba fragilis* as a potential cause of CAP in children. Despite its first description over 100 years ago there still is a lack of consensus regarding the potential pathogenicity of this anaerobe flagellate protozoa. Possible interactions with the gut microbiota by the parasite or the possibility of different virulent strains remain unidentified. Since the presence of *D. fragilis* in feces is characterized by a wide spectrum of gastro-intestinal symptoms to asymptomatic carriership, its ability to cause gastro-intestinal disease, remains debatable. Even more conflicting is that the large series of scientific reports that provided support *D. fragilis* to be a potential pathogen are opposed by a single, and only, conducted randomized controlled trial regarding the effect of antibiotic treatment on gastrointestinal symptoms caused by *D. fragilis* that showed no beneficial effect of antibiotics over placebo. As such, this lack of consensus amongst clinicians regarding the potential pathogenicity of *D. fragilis* has led towards significant heterogeneity amongst clinicians in clinical practice.

In **Chapter 5** we performed a survey amongst Dutch physicians responsible for treating children with chronic abdominal pain regarding the current clinical attitude towards *D. fragilis*. We found significant heterogeneity in clinical practice amongst Dutch physicians regarding the diagnostic- and therapeutic approach of *D. fragilis* in children. Despite the debatable pathogenicity of *D. fragilis* 80% of responding physicians considered *D. fragilis* a potential pathogen. However, only a small proportion of physicians regularly performed diagnostic tests for *D. fragilis*. The diagnostic modality of preference was a fecal polymerase chain reaction (PCR) and when a positive result for *D. fragilis* was found 63% of physicians prescribed metronidazole as antibiotic of first choice.

Second, in **Chapter 6** we performed a study in which we aimed to investigate whether concomitant alterations in gut microbiota, which have been described in other parasitic infections, may be associated with gastro-intestinal symptoms in *D. fragilis*. We found high prevalence of *D. fragilis* in feces of our healthy asymptomatic control population but microbiota of children with *D. fragilis* and gastrointestinal symptoms did not differ significantly in terms of composition and diversity compared to asymptomatic controls. Both on phylum and species level. Based on our results we concluded that the intestinal microbiota does not play a key role in the presence of clinical symptoms in children with *D. fragilis*.

Third, in **Chapter 7** we performed a systematic review which aimed to evaluate the relationship between eradication of *D. fragilis* and resolution of symptoms to evaluate the strength of evidence that *D. fragilis* in symptomatic children warrants antibiotic treatment. Included studies were of low quality of evidence due to small studied groups of pediatric patients and significant heterogeneity amongst studies. Most studies focused on abdominal pain and diarrhea as reported gastro-intestinal symptoms.

Eradication rates of *D. fragilis* and resolution of symptoms varied widely between the various prescribed antibiotic regiments. Paromomycin proved to have the highest *D. fragilis* eradication rate and clioquinol proved to have the highest success rate in resolution of symptoms. But higher eradication rates were not associated with a higher rate of resolution of symptoms which could indicate that *D. fragilis* may not be the causative factor for the displayed symptoms. We concluded that testing for *D. fragilis* in pediatric patients with persistent abdominal pain and diarrhea, and subsequent treatment, should only be performed when other causes are excluded. Furthermore, we concluded that future studies regarding causality between the presence of gastro-intestinal symptoms and *D. fragilis* and regarding eradication of *D. fragilis* after antibiotic treatment are warranted.

REFERENCES

1. Tabbers M, Benninga M, Venmans L, Rutten J, Korterink J. NVK Richtlijn functionele buikpijn bij kinderen (2015). 2015.
2. Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S, et al. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr. 2005;40(1):1–19.
3. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease. J Pediatr Gastroenterol Nutr. 2012;54(1):136–60.

9

General discussion and future perspectives

This thesis focused on diagnostic- and therapeutic dilemma's in daily clinical practice in children with chronic abdominal pain (CAP). The main focus was on celiac disease (CD) and *Dientamoeba fragilis* (*D. fragilis*) as potential organic causes for CAP. Aims of this thesis included the evaluation of guideline adherence amongst clinicians treating children with CAP, to investigate the current clinical spectrum of pediatric CD and the clinical relevance of routine biochemical tests during follow-up. Furthermore, the potential role of *D. fragilis* as cause of CAP in children was evaluated, including microbial studies and the assessment of clinicians' diagnostic and therapeutic approach.

Diagnostic work-up in children with chronic abdominal pain

Clinical decision making in children with CAP is a complex diagnostic process combining evidence-based medicine and clinical expertise. This diagnostic process is influenced by various patient-, clinician- and disease-related factors, which cannot all be incorporated in clear-cut, evidence-based guidelines for children with CAP. The vast majority of children with CAP don't have an organic cause for their symptoms, and it may be challenging for clinicians to identify the small proportion of children who do have an organic cause for CAP. The proportion of children without an organic cause for CAP are diagnosed with a "functional abdominal pain disorder" according to the Rome-IV criteria (1). These criteria state that a functional abdominal pain disorder is diagnosed when, amongst other reasons, no other medical condition can fully explain CAP after appropriate evaluation. In order to exclude the relatively small proportion of somatic diseases able to cause CAP in children, the Dutch clinical guideline for functional abdominal pain disorders recommends to perform limited additional diagnostic tests (2). This includes C-reactive protein, a complete blood count, celiac disease (CD) serology and, when inflammatory bowel disease (IBD) is suspected, fecal calprotectin. In the study described in **Chapter 2** we aimed to evaluate guideline adherence amongst Dutch pediatricians in diagnosing children with CAP without the presence of "red flags", such as weight loss, gastrointestinal bleeding, vomiting, chronic diarrhea, unexplained fever, joint pain, jaundice or a positive family history for IBD, CD or familial Mediterranean fever. In this study we observed relatively low guideline adherence and large intra- and inter-observer variation amongst pediatricians regarding the amount and content of requested diagnostic tests in children with CAP. Interestingly, the majority of pediatricians performed more diagnostic tests than recommended by the Dutch 2015 guideline regarding pediatric functional abdominal pain disorders (2). This guideline recommends not to perform additional blood tests such as serum liver- or kidney function, additional urine analysis, additional helicobacter pylori tests and abdominal ultrasounds in children with abdominal pain without "red flags" (2). An additional fecal calprotectin sample is only advised when there are signs compatible with IBD (2). We observed that 40% of pediatricians performed serum liver- or kidney function, 16% urine analysis, 21% a *Helicobacter pylori* test, 46% a fecal calprotectin sample and 19% an abdominal ultrasound in children with abdominal pain without "red flags", respectively. However, since the studied population in our study in **Chapter**

2 were exclusively children with CAP without "red flags" these diagnostic tests were abundant. This performance of additional tests leads to an increase in health care costs, an increase in unexpected- or incidental findings, which may consequently lead to even more additional tests, additional costs and possibly to anxiety in both children and their parents. Our results suggest that, despite the availability of a well-defined CAP guideline, adherence by pediatricians to the diagnostic recommendations made by the guideline is moderate. This has led to the execution of more additional diagnostic testing in children with CAP without 'red flags' than recommended by the Dutch 2015 guideline (2). These findings are in line with a recent multi-center survey study which showed that only 50% of the interviewed Dutch pediatricians were adherent to management guidelines in treating functional abdominal pain in children (3). Remarkably, self-reported guideline adherence was 85%. Both studies demonstrate a moderate adherence to the current CAP guideline in terms of additional diagnostic tests and treatment. This highlights the importance of proper implementation, dissemination and finally, evaluation of constructed guidelines.

Guideline adherence is influenced by numerous factors which involve construction, implementation and dissemination of a guideline and the intrinsic motivation of clinicians to read a guideline and adhere to it. In order to increase guideline adherence, it is essential to understand the driving factors that influence this process. Understanding these driving factors may help to construct specific interventions that are capable to improve guideline adherence. The first critical factor influencing guideline adherence involves the construction of a guideline itself and its recommendations. Clear constructed guidelines are developed by a multidisciplinary panel of experts, which aim to identify relevant subgroups of patients and compare several care options and their clinical outcomes. Quality of evidence and strengths of recommendations are provided and need to be revised or renewed when new evidence becomes available. Conflicts of interest, potential bias and other distortions should be minimized and transparent and the whole process must be prone to peer-review to increase trust and credibility (4). When these indicators are not met during construction of guidelines, newly constructed guidelines may be considered inapplicable to patients or as reducing clinician autonomy, which subsequently leads to non-adherence by clinicians (5,6). However, we did not investigate if these indicators were met in this thesis and therefore we suggest to investigate these indicators in future studies.

The second critical factor is the process of guideline implementation and dissemination. Eventually, clinicians cannot adhere to guidelines they do not know about. Haskell et al demonstrated that dissemination of new clinical guidelines rarely is sufficient to alter current practice and that targeted interventions aimed at behavioral changes may improve compliance (7). New clinical guidelines can be promoted by means of online advertisement, publication in medical journals, scientific meetings, promotion during grand rounds and could be presented as product samples in the form of summaries,

handouts or flyers (8). Furthermore, as new guidelines are implemented, local staff- or educational meetings can be performed in local hospitals in order to promote and educate clinicians regarding new guidelines and to emphasize the benefits of using them.

The third critical factor concerns the intrinsic motivation of clinicians to read guidelines and adhere to them. There are many potential reasons why clinicians could resist to adhere to guidelines. Most reported reasons include inapplicability to the patient, inability to reconcile patient preferences with guideline recommendations, lack of outcome expectancy and lack of agreement with guideline recommendations (4,9). All complex issues which need to be addressed when constructing and implementing evidence-based guidelines. For example, by means of surveys or audits.

The fourth critical factor is related to external factors. For instance, during the start of the global covid-19 pandemic, there was an enormous amount of pressure by governments and medical federations in order to quickly construct and implement treatment guidelines for covid-19 in order to improve treatment outcome, quality of care and minimize heterogeneity in clinical practice. Finally, in order to improve the quality of constructed guidelines, it is essential that guidelines are evaluated frequently. A process which must cover diagnostic performance and applicability in daily clinical practice. Unfortunately, evaluating and updating guidelines is a time-consuming and intensive process, which can be partly overcome by updating smaller parts of available guidelines. If we translate these factors to the pediatricians studied in **Chapter 2**, these pediatricians reported that their main reasons to deviate from the guideline included feelings of being insufficiently informed about the guideline, disagreement with the content of the guideline and not being convinced of the added value of the guideline. It should be emphasized that this study included only 8 pediatricians from one medical centre in the Netherlands with a varying amount of experience. Therefore, firm conclusions regarding reasons for limited guideline adherence in diagnosing children with CAP could not be drawn

In 2021, a new Dutch guideline for functional abdominal pain disorders for children aged 4-18 years was published (10). In children with CAP without 'red flags' this updated guideline suggests to perform a complete blood count, C-reactive protein and trans-tissue glutaminase type 2 (TG2A) antibodies to rule out celiac disease. This updated guideline states that there is no indication to perform urine analysis, *Helicobacter pylori* tests, radiologic examinations and endoscopy in the absence of 'red flags'. Fecal examination for *Giardia lamblia* can be considered if a child also has diarrhea in combination with CAP as presenting symptoms. These recommendations are largely comparable to the previous 2015 guideline, to which we observed low guideline adherence amongst pediatricians in a single center (2). It is unknown whether adherence to the diagnostic recommendations of the CAP guideline is also low on a nation-wide

scale and if the provided reasons not to adhere to the diagnostic recommendations are also valid for pediatricians on a nation-wide scale. Furthermore, it is also unknown which steps for national and local implementation and dissemination of the 2021 guideline were taken by the Dutch Pediatric Society. Usually, when a guideline is updated, it is published online, various scientific- and educational meetings are planned in local hospitals and short summaries of the content and recommendations are promoted online and on social media to its users. But we did not investigate these factors. The findings of our study suggest that there are several factors that influence guideline adherence by clinicians diagnosing children with CAP. Future studies are warranted which aim to evaluate which factors influence the intrinsic motivation of clinicians to adhere to the recommendations made by the guideline in order to increase guideline adherence and ultimately, to improve quality of care, minimize healthcare costs and minimize heterogeneity in clinical practice.

Celiac disease

Due to the relatively high prevalence of CD in the Western population of approximately 0.5-1% and the association between CD and CAP in the pediatric population, current guidelines recommend performing CD serology in children with CAP (2,13–17). Through the past decades the clinical spectrum of pediatric CD, in terms of the type of symptoms at diagnosis, has changed significantly due to increased awareness for CD amongst clinicians and the availability of highly sensitive and specific serological tests (17–24). This has contributed to the idea of CD as an iceberg conception (14,21,25).

Chapter 3 describes that the current clinical spectrum at diagnosis of pediatric CD in the Netherlands is shifting towards more atypical, often extra-intestinal symptoms. The classical presentation of pediatric celiac disease with a distended abdomen, failure to thrive and chronic diarrhea has gradually decreased from approximately 70% between 1975-1990 to 45% between 1993-2000 (17,26). Our study showed a further decrease to approximately 30% in the pediatric population in children below 4 years of age. The phenomenon of a changed clinical spectrum towards a more atypical presentation was particularly observed in children older than four years of age. We also observed that the average age of first presentation of the disease amongst the Dutch pediatric population has increased from approximately 3 to 6 years. In children below 4 years of age the classical triad of symptoms (chronic diarrhea, distended abdomen and failure to thrive) still is the most common form of presentation in up to 90% of our studied population. We hypothesize that the latter is due to an increased awareness amongst clinicians for CD as a potential cause of a large variety of symptoms in the pediatric population and due to certain changes in our dietary habits. The increased age of first presentation of the disease can partly be explained by these previous factors, but also partly by using highly sensitive and specific serological tests in both symptomatic and asymptomatic individuals, in individuals with a genetic predisposition, or in individuals with a positive family history for CD by means of screening. Furthermore, the prevalence

of CD in the pediatric population has increased substantially in the Netherlands through the past decades from 4.7/100.000 inhabitants in 1980 to 12.3/100.000 inhabitants in 2010 (26,27). In our study we found a prevalence of 21.1/100.000 inhabitants in 2017. However, since this was a single-center retrospective study these results cannot be compared and extrapolated to national data, but do indicate that the prevalence of pediatric CD is increasing. This has contributed to the concept of CD as an iceberg conception (14,21,25). The increase in prevalence of CD and the alteration in the clinical presentation of pediatric CD, with an increase in extra-intestinal symptoms, justifies clinicians to lower the threshold to test for CD in children with atypical or extra-intestinal symptoms, such as fatigue and iron deficiency anemia. Especially if these symptoms are present in combination with intestinal symptoms such as recurrent abdominal pain and chronic diarrhea. We also found that in approximately 10% of the pediatric patients with CD, the CD serology only became positive after at least one repeated test. Most children with CD were female and of pre-school age with numerous subjective complaints at presentation. This demonstrates that children can display clinical symptoms that prejudge positive CD serology. However, it is also known that positive CD serology can prejudge the onset of clinical symptoms. Based on the findings in this study, it seems advisable to repeatedly test for CD at least once after 1 to 2 years in children with symptoms compatible with CD but previous negative CD serology, especially in those with an increased risk for the development of CD (genetically predisposed and children with a positive family history). However, since our study was a single-centre study which included a limited number of 105 newly diagnosed children with CD, these data should be interpreted cautiously. Firm recommendations cannot be made until these findings are explored in larger future studies. If the clinical suspicion for CD remains, despite repeatedly negative CD serology, HLA-DQ2 and DQ8 analysis can be considered to try to rule out CD as potential cause. If a CD phenotype is present than a referral to a pediatric gastroenterologist for a possible esophagogastroduodenoscopy with histological evaluation of duodenal biopsies can be considered, since a histological diagnosis remains the golden standard for CD (28,29). Current 2020 European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines for the diagnosis of CD advise not to perform HLA-testing in children in whom the other criteria for CD diagnosis are fulfilled (ie. TG2A-levels >10x upper limit of normal in combination with positive anti-endomysial antibodies (EMA), confirmed in a second blood sample or TG2A-levels <10x ULN with positive duodenal biopsies)(30). HLA-testing should be considered in “high-risk” groups, such as children with type 1 diabetes mellitus or in 1st degree relatives or in Down syndrome to rule out the possibility of CD. Furthermore, HLA-testing can be used to rule out CD in certain cases where clinical suspicion remains despite negative CD serology. However, the decision to perform HLA-testing should be reserved to pediatricians or pediatric gastroenterologists. If no risk alleles are found during HLA-testing, CD is very unlikely (30). Timely recognition of children suspected of CD, an accurate diagnosis and treatment with a strict gluten free diet (GFD) are key

factors to reduce the risk of social- and physical growth retardation and can prevent further complications such as osteoporosis and vitamin- and mineral deficiencies.

In **Chapter 4**, we discussed the clinical relevance of laboratory investigations during follow-up of pediatric CD and adherence to the Dutch national guideline amongst Dutch pediatricians. The Dutch guideline recommends annual measurement of hemoglobin, hematocrit, mean corpuscular volume, folic acid, vitamin B12, calcium, alkaline phosphatase, iron levels (serum iron or serum ferritin) and trans-tissue glutaminase type 2 antibodies (TG2A) in GFD-adherent pediatric patients during follow-up (31). In GFD non-adherent patients the guideline recommends to increase the frequency of these annually recommended laboratory parameters. Our study in **Chapter 4** demonstrated that Dutch pediatricians request large amounts of laboratory investigations during follow-up of children with CD, of which most requested laboratory investigations were parameters not suggested by the guidelines. A total of 1570 laboratory investigations were performed, of which 45.4% (713/1570) was in compliance with the parameters recommended by the Dutch guideline. Interestingly, amongst these 713 laboratory investigations, relevant deviations were found in only 5%. This low proportion of deviations in laboratory investigations can be explained because GFD is the only available, but very effective treatment for CD. Consequently, absolution from gluten commonly results in complete restoration of gut function and subsequently normalization of laboratory results, concomitant deficiencies and recovery from other complications. We found deficiencies in serum iron/ferritin (7%), hemoglobin (7%) vitamin B12 (7.7%), folic acid (0%) and calcium (0%) in our pediatric population during follow-up. These results also correspond with a previous study in a Dutch pediatric CD population by Wessels et al regarding complementary serological investigations during follow-up of CD (32). During a 5-year period, they reported only mild deficiencies in a GFD-adherent pediatric population in serum iron, vitamin B12 and folic acid in only 5-10% and no deficiencies in serum calcium. In general, there is limited information regarding coincidence of vitamin- and mineral deficiencies in children treated with CD, largely due to studies in small and heterogeneous populations which only focused on certain nutritional deficiencies mainly during diagnosis (33–36). However, it can be concluded that the frequency in which nutritional deficiencies were detected in our population and the population described by Wessels et al were similar or even less frequent than described in previous studies (32–36). Furthermore, previous studies have shown that hypocalcemia, folic acid deficiency and vitamin b12 deficiency were present in only a very small proportion of children and that normalization of these deficiencies occurred in almost all patients after a year of compliance to GFD, even without supplements (32,37). These findings, challenge the clinical relevance of annual measurement of the parameters suggested by the Dutch national guideline in GFD-adherent children during follow-up (31). When this specific section of the Dutch national guideline regarding additional laboratory investigations during follow-up is updated, the clinical relevance and diagnostic yield of these additional laboratory tests should

be critically assessed. Possibly, the recommendations regarding additional laboratory investigations should be updated.

During the inclusion period of our study between 2017 and 2019 there were no available international guidelines regarding management and follow-up of pediatric CD. However, only recently, in September 2022 the ESPGHAN published a position paper on the management and follow-up of pediatric CD (38). They suggest to perform the first follow-up visit after diagnosis within 3-6 months and follow-up visits every 6 months thereafter until normalization of TG2A antibodies. After normalization of TG2A antibodies follow-up should be continued every 12-24 months thereafter. In case earlier advice is needed, symptoms persist despite adherence to GFD, concerns are present on how a family is coping with GFD, or if there are ongoing issues with growth, bloodwork can be performed earlier (38). During follow-up visits the guideline recommends to evaluate children for gastrointestinal and extra-intestinal signs and symptoms, and to obtain anthropometric measurements and growth parameters. At time of diagnosis, measurement of TG2A levels, complete blood count, micronutritional status (eg, hemoglobin, iron, vitamin B12 and vitamin D levels) and alanine aminotransferase (ALT) should be performed. Any abnormalities should be followed and deficiencies should be corrected until normalization. If no abnormalities are present at diagnosis, routine additional diagnostic tests are not advised during follow-up by this position paper (38). Screening for thyroid disease may be considered during follow-up after clinical evaluation at the discretion of the clinician (38). The recommendations made the 2022 ESGPHAN position paper for follow-up of GFD-adherent children during treatment of CD are somewhat in line with the findings of the study by Wessels et al and our study described in **Chapter 4** (32,38).

In general, the pediatricians we studied performed too many routine additional laboratory investigations during follow-up of pediatric CD, whereas the clinical relevance of these routine laboratory investigations seems limited. Whether or not this is also true for pediatricians on a national, or even international scale, cannot be answered by our study since we only studied pediatricians in a single center in the Netherlands. However, this were pediatricians of variable age and with a variable amount of clinical expertise and therefore could potentially represent a larger population of pediatricians, but this should be investigated in future studies. For assessment of the clinical relevance of additional laboratory investigations during follow-up, a future large multi-center cohort study is warranted, in which all children newly diagnosed with CD are monitored during a two-to-three year period since normalization of deficiencies and TG2A-levels generally seem to occur within two years after initiation of GFD (39–41). Based on the study by Wessels et al described above, the recently published position paper by ESPGHAN and our study described Based on our findings in **Chapter 4**, we suggest to perform laboratory measurements at diagnosis that evaluate the presence of malabsorption, including a complete blood count, iron status, folic acid, vitamin B12, calcium, vitamin D

and ALT (32,38). If deficiencies are found they should be treated and monitored during follow-up until normalization. If no deficiencies are found at diagnosis, only TG2A-levels should be monitored annually until normalization with an extra measurement 6 months after diagnosis in order to evaluate GFD-adherence. Finally, GFD- adherence should be monitored closely by a dietician and anthropometric data should be recorded during every follow-up visit. Gathering this data on a large scale should make it possible to detect the frequency of occurrence of these deficiencies in GFD-adherent and non-adherent children and should unravel the role of frequent additional testing for TG2A and deficiencies. This could lead to a reduction in laboratory investigations, health care costs and a reduction in the number of visits to outpatient clinics of children and their care givers. Ultimately, this could even lead to home-monitoring of GFD-adherent children with CD(42).

Dientamoeba fragilis

Over 100 years ago Jepps and Dobell described the first cases of *D. fragilis* in British soldiers with diarrhea and abdominal pain (43). Since then, a vast amount of scientific reports support *D. fragilis* to be a potential pathogen or to be a harmless commensal. Its ability to cause gastrointestinal disease therefore remains debatable. Described clinical presentation varies widely from asymptomatic carriership to a wide spectrum of gastrointestinal complaints, of which the most frequently reported symptoms are abdominal pain and diarrhea. Symptoms which are, however, common in the pediatric population. Most clinicians are tempted to perform an extensive diagnostic work-up in children with these complaints in order to exclude somatic disorders, including microscopic and molecular diagnosis of intestinal parasites, including *D. fragilis*, regardless of guidelines (2).

The survey in **Chapter 5** showed that there is significant heterogeneity amongst Dutch clinicians, who are responsible for the care of children with gastro-intestinal complaints regarding their attitude towards pathogenicity of *D. fragilis* and applied diagnostic- and therapeutic approach. This survey well displayed the clinical consequences when the pathogenicity of a micro-organism is debatable, high quality randomized controlled trials regarding treatment are absent and multiple treatment guidelines constructed by different professions with different strategies are available. This heterogeneity in clinical practice leads towards an increase in health care costs, execution of unnecessary diagnostic tests with potential false-positive or false-negative results and unnecessary anxiety in children and their parents.

Chapter 6 demonstrated that the presence of *D. fragilis* in the intestine of children does not lead to significant alterations in the intestinal microbiota. We aimed to evaluate intestinal microbiota in children with gastrointestinal symptoms and a positive fecal PCR for *D. fragilis* and compared them with the intestinal microbiota of healthy controls matched for age and gender. We were not able to find significant alterations

in the intestinal microbiota in terms of composition and diversity, but we did find a high percentage of 84% of *D. fragilis* carriership in the healthy control population. High prevalence of *D. fragilis,* in up to 50% of asymptomatic Dutch children, has also found been in other Dutch studies which used real-time PCR as the diagnotic method. Such a high prevalence of *D. fragilis* in an asymptomatic pediatric population could be interpreted as illustration of the non-pathogenic character of this parasite in the majority of cases. However, the stools of the studied children were only examined for *D. fragilis* by means of a fecal PCR and not by light microscopy to detect the trophozoite state of the parasite which could be considered as proof for true replication of *D. fragilis* in the intestine and could have led to an overestimate of the real prevalence in these cohorts. The abcense of alterations in intestinal microbiota in terms of composition and diversity indicates that intestinal dysbiosis is not the driving factor in causing gastrointestinal symptoms in children with *D. fragilis.* However, the availability of numerous scientific reports that document resolution of symptoms after treatment of *D. fragilis* with antibiotics and several promising rodent models trying to fulfill the Koch's postulates at least do provide some evidence that *D. fragilis* could be seen as a pathogen. But, most published scientific reports were retrospective studies which could have led to significant selection bias and significant placebo effect in reporting the outcomes of these studies. Therefore these results must be cautiously interpreted.

The systematic review in **Chapter 7** showed that treatment of *D. fragilis* with metronidazole, paromomycin or clioquinol leads to resolution of gastrointestinal symptoms in 35-90% of children with varying rates of eradication of the parasite. As such, paromomycin proved to have the best eradication rate and clioquinol proved to have the best success rate in eradication of symptoms. This suggests that, at least in a subset of children, treatment of *D. fragilis* could be beneficial, but these findings are all based upon relatively small, studied populations of children with large heterogeneity amongst the studies, of which most were observational studies and had a small number of observed events. The only conducted randomized controlled trial regarding treatment of *D. fragilis* in children showed no beneficial effect of treatment with metronidazole over placebo in resolution of symptoms (44). Although this study had some serious flaws, the results of this study limits the strength of evidence that *D. fragilis* in the pediatric population warrants treatment (43). Most studies included in the review studied abdominal pain and diarrhea as predominant symptoms. These symptoms are also predominant symptoms in the spectrum of functional abdominal pain disorders (FAPDs) and the children studied in these observational studies could have very well had one of these FAPDs.

The key questions remain, do clinicians need to test for *D. fragilis* in children with CAP and, if a positive fecal PCR for *D. fragilis* is found, does every child need to be treated with antibiotics? When the available evidence is weighted and considered, the answer to both of these questions is "no". The answer to these questions is defined by three critical

elements. First, the element of selection of children in whom to test for *D. fragilis.* There is no place to test for *D. fragilis* if a child only has CAP as a presenting symptom. Our systematic review in **Chapter** 7 and a previous study by Maas et al demonstrated that the vast majority of patients with protozoal infections, including *D. fragilis,* at least had a combination of gastrointestinal symptoms with CAP (45). If there is a combination of gastrointestinal complaints, such as for instance a combination of CAP and diarrhea or bloating, clinicians first have to rule out other potential diagnosis, such as CD or inflammatory bowel disease. Even then, clinicians should be very reserved to test for *D. fragilis* since the pathogenicity of *D. fragilis* and its ability to cause gastrointestinal complaints is still highly debatable.

The second important element, when clinicians do decide to test for *D. fragilis,* is how to make a diagnosis? Nowadays, a fecal-PCR is used to confirm the presence of a parasite. PCR is a reliable diagnostic tool with high sensitivity and specificity which can detect even the smallest amounts of parasite. However, one of the distinct disadvantages of this technique is that it is not able to differentiate between the presence of actively dividing parasites (or trophozoites) as a distinct feature of proof of true replication and inactive forms or only small amounts of *D. fragilis* DNA. This is where light microscopy (LM) comes into play. This technique can identify these trophozoites in stained stools and is able to quantify the parasitic load by means of the number of parasites per microscopic field. It therefore seems recommended to use a combination of the two, by means of a dual-feces-test, to establish the diagnosis. If LM is not available, PCR with a quantification of the PCR result could be used as an alternative.

The third and final element is the selection of children that could benefit from treatment and the selection of an antibiotic treatment regimen. When clinicians do decide to test for *D. fragilis*, after excluding other causes, and a positive fecal PCR is found (preferably in combination with LM), treatment should be discussed with the child's parents or care givers. It should be emphasized that the potential pathogenicity of *D. fragilis* is still under debate, that the causality between the presence of the parasite and the child's symptoms is questionable and that treatment with an antibiotic not always leads to resolution of symptoms. In addition our study in **Chapter 6** demonstrated that *D. fragilis* is present in a significant part of asymptomatic controls which indicates *D. fragilis* to be a commensal. Furthermore, treatment with an antibiotic is not harmless considering the potential disturbance of the gut microbiota which could lead to alterations in intestinal permeability, gut motility, intestinal inflammation and even irritable bowel syndrome (46). When both parents and clinician decide to initiate treatment, paromomycin or clioquinol seem to be the best available treatments for *D. fragilis*. Treatment with metronidazole seems inferior to these two antibiotics with a lower eradication rate and slightly lower rate of resolution of symptoms. Also metronidazole has a wider spectrum of antibiotic activity which could lead to large scale alterations of the anaerobic

intestinal microbiota and thus has more potential side effects than both paromomycin and clioquinol makes this treatment third choice.

Efficacy of treatment, by means of eradication of the parasite, should be analyzed by means of control of eradication after treatment. Preferably, control of eradication should be performed the day after the end of the course of antibiotic treatment (i.e. with paromomycin and clioquinol at day 8). However, firm recommendations cannot be made until randomized, double-blind controlled trials are performed using several registered antibiotics for treating *D. fragilis*. Until then clinicians should be restrictive in performing diagnostic tests for *D. fragilis* in children with a combination of gastrointestinal complaints and even more restrictive in performing treatment when only a positive fecal-PCR for *D. fragilis* is found.

Finally, in order to solve the clinical debate regarding the potential pathogenicity of *D. fragilis,* future studies are warranted to elucidate the mechanism of *D. fragilis* intestinal infection and to evaluate the efficacy of treatment of *D. fragilis* with antibiotics. A previous study by Caccio demonstrated that there are two different genotypes of *D. fragilis* present with a strong predominance of genotype 1, but that their individual virulence and ability to cause gastrointestinal symptoms is unclear (47). Future studies should aim at investigating different virulent strains of *D. fragilis* and at possible interactions between *D. fragilis* and the intestinal commensal flora. Not only the interactions between *D. fragilis* and bacteria should be investigated, the interactions between other commensal inhabitants such as intestinal yeasts should be investigated as well. A large double-blind randomized controlled trial in children with well-defined clinical symptoms, appropriate diagnostic testing by means of the combination of light microscopy and fecal-PCR, and follow-up should be performed. In order to try unravel the role of the gut microbiome, it would be an interesting option to investigate the gut microbiota in every child before treatment with antibiotics, directly after treatment with antibiotics and 6-8 weeks thereafter. Furthermore, various treatment regiments should be explored. Comparing paromomycin and clioquinol with placebo is mandatory and as an alternative option metronidazole could also be investigated. Paromomycin should be dosed 25-35mg/kg per day in three daily doses for 7 days and clioquinol should be dosed in 15mg/kg per day in three daily doses for 7 days. The efficacy of antibiotic treatment needs to be investigated in two ways, by means of eradication of *D. fragilis* immediately after antibiotic therapy and resolving of gastrointestinal complaints approximately 6-8 weeks after treatment with antibiotics.

Conclusion

CAP is a significant and frequent complaint in children, which can be puzzling for both children and their parents, but also for clinicians. The main challenge for clinicians remains to identify the small proportion of children with an underlying organic cause for CAP. Aims of this thesis included the evaluation of guideline adherence amongst

clinicians diagnosing children with CAP. Our results provide some indication that adherence to the Dutch CAP guideline by clinicians is a complex process influenced not only by careful construction and proper implementation and dissemination, but also by the intrinsic motivation of clinicians to adhere to them. An updated version of the Dutch CAP guideline was published with nearly the same recommendations for diagnosing CAP and various attempts for the implementation and dissemination were made. Therefore, improving the intrinsic motivation of clinicians to adhere to guidelines seems key to improve adherence. Hypothetically, this could be done by means of performing audits or surveys exploring factors why clinicians resist to adhere to guidelines despite clear construction, implementation and dissemination of the CAP guideline. Second, we aimed to investigate the current spectrum of pediatric CD and the clinical relevance of routine biochemical tests during follow-up. CD has a predominant role in CAP, but the current clinical spectrum has changed towards more display of extra-intestinal symptoms during diagnosis. This justifies clinicians to test for CD more often. Guideline adherence amongst Dutch pediatricians to the Dutch CD guideline for follow-up is low. This dated guideline recommends to much routine laboratory test during follow-up and should be updated with the recommendations made by the 2022 ESPGHAN position paper. Third, we aimed to evaluate the role of *D. fragilis* in CAP in children. Since the pathogenicity of DF is not confirmed and highly debated , clinicians should be restrictive when to test for *D. fragilis* and should only consider this in children with a combination of CAP with other gastrointestinal symptoms in whom other potential causes for CAP are excluded.

REFERENCES

1. 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.
2. Tabbers M, Benninga M, Venmans L, Rutten J, Korterink J. NVK Richtlijn functionele buikpijn bij kinderen (2015). 2015.
3. Gorka AM, Nauta F, Bijlsma MW, Taselaar P, Diederen K, Hol J, et al. Current treatment practice of functional abdominal pain disorders in children : A multicenter survey. Indian J Gastroenterol. 2022;(Online ahead of print).
4. Baron D, Metnitz P, Rhodes A, Kozek-Langenecker S. Clinical guidelines: How can we improve adherence and implementation. Eur J Anaesthesiol. 2017;34(6):329–31.
5. van der Weijden BM, Achten NB, Bekhof J, Evers EE, Berk M, Kamps AWA, et al. Multicentre study found that adherence to national antibiotic recommendations for neonatal early-onset sepsis was low. Acta Paediatr Int J Paediatr. 2021;110(3):791–8.
6. Niele N, van Houten MA, Boersma B, Biezeveld MH, Douma M, Heitink K, et al. Multi-centre study found that strict adherence to guidelines led to computed tomography scans being overused in children with minor head injuries. Acta Paediatr Int J Paediatr. 2019;108(9):1695–703.
7. Haskell L, Tavender EJ, Wilson CL, O'Brien S, Babl FE, Borland ML, et al. Effectiveness of Targeted Interventions on Treatment of Infants with Bronchiolitis: A Randomized Clinical Trial. JAMA Pediatr. 2021;175(8):797–806.
8. Gagliardi A, Brouwers M, Palda V, Lemieux-Charles L, Grimshaw J. How can we improve guideline use? A conceptual framework of implementability. Implement Sci. 2011;6(1):26.
9. Arts DL, Voncken AG, Medlock S, Abu-Hanna A, van Weert HCPM. Reasons for intentional guideline non-adherence: A systematic review. Int J Med Inform. 2016;89:55–62.
10. Rexwinkel R, de Bruijn C, Tabbers M. NvK richtlijn "Functionele buikpijn bij kinderen" 2021. 2021;
11. Rutten JMTM, Korterink JJ, Venmans LMAJ, Benninga MA, Tabbers MM. Richtlijn 'Functionele buikpijn bij kinderen.' Ned Tijdschr Geneeskd. 2017;161:D781.
12. Samaan Z, Mbuagbaw L, Kosa D, Borg Debono V, Dillenburg R, Zhang S, et al. A systematic scoping review of adherence to reporting guidelines in health care literature. J Multidiscip Healthc. 2013;6:169–88.
13. Ludvigsson JF, Leffler DA, Bai JC, Biagi F, Fasano A, Green PHR, et al. The Oslo definitions for coeliac disease and related terms. Gut. 2013;62(1):43–52.
14. Csizmadia CG, Mearin ML, von Blomberg BME, Brand R, Verloove-Vanhorick SP. An iceberg of childhood coeliac disease in the Netherlands. Lancet. 1999;353(9155):813–4.
15. Rubio-Tapia A, Ludvigsson JF, Brantner TL, Murray JA, Everhart JE. The Prevalence of Celiac Disease in the United States. Am J Gastroenterol. 2012;107(10):1538–44.
16. Mustalahti K, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S, et al. The prevalence of celiac disease in Europe: Results of a centralized, international mass screening project. Ann Med. 2010;42(8):587–95.

17. Steens RFR, Csizmadia CGDS, George EK, Ninaber MK, Hira Sing RA, Mearin ML. A National Prospective Study on Childhood Celiac Disease in the Netherlands 1993–2000: An Increasing Recognition and a Changing Clinical Picture. J Pediatr. 2005;147(2):239–43.

18. Almallouhi E, King KS, Patel B, Wi C, Juhn YJ, Murray JA, et al. Increasing Incidence and Altered Presentation in a Population-based study of Pediatric Celiac Disease in North-America. J Pediatr Gastroenterol Nutr. 2018;65(4):432–7.

19. Barker JM, Liu E. Celiac disease: pathophysiology, clinical manifestations, and associated autoimmune conditions. Adv Pediatr. 2008;55:349–65.

20. Catassi C, Gatti S, Fasano A. The New Epidemiology of Celiac Disease. J Pediatr Gastroenterol Nutr. 2014;59(July):S7–9.

21. Fasano A. Clinical presentation of celiac disease in the pediatric population. Gastroenterology. 2005;128(4 Suppl 1):S68-73.

22. Khatib M, Baker RD, Ly EK, Kozielski R, Baker SS. Presenting Pattern of Pediatric Celiac Disease. J Pediatr Gastroenterol Nutr. 2016;62(1):60–3.

23. Roma E, Panayiotou J, Karantana H, Constantinidou C, Siakavellas SI, Krini M, et al. Changing pattern in the clinical presentation of pediatric celiac disease: a 30-year study. Digestion. 2009;80(3):185–91.

24. Shahraki T. Clinical Spectrum of Celiac Disease in Children in Sistan and Baluchestan Province. Arch Iran Med. 2016;19(11).

25. Nenna R, Tiberti C, Petrarca L, Lucantoni F, Mennini M, Luparia RPL, et al. The Celiac Iceberg. J Pediatr Gastroenterol Nutr. 2013 Apr;56(4):416–21.

26. George EK, Mearin ML, Van Der Velde EA, Houwen RH, Bouquet J, Gijsbers CF, et al. Low incidence of childhood celiac disease in the Netherlands. Pediatr Res. 1995;37(2):213–8.

27. Burger JPW, Roovers EA, Drenth JPH, Meijer JWR, Wahab PJ. Rising incidence of celiac disease in the Netherlands; an analysis of temporal trends from 1995 to 2010. Scand J Gastroenterol. 2014;49(8):933–41.

28. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin M, Ribes-Koninckx C, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition guidelines for diagnosing coeliac disease 2019. J Pediatr Gastroenterol Nutr. 2019;70(1):141–56.

29. Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S, et al. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr. 2005;40(1):1–19.

30. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. J Pediatr Gastroenterol Nutr. 2020;70(1):141–56.

31. CBO. Richtlijn Coeliakie en Dermatitis Herpetiformis [Internet]. Haarlem: Nederlandse Vereniging van Maag-Darm-Leverartsen; 2008. Available from: https://www.mdl.nl/files/richlijnen/richtlijn_Coeliakie_definitief.pdf

32. Wessels M, van Veen I, Vriezinga S, Putter H, Henri E, Maria H, et al. Complementary Serologic Investigations in Children with Celiac Disease Is Unnecessary during Follow-Up. J Pediatr. 2015;169:1–6.

33. Bonamico M, Vania A, Monti S, Ballati G, Mariani P, Pitzalis G, et al. Iron Deficiency in Children with Celiac Disease. J Pediatr Gastroenterol Nutr. 1987;6(5):702–6.

34. Botero-lopez J, Araya M, Parada A, Mèndez M, Pizarro F, Espinosa N, et al. Micronutrient Deficiencies in Patients With Typical and Atypical Celiac Disease. J Pediatr Gastroenterol Nutr. 2011;53(3):265–70.

35. Haapalahti M, Kulmala P, Karttunen TJ, Paajanen L, Laurila K, Mykka H, et al. Nutritional Status in Adolescents and Young Adults with Screen-Detected Celiac Disease. J Pediatr Gastroenterol Nutr. 2005;40(5):566–70.

36. Gokce S, Arslantas E. The changing face and clinical features of celiac disease in children. Pediatr Int. 2014;57(1):107–12.

37. Deora V, Aylward N, Sokoro A, El-matary W. Serum Vitamins and Minerals at Diagnosis and Follow-up in Children With Celiac Disease. J Pediatr Gastroenterol Nutr. 2017;65(2):185–9.

38. Mearin ML, Agardh D, Antunes H, Al-toma A, Auricchio R, Castillejo G, et al. ESPGHAN Position Paper on Management and Follow-up of Children and Adolescents With Celiac Disease. J Pediatr Gastroenterol Nutr. 2022;75(3):369–86.

39. Gidrewicz D, Trevenen C, Lyon M, Decker Butzner Ã. Normalization Time of Celiac Serology in Children on a Gluten-free Diet. J Pediatr. 2017;64(3):362–7.

40. Sansotta N, Alessio MG, Norsa L, Previtali G, Ferrari A, Guerra G, et al. Trend of Antitissue Transglutaminase Antibody Normalization in Children With Celiac Disease Started on Gluten-free Diet: A Comparative Study Between Chemiluminescence and ELISA Serum Assays. J Pediatr Gastroenterol Nutr. 2020 Jan;70(1):37–41.

41. Blansky BA, Hintze ZJ, Alhassan E, Leichtner AM, Weir DC, Silvester JA. Lack of Follow-up of Pediatric Patients With Celiac Disease. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2019 Nov;17(12):2603–4.

42. Vriezinga S, Borghorst A, van den Akker-van Marle E, Benninga M, George E, Hendriks D, et al. E-Healthcare for Celiac Disease—A Multicenter Randomized Controlled Trial. J Pediatr. 2018;195:154-160.e7.

43. Jepps MW, Dobell C. Dientamoeba fragilis n.g., n.sp., new intestinal amoeba from man. Parasitology. 1918;10:352–67.

44. Röser D, Simonsen J, Stensvold CR un., Olsen KEP, Bytzer P, Nielsen H V., et al. Metronidazole therapy for treating dientamoebiasis in children is not associated with better clinical outcomes: a randomized, double-blinded and placebo-controlled clinical trial. Clin Infect Dis. 2014;58(12):1692–9.

45. Maas L, Dorigo-Zetsma JW, de Groot CJ, Bouter S, Plötz FB, Van Ewijk BE. Detection of intestinal protozoa in paediatric patients with gastrointestinal symptoms by multiplex real-time PCR. Clin Microbiol Infect. 2014;20(6):545–50.

46. Canakis A, Haroon M, Weber HC. Irritable bowel syndrome and gut microbiota. Curr Opin Endocrinol Diabetes Obes. 2020 Feb;27(1):28–35.

47. Cacciò S. Acta Tropica Molecular epidemiology of Dientamoeba fragilis. Acta Trop. 2018;184:73–7.

10

Nederlandse samenvatting

Chronische buikpijn bij kinderen is een van de meest voorkomende aandoeningen bij kinderen en jongvolwassenen wereldwijd met hoge prevalentie onder de westerse bevolking. Ondanks dat het frequent voorkomt wordt slechts een klein percentage veroorzaakt door een chronische ziekte en de overige 90% van de kinderen voldoet aan de diagnostische criteria van zogeheten functionele buikpijn syndromen. Functionele buikpijn syndromen zijn aandoeningen waarbij er geen lichamelijke oorzaak kan worden vastgesteld voor de klachten. Veelal zit hier een psychische component in. Het diagnosticeren van dat kleine aantal kinderen met een chronische ziekte als oorzaak voor de chronische buikpijn is van groot belang vanwege de forse invloed op zowel de groei als de ontwikkeling van het kind. Echter kan het identificeren van deze kleine groep kinderen met een chronische ziekte ingewikkeld zijn voor (kinder)artsen wat veelal leidt tot het uitvoeren van veel diagnostiek. Duidelijke richtlijnen ontbreken hiervoor of worden onvoldoende nageleefd. Dit leidt veelal tot het inzetten van niet noodzakelijke diagnostiek, mogelijke vals-positieve of vals-negatieve uitslagen, oplopende kosten in de gezondheidszorg en tot uitslagen waarvan de klinische relevantie soms onduidelijk is. Wel duidelijk is dat, door het frequente voorkomen in de westerse bevolking, bij kinderen met chronische buikpijn te allen tijde op gluten allergie getest dient te worden vanwege de grote consequenties hiervan. Echter, is gedurende de afgelopen tientallen jaren het klinisch beeld van de symptomen waarmee kinderen met gluten allergie zich presenteren sterk veranderd. Een voorbeeld van wat onduidelijk is, is de rol van de parasiet *Dientamoeba fragilis* in het veroorzaken van maag-darm klachten bij kinderen. Dit heeft er toe geleid dat er forse veranderingen zijn opgetreden in hoe artsen omgaan met de diagnostiek en behandeling van kinderen met glutenallergie en *Dientamoeba fragilis.*

Middels dit proefschrift pogen wij een aantal zaken te onderzoeken: 1) het volgen van richtlijnen door kinderartsen die kinderen met chronische buikpijn of gluten allergie behandelen; 2) de verandering in het klinische beeld van kinderen met glutenallergie; 3) de relevantie en opbrengst van de voorgestelde diagnostiek in de huidige richtlijn glutenallergie; en 4) de rol van de parasiet *Dientamoeba fragilis* in het ontstaan van chronische buikpijn bij kinderen.

Diagnostiek bij kinderen met chronische buikpijn

Het eerste deel van dit proefschrift richt zich op het inzetten van diagnostiek bij het evalueren van kinderen met chronische buikpijn. Chronische buikpijn is een frequente reden voor een verwijzing van een kind of jongvolwassene naar een kinderarts. Het evalueren van kinderen en jongvolwassenen met chronische buikpijn kan ingewikkeld zijn om meerdere redenen: chronische buikpijn kan veroorzaakt worden door een grote diversiteit aan aandoeningen, jonge kinderen zijn nog onvoldoende in staat om hun klachten goed te beschrijven, ouders kunnen soms de klachten van hun kind moeilijk interpreteren en zowel ouders als artsen willen geen aandoeningen missen als oorzaak voor de chronische buikpijn. Al deze factoren gecombineerd kunnen leiden tot

onzekerheid bij artsen wat vervolgens leidt tot het uitvoeren van een grote diversiteit aan aanvullende onderzoeken. Dit leidt op zichzelf tot een grote heterogeniteit in de diagnostiek en behandeling van kinderen met chronische buikpijn en tot de uitvoering van nog meer aanvullende diagnostiek indien er onverwachte uitslagen gevonden worden. Om artsen richting te geven wordt er gebruik gemaakt van richtlijnen waarin uiteengezet wordt hoe problemen aan te pakken. Het doel van deze richtlijnen is om de zorg zo uniform mogelijk te houden en het inzetten van diagnostiek zoveel mogelijk te beperken tot het noodzakelijke en zodoende de kwaliteit van zorg te verbeteren en de ontwikkeling van zorgkosten te beperken. Het is echter bekend dat het volgen van deze richtlijnen door artsen beperkt is door zowel aan de richtlijn gebonden factoren als door arts-gebonden factoren. Daarom is het van belang om periodiek deze richtlijnen te beoordelen op bruikbaarheid, functioneren en “up-to-date zijn” en zo nodig aan te passen om de kwaliteit van zorg voor kinderen met chronische buikpijn te optimaliseren

Hoofdstuk 2 beschrijft een studie waarin wij retrospectief het naleven van de richtlijn chronische buikpijn bij kinderen door kinderartsen hebben onderzocht en hebben gekeken naar de variatie in verrichte diagnostiek door dezelfde kinderarts en tussen verschillende kinderartsen in een groot Nederlands ziekenhuis. De kinderen die door deze kinderartsen werden behandeld waren tussen de 4 en 18 jaar oud en waren verwezen voor chronische buikpijn zonder de aanwezigheid van alarmsymptomen. We vergeleken de door de kinderartsen verrichte diagnostiek met de diagnostiek die door de richtlijn wordt aanbevolen. In 26% van de kinderen werd daadwerkelijk een aandoening vastgesteld. Geen enkele kinderarts verrichte enkel de door de richtlijn aanbevolen bepalingen zonder het uitvoeren van extra onderzoek, maar het aanvullend onderzoek van 67% van de kinderartsen bevatte wel de aanbevolen bepalingen volgens de richtlijn. Er werd een grote variatie gevonden in verrichte diagnostiek tussen de kinderartsen maar ook binnen dezelfde kinderarts. Redenen om af te wijken van de richtlijn werden onderzocht en kinderartsen gaven aan dat zij afweken van de richtlijn vanwege: het onvoldoende geïnformeerd zijn over de inhoud van de richtlijn, (gedeeltelijke) onenigheid over de inhoud van de richtlijn en het niet overtuigd zijn van de toegevoegde waarde van de richtlijn. Deze resultaten suggereren dat kinderartsen meer lijken te vertrouwen op hun klinische ervaring dan op bestaande op wetenschap gebaseerde richtlijnen bij de behandeling van kinderen met chronische buikpijn.

Gluten allergie

Het tweede deel van dit proefschrift richt zich op twee aspecten van gluten allergie als een van de mogelijke oorzaken van chronische buikpijn in kinderen. Voornamelijk omdat het uitsluiten van gluten allergie een kernelement is in de diagnostiek naar chronische buikpijn bij kinderen. In de afgelopen jaren is de bewustwording en alertheid met betrekking tot glutenallergie sterk gegroeid, ook in Nederland. De ontwikkeling en het gebruik van zeer gevoelige bloedonderzoeken in kinderen met- of zonder klachten of genetisch belastbare kinderen heeft er toe geleid dat er steeds meer kinderen met

gluten allergie gediagnosticeerd zijn en dat het palet aan symptomen waarmee zij zich presenteren sterk is veranderd. Het is onbekend of dit ook geldt voor de Nederlandse kinderen gezien dit al meer dan 10 jaar niet is onderzocht. Indien dit ook voor de Nederlandse kinderen geldt zou dit impliceren dat artsen veel vaker alert zouden moeten zijn op glutenallergie, ook bij vage symptomen. Een ander belangrijk aspect van de behandeling van kinderen met glutenallergie is de follow-up. Bij glutenallergie kunnen verschillende tekorten in vitamines en mineralen optreden, zowel tijdens de diagnose als tijdens de follow-up indien zij zich niet strikt houden aan een glutenvrij-dieet. Het is verwonderlijk dat er geen internationale richtlijnen zijn die aangeven hoe de follow-up van kinderen met glutenallergie vorm gegeven dient te worden in termen van hoe vaak zij gezien dienen te worden en welke bloedafnames er nodig zijn om tekorten te monitoren. Wel is er een gedateerde Nederlandse richtlijn, maar deze is niet eerder geëvalueerd en beoordeeld op zijn werking.

Hoofdstuk 3 beschrijft een studie waarin wij het huidige klinische beeld van kinderen met glutenallergie onderzoeken. Hierin bekeken wij alle kinderen die tussen 2007 en 2017 in het Tergooi ziekenhuis zijn gediagnosticeerd met glutenallergie volgens de geldende Europese richtlijnen en vergeleken deze kinderen met een controle groep die verwezen waren vanwege dezelfde soort symptomen maar zonder glutenallergie om te beoordelen of er bepaalde symptomen typisch zijn voor glutenallergie. Er werden 105 kinderen met glutenallergie gediagnosticeerd wat past bij een incidentie van 21.09 per 100.000 inwoners onder de 18. Zo'n 40% waren peuters en kleuters met voornamelijk typische maag-, darmklachten. De basis- en hogere school kinderen hadden vaker atypische klachten dan typische klachten. Chronische buikpijn en een opgezette buik waren de meest voorkomende symptomen, maar de klassieke trias kwam slechts in 32.6% van de onderzochte populatie voor (voornamelijk bij peuters en kleuters). De klassieke presentatie blijkt verschoven van zo'n 90% bij peuters en kleuters naar een atypische presentatie bij zo'n 40-50% bij basis- en hogere school kinderen.

Hoofdstuk 4 beschrijft een studie waarin wij het volgen van de coeliakie richtlijn door kinderartsen en de diagnostische opbrengst van de gesuggereerde aanvullende onderzoeken van deze richtlijn onderzoeken. Het strikt opvolgen van deze richtlijn door kinderartsen blijkt beperkt te zijn en dat de opbrengst van de gesuggereerde aanvullende onderzoeken ook beperkt is. Dit onderstreept dat de huidige Nederlandse richtlijn gedateerd is en aan vernieuwing toe is.

Dientamoeba fragilis

Het derde deel van dit proefschrift richt zich op de parasiet *Dientamoeba fragilis* als een potentiële oorzaak van chronische buikpijn bij kinderen. Ondanks dat deze parasiet ruim 100 jaar geleden voor het eerst werd beschreven is er nog steeds veel discussie over de pathogeniciteit van deze parasiet. Mogelijke oorzaken als verstoringen van de darmflora door de parasiet of het bestaan van verschillende virulente stammen

blijven in het ongewis. De discussie wordt verder aangewakkerd door het feit dat de aanwezigheid van de parasiet in de darm bij kinderen gepaard gaat met zowel een breed spectrum aan symptomen als met asymptomatisch dragerschap. Het is intrigerend dat er veel wetenschappelijke artikelen zijn gepubliceerd die bewijs leveren dat de parasiet ziekmakend kan zien, maar dat de enige placebo gecontroleerde gerandomiseerde studie (wat als de hoogst mogelijke bewijsgraad geldt) die de behandeling van de parasiet met antibiotica onderzoek dit tegenspreekt. Deze tegenstrijdigheid heeft vanzelfsprekend geleid tot een grote diversiteit in hoe artsen tegen deze parasiet aankijken en hoe zij vervolgens omgaan met de diagnostiek en behandeling hiervan.

Hoofdstuk 5 beschrijft een vragenlijst studie onder Nederlandse huisartsen en kinderartsen hoe zij tegen *Dientamoeba fragilis* aankijken. Er blijkt veel verscheidenheid te zijn in de klinische praktijk in hoe Nederlandse artsen diagnostiek verrichten naar de parasiet en in hoe zij de parasiet behandelen. Ondanks dat het onduidelijk is of de parasiet ziekmakend is beschouwd 80% van de ondervraagde artsen de parasiet als een mogelijke veroorzaker van maag- en darmklachten. Echter test maar een klein deel van de ondervraagde artsen gericht op de parasiet. Bij een positieve ontlastingstest voor de parasiet blijkt metronidazol het antibioticum van eerste keus.

Hoofdstuk 6 beschrijft een hypothese bevestigende studie waarin onderzocht wordt of veranderingen in de darmflora, mogelijk veroorzaakt door de aanwezigheid van *Dientamoeba fragilis* in de darm de oorzaak is van maag-, darmklachten ontstaan. Er werden geen significante verschillen aangetoond in de darmflora tussen kinderen met *Dientamoeba fragilis* en gezonde controles. Hierdoor lijkt het onwaarschijnlijk dat verstoring van de darmflora door *Dientamoeba fragilis* de oorzaak is van maag-, darmklachten bij kinderen met de aanwezigheid van *Dientamoeba fragilis* in de darm.

Tot slot beschrijft **Hoofdstuk 7** een systematische review van de beschikbare literatuur waarin wij de relatie tussen het behandelen van *Dientamoeba fragilis* en het verdwijnen van symptomen onderzoeken en de sterkte van het bewijs wegen dat kinderen met een symptomatische *Dientamoeba fragilis* infectie behandeld moeten worden met antibiotica. De beschikbare literatuur richt zich met name op buikpijn en diarree als aanwezige symptomen en bevat enkel studies met kleine aantallen patiënten, die allemaal verschillend van opzet zijn waardoor er een lage bewijskracht is. Het eradiceren van de parasiet uit de ontlasting en het daadwerkelijk verdwijnen van aanwezige symptomen verschilde sterk tussen de verschillende studies met verschillende behandelschema's met verschillende antibiotica. Tussen de verschillende middelen blijkt paromomycine het meest effectief te zijn om de parasiet uit de ontlasting te krijgen en blijkt clioquinol het meest effectief te zijn in het oplossen van de aanwezige symptomen. Echter blijkt ook dat als de effectiviteit toeneemt om de parasiet uit de ontlasting te krijgen er niet meer kinderen van hun symptomen af komen. Dit suggereert dat de aanwezigheid van de parasiet niet de (enige) oorzaak van de klachten

is. Hierdoor concluderen wij dat het testen voor *Dientamoeba fragilis* bij kinderen met chronische buikpijn en diarree enkel geïndiceerd is als andere (meer voor de hand liggende oorzaken) zijn uitgesloten. Toekomstige studies zijn nodig om een oorzakelijk verband aan te tonen tussen de aanwezigheid van *Dientamoeba fragilis* en maag- en darmklachten en om duidelijk te krijgen welk antibiotisch regime het best geschikt is voor de behandeling.

APPENDICES

List of publications
List of co-authors
Author contributions
PhD portfolio
About the author
Dankwoord (Acknowledgements in Dutch)
Supplementals (Chapter 3 and 4)

LIST OF PUBLICATIONS

In this thesis

van Kalleveen MW, de Meij T, Plötz FB (2018), Current Clinical Spectrum of Paediatric Coeliac Disease: a 10-year single-centre experience, Eur J Pediatr 2018 Apr;177(4):593-602. doi: 10.1007/s00431-018-3103-4.

van Kalleveen MW, Noordhuis EJ, Lasham C, Plötz FB (2019), Large Variation Amongst Paediatricians in Clinical Practice in Children with Recurrent Abdominal Pain, Pediatr Gastroenterol Hepatol Nutr. 2019 May;22(3):225-232. doi: 10.5223/pghn.2019.22.3.225

van Kalleveen MW, van Gool T, Klarenbeek NN, Benninga MA, Savelkoul PHM, de Meij T, Plötz FB (2020), Dientamoeba fragilis in children: A systematic review on diagnostic considerations and efficacy of treatment. Expert Rev Gastroenterol Hepatol. 2020 Mar;21:1-12. doi: 10.1080/17474124.2020.1739520.

van Kalleveen MW, Budding AB, Benninga MA, Savelkoul PHM, van Gool T, van Maldeghem I, Dorigo-Zetsma JW, Bart A, Plötz FB, de Meij TGJ (2021), Intestinal Microbiota in Children with Symptomatic Dientamoeba fragilis Infection: A Case-control Study. Ped Infect Dis J 2021 Apr 1;40(4):279-283, doi: 10.1097/INF.0000000000002975.

van Kalleveen MW, Dykstra TC, de Meij TGJ, Plötz FB (2021), Guideline adherence and clinical relevance of laboratory investigations during follow-up in paediatric coeliac disease: a Dutch single-centre cohort study. Acta Paediatrica 2021 Sep;110(9):2641-2647 doi: 10.1111/apa.15967

van Kalleveen MW, van Bergen M, Benninga MA, Savelkoul PHM, Plötz FB, de Meij T (2021), Diagnostic and Therapeutic Considerations Towards Dientamoeba fragilis in Children: A Survey Amongst General Practitioners and Pediatricians in the Netherlands. J Pediatr Gastroenterol Nutr. 2021 Dec 1;73(6):e121-e125 doi: 10.101097/MPG.0000000000003297

Other publications

van Kalleveen MW, Walraven M, Hendriks MP (2018), Pazopanib-related tumor lysis syndrome in metastatic renal cell carcinoma: a case report, Invest New Drugs 2018 Feb 20. doi: 10.1007/s10637-018-0576-y.

van Kalleveen MW, de Meij T, Plötz FB (2018), Paediatric coeliac disease: increased incidence or increased awareness?, J Pediatr Gastroenterol Nut 2018 Aug;67(2):e42. doi: 10.1097/MPG0000000000002046

van Kalleveen MW, de Meij T, Plötz FB (2019), Coeliakie op de kinderleeftijd: Een veranderend klinisch spectrum. Ned Tijdschr Geneeskd. 2019 Mar 19;163. Pii: D3059

van Kalleveen MW, de Meij T, Plötz FB (2022), Microbiota in Children with Dientamoeba Fragilis; A Player to Take into Account? J Pediatr Gastroenterol Nutr. 2022 Feb 1;74(2):e40 doi: 10.1097/MPG.0000000000003267

LIST OF CO-AUTHORS

Aldert Bart - Department of Medical Microbiology, Tergooi Hospital, Blaricum, The Netherlands.

Marc Benninga - Department of Pediatric Gastroenterology, Emma Children's Hospital, Amsterdam UMC, Amsterdam, The Netherlands.

Merel van Bergen - Department of Pediatric Gastroenterology, Emma Children's Hospital Amsterdam UMC, Amsterdam.

Andries Budding - Department of Medical Microbiology & Infection Control, Amsterdam UMC, Amsterdam, The Netherlands

Wendelien Dorigo-Zetsma - Department of Medical Microbiology, Tergooi Hospital, Blaricum, The Netherlands.

Tim Dykstra - Department of Paediatrics, Tergooi Hospital, Blaricum, The Netherlands.

Tom van Gool - Department of Medical Microbiology, Section Clinical Parasitology, Amsterdam UMC, Amsterdam, The Netherlands.

Nikki Klarenbeek - Department of Pediatrics, Tergooi Hospital, Blaricum, The Netherlands.

Carole Lasham - Department of Pediatrics, Tergooi Hospital, Blaricum, the Netherlands.

Iris van Maldeghem - Department of Pediatrics, Tergooi Hospital, Blaricum, The Netherlands.

Tim de Meij - Department of Pediatric Gastroenterology, Emma Children's Hospital, Amsterdam UMC, Amsterdam, The Netherlands.

Elise Noordhuis - Department of Pediatrics, Tergooi Hospital, Blaricum, the Netherlands.

Frans Plötz - Department of Pediatrics, Tergooi Hospital, Blaricum, The Netherlands and Department of Pediatrics, Emma Children's Hospital, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.

Paul Savelkoul - Department of Medical Microbiology & Infection Control, Amsterdam UMC, Amsterdam, The Netherlands and Department of Medical Microbiology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center, Maastricht, The Netherlands.

AUTHOR CONTRIBUTIONS

Chapter 2, Large variation in clinical practice amongst pediatricians in treating children with recurrent abdominal pain

Contributions

Study design: EN, FP, MK; Data collection: EN; Data analysis: EN, MK; Writing manuscript: MK, FP, CL; Revision of manuscript: FP, CL; Approval of manuscript: All authors; Supervision: FP;

Chapter 3, Clinical spectrum of paediatric coeliac disease: a 10-year single-centre experience

Contributions

Study design: MK, FP; Data collection: MK; Data analysis: MK, FP; Writing manuscript: MK; Revision of manuscript: MK, FP; Approval of manuscript: All authors; Supervision: FP, TM;

Chapter 4, Guideline adherence and clinical relevance of laboratory investigations during follow-up of paediatric coeliac disease: a Dutch single-centre cohort study

Contributions

Study design: MK, TD, FP; Data collection: TD; Data analysis: MK, TD, FP; Writing manuscript: TD, MK; Revision of manuscript: FP, TM; Approval of manuscript: All authors; Supervision: FP, TM;

Chapter 5, Diagnostic and therapeutic considerations towards *Dientamoeba fragilis* in children: a survey amongst general practitioners and pediatricians in the Netherlands

Contributions

Study design: MK, MB, TM, MB; Data collection: MB; Data analysis: MK, MB; Writing manuscript: MB, MK; Revision of manuscript: FP, TM, MB, PS; Approval of manuscript: All authors; Supervision: FP, TM, MB, PS;

Chapter 6, Intestinal microbiota in children with symptomatic *Dientamoeba fragilis* infection: a case-control study

Contributions

Study design: MK, AB, IM; Data collection: IM, MK, AB; Sample processing: IM, AB; Sample analysis: MK, IM, AB; Data analysis: MK, AB; Writing manuscript: MK; Revision of manuscript: AB, MB, PS, TG, JD, AB, FP, MB; Approval of manuscript: All authors; Supervision: FP, TM, MB;

Chapter 7, *Dientamoeba fragilis* in children: a systematic review on diagnostic considerations and efficacy of treatment

Contributions:

Study design: MK, NK, FB; Data collection: MK, NK; Data analysis: MK, NK, FP, TM; Writing manuscript: MK, NK, TG; Revision of manuscript: TG, MB, PS, TM, FP; Approval of manuscript: All authors; Supervision: TG, MB, PS, TM, FP;

PHD PORTFOLIO

1. PhD training	Year	ECTS
General courses		
-	-	-
Seminars, workshops and master classes		
-	-	-

Presentations	Year	ECTS
Periodiek regional congress kindergeneeskunde, VUmc	2017	-
Research Symposium Tergooi Hospitals, Blaricum. "Current clinical spectrum of paediatric coeliac disease"	2017	-
Research Symposium Noordwest Hospital, Alkmaar. "Diagnostic and therapeutic considerations in children with *Dientamoeba fragilis*"	2021	-
Conferences		
-	-	-

2. Teaching	Year	ECTS
Supervision of Scientific Internship student, Amsterdam UMC, Amsterdam	2019	-
Supervision of Scientific Internship student, Tergooi Hospitals, Blaricum. (16 weeks)	2020	-
Supervision of Scientific Internship student, Amsterdam UMC, Amsterdam (16 weeks)	2021	-

3. Grants	Year	
Tergooi Research Support Grant	2018	-

4. Publications	Year
Current Clinical Spectrum of Paediatric Coeliac Disease: a 10 year single-centre experience. *European Journal of Pediatrics 2018;177(4):593-602*	2018
Paediatric coeliac disease: increased incidence or increased awareness? *Journal of Pediatric Gastroenterology Hepatology and Nutrition 2018;67(2):e42*	2018
Coeliakie op de kinderleeftijd: Een veranderd klinisch spectrum. *Nederlands Tijdschrift voor Geneeskunde 2019;163(0):D3059*	2019

Large Variation Amongst Paediatricians in Clinical Practice in Children with Recurrent Abdominal Pain *Pediatric Gastroenterology Hepatology and Nutrition 2019;22(3):225-232*	2019
Dientamoeba fragilis in children: A systematic review on diagnostic considerations and efficacy of treatment. *Expert Reviews in Gastroenterology and Hepatology 2020;14(4):231-242*	2020
Intestinal Microbiota in Children with Symptomatic Dientamoeba fragilis Infection: A Case-control Study. *The Pediatric Infectious Disease Journal 2021;40(4):279-283*	2021
Guideline adherence and clinical relevance of laboratory investigations during follow-up in paediatric coeliac disease: a Dutch single-centre cohort study. *Acta Paediatrica 2021;110(9):2641-2647*	2021
Diagnostic and Therapeutic Considerations Towards Dientamoeba fragilis in Children: A Survey Amongst General Practitioners and Pediatricians in the Netherlands. *Journal of Pediatric Gastroenterology Hepatology and Nutrition 2021;73(6):e121-e125*	2021
Microbiota in Children with Dientamoeba fragilis; A Player to Take into Account? *Journal of Pediatric Gastroenterology Hepatology and Nutrition 2022;74(2):e40*	2022

ABOUT THE AUTHOR

Michael van Kalleveen was born in Utrecht on the 10th of July 1992. He was raised in Leusden, near Amersfoort, together with his little brother. He went to “De Rossenberg” primary school, which he completed in 2004. In 2004 he went to Amersfoort to the “Johan van Oldenbarnevelt gymnasium” which he completed in 2010. At the age of 15 he started working at the Albert Heijn and was promoted to shiftleader and eventually teamleader before the age of 18. After being drawn out for medical school he started to study pharmaceutical sciences between 2010 and 2011 at the Vrije Universiteit of Amsterdam where he completed his propaedeutic year with an average of 8,4. During the summer of 2011 he received the joyful message that he was accepted for medical school and decided to quit his study of pharmaceutical sciences. During his first year he did his nursing internship at the Horacio Oduber Hospital in Oranjestad Aruba. Throughout his entire Bachelor’s and Master’s degree he kept working as teamleader for Albert Heijn on Sundays and during days off and holidays. During his medical study he wanted to become an oncologist and during his final year he completed an internship at the hematology- and oncology department of Noordwest Ziekenhuis in Alkmaar. For his Master thesis, he went to Tergooi Hospitals Blaricum to study the current clinical spectrum of paediatric coeliac disease where he met with Dr. Frans Plötz and Dr. Tim de Meij again and wrote his first publication for the European Journal of Pediatrics. His master thesis was rated with a 9,7. After finishing his medical study in 2017 with an average grade of 8,4, he started working as an resident at the department of internal medicine at Noordwest ziekenhuisgroep in Alkmaar. During his first year of residency, he applied for a PhD-trajectory, supervised by dr. Plötz, dr. de Meij, prof. Benninga and prof. Savelkoul. After several months he altered his plans to become an oncologist and became resident at the department of Gastroenterology in Alkmaar where he worked for 2 subsequent years after which he went to the department of Gastroenterology and Hepatology of Leiden University Medical Centre where he worked as a resident for nearly a year. Finally, after 3 years of working as a gastroenterology and hepatology resident, he finally decided he did not want to become a gastroenterologist and returned to the department of internal medicine at the Noordwest ziekenhuis in Alkmaar to chase his dream to become an oncologist. In October 2022 he was accepted for a residency in internal medicine at the Amsterdam University Medical Centre. In February 2023 he will start as a resident internal medicine at Zaandam Medical Centre where he can further chase his dream to become an oncologist. Michael loves playing football, watching movies and series, going out to music festivals, long walks on the beach or in the forrest with his dog Ollie and playing videogames. He lives with his dog Ollie in Alkmaar.

DANKWOORD (ACKNOWLEDGEMENTS)

Het schrijven van een proefschrift in deeltijd verband, naast een klinische baan als arts-assistent, is een uitdaging gebleken waarin ik mij van tevoren toch wel enigszins heb vergist. Nu ik, bijna 5 jaar later, hierop kan terug kijken zou ik het niet anders hebben willen doen, maar zou ik anderen die hetzelfde overwegen wel willen meegeven hier zeer goed over na te denken. Misschien zou ik het ze zelfs wel afraden. Echter was het tot stand komen van dit proefschrift een fantastische reis. Een reis die mij de rest van mijn leven bij zal blijven en veel waardevolle lessen heeft geleerd. Het was een reis die niet zonder horden en stoten ging. Een reis met veel ups en downs, keihard werken waarbij eindeloze energie, inzet en doorzettingsvermogen werd geëist. Een reis waarbij leren plannen en organiseren misschien wel het allerbelangrijkste werd, maar vooral ook een reis waarin ik een aantal zeer inspirerende en fijne mensen ben tegen gekomen waar ik verbindingen mee ben aangegaan die ik nog lang hoop te behouden.

Allereerst, copromotor, mentor, inspirator en vertrouwenspersoon prof. dr. Frans Plötz. Lieve Frans, man van het allereerste uur, de man door wie mijn interesse in de wetenschap ontstond, initiator van mijn reis. In de afgelopen vijf tot zes jaar dat wij elkaar kennen kan ik inmiddels wel zeggen dat ik toch een vriend rijker ben geworden. Vanaf de allereerste letter van mijn allereerste onderzoeksvoorstel, wat uiteindelijk meteen mijn allereerste publicatie werd, tot de allerlaatste letter van dit proefschrift ben jij altijd meer dan zeer betrokken geweest. Altijd wist jij mij de prikkeling te geven die ik nodig heb. Altijd was je bereikbaar. Altijd gaf jij binnen de kortste keren commentaren op mijn ingestuurde stukken. Je voelde altijd feilloos aan wanneer ik ergens mee in de knoop zat en meer sturing nodig had, maar daarentegen voelde je ook altijd feilloos aan wanneer ik meer ruimte nodig had. Je hebt de afgelopen jaren veel voor mij betekend, al mijn ups en downs en tegenslagen meegemaakt, altijd een luisterend oor geboden daar waar ik dat soms nodig had, maar mij vooral ook altijd voorzien van goede adviezen en (levens)lessen. “Houd moed” en “houd vertrouwen” spiegelde je mij altijd en dat is precies wat ik daardoor altijd ben blijven doen. Ik ben je meer dan zeer dankbaar voor alles wat je voor mij hebt betekend, voor alles dat je voor mij hebt weten te regelen, voor al jouw begeleiding en jouw eindeloze bron van inspiratie. Ik hoop dan ook dat ik in de toekomst nog lang met je mag blijven samenwerken.

Mijn promotor, prof. dr. Marc Benninga. Beste Marc, dankzij Frans en Tim leerde ik jou kennen. Ik had nog geen promotor, maar jij was gelijk bereid om deze rol op je te nemen. Ik zal je herinneren om je humor. Humor die ik soms in de droge emails niet altijd in één keer door had. Altijd was je op een positieve manier kritisch op dat wat ik schreef. Het leverde meer dan eens leuke discussies op. Ik zal zeker de discussie over de pathogeniciteit van de Dientamoeba herinneren, daar waar ik in de begin stadia van dit proefschrift nog overtuigd was en samen met Frans en Tom van Gool als “de drie dientamoeba musketiers” jou en Tim probeerde te overtuigen, werd ik dat naar mate

het proefschrift vorderde minder en minder. Ik zal je herinneren om de gastvrijheid en de uitnodigingen bij je thuis met Frans en Tim samen. Bedankt dat je de rol van promotor op je wilde nemen en bedankt dat jij mijn reis mede mogelijk hebt gemaakt.

Mijn tweede copromotor, mede-mentor en mede-inspirator, dr. Tim de Meij. Lieve Tim, net als Frans man van het eerste uur. Ik weet nog goed hoe zenuwachtig ik was toen Frans en ik jou om jouw mening en samenwerking vroegen toen ik mijn master thesis aan het schrijven was. Sindsdien heb jij een belangrijke rol vervuld in mijn reis naar het voltooien van dit proefschrift. Altijd ben je meer dan betrokken geweest. Jouw scherpzinnige commentaren tilde de manuscripten altijd naar een hoger niveau. Ook jij was altijd razendsnel met jouw commentaren waardoor ik snel weer verder kon. Jij maakte ook veel van mijn ups en downs en mijn tegenslagen mee, maar wist mij altijd het vertrouwen te geven dat er weer betere tijden zouden komen. Bedankt voor je tomeloze inzet, bedankt voor alles dat je voor mij hebt betekend en geregeld. Bedankt voor jouw rol in mijn reis naar het voltooien van dit proefschrift. Ik hoop dat we in de toekomst contact zullen blijven houden.

Mijn tweede promotor, prof. dr. Paul Savelkoul. Beste Paul, ik was op zoek naar een tweede promotor om het microbiologie deel van mijn proefschrift te kunnen leiden en met dank aan Tim kwam ik bij jou uit. Jij zag heil in mijn avontuur en was gelijk bereid om deze rol op je te nemen. Ik wil je bedanken voor jouw begeleiding op mijn reis, dankbaar dat jij het voor mij misschien wel meest ingewikkelde deel van dit proefschrift wilde begeleiden.

Aan de leden van de beoordelingscommissie prof dr. Mearin Manrique, prof. dr. Heymans, prof. dr. Wijburg, prof. dr. Frenkel, prof. dr. Pajkrt en dr. Koot: hartelijk dank voor de vlotte en unanieme beoordeling van het proefschrift. Ook wil ik graag mijn dank betuigen aan alle co-auteurs die hebben bijgedragen aan de uitvoering en verbetering van de verschillende studies in dit proefschrift: dr. Aldert Bart, Merel van Bergen, dr. Andries Budding, dr. Wendelien Dorigo-Zetsma, Tim Dykstra, dr. Tom van Gool, Nikki Klarenbeek, Carol Lasham, Iris van Maldeghem en Elise Noordhuis.

De vakgroepen maag-, darm- en leverziekten en interne geneeskunde van het Noordwest ziekenhuis, en in het bijzonder opleider MDL dr. Michael Klemt-Kropp, wil ik graag bedanken voor hun flexibiliteit en de geboden mogelijkheid om parttime aan mijn proefschrift te kunnen werken naast mijn klinische werk als arts-assistent. Het was altijd mogelijk om extra vrije dagen te krijgen indien dit voor de voortgang van het proefschrift noodzakelijk was. Van Tergooi Ziekenhuizen locatie Blaricum wil ik ook alle kinderartsen bedanken voor de warme omgeving die heeft bijgedragen aan het opstarten van dit proefschrift.

Aan mijn dierbare beste vrienden, ik realiseer mij dat ik de afgelopen jaren minder beschikbaar ben geweest voor jullie dan ik misschien zou willen. Ondanks dat is onze vriendschap even krachtig als altijd gebleven en is het niet verwaterd. Nu mijn dubbelrol als arts-assistent en PhD-student eindelijk voorbij is hoop ik dat we vooral door kunnen gaan waar we gebleven waren. Het is een onwijs geluk en een onwijze eer zulke fijne vrienden te mogen hebben.

Lieve papa en mama, tot slot aan mij de eer jullie te bedanken voor jullie onverzettelijke liefde en steun aan mij. Waar ik ook ga, wat ik ook doe en wat er ook gebeurd, jullie zullen nooit van mijn zijde wijken en mij onvoorwaardelijk steunen. Nooit is iets voor jullie te gek. Ik prijs mij meer dan heel gelukkig met ouders zoals jullie. Weet dat ik enorm tegen jullie op kijk en dat jullie erg belangrijk zijn voor mij. Jullie hebben mij geleerd om altijd het beste uit mezelf te willen halen en mij geleerd om altijd door de wolken heen de zon te blijven zien. Dat ik hier nu sta is mede dankzij jullie. Tot slot, lieve opa Jan, ook al ben jij al hele lange tijd niet meer onder ons, ik ben mijn belofte als kleine jongen aan jou nooit vergeten. Op jouw sterfbed beloofde ik jou ervoor te zorgen dat er nooit meer iemand zou lijden aan kanker zoals jij daar aan leed. Jij bent mijn ultieme inspirator om elke dag het beste uit mezelf te halen. En met het afronden van dit proefschrift en het behalen van een opleidingsplek tot internist ben ik weer een stapje dichterbij gekomen bij het inlossen van mijn belofte aan jou. Ik hoop dat je trots op mij bent.

Michael van Kalleveen

SUPPLEMENTAL MATERIAL (Chapter 3)

Supplementary table 1. Diagnostic features of patients with positive CD serology after repeated testing.

Gender	Age (years)	Initial tTG (U/mL)	First positive tTG (U/mL)	# days to become positive	Marsh-score	HLA DQ2/DQ8 positive?	Serum IgA (g/L)	Positive family history?
M	1.0	<0.1	67	521	3	Yes/No	0.52	No
M	2.1	0.0	685	524	3	Yes/No	<0.05	No
F	5.9	0.3	106	609	-	Yes/No	0.56	Yes
M	4.3	0.1	>128	922	-	Yes/No	0.78	No
F	6.1	3.7	24	140	3	Yes/No	0.55	No
F	5.2	0.0	>128	1054	-	Yes/No	0.65	Yes
F	8.0	6.9	>128	916	3	Yes/Yes	1.66	No
F	9.5	2.8	>128	336	3	Yes/No	2.1	No
F	5.4	0.6	18	646	3	?/?	1.13	No

Patient number 2 used inhalation corticosteroids and had a CD associated IgA deficiency.

Table S1. Haematology results of the studied population.

Test	Mean [Range] ± std.	95% CI	Patients/cases + (mean)	Reference values
ESR (N = 13) N = 2 N = 11	3.00 [2 – 4] ± 1.41 7.00 [2 – 31] ± 8.24	-10 – 16 2 – 12	N = 0 N = 1/1 (31)	mm/hour <15 (<50 years male) <20 (<50 years female)
Haemoglobin (N = 130) N = 92 N = 27 N = 8 N = 3	7.80 [6.9 – 8.7] ± 0.45 8.37 [7.5 – 9.9] ± 0.59 8.55 [7.7 – 9.2] ± 0.52 8.87 [8.3 – 9.7] ± 0.74	7.7 – 7.9 8.1 – 8.6 8.2 – 8.9 7.0 – 10.7	N = 2/2 (6.9) N = 0 N = 0 N = 1 (8.3)	mmol/L 7.0 – 9.0 (2 – 12 years) 7.0 – 10.0 (12 – 15 years) 7.5 – 10.0 (female > 15 years) 8.5 – 11.0 (male > 15 years)
Haematocrit (N = 130) N = 24 N = 66 N = 27 N = 13	0.38 [0.34 – 0.40] ± 0.02 0.37 [0.33 – 0.42] ± 0.02 0.40 [0.35 – 0.45] ± 0.03 0.41 [0.37 – 0.48] ± 0.04	0.37 – 0.38 0.37 – 0.38 0.39 – 0.41 0.38 – 0.43	N = 0 N = 3/3 (0.34) N = 0 N = 5/5 (0.38)	L/L 0.32 – 0.42 (7 days – 6 years) 0.35 – 0.45 (6 – 12 years) 0.35 – 0.45 (female >12 years) 0.40 – 0.50 (male >12 years)
Erythrocytes (N = 130) N = 90 N = 28 N = 9 N = 3	4.60 [3.88 – 5.36] ± 0.31 4.66 [4.08 – 5.35] ± 0.32 4.69 [4.17 – 5.10] ± 0.37 4.91 [4.46 – 5.60] ± 0.61	4.54 – 4.66 4.55 – 4.77 4.42 – 4.95 3.40 – 6.41	N = 0 N = 0 N = 0 N = 0	X 10^9/L 3.50 – 5.50 (181 days – 12 years) 4.00 – 5.50 (12 – 15 years) 4.00 – 5.00 (female > 15 years) 4.50 – 5.50 (male > 15 years)
MCH (N = 130) N = 11 N = 78 N = 41	1576 [1457 – 1674] ± 66.09 1717 [1434 – 1959] ± 106.48 1804 [1624 – 1991] ± 78.08	1534 – 1618 1694 – 1740 1781 - 1828	N = 0 N = 4/5 (1824) N = 4/4 (1660)	amol/L 1400 – 1800 (0.5 – 4 years) 1500 - 1900 (4 – 12 years) 1700 - 2100 (> 12 years)
MCV (N = 130) N = 90 N = 40	82.04 [73 – 92] ± 4.30 86.36 [80 – 96] ± 3.89	81 – 83 85 – 88	N = 3/3 (92) N = 2/2 (96)	fl 70 – 90 (< 12 years) 75 – 95 (> 12 years)
RDW (N = 130) N = 130	12.61 [11.2 – 16.2] ± 0.81	12.5 – 12.7	N = 4/4 (15.08)	% 11.0 – 14.5
Thrombocytes (N = 84) N = 57 N = 27	307.10 [202 – 602] ± 80.73 277.76 [207 – 352] ± 34.15	286 – 327 265 – 291	N = 1/1 (602) N = 0	X10^9/L 150 – 600 (<12 years) 150 – 450 (>12 years)

Table S1. Continued.

Test	Mean [Range] ± std.	95% CI	Patients/cases + (mean)	Reference values
Leukocytes (N = 84) N = 16 N = 60 N = 8	9.14 [5.3 – 12.3] ± 2.12 7.31 [3.9 – 14.3] ± 2.26 5.76 [3.5 – 10.8] ± 2.51	8.1 – 10.2 6.7 – 7.9 3.7 – 7.9	N = 1/1 (5.30) N = 3/3 (10.8) N = 2/2 (3.60)	X10^9/L 5.5 – 17.0 (1 – 6 years) 4.0 – 14.0 (6 – 15 years) 4.0 – 11.0 (> 15 years)
Neutrophils (N = 72) N = 12 N = 53	3.93 [2.3 – 6.1] ± 1.47 3.40 [1.4 – 11.0] ± 1.92	3.0 – 4.9 2.9 – 3.9	N = 0 N = 3/3 (7.40)	X10^9/L 1.5 – 9.0 (1 – 6 years) 1.5 – 8.0 (> 6 years)
Lymphocytes (N = 72) N = 12 N = 49 N = 4	3.83 [2.1 – 5.1] ± 0.97 2.71 [1.6 – 5.5] ± 0.72 1.88 [1.4 – 2.3] ± 0.40	3.2 – 4.5 2.5 – 2.9 1.2 – 2.5	N = 0 N = 1/1 (5.50) N = 0	X10^9/L 1.0 – 8.0 (1 – 6 years) 1.0 – 5.0 (6 – 15 years) 1.0 – 4.0 (> 15 years)
Eosinophils (N = 72) N = 12 N = 53	0.47 [0.11 – 1.7] ± 0.47 0.43 [0.10 – 4.20] ± 0.60	0.2 – 0.8 0.3 – 0.6	N = 1/1 (1.70) N = 6/8 (1.17)	X10^9/L < 1.0 (181 days – 6 years) < 0.5 (>6 years)
Monocytes (N = 72) N = 12 N = 53	0.56 [0.18 – 0.83] ± 0.18 0.55 [0.25 – 1.00] ± 0.16	0.4 – 0.7 0.5 – 0.6	N = 0 N = 1/1 (0.25)	X10^9/L 0.10 – 1.0 (1 – 6 years) 0.30 – 1.0 (>6 years)
Basophils (N = 72) N = 65	0.10 [0.10 – 0.18] ± 0.01	0.99 – 0.10	N = 0	X10^9/L <0.2 (>1 day)
Reticulocytes (N = 1) N = 3 N = 0	43.33 [39 – 51] ± 6.66 -	27 – 60 -	N = 0 -	X10^9/L 30 – 100 (3 months – 12 years) 50 – 100 (>12 years)

Table S2. Chemistry result of the studied population.

Test	Mean [Range] ± std.	95% CI	Patients/Deviations + mean	Reference value limit
CRP (N = 15) N = 15	1.50 [1.00 – 7.00] ± 1.55	1 – 2	N = 1/1 (7)	mg/L <5
Sodium (N = 14) N = 11 N = 3	139.64 [138 – 143] ± 1.80 142.00 [141 – 143] ± 1.00	138 – 141 140 – 145	N = 0 N = 1/1 (143)	mmol/L 137 – 144 (reference before 12/02/2019) 133 – 142 (reference after 12/02/2019)
Potassium (N = 14) N = 14	4.20 [3.7 – 4.6] ± 0.28	4.0 – 4.4	N = 0	mmol/L 3.5 – 5.0
Calcium (N = 52) N = 33 N = 19	2.39 [2.30 – 2.59] ± 0.07 2.41 [2.32 – 2.55] ± 0.06	2.37 – 2.42 2.39 – 2.45	N = 0 N = 0	mmol/L 2.15 – 2.60 (Reference before 12/02/2019) 2.10 – 2.55 (Reference after 12/02/2019)
Phosphate (N = 30) N = 17 N= 2 N= 11	1.49 [1.30 – 1.70] ± 0.12 1.27 [1.15 – 1.39] ± 0.17 1.48 [1.24 – 1.72] ± 0.14	1.43 – 1.55 -0.25 – 2.79 1.38 – 1.57	N = 0 N = 0 N = 4/4 (1.63)	mmol/L 1.00 – 2.00 (1–16 years, reference before 12/02/2019) 0.70 – 1.40 (>16 years, reference before 12/02/2019) 0.74 – 1.52 (reference after 12/02/2019)
Magnesium (N = 8) N = 8	0.93 [0.80 – 1.30] ± 0.16	0.8 – 1.1	N = 1/1 (1.30)	mmol/L 0.7 – 1.0
Urea (N = 13) N = 8 N = 0 N = 4 N = 1	3.68 [2.6 – 4.6] ± 0.79 - 3.78 [3.2 – 4.4] ± 0.61 2.2	3.0 – 4.3 - 2.8 – 4.8 -	N = 0 - N = 0 N = 1/1 (2.2)	mmol/L <6.4 (<18 years, reference before 12/02/2019) 1.8 – 6.0 (1 – 3 years, reference after 12/02/2019) 2.5 – 6.0 (3 – 14 years, reference after 12/02/2019) 3.0 – 7.5 (14 – 19 years, reference after 12/02/2019)
Creatinine (N =17) N = 5 N = 12	42.40 [39 – 55] ± 7.06 44.92 [28 – 66] ± 12.84	34 – 51 37 - 53	N = 5/5 (42.40) N = 8/8 (37.50)	umol/L 65 – 115 (male) 50 – 95 (female)
ALP (N = 35) N = 19 N = 3 N = 0 N = 13	234.55 [45 – 371] ± 81.71 81.67 [80 – 85] ± 2.89 - 288.40 [173 – 478] ± 89.28	196 – 272 75 – 89 - 237 – 340	N = 0 N = 0 - N = 13/15 (288.40)	U/L <400 (1-17 years, reference before 12/02/2019) <100 (>17, female, reference before 12/02/2019) <115 (>17, male, reference before 12/02/2019) 30 – 120 (reference after 12/02/2019)

Table S2. Continued.

Test	Mean [Range] ± std.	95% CI	Patients/Deviations + mean	Reference value limit
GGT (N = 12) N = 12	11.31 [9 – 13] ± 1.11	11 – 12	N = 0	U/L <40
ASAT (N = 19) N = 14 N = 5	28.93 [21 – 48] ± 7.65 34.40 [28 – 44] ± 6.19	25 – 33 27 – 42	N = 3/3 (41.67) N = 1/1 (44)	U/L <30 (female) <35 (male)
ALAT (N = 21) N = 15 N = 6	16.88 [11 – 26] ± 4.83 22.50 [14 – 34] ± 7.45	14 – 19 15 – 30	N = 0 N = 0	U/L <35 (female) <45 (male)
LD (N = 8) N = 8	244.75 [167 – 353] ± 60.90	194 – 296	N = 1/1 (333.50)	U/L <250 (>6 months)
Glucose (N = 1) N = 11	5.0 [4.4 – 6.0] ± 0.47	4.7 – 5.3	N = 0	mmol/L 3.3 – 7.8
Vitamin B12 (N = 26) N = 26	489.81 [222 – 935] ± 164.28	434 – 556	N = 2/3 (829.33)	Pmol/L 140 – 640
Folic Acid (23) N = 15 N = 8	27.07 [12.4 – 45.4] ± 10.67 28.05 [15.7 – 37.7] ± 8.28	21.2 – 33.0 21.1 – 35.0	N = 0 N = 0	nmol/L >10.0 (reference before 12/02/2019) 7.8 – 45.3 (reference after 12/02/2019)
Iron (N = 42) N = 42	15.57 [3.6 – 31.8] ± 5.88	14 – 17	N = 11/17 (10.32) < N = 1/1 (31.80) >	umol/L 14 – 30
Transferrin/TYBC (N = 42) N = 42	70.16 [52 – 95] ± 70.16	67 – 73	N = 1/1 (95)	umol/L 50-90
Iron saturation (N = 42) N = 42	0.22 [0.05 – 0.45] ± 0.09	0.20 – 0.25	N = 9/15 (0.15)	fraction 0.20 – 0.45
Ferritin (N = 51) N = 24 N = 9 N = 13 N = 5	42.84 [21.0 – 99.0] ± 18.51 35.00 [18.0 – 80.0] ± 19.58 33.77 [15.0 – 55.0] ± 12.06 30.80 [16.0 – 59.0] ± 16.69	35 – 50 20 – 50 26 – 41 10 – 52	N = 0 N = 2/3 (19.33) N = 0 N = 1 (16.0)	ug/L 15 – 150 (female, reference before 12/02/2019) 25 – 400 (male, reference before 12/02/2019) 15 – 204 (female, reference after 12/02/2019) 22 – 275 (male, reference after 12/02/2019)

Table S2. Continued.

Test	Mean [Range] ± std.	95% CI	Patients/Deviations + mean	Reference value limit
Total protein (N = 2) N = 2	70	70	N = 0	g/L 64 – 83 (reference after 12/02/2019)
Albumin (N = 60) N = 60	45.68 [40 – 74] ± 4.83	44 – 47	N = 4/4 (58.75)	g/L 35 – 50
IgA (N = 155) N = 43 N = 66 N = 46	0.83 [0.05 – 2.25] ± 0.41 1.02 [0.05 – 3.30] ± 0.45 1.35 [0.58 – 2.19] ± 0.35	0.71 – 0.95 0.92 – 1.13 1.25 – 1.45	N = 2/3 (0.05) < N = 1/1 (2.25) > N = 1/1 (0.05) < N = 1/1 (3.3) > N = 1/1 (0.58) <	g/L 0.10 – 1.60 (1 – 7 years) 0.30 – 2.00 (7 – 12 years) 0.70 – 4.00 (> 12 years)
Vitamin D (N = 40) N = 40	76.06 [12 – 167] ± 33.60	65 – 87	N = 5/6 (29.57)	nmol/L 50 – 250
IgG total (N = 3) N = 1 N = 2 N = 0	6.71 8.91 -	- - -	N = 0 N = 0 -	g/L 4.00 – 11.00 (1 – 7 years) 6.00 – 12.00 (7 – 12 years) 7.00 – 16.00 (>12 years)

Table S3. Endocrinology results of the studied population.

Test	Mean [Range] ± std.	95% CI	Deviation frequency	Reference values
TSH (N = 45) N = 45	1.97 [0.30 – 5.20] ± 0.09	1.71 – 2.23	N = 2/2 (0.34) < N = 1/1 (5.20) >	mU/L 0.50 – 4.5 (1 – 18 years)
FT4 (N = 29) N = 8 N = 12 N = 9	15.63 [13 – 19] ± 1.85 15.50 [10 – 19] ± 2.38 12.89 [11 – 17] ± 1.83	14 – 17 14 – 17 11.48 – 14.30	N = 0 N = 1/1 (10) N = 0	pmol/L 6 – 30 (1 – 12 years, reference before 12/02/2019) 12 – 22 (>12 years, reference before 12/02/2019) 9.01 – 19.05 (>1-year, reference after 12/02/2019)
LH (N = 2) N = 2	2.65 [1.3 – 4.0] ± 1.90	-14.5 – 19.8	N = 1/1 (1.7)	U/L 1.7 – 8.6 (male)
FSH (N = 2) N = 2	3.20 [2.6 – 3.8] ± 0.85	-4.4 – 10.8	N = 0	IU/L 1.5 – 12.4 (male, reference before 12/02/2019)
Testosterone (N = 2) N = 2	7.25 [0.5 – 14.0] ± 9.55	-78.5 – 93.0	N = 1/1 (0.5)	Nmol/L 1 – 38.5 (12 – 15 years, male, reference before 12/02/2019)
SHBG (N = 2) N = 2	85.50 [60 – 111] ± 36.06	-239 – 410	N = 0	Nmol/L 15 – 49 (male, reference before 12/02/2019)
IGF-1 (N = 5) N = 5	25.28 [9.5 – 43.9] ± 12.69	10 – 41	N = 0	Nmol/L 8 – 60 (all ages, highest during puberty)
Anti-TPO (N = 1) N = 1	3.72 [2.64 – 4.80] ± 1.52	-10.00 – 17.44	N = 0	X 10^9/L 4.00 – 5.50 (12 – 15 years)

Table S4. CD serology results of the studied population.

Test	Mean [Range] ± std.	Reference values
TG2A IgA (N = 151)	1.64 [0.0 – 4.3] ±1.23	U/ml – corrected for IgA <0.10
N = 59	7.27 [5.2 – 9.3] ± 1.40	<5 (negative, reference before 01/07/2018)
N = 10	18.64 [10.0 – 42.0] ± 10.07	5-10 (weakly positive, reference before 01/07/2018)
N = 12	6.27 [1.9 – 17.6] ± 5.17	>10 (positive, reference before 01/07/2018)
N = 47	23.25 [20.4 – 27.7] ± 2.52	<20 (negative, reference after 01/07/2018)
N = 8	77.32 [30.1 – 334.4] ± 67.78	20 – 30 (weakly positive, reference after 01/07/2018)
N = 25		>30 (positive, reference after 01/07/2018)
TG2A IgG (N = 7)	0.78 [0.60 – 1.00] ± 0.17	U/ml
N = 6	21.0	<7 (negative)
N = 1		>= 7 (positive)
Endomysium IgA (N = 49)	N = 21	Negative
N = 21	N = 27	Weakly positive
N = 27	N = 1	Positive
N = 1		
DGP-IgG (N = 16)	3.20 [0.80 – 6.70] ± 2.03	U/ml
N = 9	8.9	<7 (negative, reference before 01/07/2018)
N = 1	15	7 – 10 (weakly positive, reference before 01/07/2018)
N = 1	7.20 [2.8 – 16.4] ± 5.43	>10 (positive, reference before 01/07/2018)
N = 5	-	<20 (negative, reference after 01/07/2018)
N = 0	-	20 – 30 (weakly positive, reference after 01/07/2018)
N = 0		>30 (positive, reference after 01/07/2018)