CHRONIC ABDOMINAL PAIN, FATIGUE and INFLAMMATORY BOWEL DISEASE in CHILDREN

Els Van de Vijver

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CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract with two distinct phenotypes: Crohn's disease (CD) and ulcerative colitis (UC). Approximately 10% of all patients with IBD manifestations in the colon have overlapping features of both phenotypes and are categorized as 'IBD unclassified' (IBDU).(1)

CD has a classic triad of presenting symptoms: abdominal pain, weight loss and diarrhoea. In contrast, weight loss is relatively rare as presenting symptom in patients with UC, in which rectal bleeding tends to be more prominent at the time of diagnosis. Clinical presentation of either of the two types of IBD varies according to the location, severity and chronicity of inflammation.(2) About 10% of all new patients with IBD are younger than 19 years of age at the time of diagnosis.(3) Compared to adults, children are more likely to present with extensive disease and to be at greater risk of complications.(2)

Strategies for diagnosing IBD, alleviating symptoms and improving well-being have been subjects of intensive research. Nevertheless, conclusive answers have yet to be provided regarding important research questions concerning the management of paediatric IBD (see **Table 1** for current knowledge gaps).

Table 1: Knowledge gaps concerning paediatric IBD

Diagnostics
Appropriate triage for patients with gastrointestinal complaints for endoscopic evaluation
Reliable monitoring of disease activity in ambulatory patients
Treatment
Understanding the effect of enteral nutrition on inflammation (and predicting success/failure)
Comparison between novel and currently available medication for children: long-term safety and efficacy
Use of genetic profiling to develop precision medicine
Effect of altering the gut microbiome with anti/pro/prebiotics
Education of patients
Optimizing drug adherence
Achieving treatment self-competence and autonomy in adolescents
Improving quality of life
Supporting psychological growth
Physical and psychological impact of lifelong therapy
Quantification and characterisation of fatigue in IBD
Identifying factors associated with fatigue

Inflammatory bowel disease is a chronic illness that is frequently characterized by periods of exacerbation and remission, and that often follows a progressive course. Unpredictable flares, frequent hospitalizations and chronic medication use affect psychosocial functioning and limit social activities.(4) Early recognition of IBD can reduce the inflammation in an early

phase of the disease, what could subsequently alter the course of the disease and prevent long-term complications.

In the absence of full curative treatment, the current ultimate goal of paediatric IBD care is to minimize the burden of disease for patients. This thesis focuses on the triage of children with abdominal complaints with regard to endoscopic evaluation, as well as on recognizing fatigue in IBD patients. In the first part of this thesis, we investigate strategies to improve the diagnostic pathway to identify children with gastrointestinal complaints due to IBD for whom endoscopic evaluation is indicated. In the second part, we address quality-of-life issues following the diagnosis of IBD and, more specifically, persistent fatigue in clinically inactive or mild/moderate IBD.

Part I **- Triage for endoscopy**

Endoscopy of the upper and lower gastrointestinal tract with biopsies is the reference standard for diagnosing IBD.(1) At the same time however, many patients with gastrointestinal complaints do not appear to have IBD. Since endoscopy is an invasive diagnostic procedure with the possibility of harmful complications, only patients with the highest risk of inflammatory disorders should be exposed to endoscopy. For general paediatricians who see many patients with gastrointestinal complaints (e.g. recurrent abdominal pain, diarrhoea), it may be a challenge to decide which child should be referred for endoscopy. A non-invasive test could enhance the process of triage for children with regard to referral for endoscopy. This triage test could be very helpful if it would be reliable in preventing children at low risk of IBD from undergoing endoscopy.

As stated above, IBD is characterized by chronic inflammation of parts of the intestine. For this reason, blood and urine markers have traditionally been assessed as indicators of intestinal inflammation in patients suspected of having IBD.(5) In current practice, C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) continue to be the most frequently used blood markers for inflammation. Despite the common use of such blood markers, their specificities and sensitivities for IBD are low, making them less suitable for decision making regarding which patients with gastrointestinal complaints should or should not be referred for endoscopy.(6) Substances that are excreted in urine (e.g. neopterin,

leukotriene E-4 and prostaglandine E metabolite) have also been investigated as markers of inflammation. To date, however, none of these markers has been validated for clinical use.(5)

Faecal markers

Before a biomarker can be safely used as a clinical triage test, its discriminatory power must be defined upon the intended patient population and in a specific clinical setting within the current diagnostic pathway. This is because the sensitivity and specificity of a test can vary according to the patient population and clinical setting.(7)

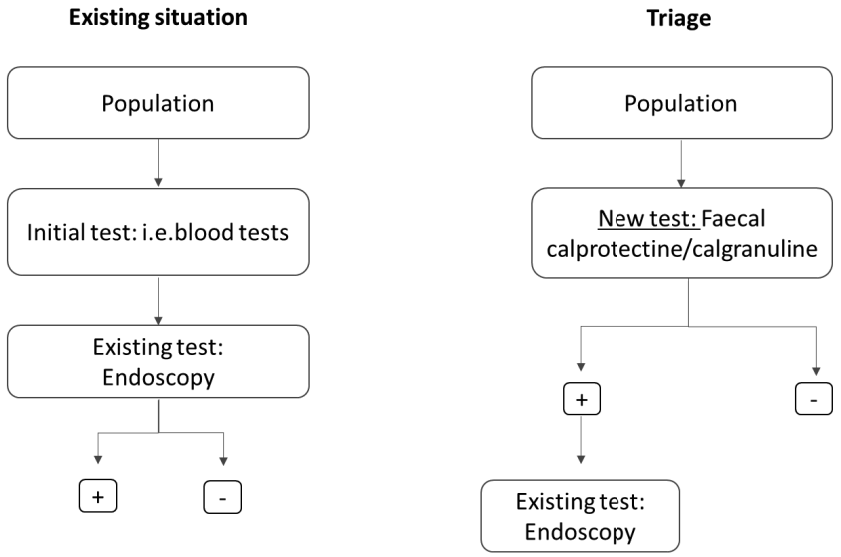

Figure 1 l Roles of tests and positions in existing diagnostic pathways. Figure adapted from (7)

Given that the inflammation in IBD is an intestinal process, it is logical that faecal parameters have also been suggested as being valuable for the assessment of intestinal inflammation. In practice, faecal markers have indeed become very popular for assessing the presence of inflammation in the bowel. During mucosal inflammation, various substances are actively released or passively leaked into the intestinal lumen, and they are subsequently excreted in the stool. An overview of the faecal parameters that have been evaluated is presented in **Table 2**.(8, 9)

Table 2: Faecal markers of inflammation (8, 9)

Faecal marker	**Molecular function/cellular source**	**Number of articles in paediatric literature (1990 - June 2019)**
Lysozyme	Released with degranulation of Paneth cells, macrophages and granulomas	20
Polymorphonuclear elastase	Released with degranulation of polymorfonuclear granulocytes; plays a role in the first-line host defence	43
Myeloperoxidase	Cytotoxic lysosomal protein released with degranulation of activated neutrophils	47
Metalloproteinase-9	Protein involved in neutrophil migration process, released by a variety of cell types, including activated neutrophils	3
Neopterin	Synthesized by activated macrophages; serves as marker of cellular immune-system activator	23
Lactoferrin	Cytoplasmic iron binding glycoprotein secreted by neutrophils and mucosal epithelial cells	21
Calprotectin (S100A8/S100A9)	Cytoplasmic calcium binding protein released by neutrophils, monocytes and epithelial cells	154

All substances listed in **Table 2** are found in abundance in the faeces of patients with active IBD, and concentrations are significantly lower in patients in remission.(5, 10) Calprotectin has been studied more extensively in children than any other faecal marker, and this biomarker test is now readily available in clinical laboratories.

Evaluating the accuracy of the calprotectin stool test

In 2010, we wrote a diagnostic meta-analysis (11) on the applicability of calprotectin as a triage test. All the articles included in that meta-analysis were based on the fully paired design that is typical of Phase II diagnostic accuracy studies. In these studies, a group of patients suspected of having IBD underwent faecal calprotectin testing followed by the reference standard: endoscopy. These studies estimated the diagnostic accuracy of faecal calprotectin under ideal experimental conditions.

In the next phase, a triage test (e.g. faecal calprotectin) needs to be used as means of triage before performing the existing diagnostic test (i.e. endoscopy), and only patients with positive results on the triage test will continue along the diagnostic pathway (see **Figure 1**). In 'Phase III' diagnostic accuracy studies, accordingly, not all suspected patients would need to undergo the reference standard.(12) To date, no Phase III diagnostic accuracy studies have been performed with regard to the potential value of faecal calprotectin. Therefore, we set ourselves to test the accuracy of faecal calprotectin with respect to the identification of patients with gastrointestinal complaints that should be referred for endoscopy because of IBD.

In **Chapter 2** we assess the role of faecal calprotectin as a triage test in the diagnostic work-up of children with gastrointestinal complaints (such as chronic abdominal pain, diarrhoea) in a Phase III diagnostic accuracy study. All children will have a faecal calprotectin test, but not all suspected patients will undergo endoscopy. The decision to expose a patient to endoscopy will be based on the physicians' clinical gut feeling. They are blinded to the FC result and consequently cannot take this result into account.

Faecal calprotectin is not the only marker for intestinal inflammation. Another candidate marker that could aid in differentiating IBD from IBS is Calgranulin-C (S100A12). Calgranulin-C is released almost exclusively by activated granulocytes, and has hardly been investigated as a marker of intestinal inflammation.(13) In previous case control studies, calgranulin-C showed diagnostic promise, exhibiting better specificity for intestinal inflammation relative to calprotectin.(14-16) In **Chapter 3**, we investigate the use of calgranulin-C to predict IBD in children and teenagers with chronic abdominal pain and diarrhoea, and we compare its accuracy to that of the calprotectin stool test.

Chapter 4 describes a further refinement of the formulated diagnostic test strategy of chapter 2, based on repeating the study in a separate and large validation cohort. We use an optimized cut off for calprotectin in combination with blood markers, and modify the inclusion criteria of the study cohort.

Part II **- Quality of life beyond clinical remission: Fatigue in paediatric IBD**

Treatment goals in IBD have changed considerably over the years. In the past, treatment was limited to controlling exacerbation, and it was aimed at alleviating clinical symptoms. The introduction of biological agents as anti-inflammatory therapy marked the beginning of a new era. Mucosal healing became the primary goal of treatment. The use of these agents have decreased rates of surgery and hospitalization.(17) In current practice, the focus of IBD treatment is typically on anti-inflammatory agents and therapies that modulate the immune system. However, many symptoms may not be directly caused by active inflammation, but may involve other mechanisms, including disease-associated symptom experience and psychological processes.(18) Moreover, the increasing recognition of, and appreciation for, the importance of health-related quality-of-life has spurred the use of such patient-important outcomes in clinical trials.

Quality of life in IBD

The World Health Organization defines quality of life as 'an individual's perception of their position in life, in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns'.(19) In IBD, stress and psychological health are likely to play an important role in symptom experience. For this reason, an exclusive focus on anti-inflammatory therapies is not expected to fully serve the needs of the patient. One important symptom that is frequently reported by IBD patients is fatigue.

Fatigue

Fatigue refers to a subjectively overwhelming sense of tiredness, lack of energy and a feeling of exhaustion that decreases an individual's capacity for physical and mental activity.(20) It is

a common, independent and nonspecific symptom that has been identified in numerous chronic health conditions in childhood, including IBD.(21)

Fatigue can be a major source of disablement in patients with a chronic disease, and it is often reported as being amongst the most severe and distressing symptoms.(22) Fatigue affects physical, emotional, cognitive and social functioning, and it has an impact on the quality of life. Despite its importance, researchers have rarely included and quantified fatigue in assessments of symptom severity or outcomes of chronic diseases in which it is observed, including chronic obstructive pulmonary disease, cystic fibrosis, chronic renal disease, cancer and heart failure.(22)

The quantification of fatigue poses several challenges, due to the lack of a consensus framework, vague terminology and a multidimensional nature of symptoms. Although subjective methods (e.g. self-reported or parent-reported surveys)(23, 24) are commonly used, they can be distorted by response and recall bias. More objective methods (e.g. polysomnography and performance tests)(25-27) are expensive and time-consuming, and the prevalence of fatigue varies by age group. For example, it is common in infancy, early childhood and late adolescence, while being less frequently observed in mid-childhood, and it is more common in girls than it is in boys.(26) Such variations in fatigue amongst healthy children makes it difficult to quantify fatigue in patients.

Fatigue is determined by a variety of factors. In adult studies, active inflammation, depression and other factors are implied in fatigue. A recent review on fatigue in IBD patients classifies factors into two categories: modifiable and non-modifiable.(28) The modifiable factors include physical factors (e.g. disease activity, anaemia, physical functioning and fitness), as well as psychosocial factors (e.g. anxiety, depression and self-directed personality). Non-modifiable factors include disease duration, gender and extra-intestinal manifestations. All studies in the review describe adult patients, and it is unclear whether a similar classification would apply to children with IBD. For this reason, we performed a systematic review of the existing literature aimed at exploring the prevalence of fatigue in paediatric IBD and identifying elements that contribute to fatigue (**Chapter 5**). Several studies have suggested that disease activity, anaemia and physical functioning affect the experience of fatigue.(24, 29-31)

In **Chapter 6**, we evaluate the effect of anaemia, subclinical inflammation and physical fitness on the sense of fatigue. For that, we conducted a cross-sectional observational study in children and adolescents with IBD. This study is based on validated questionnaires for fatigue and quality of life, endurance testing, blood testing and stool analysis to delineate fatigue in IBD. The study is intended to assess the extent to which the severity of fatigue is correlated with disease-related factors and biochemical parameters.

In **Chapter 7**, we discuss the implications of our different studies for clinical practice, a few remaining unresolved issues and we present suggestions for future research.

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CHAPTER 2

SAFELY RULING OUT IBD IN CHILDREN AND TEENAGERS WITHOUT REFERRAL FOR ENDOSCOPY

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ABSTRACT

BACKGROUND: Up to 70% of children and teenagers referred to a paediatric gastroenterology center with suspected inflammatory bowel disease (IBD) do not have the disease.

OBJECTIVE: To evaluate whether faecal calprotectin (fCal) as an „add-on test" improves the specificity of the clinical case definition for suspected IBD in a general paediatric practice.

METHODS:

DESIGN: A prospective diagnostic accuracy study.

SETTING: Six outpatient clinics for general paediatrics and one tertiary care hospital in the Netherlands.

PATIENTS: 117 children and teenagers with a clinical suspicion of IBD

DIAGNOSTIC TESTS: Faecal calprotectin was measured (index test) in all patients. Patients with a high index of suspicion on the basis of the paediatrician's global assessment, physical examination and blood results were referred for endoscopy (reference standard). Children and teenagers who were not selected for endoscopy initially, were followed for half a year for the appearance of possible additional symptoms (delayed type reference standard).

PRIMARY OUTCOME: The proportion of referred patients with confirmed IBD.

RESULTS: The mean age of the included patients was 14 years (range 6 - 18). A total of 42 (36%) had confirmed IBD. The paediatricians, who were blinded to the faecal calprotectin result, referred 68 children and teenagers for endoscopy. If they had referred only those patients with a positive faecal calprotectin result (> 50 µg/g), 54 patients would have undergone endoscopy.

LIMITATION: The study relied on clinical follow-up to detect missed IBD.

CONCLUSION: A diagnostic strategy in general paediatric practice by using a simple clinical case definition for suspected IBD in combination with a positive fC result, increases the specificity to detect IBD and reduces the need for referral to a paediatric gastroenterology center with a very low risk of missing a case.

INTRODUCTION

For the paediatrician treating a child with recurrent abdominal pain and diarrhoea it is often difficult to clinically distinguish between those who need an endoscopy and those who do not. Identification of children with low likelihood of organic disease would justify a non-invasive "watchful waiting" strategy, while a high likelihood of inflammatory bowel disease (IBD) would justify referral to specialist services for endoscopy. In a recently published diagnostic meta-analysis(1) we found that increased levels of calprotectin in the stool can identify children who are most likely to have IBD. All included studies used the fully paired design in which a group of patients first undergo faecal calprotectin testing and then endoscopy. These studies estimated the diagnostic accuracy of faecal calprotectin under ideal experimental circumstances. We conducted a phase III diagnostic accuracy study to determine whether faecal calprotectin could serve as a screening test to identify children with a high likelihood for IBD and reduce the number of children and teenagers undergoing invasive endoscopy.

We aimed to determine an objective diagnostic strategy to minimise the number of children and adolescents with negative endoscopy results without missing any case of IBD.

PATIENTS AND METHODS

Study setting and participants

This study was performed at the paediatric outpatient clinic of six general hospitals and one tertiary care hospital (University Medical Center Groningen (UMCG)) in the northern region of the Netherlands. Most paediatricians in the general hospitals were trained as fellows in paediatric gastroenterology and half of them had their training in the UMCG. Children and teenagers between 6 and 18 years of age with abdominal complaints were eligible for participation. Younger children have higher normal values of faecal calprotectin and were excluded for this reason.(2,3) Patients were included when they fulfilled the clinical case definition for suspected IBD. (**box 1**)

Box 1. Criteria used to define a population of children and teenagers with a clinical suspicion of IBD.

Persisting diarrhoea (>4 weeks)
OR recurrent (≥ 2 episodes in 6 months) abdominal pain and diarrhoea
AND at least one of the following criteria:
rectal blood loss
unintended weight loss or linear growth retardation
peri-anal symptoms (skin tag, fistula, fissure, abscess)
Anaemia (Hb cut-off point in mmol/L): < 12 y: 7.1; girls ≥ 12 y: 7.4; boys ≥ 12 y: 8.1 [15] or other extra-intestinal manifestations (erythema nodosum, arthritis, uveitis)
Increased markers of inflammation: ESR > 20 mm/hr; C-reactive protein > 10 mg/L

Data collection

After the first presentation at the outpatient clinic, all patients provided a stool sample collected at home. The faecal samples were analysed at the Department of Laboratory Medicine in the UMCG by a commercially available quantitative assay (CALPRO®ELISA test (ALP), Calpro AS, Lysaker, Norway). The manufacturer cut-off point is 50 µg/g stool. Laboratory technicians were blinded to the final diagnosis of the patients. The decision to perform any other diagnostic tests (including endoscopy) was left to the paediatric gastroenterologist's discretion and was independent of the faecal calprotectin result. Confirmation of IBD was obtained after endoscopic and histological evaluation according to the ESPGHAN Porto Criteria.(4) The diagnosis "non-IBD" was made when other test results gave a convincing explanation for the symptoms, or when the symptoms had completely resolved at 6-month follow-up. An experienced paediatric histopathologist, who was blinded to the results of other diagnostic tests, assessed each biopsy specimen. Stool cultures were evaluated for Salmonella, Shigella, Yersinia, Campylobacter, Clostridium difficile (inclusive of toxin A and B), Giardia Lamblia, Entamoeba histolytica an parasites. (**Fig 1**)

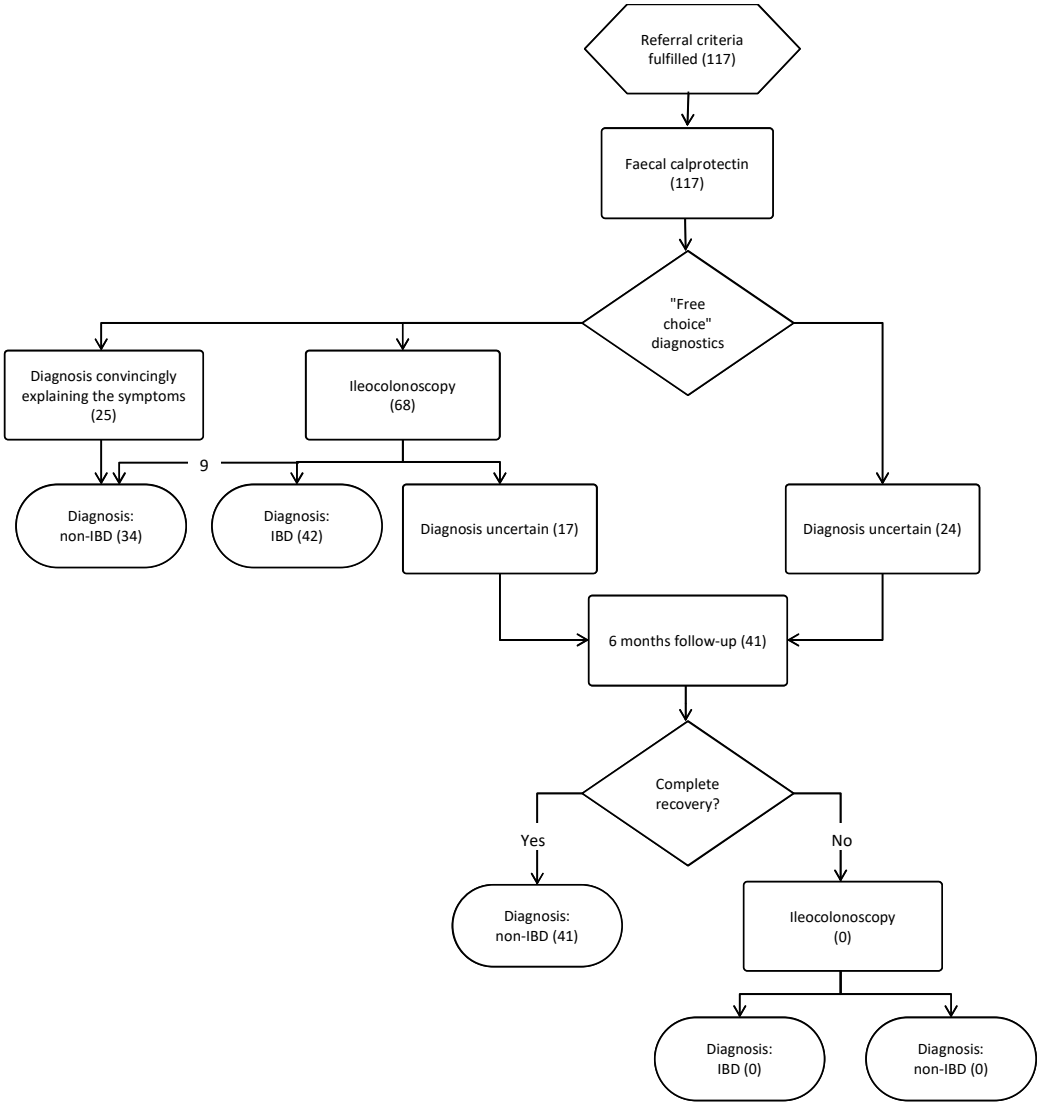

Figure 1 l Study design and patient flow (n)

Statistics

Parameters of test reliability including sensitivity, specificity, likelihood ratios and post-test probabilities with their 95% CIs were calculated with the use of the MedCalc statistical software package for biomedical research, (V.12.1.4). To compare the specificities of the different diagnostic pathways the McNemar test for paired data was used. Student's t-tests and chi-square tests were used to compare baseline characteristics between groups. All tests were two sided and the level of significance used was a p value < 0.05. Data were analysed in SPSS (version 19.0 for windows).

Ethical approval and informed consent

This study was exempted from Institutional Review Board approval as it involved the study collection of data generated by routine medical care. The data were collected and recorded by the investigators in such a manner that subjects could not be identified, directly or through identifiers linked to the subjects.

RESULTS

Between February 2009 and June 2010, 117 children and teenagers were prospectively included in the study. Patient characteristics are presented in **Table 1**.

Table 1: Patient characteristics

	Confirmed IBD (n=42)	**Non-IBD (n=75)**
Age in years, mean (SD; range)	**14 (3,3; 6-18)**	**12,9 (3,8; 6-18)**
Male (%)	**45**	**50**
Presenting Symptoms (% (95% CI))		
Rectal blood loss	**62% (47 to 77)**	**41% (30 to 52)**
Unintended weight loss or linear growth retardation	**55% (40 to 70))**	**46% (35 to 57)**
Peri-anal symptoms (skin tag, fistula, fissure, abscess)	**19% (7 to 31)**	**9% (3 to 15)**
Anaemia: < 12 y: 7.1; girls ≥ 12 y: 7.4; boys ≥ 12 y: 8.1 (Hb cut-point in mmol/L) or other extra-intestinal manifestations (erythema nodosum, arthritis, uveitis)*	**83% (72 to 94)**	**46% (35 to 57)**
Increased markers of inflammation: ESR > 20 mm/hr; Creactive protein > 10 mg/L *	**83% (72 to 94)**	**28% (18 to 38)**

* $p < 0.05$

IBD was confirmed in 42 patients (36%). Twenty-four were diagnosed with Crohn's disease, 16 with ulcerative colitis and two with IBD unclassified. There were no differences in age and sex between the groups with confirmed IBD and non-IBD. The paediatricians, who were blinded to the faecal calprotectin result, referred 68 children and teenagers for endoscopy on the basis of a high index of suspicion for IBD. They were evaluated according to the Porto criteria with gastroscopy and ileocolonoscopy (97% ileal intubation) with segmental biopsies for histological evaluation. One child was diagnosed with Crohn's Disease after a negative gastroscopy and ileocolonoscopy but positive MR enterography.

Table 2: Non IBD diagnoses (N=75)

Diagnosis	**n (%)**	**Faecal calprotectin ≥50 µg/g, n**
Infectious diseases	**13 (17)**	
Viral gastroenteritis	2	0
Bacterial gastroenteritis (Yersinia, Campylobacter and Salmonella)	7	6
Parasites(Blastocystis, Giardia, Entamoeba coli, Enterobius)	4	2
Nutrition related diarrhoea	**2 (3)**	
Toddler diarrhoea	2	0
Miscellaneous gastrointestinal diseases	**18 (24)**	
Constipation	9	0
H.Pylori gastritis	1	0
Meckel's diverticulum	1	0
Solitary rectal ulcer	1	0
Spontaneous reduction of invagination	1	0
Eosinophilic gastro-enteritis	1	1
Haemorrhoids	1	0
Physical activity induced intestinal ischemia	1	1
Superior Mesenteric Artery syndrome	1	0
Celiac disease	1	0
Non organic gastrointestinal diseases	**34 (45)**	
Functional abdominal pain	32	7
Anorexia nervosa	2	0
Other	**1(0,1)**	
Juvenile dermatomyositis	1	1
Spontaneous recovery, no definite diagnosis	**7 (9)**	2

Table 2 shows the diagnoses of patients without IBD. The majority of these patients did not need endoscopy to exclude IBD and had other tests including stool analyses for bacteria, ova and parasites, gastroscopy, abdominal ultrasound, CT-scan, Meckel scan, serology and dietary measurements leading to the diagnosis. Thirty-two children and teenagers were diagnosed with functional abdominal pain and were followed for at least 6 months to confirm this diagnosis. Seven of these patients had elevated calprotectin (range 97-400 µg/g stool) and 11 underwent endoscopy (including ileocolonoscopy) with negative results. Sixteen patients without IBD had abdominal ultrasound, six CT-enteroclysis and three abdominal MRI as part of their diagnostic work up. Seven patients had no definite diagnosis despite several tests and had spontaneous recovery of their ailments during follow up.

Median time between faecal sampling for calprotectin and endoscopy was 22 days (range 0-79) in the confirmed IBD-group and 42 days (0-164) in the non-IBD group (p=0.105).

Diagnostic accuracy

The pre-test probability of IBD in our study population was 36%. Calprotectin (cut-off point 50 µg/g) was elevated in all children and teenagers with IBD (sensitivity 100%, 95% CI 92% to 100%), and in 20 out of 75 patients without IBD (specificity 73%, 95% CI 62% to 83 %). Table 3 shows that a value above the cut-off point (in the absence of gastrointestinal infection) gives a specificity of 81% (95% CI 69% to 90%).

Table 3: Added value of the "clinical eye" or faecal calprotectin testing in a population of children and teenagers suspected of IBD

Characteristics	**"Clinical eye" of the paediatrician**	**Faecal calprotectin**	**Faecal calprotectin (gastrointestinal infections excluded)**
n	117	117	104
True positives	42	42	42
True negatives	49	55	50
False positives	26	20	12
False negatives	0	0	0
Pre-test probability	36%	36%	40%
Sensitivity (95% CI)	100% (92 to 100%)	100% (92 to 100%)	100% (92 to 100%)
Specificity (95% CI)	65% (53 to 76%)	73% (62 to 83%)	81% (69 to 90%)
Positive likelihood ratio (95% CI)	2.9 (2.1 to 3.9)	3.8 (2.6 to 5.5)	5.2 (3.1 to 8.6)
Negative likelihood ratio (95% CI)	0	0	0
Post-test probability given a positive test result (95% CI)	62% (49 to 73%)	68% (55 to 79%)	78% (64 to 88%)
Post-test probability given a negative test result (95% CI)	100% (93 to 100%)	100% (93 to 100%)	100% (93 to 100%)

Scenario analysis

When the decision to schedule patients for endoscopic evaluation is left to the paediatrician's discretion (as was the case in our study), 38% of the children and teenagers subjected to ileocolonoscopy had a negative result (**Table 4**).

Table 4. Scenario analysis

Referral based on:	Patients subjected to ileocolonoscopy (n)	IBD-negative ileocolonoscopy (n)	Proportion with IBDnegative ileocolonoscopy
"Clinical eye" of the paediatrician	68	26	38%
Faecal calprotectin > 50 µg/g	62	20	32%
Faecal calprotectin > 50 ug/g (in absence of gastrointestinal infection)	54	12	22%

If the decision for referral were to be solely based on the faecal calprotectin result, 32% of the children subjected to ileocolonoscopy would have had a negative result. Basing referral on a positive faecal calprotectin result in the absence of gastrointestinal infection would have resulted in 22% negative ileocolonoscopies, and no missed IBD cases. The reduction of negative endoscopies is significant (p= 0.001).

DISCUSSION

Implications of key findings

To our knowledge, this is the first phase III study that evaluates the usefulness of faecal calprotectin in routine paediatric practice. The analytical eye of the paediatrician selected 68 children and teenagers for endoscopy to confirm IBD, of which 26 (38%) had a negative result. A referral based on a faecal calprotectin level above 50 µg/g without excluding a gastrointestinal infection would wrongly select 20 out of 62 patients (32%). When patients with proven gastrointestinal infections are excluded beforehand the number of IBD-negative endoscopies would be significantly reduced to 22% (12 out of 54 suspected patients) without missing one case of IBD.

Comparison with other studies

We recently published a diagnostic meta-analysis (1) in which we summarised the best available evidence on the diagnostic accuracy of faecal calprotectin for screening. We included the reports of six adult studies and seven Phase II studies in children and teenagers. We concluded that calprotectin is accurate when screening for suspected IBD in adults, but less so in children. The pooled sensitivity and specificity in children and teenagers was 0.92 (95% CI 0.84 to 0.96) and 0.76 (95% CI 0.62 to 0.86), respectively. Gastrointestinal infections, especially bacterial infections with Shigella, Yersinia or Salmonella, can mimic the onset of IBD in children and teenagers and cause increased faecal calprotectin levels. Since the publication of our meta-analysis two new paediatric phase II study have been published.(5,6) In these studies a fully paired design was used in which all included children were subjected to endoscopic evaluation. In "real life", when another diagnosis than IBD is more likely, paediatricians may wish to avoid this invasive and uncomfortable procedure. The major difference between the Diamanti study and ours was a remarkable high pre-test probability of 60 % that can be explained by the tertiary care setting in which a highly selected group of patients with suspected IBD was seen. In our study pretest probability was 36%. In the Henderson study the patients with a high calprotectin result and no endoscopy were not evaluated and followed up.

Limitations of this study

Although phase II studies give cause to overestimate diagnostic accuracy, the validity of a phase III study like ours is also compromised. As not all patients underwent the reference test, uncertainty remains about the correct diagnosis in some patients. We therefore decided to add another prognostic dimension to the reference standard, namely, the clinical course of patients. IBD is not a self-limiting disease and will usually become clinically manifest within a few months after the first diagnostic suspicion, in this study the moment of enrolment. Patients who remained free of disease for 6 months were believed not to have IBD. This "delayed type" diagnosis may not be ideal, but is the best achievable solution closely connected with the reality of clinical care.(7)

Faecal calprotectin can give false-positive results when patients use non-steroidal anti-inflammatory drugs (8-10) or proton pump inhibitors.(11) We did not collect information

about the use of these drugs in the study population, which could be the reason why some children with functional abdominal pain had elevated faecal calprotectin. In a cross-sectional study of school-aged children with functional abdominal pain and irritable bowel syndrome, slightly elevated calprotectin concentrations were found in their stools compared with control children.(12) However, the concentrations were clearly in the lower range (65+/- 75 µg/g) while the majority of confirmed IBD cases in our population had concentrations way above 500 µg/g on their first presentation.

Applicability of findings to paediatric care

Pre-test probability of IBD in our study population was 36%. The study population was a combination of patients from secondary and tertiary care hospitals, where the emphasis is usually on "ruling in": increasing the probability of IBD before carrying out more expensive, time-consuming and invasive procedures; establishing a firm diagnosis; and starting appropriate treatment. A diagnostic test with a high specificity is therefore preferred.(13) In this study we tested the "ruling out" properties of faecal calprotectin in paediatric practice. We found that a normal calprotectin level reduces the probability of IBD to zero. In other words, the diagnosis IBD can be ruled out with confidence. The paediatrician does not need to refer the patient for endoscopic evaluation, but instead can provide reassurance or adopt a " watchful waiting" strategy.

C-reactive protein (CRP) is a far less reliable test to rule out IBD than calprotectin. In a recently published paper a large number of children and teenagers with newly diagnosed IBD proved to have normal CRP levels.(14)

The conclusions of this study cannot simply be translated into the practice of the general practitioner or family doctor. IBD prevalence in the general population is much lower. In the first-line setting the number of false-positive calprotectin results will therefore increase, exposing too many patients without IBD to unnecessary endoscopic procedures. At present, there is no evidence of how the test performs in primary care. If studies conducted in primary care find a high diagnostic accuracy of the faecal calprotectin test it, will be an important step forward in how IBD is diagnosed.

CONCLUSION

A diagnostic strategy in general paediatric practice of using a simple clinical case definition for suspected IBD in combination with a positive faecal calprotectin result increases the specificity to detect IBD and reduces the need for referral to a paediatric gastroenterology center with a very low risk of missing any cases. At the same time normal calprotectin levels confidently rule out intestinal inflammation and further investigations can be tailored appropriately without referring the patient for endoscopy. This is good news for patients (less invasive tests) and clinicians (shorter waiting lists for endoscopy).

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CHAPTER 3

PREDICTING INFLAMMATORY BOWEL DISEASE IN CHILDREN WITH ABDOMINAL PAIN AND DIARRHOEA: CALGRANULIN-C VERSUS CALPROTECTIN STOOL TEST

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ABSTRACT

BACKGROUND: Calgranulin-C (S100A12) is a new faecal marker of inflammation that is potentially more specific for inflammatory bowel disease (IBD) than calprotectin, since it is only released by activated granulocytes. We compared calgranulin-C and calprotectin to see which of the two tests best predicted IBD in children with chronic abdominal pain and diarrhoea.

METHODS:

DESIGN: Delayed-type cross-sectional diagnostic study.

SETTING AND PATIENTS: Previously undiagnosed patients aged 6 to 17 years, who were seen in paediatric clinics in the Netherlands and Belgium, sent in a stool sample for analysis. Patients with a high likelihood of IBD underwent upper and lower endoscopy (i.e. preferred reference test), while those with a low likelihood were followed for 6 months for latent IBD to become visible (i.e. alternative reference test). We used Bayesian modeling to correct for differential verification bias.

MAIN OUTCOME MEASURES: Primary outcome was the specificity for IBD using predefined test thresholds (calgranulin-C 0.75 µg/g, calprotectin 50 µg/g). Secondary outcome was the test accuracy with thresholds based on receiver operating characteristics (ROC) analysis.

RESULTS: IBD was diagnosed in 93 of 337 patients. Calgranulin-C had significantly better specificity than calprotectin when predefined thresholds were used (resp. 97% [95% CI 94-99%] vs. 71% [95% CI 63-79%]). When ROC-based thresholds were used (calgranulin-C 0.75 µg/g, calprotectin 400 µg/g), both tests performed equally well (specificity 97% [95% CI 94-99] vs. 98% [95% CI 95-100%]).

CONCLUSIONS: Both calgranulin-C and calprotectin have excellent test characteristics to predict IBD and justify endoscopy.

INTRODUCTION

Inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis, are lifelong conditions that often begin in childhood. Suspicion is raised in children and teenagers with chronic abdominal pain and diarrhoea. Additional red flag symptoms including rectal bleeding, weight loss, and anaemia increase the suspicion of the condition. Endoscopic evaluation of the upper and lower gastrointestinal tract with biopsies for histology is essential to diagnose IBD and to differentiate Crohn's disease from ulcerative colitis and IBD-unclassified, start appropriate therapy and prevent progressive bowel damage.(1) Many children consider endoscopy and the required bowel preparation to be uncomfortable.(2) Identification of children with a low likelihood of IBD would justify a non-invasive "watchful waiting" strategy, while on the other hand identification of those with a sufficiently high likelihood of IBD would justify urgent referral to specialist services for endoscopy.

In recent years the stool calprotectin test has been promoted as a safe and easy interpretable triage tool for endoscopy.(3,4) Calprotectin is mainly released by neutrophil granulocytes, but other cells including monocytes and epithelial cells do also excrete this protein.(5) To date, a calprotectin concentration below 50 µg/g has been proposed to rule out IBD and not to proceed to endoscopy.(6,7) However, there are concerns about the mediocre specificity of the test at this threshold, which may give rise to a considerable proportion of children and teenagers proceeding to a pointless invasive procedure.

Calgranulin-C (S100A12) is a less frequently investigated marker of intestinal inflammation that is almost exclusively released by activated granulocytes.(5) In previous case-control studies calgranulin-C showed diagnostic promise with better specificity compared to calprotectin,(8-10) but large studies in a prospective cohort with chronic abdominal pain and diarrhoea are lacking.

The aim of this study was to compare calprotectin and calgranulin-C to see which of the two markers best predicted IBD in children and teenagers with chronic abdominal pain and diarrhoea.

METHODS

Design

This was an international multicenter, delayed-type cross-sectional diagnostic accuracy study with a paired design.(11) Previously undiagnosed children and teenagers presenting with persistent diarrhoea for more than 4 weeks or chronic or recurrent abdominal pain were screened with the calprotectin stool test (existing test) and with the calgranulin-C test (new test). Confirmation of the target condition (IBD) was based on endoscopy with biopsies (reference standard) or clinical follow-up (alternative reference standard). The study was registered before recruitment of the first participant, and the study protocol has been published in BMJ Open.(12)

Patients

Patients were recruited from sixteen secondary and three tertiary level hospitals in the Netherlands and Belgium. They were eligible when aged between 6 and 17 years. The flow of patients from the first hospital visit to the choice of the reference test was described comprehensively in our published study protocol.(12) In brief, during the first hospital visit baseline characteristics, date of birth, presence of major and minor red flag signs and symptoms for IBD and use of non-steroidal anti-inflammatory drugs were entered on the study website (www.cacatustudie.eu). A stool specimen was collected at home and sent to the hospital laboratory of the coordinating study center, where it was immediately tested for calprotectin and colon pathogens (including *Shigatoxin-producing Escherichia coli, Salmonella, Shigella, Enteroinvasive Escherichia coli, Campylobacter*, and *Giardia lamblia*) with a real-time multiplex PCR technique. Residual faeces was stored at −80°C for calgranulin-C batch testing at a later stage.

Assays

Stool calprotectin concentrations (µg/g) were measured with the fCAL ELISA (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland) and stool calgranulin-C concentrations (µg/g) with the commercially available Inflamark ELISA (CisBio Bioassays, Codolet, France), both on a Dynex DS2 Automated ELISA System (Alpha Labs, Easleigh, UK) in the same laboratory. The extraction and measuring technique of calgranulin-C was previously described in detail.(13) In discordant pairs (i.e. increased calprotectin and normal calgranulin-C, or vice versa) we did a posthoc analysis of potential viral causes (adeno, entero, astro, rota, noro, parecho and

sapovirus). Laboratory technicians were blinded to symptoms filled in on the website. The attending paediatricians were informed of the calprotectin and PCR result for bacteria and Giardia lamblia, but they were blinded to the calgranulin-C and PCR result for viruses. The predefined thresholds used in this study were 50 µg/g for calprotectin and 0.75 µg/g for calgranulin-C.

Reference tests

We used an automated IBD Risk Stratifier (**figure 1**) to advice paediatricians whether patients should proceed to endoscopy (the preferred reference standard) for verification of IBD, or whether they should be followed up clinically for possible latent IBD to become visible (the alternative reference standard).

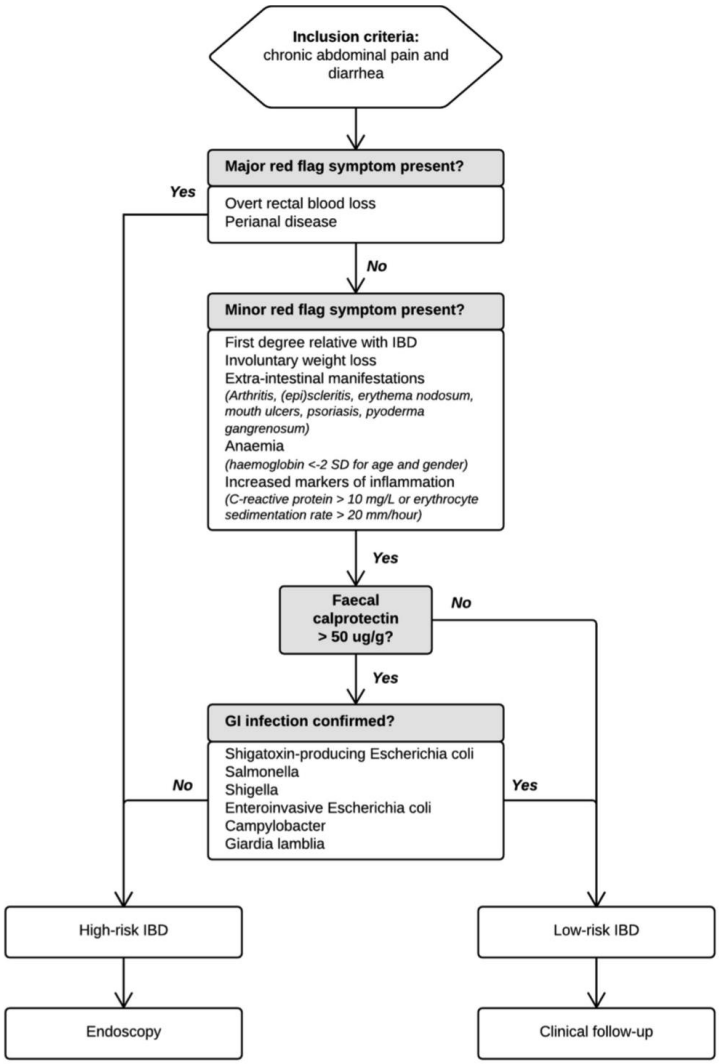

Figure 1 I Algorithm explaining the multi-step IBD Risk Stratifier used to standardize the assignment of patients to either endoscopy or clinical follow-up

Paediatricians could deviate from this advice for documented clinical reasons. Endoscopy was performed under general anesthesia by an experienced paediatric gastroenterologist in one of six participating centers. Both upper and lower gastrointestinal tract were evaluated according to the revised Porto criteria,(14) and biopsies were taken from every bowel segment. Histopathological examination was performed by experienced histopathologists. Endoscopists and histopathologists had access to clinical information and calprotectin results,

but were blinded to the results of the calgranulin-C test. In case patients were assigned to the alternative reference standard, they were re-evaluated using the IBD Risk Stratifier until six months after inclusion. In case the initial risk stratum changed to 'high-risk', endoscopy was performed ultimately.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, version 22.0 for Windows) and presented with GraphPad Prism (version 5 for Windows (San Diego, California, USA)). Diagnostic accuracy measures (sensitivity, specificity, positive predictive value, negative predictive value) were calculated for both the high-risk and low-risk stratum using predefined thresholds, as well as optimal thresholds (defined as the most upper left data point in the receiver operating characteristic (ROC) curve). Since we used both endoscopy and clinical follow-up as reference standard -with the latter being less accurate-, we used a Bayesian correction method to adjust for differential verification bias.(15-17) This method takes into account the verification pattern as well as bias due to imperfection of the clinical follow-up in a single model. The method requires specifying the verification pattern and giving a best guess of the accuracy of both reference standards in the form of a prior distribution. We assumed that endoscopy had 95–100% sensitivity and 95–100% specificity to diagnose IBD, and that clinical follow-up had a sensitivity of 80–100% and a specificity of 60–80% to diagnose latent IBD. Our inferences are based on the posterior distributions calculated using JAGS ('Just Another Gibbs Sampler'), a free program licensed under GNU General Public License.(18) The R-package script is provided in supplementary data 1. The sample size calculation was previously described.(12)

Human Subjects Protection

This study was performed according to the Declaration of Helsinki. This study was conducted with the approval of the Medical Ethical Committee of the University Medical Center in Groningen (METc 2013/503) and Antwerp University Hospital (14/40/407). All participants aged 12 and above and their legal guardians gave informed consent to use data generated by routine medical care. The data were collected and recorded by the investigators in such a manner that subjects could not be identified, directly or through identifiers linked to the subjects.

RESULTS

A total number of 354 children and teenagers with chronic abdominal pain and diarrhoea were recruited into the study between September 2014 and September 2016, and 337 were included in the final analysis. In the early stages 142 patients proceeded to endoscopy, while 195 were assigned to clinical follow-up. Another 19 children from the low-risk group were referred for endoscopy at a later stage. Eventually 48% of patients in the study cohort (161 of 337) underwent endoscopy, of which 93 were diagnosed with IBD. The patient study flow is shown in **Figure 2**.

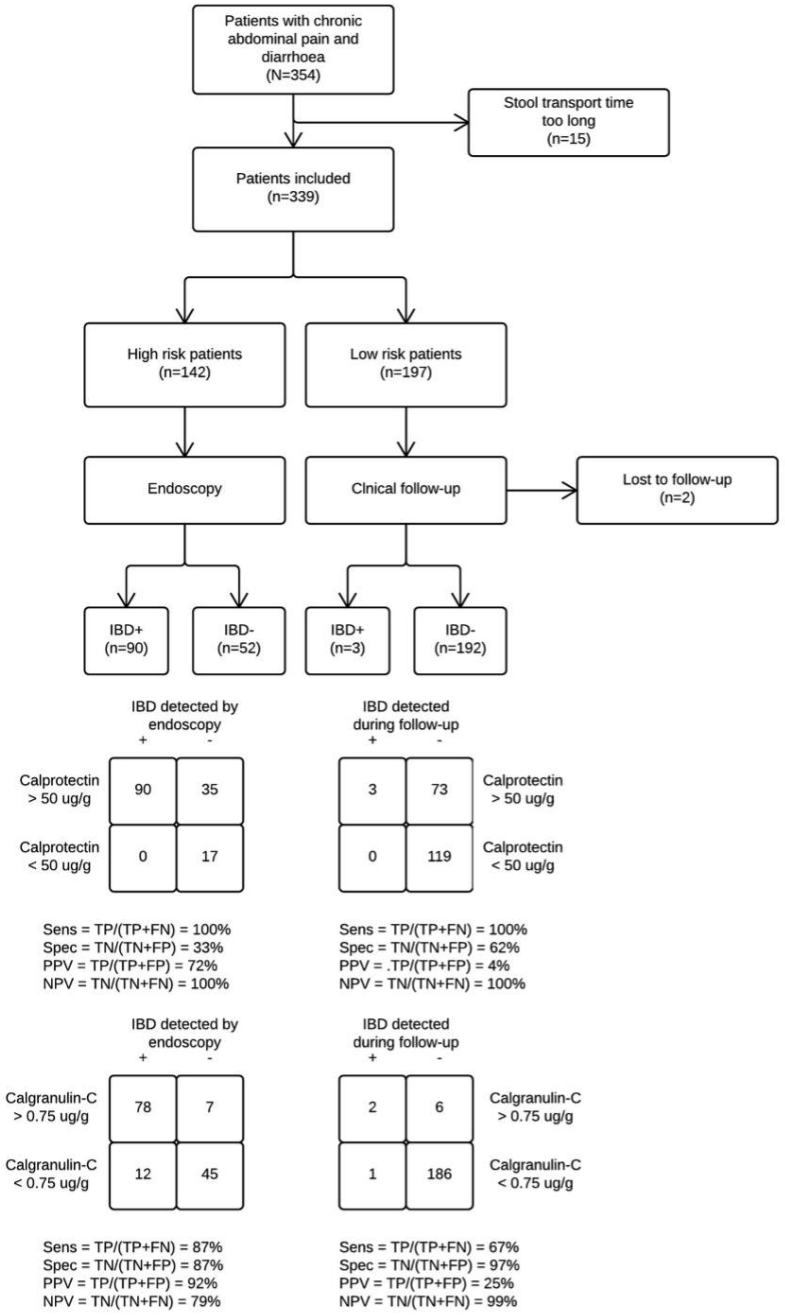

Figure 2 I Study flow diagram showing differential verification bias
IBD = inflammatory bowel disease; Sens = sensitivity; Spec = specificity; NPV = negative predictive value; PPV = positive predictive value.

Baseline characteristics are presented in **Table 1**. The patients in the high risk stratum were older, had more red flag symptoms and higher calprotectin concentrations than those in the low risk stratum. Three patients who were initially in the low risk stratum were later found to have IBD. All three had elevated faecal calprotectin concentrations (range 340 to 480 μg/g) and a positive PCR result.

Table 1: Baseline characteristics of patients with chronic abdominal pain and diarrhoea stratified into high and and low risk for inflammatory bowel disease (IBD).
Values are number (%) unless otherwise stated.

Characteristics	**High risk (n=142)**	**Low risk (n=195)**
Reference test	**Endoscopy**	**Clinical follow-up**
Demographics		
Median age in years (interquartile range)	14 (11-15)	12 (9-14)
Male gender	67 (47%)	112 (57%)
Major red flag symptoms		
Overt rectal blood loss	90 (63%)	0 (0%)
Perianal disease (superficial anal fissures excluded)	20 (14%)	0 (0%)
Minor red flag symptoms		
Weight loss or linear growth deceleration	52 (37%)	47 (24%)
Extra-intestinal symptoms (including arthritis)	20 (14%)	13 (7%)
Family history of IBD	12 (9%)	18 (9%)
Anaemia (haemoglobin <-2 SD for age and gender)	56 (39%)	19 (10%)
Increased markers of inflammation (C-reactive protein> 10 mg/L or erythrocyte sedimentation rate >20 mm/hr)	58 (41%)	10 (5%)
Stool test		
Increased calprotectin (>50 μg/g)	125 (88%)	76 (39%)

Ongoing or worsening symptoms despite eradication of the pathogen made the clinician decide to proceed to endoscopy. The distributions of calprotectin and calgranulin-C values per final diagnosis are shown in **Figure 3**.

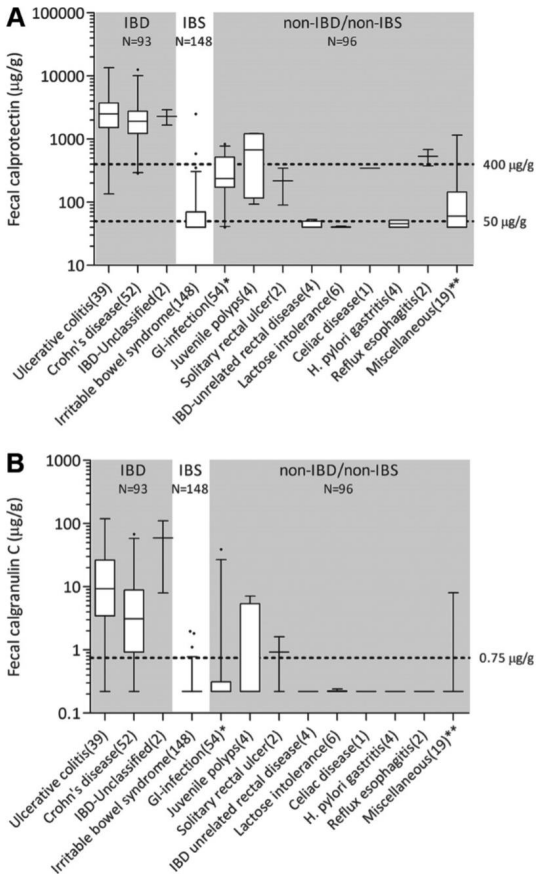

Figure 3 I Box- and whisker plot for calprotectin (A) and calgranulin-C (B) concentrations per diagnosis. Whiskers represent the 95% confidence interval. Number of cases in brackets.
* The GI-infection group had either bacterial colon pathogens or G. lamblia.
**The miscellaneous group included bile-salt diarrhoea (n=1), hemolytic uremic syndrome (n=1), mediterranean fever (n=1), fructose overload (n=1), spondylarthropathy (n=1), Hirschsprung's disease (n=1) and allergic enterocolitis (n=1). The remaining 12 were "non-IBD, not otherwise specified".

Predefined thresholds

Figure 4A shows the diagnostic accuracy measures based on predefined thresholds for calprotectin (50 µg/g) and calgranulin-C (0.75 µg/g), calculated with the Bayesian correction method. In this analysis calgranulin-C has significantly better specificity (97.3% (95% CI: 94.1 to 99.4) vs. 71.3% (CI: 63.3 to 79.0)) and better positive predictive value (92.7% (CI: 84.6 to

98.4) vs. 72.7% (CI: 63.8 to 81.0) compared to calprotectin. The numerical data are shown in supplementary file 2.

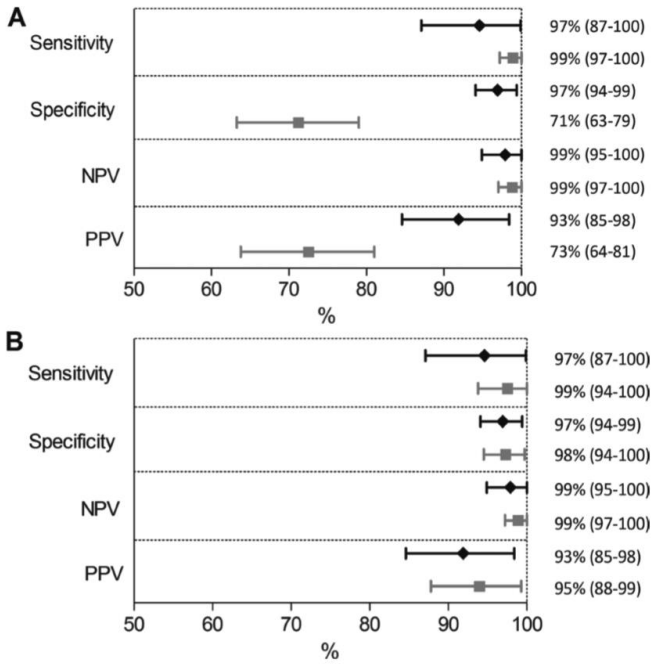

Figure 4 I Diagnostic accuracy measures of the calprotectin (grey square) and calgranulin-C test (black diamond) to diagnose IBD in children.
Graph A shows the results when predefined thresholds are used (resp. 50 µg/g and 0.75 µg/g).
Graph B shows the results when ROC-based optimal thresholds (resp. 400 µg/g and 0.75 µg/g) are used. Whiskers represent the 95% credible interval.

Optimal (ROC-based) thresholds

The optimal (ROC-based) threshold for calprotectin was 400 µg/g, while the optimal threshold of calgranulin-C was equal to the pre-defined threshold (0.75 µg/g). The difference in specificity and positive predictive value disappeared when optimal thresholds were compared. A graphical representation of the equivalence between calprotectin and calgranulin-C for the complete study cohort (verified with either reference test) is shown in **figure 4B**.

Concordant vs. discordant pairs

Figure 5 shows that 306 of 337 pairs of calprotectin and calgranulin-C results were concordant (91%). Discordant pairs (n=31 (9%)) are described in detail in supplementary file 4. Thirteen

children with a discordant result were diagnosed with IBD. Two cases were missed with the calprotectin test (threshold 400 µg/g) and 11 cases were missed with the calgranulin-C test (threshold 0.75 µg/g).

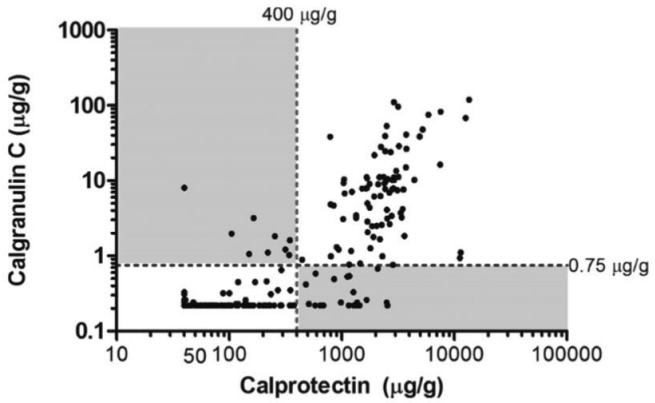

Figure 5 I Scatter plot showing concordant and discordant pairs of calprotectin and calgranulin-C measurements.
The broken lines represent the ROC-based optimal thresholds for calprotectin (400 µg/g) and calgranulin-C (0.75 µg/g). White fields represent concordant pairs (91%), grey fields represent discordant pairs (9%).

DISCUSSION

The clinical presentation of paediatric IBD is frequently non-specific and overlaps with irritable bowel syndrome (IBS). Early differentiation is important to avoid delay in proceeding to endoscopy on the one hand and to avoid unnecessary invasive procedures on the other. The mere existence of this trade-off means that a non-invasive and highly discriminative test is needed. We compared the calprotectin and calgranulin-C stool test to see which of the two markers best predicted IBD in children and postulated that the latter probably had better specificity. In this large-scale paediatric diagnostic accuracy study on markers of intestinal inflammation, we show that calgranulin-C has better specificity for IBD than calprotectin, provided the use of common thresholds. When optimal (ROC-based) thresholds are used (i.e. calprotectin 400 µg/g; calgranulin-C 0.75 µg/g), both tests have exceptionally high sensitivity and specificity to diagnose IBD.

Comparison with existing literature

Well-designed studies on the discriminative power of calgranulin-C are scarce. An Australian research team previously reported on a study comparing calprotectin and calgranulin-C.(9) They obtained stool samples from 61 children (2-16 years old) who presented with gastrointestinal symptoms prior to admission for gastrointestinal endoscopy. The predefined threshold used for calgranulin-C in their study cohort (10 µg/g)(8) was substantially higher than the one we used (0.75 µg/g).(13) The difference is likely to be explained by differences in assays and selection of patients. We included a fair amount of patients that did not proceed to endoscopy, which increases the applicability of our results for populations seen in non-specialized centers. An important methodological flaw in the Australian study was the omission of a fair comparison of optimal thresholds for both markers, which may have resulted in an overinterpretation of calgranulin-C test accuracy.

Several recently published meta-analyses have shown that the calprotectin stool test has good negative predictive ("rule-out") value at the commonly used threshold (50 µg/g).(3,4,6) A large share of the studies included in these meta-analyses had a case-control design which gives rise to spectrum bias and overestimation of test accuracy relative to the real-life practice.(19) We avoided spectrum bias and therefore expected to find more modest accuracy measures than previously reported. Contrary to our expectations, we found that the good rule-out value of calprotectin still holds in a heterogeneous study population with chronic abdominal pain and diarrhoea.

At the threshold of 50 µg/g, the specificity of the calprotectin test for the detection of IBD (71%) was comparable with previously reported values. The ROC-based optimal threshold was higher than in previously reported papers. We used the calprotectin ELISA assay of BÜHLMANN Laboratories, which is known to report higher concentrations than the Immunodiagnostik and Eurospittal assays.(20) This so-called between-assay variability indicates the need for assay standardization. In the meantime, each laboratory should investigate transferability of the manufacturer's thresholds to its patient population and if necessary, determine its own local thresholds to optimally identify IBD and avoid the need for further costly and invasive investigations.

Strengths and limitations

This large-scale multicenter cross-border accuracy study better reflects 'real-life' practice than any other previously published study on stool tests for screening and selecting children for endoscopy. We used an automated IBD Risk Stratifier to standardize the assignment of patients to either the high or low risk stratum. The cooperation of both secondary and tertiary level hospitals in this study promotes the generalizability of our results and conclusions.

The attending paediatricians were not blinded to the calprotectin results. This led to a deviation from the automated advice of the IBD Risk Stratifier in 25% of cases. In supplementary file 5 we show that this especially happened in the calprotectin grey zone between 50 and 400 ug/g. Knowledge of the calprotectin concentration also led to a diagnostic work-up bias that is usually the case in screening studies where only patients with a positive index test result move on to the reference standard. We reduced this bias by following the low-risk patients for 6 months for possible latent IBD to become visible. One might argue that this observation period was not sufficiently long, but we are confident that the majority of initially missed cases with IBD would become apparent within this time.

Clinical implications

Both calprotectin and calgranulin-C have excellent test characteristics to predict IBD in children and teenagers with chronic abdominal pain and diarrhoea and justify endoscopy. In this inception cohort the calprotectin action threshold for proceeding to endoscopy is around 400 µg/g, and this underlines the relevance of using a 'two-threshold strategy' as proposed in several publications.(7,21-23) The grey zone between the commonly used threshold of 50 µg/g that demarcates the normal range and the action threshold gives room for shared decision making with the patient and his/her parents, in which presence of major red flag symptoms and impact on daily functioning of the child may additionally guide management. One can opt for watchful waiting with monthly monitoring of stool calprotectin or decide to move on to endoscopic evaluation. When calprotectin concentrations are truly out of range, and gastrointestinal infections and nonsteroidal anti-inflammatory drug use are excluded, the patient should proceed to endoscopy to rule in IBD. A two-threshold strategy does not seem to be of added value when the calgranulin-C stool test is picked as the triaging tool of preference.

Stool markers are of great help to distinguish IBD from IBS in children with only minor red flag symptoms. When children present with major red flag symptoms of IBD they will be referred for endoscopy regardless of the stool marker result. There is no added value of stool testing for triaging purposes in this category, although the knowledge of a baseline calprotectin concentration is useful for monitoring the response to treatment. Physicians should take note that different patient populations and different test assays may lead to variations in thresholds.(20,21,24)

CONCLUSIONS

Measuring calprotectin or calgranulin-C concentrations in stool is a useful triage tool for identifying patients who are most likely to need endoscopy for suspected inflammatory bowel disease. The discriminative power to safely exclude the disease (specificity) is significantly better than previously reported. When the optimal ROC-based thresholds are used (calprotectin 400 µg/g; calgranulin-C 0.75 µg/g), both tests perform equally well in secondary and tertiary level hospitals.

ACKNOWLEDGEMENTS

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Supplementary data 1: R scripts added as separate files

JAGS script:

```
###Model correcting for Differential Verification Bias###
model
{
  ###Prior distributions###
  phi ~ dbeta(1,1)
  ST ~ dbeta(1,1)
  CT ~ dbeta(1,1)
  SR1 ~ dbeta(151.125,3.875)# Sensitivity range R1: 95-100%
  CR1 ~ dbeta(151.125,3.875)  # Specificity range R1: 95-100%
  SR2 ~ dbeta(31.5,3.5)  # Sensitivity range R2: 80-100%
  CR2 ~ dbeta(58.1,24.9)    # Specificity range R2: 40-60%

  VT1R1 ~ dunif(0,1)
  VT0R1 ~ dunif(0,1)
  VT1R2 ~ dunif(0,1)
  VT0R2 ~ dunif(0,1)

  ###Probability data###
  p1 <- phi*ST+(1-phi)*(1-CT)

  p2 <- (SR1*phi*ST/(phi*ST+(1-phi)*(1-CT))+(1-CR1)*(1-phi)*(1-CT)/(phi*ST+(1-phi)*(1-CT)))
  p3 <- (SR1*phi*(1-ST)/(phi*(1-ST)+(1-phi)*CT)+(1-CR1)*(1-phi)*CT/(phi*(1-ST)+(1-phi)*CT))

  p4 <- (SR2*phi*ST/(phi*ST+(1-phi)*(1-CT))+(1-CR2)*(1-phi)*(1-CT)/(phi*ST+(1-phi)*(1-CT)))
  p5 <- (SR2*phi*(1-ST)/(phi*(1-ST)+(1-phi)*CT)+(1-CR2)*(1-phi)*CT/(phi*(1-ST)+(1-phi)*CT))

  ###Likelihood observed data###
  #Stage 1
  T1 ~ dbin(p1,n)
```

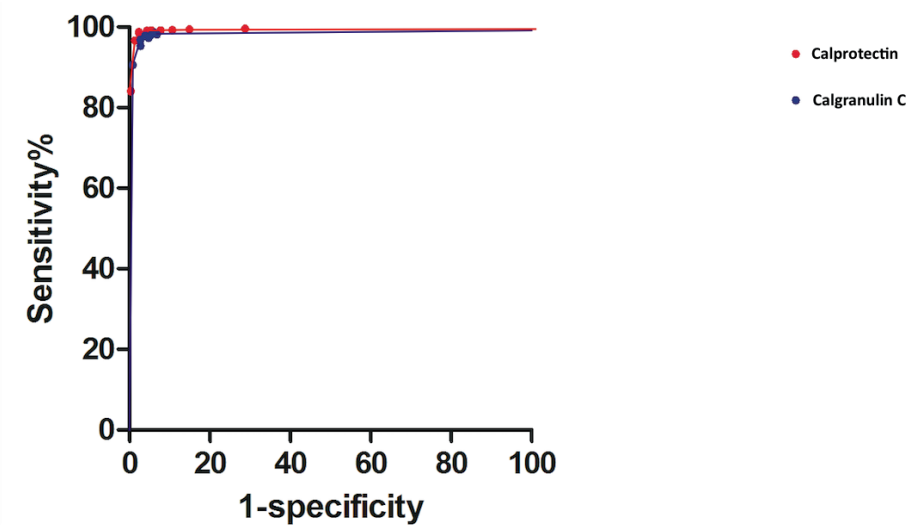
```
  #Verification Stage
  nT1R1 ~ dbin(VT1R1,T1)
  n0 <- n-T1
  nT0R1 ~ dbin(VT0R1,n0)
  nT1R2 ~ dbin(VT1R2,T1)
  nT0R2 ~ dbin(VT0R2,n0)

  #Stage 2
  T1R11 ~ dbin(p2,nT1R1)
  T0R11 ~ dbin(p3,nT0R1)
  T1R21 ~ dbin(p4,nT1R2)
  T0R21 ~ dbin(p5,nT0R2)

  #Predictive values
  PPVT <- ST*phi/(ST*phi+(1-CT)*(1-phi))
  NPVT <- CT*(1-phi)/(CT*(1-phi)+(1-ST)*phi)

  #Accuracy measures T with respect to Reference test 1
  STR1 <- p2*p1/(p2*p1+p3*(1-p1))
  CTR1 <- (1-p3)*(1-p1)/((1-p2)*p1+(1-p3)*(1-p1))
  PPVTR1 <- p2
  NPVTR1 <- 1-p3

  #Accuracy measures T with respect to Reference test 2
  STR2 <- p4*p1/(p4*p1+p5*(1-p1))
  CTR2 <- (1-p5)*(1-p1)/((1-p4)*p1+(1-p5)*(1-p1))
  PPVTR2 <- p4
  NPVTR2 <- 1-p5
}
```

Supplementary file 2: Diagnostic accuracy measures of the calprotectin and calgranulin-C test to diagnose IBD in children using predefined thresholds (respectively 50 µg/g and 0.75 µg/g) and optimal thresholds (respectively 400 µg/g and 0.75 µg/g).

Estimates calculated with the Bayesian statistical method are shown with the 95% credible interval

Patient spectrum	Diagnostic accuracy characteristics	Calprotectin (50 µg/g)	Calgranulin-C (0.75 µg/g)	Calprotectine (400 µg/g)
High risk for IBD (endoscopic verification)	Sensitivity	97.4 (94.1-99.1)	81.7 (73.1-88.7)	92.0 (85.5-96.3)
	Specificity	69.9 (62.2-77.1)	96.4 (93.4-98.3)	96.6 (93.6-98.5)
	Negative predictive value	97.2 (93.7-99.0)	92.2 (88.1-95.3)	96.3 (93.1-98.3)
	Positive predictive value	71.6 (63.2-79.0)	91.0 (83.7-95.6)	92.7 (86.2-96.6)
Low risk for IBD (verification by follow up)	Sensitivity	88.7 (83.8-92.5)	74.8 (65.2-82.7)	79.2 (71.2-85.7)
	Specificity	65.3 (56.5-73.8)	93.3 (87.9-97.0)	93.1 (87.1-97.1)
	Negative predictive value	87.2 (82.2-91.3)	89.4 (84.9-92.8)	89.7 (85.6-93.0)
	Positive predictive value	68.5 (57.5-77.8)	83.3 (69.5-92.7)	85.5 (72.4-94.1)
Complete cohort (verification with either reference test)	Sensitivity	99.5 (97.2-100)	96.8 (87.1-99.9)	98.7 (93.8-100)
	Specificity	71.3 (63.3-79.0)	97.3 (94.1-99.4)	97.7 (94.5-99.7)
	Negative predictive value	99.4 (97.0-100)	98.8 (94.9-100)	99.4 (97.2-100)
	Positive predictive value	72.7 (63.8-81.0)	92.7 (84.6-98.4)	94.8 (87.8-99.3)

Supplementary figure 3: ROC curves of calprotectin and calgranulin C.



Supplementary file 4: Detailed description of discordant pairs of calprotectin and calgranulin-C results (n=31).
Attending paediatrians were blinded to the viral PCR result, which explains the discrepancy in diagnosis in the lowest five patients.

DISCORDANT RESULTS LOW CALPROTECTIN - HIGH CALGRANULIN-C

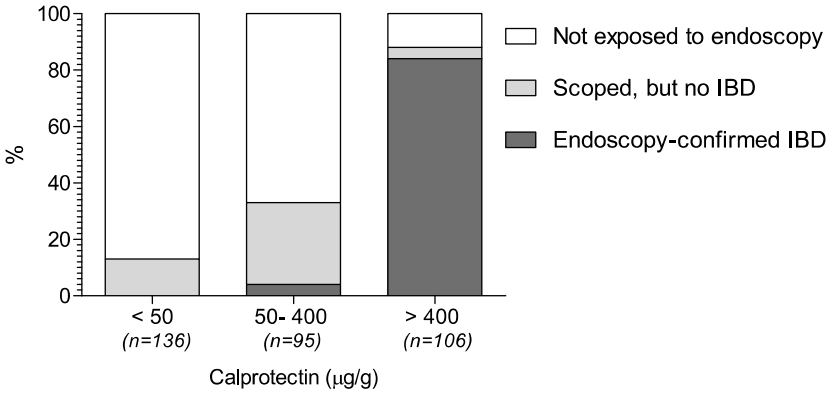
Calprotectin (µg/g)	Calgranulin-C (µg/g)	Bacterial and parasite PCR	IBD risk stratum	Diagnosis	Post-hoc viral PCR
315	1.22	negative	High	CD	negative
340	1.03	D. fragilis	High	CD	negative
150	1.06	Giardia lamblia	High	GI-infection	negative
165	3.17	D. fragilis	High	GI-infection	negative
105	1.98	negative	High	IBS	negative
220	1.11	negative	High	IBS	negative
255	1.82	D. fragilis	Low	IBS	negative
40	8.04	negative	Low	HUS	negative
345	1.62	D. fragilis	High	solitary rectal ulcer	negative

DISCORDANT RESULTS HIGH CALPROTECTIN - LOW CALGRANULIN-C

Calprotectin (µg/g)	Calgranulin-C (µg/g)	Bacterial and parasite PCR	IBD risk stratum	Diagnosis	Post hoc viral PCR
480	0.42	D. fragilis	High	UC	negative
510	0.23	STEC	High	CD	negative
855	0.49	negative	High	CD	negative
980	0.24	negative	High	CD	negative
1170	0.54	negative	High	UC	negative
1270	0.33	negative	High	CD	negative
1280	0.22	negative	High	CD	negative
1410	0.22	D. fragilis	High	UC	negative
1440	0.22	negative	High	CD	negative
1660	0.26	negative	High	CD	negative
2080	0.68	negative	High	CD	negative
645	0.22	STEC	High	GI-infection	negative
1120	0.53	negative	High	spontaneous recovery	negative
1370	0.24	Giardia lamblia	Low	GI-infection	negative
1380	0.22	STEC	Low	GI-infection	negative
2550	0.22	negative	Low	GI-infection	negative
580	0.22	negative	Low	allergy	negative
585	0.58	STEC, D. fragilis,	High	IBS	parechovirus
1160	0.22	D. fragilis	High	IBS	adenovirus
685	0.22	negative	Low	reflux oesophagitis	adenovirus
1150	0.22	negative	High	spontaneous recovery	norovirus
2510	0.24	D. fragilis	Low	spontaneous recovery	Norovirus

CD, Crohn;s disease; GI-infection, gastrointestinal infection; HUS, haemolytic uraemic syndrome; IBS, irritable bowel syndrome; STEC, shiga toxin-producing E. coli; UC, ulcerative colitis

Supplementary file 5: Stacked bars representing percentage of patients referred to specialist services for endoscopy and diagnosed with IBD per calprotectin range.



CHAPTER 4

TEST STRATEGIES TO PREDICT INFLAMMATORY BOWEL DISEASE AMONG CHILDREN WITH NON-BLOODY DIARRHOEA

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Pediatrics, 2020, epub ahead of print

ABSTRACT

OBJECTIVE: We evaluated four diagnostic strategies to predict the presence of inflammatory bowel disease (IBD) in children who present with chronic non-bloody diarrhoea and abdominal pain.

METHODS: We conducted a prospective cohort study, including 193 patients aged 6–18 years, who underwent a standardised diagnostic work-up in secondary or tertiary care hospitals. Each patient was assessed for symptoms, c-reactive protein (>10 mg/L), haemoglobin (<-2 SD for age and gender) and faecal calprotectin (≥250 μg/g). Patients with rectal bleeding or perianal disease were excluded because the presence of these findings prompted endoscopy regardless of their biomarkers. Primary outcome was IBD confirmed by endoscopy, or IBD ruled out by endoscopy or uneventful clinical follow-up for 6 months. We measured the predictive performance of each strategy with AUC and decision curves.

RESULTS: 22 of 193 (11%) children had IBD. The basic prediction model was based on symptoms only. Adding blood or stool markers increased the AUC from 0.718 [95%CI: 0.604-0.832] to 0.930 [95%CI: 0.884-0.977] and 0.967 [95%CI: 0.945-0.990]. Combining symptoms with blood and stool markers outperformed all other strategies (AUC 0.997 [95%CI: 0.993-1.000]). Triaging with a strategy that involves symptoms, blood markers and calprotectin will result in 14 of 100 patients being exposed to endoscopy. Three of them will not have IBD, and no IBD-affected child will be missed.

CONCLUSION: Evaluating symptoms plus blood and stool markers in patients with non-bloody diarrhoea is the optimal test strategy that allows paediatricians to reserve a diagnostic endoscopy for children at high-risk for IBD.

INTRODUCTION

Persistent rectal bleeding or perianal disease in children and teenagers justifies endoscopy to evaluate the presence of inflammatory bowel disease (IBD).(1-2) When the indication for endoscopy is less obvious, as in the case of patients with chronic abdominal pain and non-bloody diarrhoea, a triage test may help to distinguish who are in need of immediate referral to endoscopy.

Several meta-analyses (3-6) have shown that measuring a single faecal calprotectin level can help to distinguish IBD from functional abdominal disorders. Calprotectin concentrations above 50 µg/g predict the presence of IBD with high sensitivity [99% (range 92 to 100%)], but the mediocre specificity [65% (range 54 to 74%)](6) is the reason that a substantial number of children are wrongly exposed to endoscopy. A refinement of the cut-point to 250 µg/g was insufficient to reduce the rate of unnecessary endoscopies.(7-9)

Complications of endoscopy, related to the invasiveness of the procedure itself (colonic perforation or tear) or to anaesthesia, may be rare but could cause severe morbidity.(10-12) A diagnostic strategy that includes a combination of tests would potentially further reduce the number of children exposed to this invasive and costly procedure.

We evaluated four diagnostic strategies to predict the presence of IBD: [1] symptoms alone, [2] symptoms plus blood markers, [3] symptoms plus faecal calprotectin, and [4] symptoms plus blood markers plus faecal calprotectin.

PATIENTS AND METHODS

Study design

This international multi-centre study was a planned ancillary study of the prospective CACATU cohort (clinicaltrials.gov NCT02197780). The cohort and the calprotectin results have previously been described (11) and are replicated here for the subgroup of previously undiagnosed children and teenagers presenting with persistent or recurrent non-bloody diarrhoea and abdominal pain. Patients were assessed by a local clinician and data collected during history taking and physical examination were entered on a secured study website (www.cacatustudie.eu). Blood tests were performed at the local hospital and the results were uploaded to the study website. Stool samples were sent to the

Department of Laboratory Medicine of the University Medical Centre Groningen. Immediately after arrival, the faecal calprotectin concentration was measured and the result was made visible to the local clinician by an e-mail notification that included an automated advice on the next best move. Patients with a faecal calprotectin concentration ≥250 μg/g moved on to endoscopy with biopsies (reference standard). Patients with a faecal calprotectin concentration <250 μg/g were re-evaluated at 6 month follow-up after inclusion for possible latent IBD to become visible (alternative reference standard). Deviation from the automated advice on the next best move was considered a protocol violation. The study protocol has been published in BMJ Open.(13)

Participants

Patients were recruited from paediatric outpatient clinics of sixteen general hospitals and three tertiary care hospitals in the Netherlands and Belgium. The clinicians at the various sites were general paediatricians or paediatric gastroenterologists. Six participating centres had a paediatric endoscopy unit. Patients eligible for inclusion in this ancillary study were aged 6 to 18 years, showing persistent or recurrent non-bloody diarrhoea and abdominal pain. Patients with rectal bleeding or perianal disease were not analysed in this ancillary study, as their symptoms prompted colonoscopy regardless of any biomarker result.(1)

Outcome

Primary outcome was IBD confirmed by endoscopy of the upper and lower gastrointestinal tract, or IBD ruled out by either endoscopy or uneventful clinical follow-up for 6 months. In case of macroscopic and histological absence of inflammation, imaging of the small intestine was encouraged.

Statistical methods

Dichotomous data collected at baseline (including presence of chronic non-bloody diarrhoea, weight loss, first degree relatives with IBD and extra-intestinal symptoms) were used to construct a basic logistic regression model to predict the presence of IBD. The incremental value of blood markers (increased C-reactive protein (CRP) and haemoglobin (Hb) below -2 standard deviations) and increased faecal calprotectin (≥250 μg/g) were evaluated by adding them to the basic prediction model (**table 1**).

Table 1: Overview of predictors

Test	Measurement	Definition of positive result
Symptoms		
Persistent non-bloody diarrhoea	History	Duration ≥4 weeks
Recurrent non-bloody diarrhoea and abdominal pain	History	≥2 episodes in 6 months
Unintended weight loss	History and physical examination	> 1 kg
First degree relative with IBD	History	Affected father, mother, sibling
Extra-intestinal symptoms	Physical examination	Episcleritis, uveitis, erythema nodosum, psoriasis, finger clubbing, arthritis
Blood markers		
Increased C-reactive protein	Local laboratory	>10 mg/L
Anaemia (haemoglobin <−2 SD for age and gender)	Local laboratory	4-12 years < 7.1 mmol/l boys 12-18 years < 8.1 mmol/l girls 12-18 years < 7.4 mmol/l
Stool markers		
Increased faecal calprotectin	Central laboratory[1]	≥250 μg/g

[1] fCAL enzyme-linked immunosorbent assay, BÜHLMANN Laboratories AG, Schönenbuch, Switzerland

We estimated the performance of the four diagnostic strategies by calculating (1) the area under the receiver-operating-characteristics curve (AUC), and (2) the net benefit of each strategy through decision curve analysis. Net benefit combines the number of children that were correctly triaged for endoscopy (true positives) and the number of children exposed to an unnecessary endoscopic procedure (false positives) into a single number. We show the net benefit of each strategy through a range of risk thresholds. Finally, we calculated sensitivity and specificity with 95% confidence intervals (CIs) of the optimal diagnostic strategy. Computations were carried out with R (version 3.5.1).

Human Subjects Protection

The study was conducted according to the principles of the Declaration of Helsinki. The Medical Ethics Review Committee of the University Medical Center in Groningen (METc 2013/503) and Antwerp University Hospital (14/40/407) approved the study protocol. The legal guardian(s) of all participants, as well as children aged 12 and above, gave informed consent to use data generated by routine medical care. The investigators collected and recorded data in such a manner that subjects could not be identified, directly or through identifiers linked to the subjects.

RESULTS

Between September 2014 and September 2016, we prospectively included 354 children and teenagers in the CACATU cohort. Of these, 135 had overt rectal bloodloss or perianal disease, which justified immediate endoscopic evaluation for the presence of IBD. Fifteen patients were excluded as their stool samples arrived at the hospital laboratory after an unacceptable delay that may have caused calprotectin degradation.(14) A total of 204 patients were included for this ancillary study, of which 193 continued down the decision tree until a final diagnosis was made (**figure 1**).

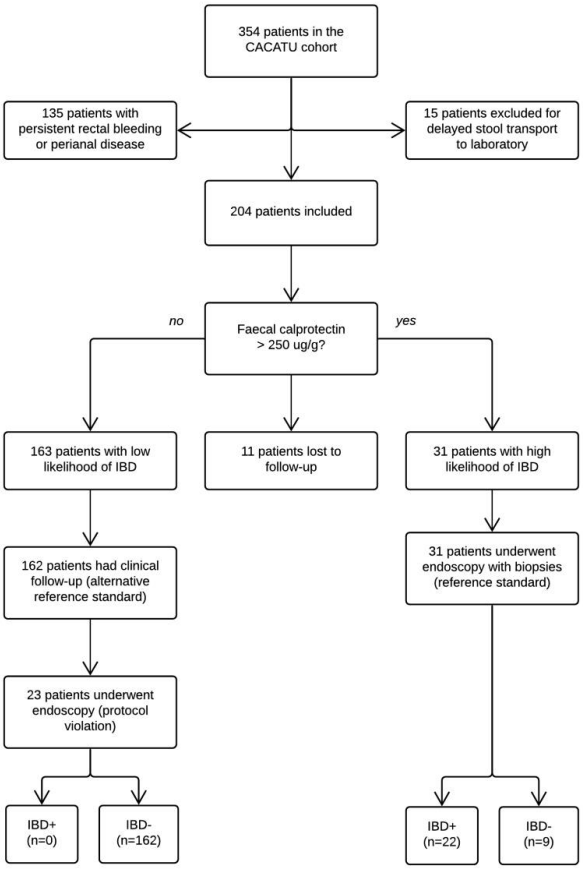

Figure 1 l Flow of participants

Baseline characteristics are shown in **table 2**. IBD was confirmed in 22 of 193 patients (11%), of whom 8 had ulcerative colitis and 14 Crohn's disease.

Table 2: Baseline characteristics of 193 patients

Characteristics	IBD (n=22)	non-IBD (n=171)	p-value
Demographics			
Median (IQR) age in years	14 (8-17)	12(6-17)	
Male gender	9 (41%)	98 (57%)	
Symptoms			
Persistent non-bloody diarrhoea (>4 weeks)	15 (68%)	58 (34%)	0.004
Recurrent non-bloody diarrhoea and abdominal pain	15 (68%)	149 (87%)	0.043
Unintended weight loss	10 (46%)	48 (28%)	0.154
First degree relative with IBD	1 (5%)	17 (10%)	0.667
Extra-intestinal symptoms	3 (14%)	11 (6%)	0.430
Blood markers			
Increased C-reactive protein	13 (59%)	9 (5%)	<0.001
Anaemia	15 (68%)	14 (8%)	<0.001
Stool markers			
Faecal calprotectin ≥250 μg/g	22 (100%)	18 (11%)	<0.001

Data are number (%) of patients unless stated otherwise.
Abbreviations: IQR, inter quartile range; SD, standard deviation

Area under the receiver-operating-characteristics curve (AUC)

Receiver-operating-characteristics (ROC) curve analysis revealed an AUC of 0.718 [95% CI: 0.604-0.832] for the basic model to predict IBD. In comparison, the ROC curve analyses of strategy 2 (symptoms + blood markers), strategy 3 (symptoms + calprotectin) and strategy 4 (symptoms + blood markers + calprotectin) revealed AUCs of 0.930 [95% CI: 0.884-0.977], 0.967 [95%CI: 0.945-0.990] and 0.997 [95%CI: 0.993-1.000], respectively (**Figure 2**).

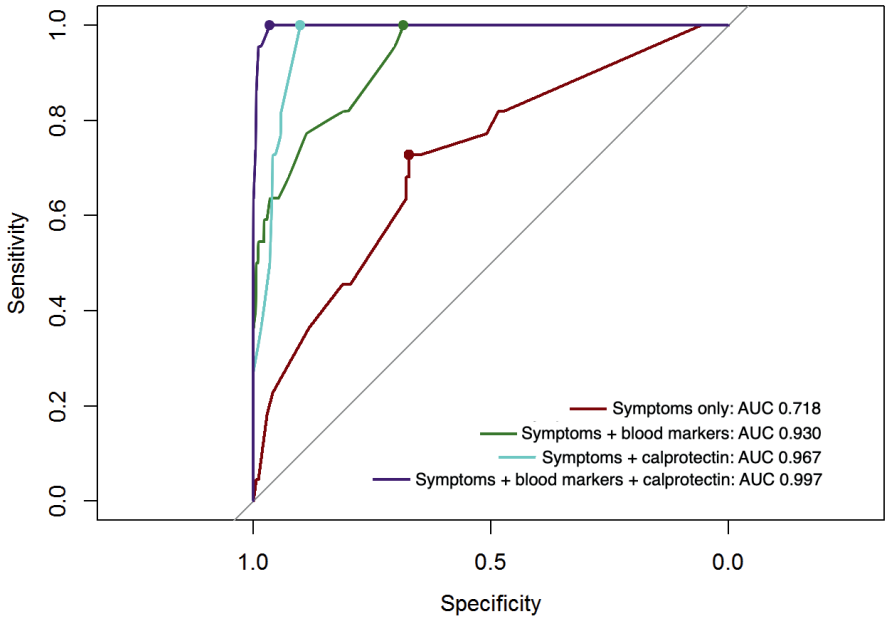

Figure 2 I ROC-curves representing the accuracy for detecting IBD in children with chronic non-bloody diarrhoea. Abbreviation: AUC, area under the curve.

The accompanying changes in sensitivity and specificity are shown in table 3. The sensitivity was 100% for strategy 2, 3 and 4, and the specificity increased from 68.4% to 90.1% and 96.5%, respectively.

Table 3: Accuracy measures for four diagnostic strategies to predict inflammatory bowel disease.

Diagnostic strategy	Sens	Spec	Number per 100 patients (IBD prevalence 11%)			
			TP	**TN**	**FP**	**FN**
1. Symptoms only	72.7%	67.3%	8	60	29	3
2. Symptoms + blood markers	100%	68.4%	11	61	28	0
3. Symptoms + calprotectin	100%	90.1%	11	80	9	0
4. Symptoms + blood markers + calprotectin	100%	96.5%	11	86	3	0

Abbreviations: Sens, sensitivity; Spec, specificity; TP, true positives; TN, true negatives; FP, false positives; FN, false negatives

Regardless of whether strategy 2, 3 or 4 was used, all IBD-affected patients were correctly exposed to endoscopy. Strategy 2, 3 and 4 correctly advised against referring 61%, 80% and 86% of patients for endoscopy, respectively.

The pre-test probability of IBD in the study cohort was 11%; a positive result of strategy 4 produced a post-test probability of IBD of 78% [95% CI: 60-87%]. The probability of IBD, if strategy 4 was negative, was reduced to 0% [95% CI 0-4%].

Decision curve analysis

In the decision curve analysis, strategy 4 (symptoms + blood markers + calprotectin) had the greatest net benefit for predicting IBD across the range of risk thresholds up to 70% (**figure 3**).

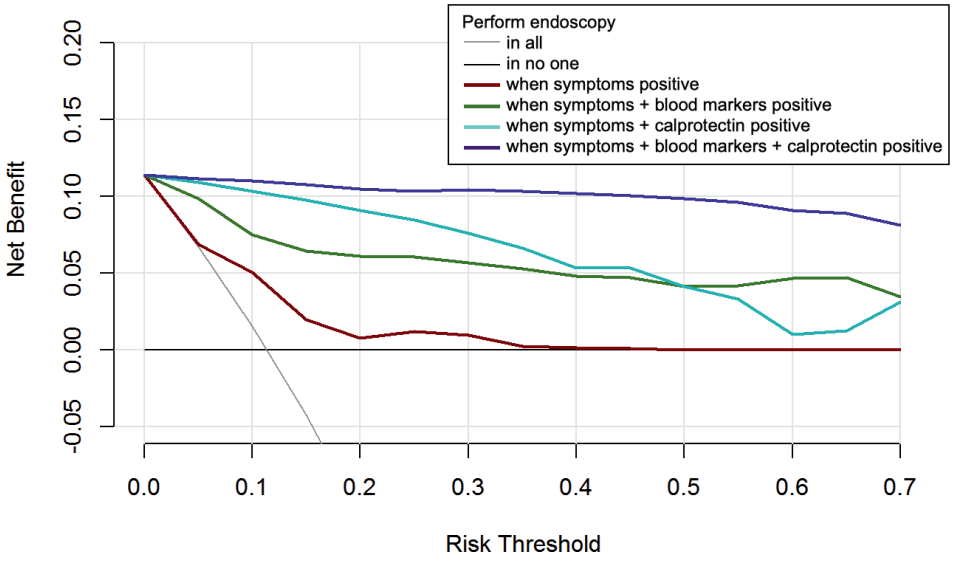

Figure 3 I Decision curves for four diagnostic strategies to predict IBD.
The default strategies were to perform endoscopy in all patients or in none. A diagnostic strategy is clinically useful if it has a greater net benefit than the default strategies.

Strategy 3 (symptoms + calprotectin) provided greater net benefit than strategy 2 (symptoms + blood markers) up to a risk threshold of 50%. When the risk threshold was 50 to 70%, strategy 2 had greater net benefit. The basic model (symptoms only) provided hardly any greater net benefit than performing endoscopy in all patients, or alternatively, performing endoscopy in no one.

Box. How to read figure 3?

Assume that a clinician does not want to expose more than 2 children to endoscopy to detect one case with IBD. In this instance the "harm-to-benefit" ratio is 1:1 (or a risk threshold of 50%). At this risk threshold the net benefit of 0.10 means that strategy 4 leads to exposing 100 per 1000 children at risk, with all of the exposed having IBD.

DISCUSSION

In this international prospective, multicentre cohort study, we demonstrate that a decision strategy based on symptoms, c-reactive protein, haemoglobin and faecal calprotectin offers physicians an opportunity to reliably screen children and teenagers with abdominal pain and non-bloody diarrhoea for IBD before referring them for endoscopy. This strategy indicates with high reliability which patients are at negligible risk for IBD and therefore should not undergo endoscopy. Prompt and accurate prediction of IBD enables paediatricians to efficiently allocate resources in endoscopy units, by reassuring those with a low risk for IBD, and at the same time prioritize those with a high risk for IBD. The time saved by refraining from unnecessary endoscopies may be better used elsewhere in the health care system, such as for offering gut-directed hypnotherapy to those with functional abdominal pain.(15, 16)

Comparison with other studies

The outcome – IBD – identified by strategy 4 was assessed in a large group of previously undiagnosed children and teenagers presenting with persistent or recurrent non-bloody diarrhoea and abdominal pain. They represented a spectrum of patients that is commonly seen in general paediatric practice. Previous studies on calprotectin included patients with perianal symptoms or overt rectal bleeding.(3,4,17) These red flag symptoms give sufficient reasons for immediate endoscopic evaluation. Inclusion of these patients causes overestimation of the discriminating power relative to the practical situation, where a test or diagnostic strategy is necessary to distinguish those with functional abdominal pain from those with IBD who lack the red flag symptoms.

Study limitations

Although the estimated sensitivity of strategy 4 to predict IBD was 100%, the 95% confidence interval suggests that IBD may occasionally be missed. Performing careful physical and laboratory examinations and arranging for follow-up will protect the patient from the sequelae of missing a case. For children and teenagers who are categorized as “low-risk” patients, but whose abdominal pain and non-bloody diarrhoea have not improved after one month, we recommend to repeat the faecal calprotectin test.

We did not demonstrate yet that following diagnostic strategy 4 has an impact on actual clinical practice. A randomised controlled trial is necessary to measure the impact of

applying the decision strategy in a clinical setting in terms of patient outcome, health professionals' behaviour, and resource use.

In this study we used the enzyme-linked immunosorbent assay of one manufacturer. Although other test kits have an acceptable agreement in the lower range (below 250 µg/g),(18) inter-assay variability is considerable above this cut-off point. We emphasize the need for assay standardisation, but in its absence assay-specific cut-offs may improve diagnostic performance.

In the strategy with blood markers, we relied on a subgroup of commonly used laboratory data, that is CRP and Hb. We did not include erythrocyte sedimentation rate (ESR), as an inverse correlation exists between Hb and ESR that could hamper the interpretation of our statistical model. Neither did we include albumin, which is known to be abnormal in a considerable proportion of paediatric patients with severe IBD,(19,20) but was a highly unusual clinical presentation in our study cohort.

Implications for practice

In many decision curves there is a trade-off in net benefit when risk thresholds increase. This is hardly the case with the optimal decision strategy in this study, where the graph takes an almost horizontal course. Paediatricians can be reassured that properly evaluating children using clinical findings, CRP, haemoglobin and calprotectin is a highly accurate non-invasive approach to investigation of possible IBD in any clinical setting.

CONCLUSION

Evaluating symptoms plus blood and stool markers in patients with non-bloody diarrhoea is the optimal test strategy that allows paediatricians to reserve a diagnostic endoscopy for children at high-risk for IBD.

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CHAPTER 5

FATIGUE IN CHILDREN AND ADOLESCENTS WITH INFLAMMATORY BOWEL DISEASE

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ABSTRACT

AIM: To identify factors other than active disease and anaemia that contribute to fatigue in paediatric inflammatory bowel disease (IBD).

METHODS: We performed an electronic search in Medline and EMBASE from their inception to May 2017 using the search term “fatigue” or the related keywords “physical impairment” and “inflammatory bowel disease” with the filter “child” (age 0-18 years). Cross-sectional and case-control studies were included. We restricted our search to studies published in English. We used the PRISMA checklist and flow diagram. Duplicate articles were manually deleted in End Note. To identify further relevant studies, we checked the reference lists of the selected articles.

RESULTS: We identified 149 papers, of which 19 were retrieved for full text review. Eleven studies were subsequently excluded because fatigue was not evaluated as an outcome measure. Eight papers focused on the desired topic and were discussed in the final analysis. A lack of uniformity of outcome measures made the pooling of data impossible. In all but one study, questionnaires were used to evaluate fatigue. In the remaining study, an accelerometer was used to measure daily activities, sleeping time and their relationships with fatigue in a more quantifiable manner.

Adolescents with IBD are significantly more fatigued than healthy controls. In addition to active disease, increased anxiety or depression and disturbed family relationships were frequently reported predictors of fatigue. Quantitative measurement of physical activity in patients with Crohn’s disease showed a reduction in the number of steps per day, and patients with ulcerative colitis had a shorter duration of physical activity during the day.

CONCLUSION: Fatigue in paediatric IBD is related to a combination of biological, functional and behavioral factors, which should all be taken into account when managing fatigue.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic disease of the gastrointestinal tract. The disease is characterized by relapsing periods of inflammation and remission and usually presents with abdominal pain, diarrhoea, rectal bleeding and weight loss.(1) The ultimate goal in IBD treatment is to reach clinical remission as quickly as possible. Fatigue and decreased physical fitness may continue to affect a patient's daily life despite disease remission. Ten percent of patients with IBD are diagnosed before the age of 19 years.(2)

Fatigue refers to a subjectively overwhelming sense of tiredness, lack of energy, and feeling of exhaustion that decreases one's capacity for physical and mental activity.(3) It is a common, independent, and nonspecific symptom identified in numerous chronic health conditions in childhood.(4) In adults with chronic disease, fatigue can be a major source of disablement and is often reported as being among the most severe and distressing symptoms.(5) It affects physical, emotional, cognitive, and social functioning, impacting quality of life. Nevertheless, fatigue has typically been ignored in the assessment of symptom severity or outcome in many diseases in which it is observed.(5)

The quantification of fatigue is challenging due to the lack of a consensus framework, vague terminology, and the multidimensional nature of symptoms. Subjective methods, such as self-reported or parent-reported surveys (6, 7), are commonly used but can be distorted by response and recall bias. More objective methods, such as polysomnography and performance tests (8-10), are expensive and time-consuming. Furthermore, the prevalence of fatigue varies among healthy paediatric age groups; it is common in infancy, early childhood, and late adolescence and less frequently observed during mid-childhood; it is more common in girls than in boys.(9)

We aimed to systematically review the literature to identify factors that contribute to fatigue in children and adolescents with IBD.

MATERIALS AND METHODS

Identification and selection of studies

We searched for studies published in Medline and EMBASE up to May 2017. The search strategy for Medline was as follows: (“fatigue” [MeSH Terms] OR “fatigue” [All Fields]) AND (“inflammatory bowel diseases”[MeSH Terms] OR (“inflammatory” [All Fields] AND “bowel” [All Fields] AND “disease” [All Fields] OR “inflammatory bowel disease” [All Fields]); (“physical examination” [MeSH Terms] OR (“physical” [All Fields] AND “examination”[All Fields]) OR “physical examination” [All Fields] OR “physical” [All Fields]) AND impairment [All Fields]) AND ("inflammatory bowel diseases"[MeSH Terms] OR ("inflammatory"[All Fields] AND "bowel"[All Fields] AND "diseases"[All Fields]) OR "inflammatory bowel diseases"[All Fields] OR ("inflammatory"[All Fields] AND "bowel"[All Fields] AND "disease"[All Fields]) OR "inflammatory bowel disease"[All Fields]) , with the filter “child” (age 0-18 years). For EMBASE, the search strategy was as follows: (“fatigue”/exp OR fatigue) AND Inflammatory AND (“bowel”/exp OR bowel) AND (“disease”/exp OR disease). We restricted our search to studies published in English. Duplicate articles identified in both Medline and EMBASE were manually deleted in End Note. To identify additional relevant studies, we checked the reference lists of the selected articles.

We selected cross-sectional or case-control studies reporting on fatigue (or its synonyms) in patients under the age of 19 years with IBD. Two reviewers (AvG and EVdV) independently screened the abstracts of all identified articles to determine their eligibility. Any disagreements regarding the inclusion of articles were solved by discussion until consensus was reached.

Quality assessment and data extraction

Study quality was assessed using the online criteria for case-control and cross-sectional studies.(36) Each item was scored as “yes”, “no”, or “not reported”.

The guidelines of the PRISMA 2009 Statement were adopted.

RESULTS

Study Selection

This study includes papers retrieved by electronic searches up to May 2017. In total, 149 papers were identified, of which 19 were retrieved for full-text review. Eleven were subsequently excluded because fatigue was not evaluated as an outcome measure. Eight focused on the desired topic and were discussed in the final analysis (**Fig 1**).

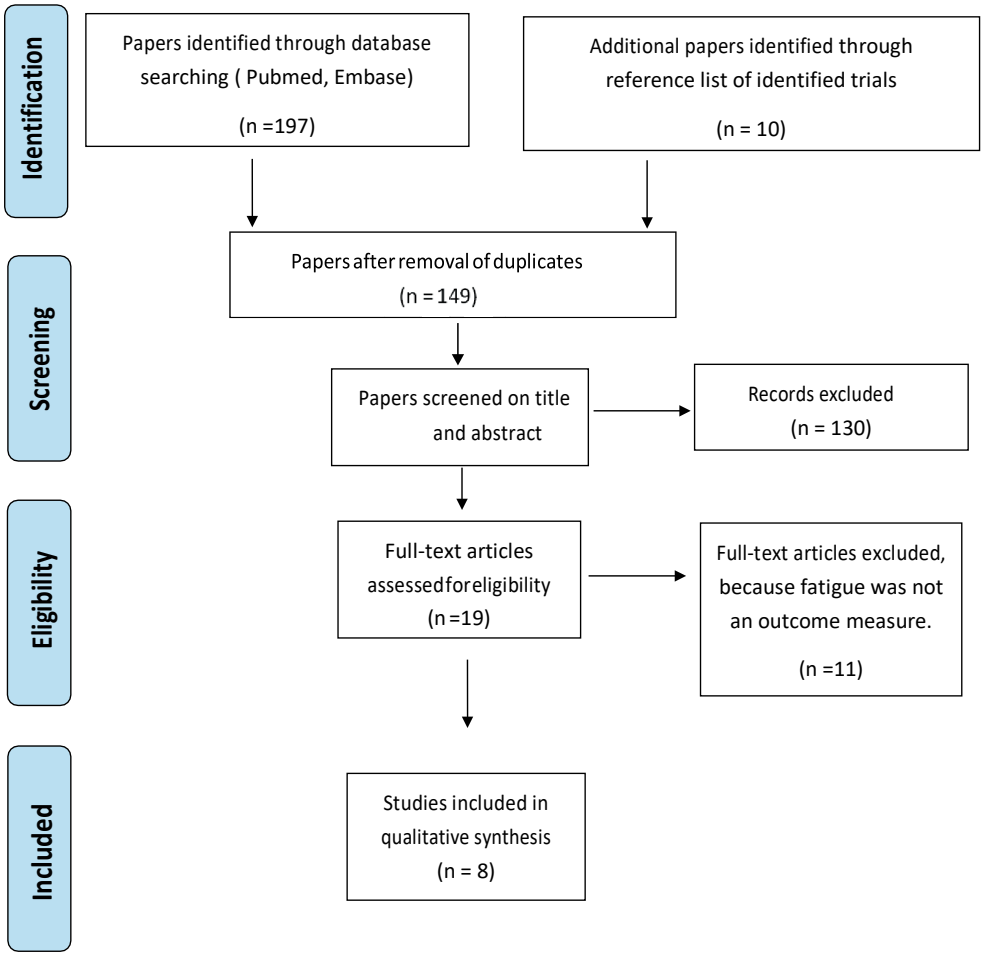

Figure 1 I Study selection

The selected studies varied considerably with regard to the fatigue assessment method, which made pooling of data impossible (see **Table 1**).

Table 1: General characteristics of the included studies.

First author (year of publication)	Study objectives	Age (years)	Patient population	Percentage of patients with active disease	Main findings related to fatigue
Marcus (2009) [6]	To evaluate the degree of fatigue and health-related quality-of-life in children with IBD	10-17	52 CD 13 UC 5 IBD-U 157 healthy controls	Remission 56% Mild 22% Moderate 17% Severe 5%	-adolescents with IBD have significantly more fatigue than healthy controls -PedsQL total fatigue, general fatigue, and sleep/rest fatigue were all impaired in patients with IBD - adolescents with IBD are fatigued even when clinical remission is reached
Nicholas (2007) [13]	To understand the lived experience and elements of quality-of-life in adolescents and adolescents with IBD	7-19	61 CD 19 UC	Not reported	- Young patients with IBD commonly feel 'sick and tired' and have 'no energy'
Pirinen (2010) [16]	To evaluate the effect of disease severity on (the frequency of) sleep problems and daytime-tiredness among adolescents with IBD	10-18	53 CD 83 UC 24 IBDU 236 healthy controls	Not reported	- Adolescents with IBD do not report more sleeping problems or overtiredness than their healthy peers -Adolescents with active disease have significantly more trouble sleeping, more daytime sleepiness and are overtired compared to adolescents with mild IBD symptoms - Adolescents with severe IBD symptoms have worse quality of sleep and more sleep disturbances than those with less severe IBD

Werkstetter et al. (2012) [8]	To evaluate whether physical activity is reduced in patients with IBD compared to control subjects	6-20	27 CD 12 UC 39 healthy controls	Remission 66% Mild 34%	-Patients with IBD show a trend toward less physical activity, especially among girls and those with mild disease activity -There is no relation between inflammatory markers (CRP) and physical activity
Rogler (2013)[7]	To examine the determinants of health-related quality-of-life in adolescents and adolescents with IBD	11-15	64 CD 46 UC	PCDAI> 15 36% PUCAI≥ 10 28%	-Patients with IBD (in particular boys) have moderate impairments in physical well-being -Impairment in physical well-being is associated with active inflammation and its symptoms
Loonen (2002) [12]	To evaluate the impact of IBD on health-related quality of life	8-18	41 CD 40 UC 2 IBD-U	Mild 60% Moderate 23% Severe 15% Missing 2%	- Adolescents with IBD have impairments in motor functioning (running, walking, playing) and complain more of tiredness, especially those with Crohn's disease
Tojek et al. (2002) [14]	To examine family dysfunction, maternal physical symptoms and maternal positive affect as correlates of health status in adolescents with IBD	11-18	36 CD 26 UC	Not reported	-Family dysfunction is related to an increased frequency of fatigue in adolescents -Maternal positive affect is inversely related to fatigue (not significant) -Fatigue is independent of maternal negative affect
Ondersma et al. (1996) [15]	To examine how psychological factors relate to disease severity among adolescents with IBD	11-17	34 CD 22 UC	Not reported	There is a relationship between negative affect and physical symptoms of fatigue

CD Crohn's disease, IBD inflammatory bowel disease, IBD-U IBD-unclassified, PCDAI paediatric Crohn's disease activity, PedsQL paediatric quality of life, PUCAI paediatric ulcerative colitis activity index, UC ulcerative colitis

Six papers reported fatigue or physical activity related to IBD as their primary outcome. The remaining two studies reported quality-of-life as the primary outcome; one used a quality-of-life questionnaire and evaluated the domain “motor functioning” separately, while the other conducted a semi structured interview with questions about the functional impact of the disease. The methodological quality of the studies is summarized in **Table 2**.

Table 2: Methodology and quality assessment

First author (year of publication) and study type	Patient selection	Disease activity score	Fatigue score	Study quality
Marcus (2009)[6] Case-control study	Patients: recruited during scheduled clinical appointments at University Hospital, USA. Healthy controls: adolescent children of hospital employees	CD: PCDAI CU and IBDU: PGA	PedsQL Multidimensional Fatigue Scale, IMPACT-III, PedsQL 4.0 Generic Core Scales Children's Depression Inventory: Short Form	Good; no sample size justification
Nicholas (2007) [13] Cross-sectional study	Patients: recruited from the database of Reference Children's Hospital, Canada	No distinction made	Semi structured interview designed by author	Poor; Patients purposively selected, questionnaires not validated, participation rate not reported
Pirinen (2010)[16] Case-control study	Patients: recruited from the database of the Population Register Center, Finland Healthy controls: matched	VAS disease severity	Youth self-reported questionnaire, Sleep Self Report, child behavior checklist	Medium; Subjective score to assess disease severity, exact sleep duration unknown
Werkstetter (2012) [8]Case-control study	Patients: recruited from University Hospital, Germany Healthy controls: matched	CD: PCDAI UC: PUCAI	SenseWear Pro2 accelerometer, German KINDL, IMPACT III	Good; no sample size justification
Rogler (2013)[7] Cross-sectional study	Patients: recruited from Swiss IBD cohort study, Switzerland	CD: PCDAI UC: PUCAI	KIDSCREEN-27	Medium; numbers in text and table do not match
Loonen (2002)[12] Cross-sectional study	Patients: recruited from a database of two large tertiary referral centers, The Netherlands	5-item symptom card (completed by patients)	TACQOL, IMPACT-II	Good; validated questionnaires, the results compared with healthy controls
Tojek (2002)[14] Cross-sectional study	Patients: recruited from routine outpatient visit in 2 urban paediatric gastroenterology hospitals, USA	No distinction made	Questions designed by author	Medium; parental factors can influence adolescent's health, the converse remains possible, only mothers investigated, questionnaires not validated
Ondersma (1996)[15] Cross-sectional study	Patients: recruited from 2 paediatric gastroenterology hospitals, USA	No distinction made	10-item Subjective Illness Questionnaire (parts or RCMAS and CDI)	Medium; no sample size justification, parts of validated questionnaires

CDI: Children's Depression Inventory, CD: Crohn's disease, IBD: inflammatory bowel disease, PCDAI: paediatric Crohn's disease activity index, PedsQL: paediatric quality of life, PGA: Physician Global Assessment, PUCAI: paediatric ulcerative colitis activity index, RCMAS: Revised Children's Manifest Anxiety Scale, UC: ulcerative colitis.

Assessment of fatigue

Seven of eight papers used subjective methods, such as questionnaires, to evaluate fatigue.(6,7,12-16) Most research teams used self-reported surveys (IMPACT-III, semi structured interviews, YSR, SSR, RCMAS, KINDL, KIDSCREEN and TACQOL) (7, 8, 12, 13, 15, 16), while others used a combination of parent proxy-reported and self-reported surveys (PEDSQL multidimensional fatigue scale and PedsQL 4.0 generic care scale).(6) Only one paper used a parent proxy-reported questionnaire (CBCL).(16) **Table 3** describes the myriad of fatigue-related diagnostic tests that were used in the included studies

Table 3: Description of fatigue-related diagnostic tests

Abbreviation	Full name	Details
CBCL	Child behavior checklist	Caregiver report form that categorizes problem behaviors in preschool and school-aged children in the following 8 syndromes: aggressive, anxious-depressed, attention, rule-breaking, somatic complaints, social, thought, withdrawn-depressed.
CDI	Children's Depression Inventory	Adolescent self-reported assessment. For each of 26 items, respondents endorsed one of three sentences indicating varying levels of depression.
IMPACT-III	Not applicable	IBD disease-specific health-related quality-of-life questionnaire for paediatric patients. It is composed of 35 items in the following 6 domains: IBD-related symptoms (7 items), systemic symptoms (3), emotional functioning (7), social functioning (12), body image (3) and treatment/intervention-related concerns (3). Each item is scored on a 5-point Likert scale, coded from 0 to 4 points. Higher scores indicate better quality of life.
KIDSCREEN 27	Not applicable	Self-reported survey is a quality of life questionnaire consisting of 27 items measuring physical well-being, psychological well-being, autonomy and parent relations, peers and social support, and school environment.
KINDL	Not applicable	Adolescent self-reported survey consists of 24 Likert-scaled items, which are subdivided into the following six dimensions (subscales) of quality of life: physical well-being, emotional well- being; self-worth, well-being in the family, well-being regarding friendships and well-being at school.
McMaster Family Assessment Device	Not applicable	Adolescent self-reported 60-item instrument that assesses six domains, namely, problem solving, communication, roles, affective responsiveness, affective involvement, behavior control and general functioning of family functioning as well as general family dysfunction.
PedsQL generic scale	Paediatric Quality of Life Inventory	Parent reported and self-reported assessment. A modular approach to measuring health-related quality of life (HRQOL) in healthy children and adolescents and those with acute and chronic health conditions. It contains the following four multidimensional scales: physical functioning, emotional functioning, social functioning, school functioning.

PedsQL Multidimensional Fatigue Scale	Paediatric Quality of Life Inventory Multidimensional Fatigue Scale	Age-appropriate versions and parallel forms for children and parents. It measures the perceptions of fatigue by children and their parents and has been validated in a variety of paediatric chronic diseases.
RCMAS	Revised Children's Manifest Anxiety scale	Adolescent self-reported assessment that is a true/false anxiety measure containing 28 items. The measured key areas are physiological anxiety, worry, social anxiety and defensiveness. The scale differentiates between anxiety-disordered and normal children.
SSR	Sleep Self Report	Adolescent self-reported assessment to discern sleep patterns and possible difficulties with sleep.
TACQOL	TNO-AZL Children's Quality of life Questionnaire	Generic health-related quality of life questionnaire enabling comparisons between groups of children with varying chronic diseases. It includes 7 scales, involving general physical function, motor function, daily function, cognitive function, social contact, and positive and negative moods.
YSR	Youth Self Report	Adolescent self-reported assessment with the following eight empirically-based syndrome scales: anxious/depressed, withdrawn/depressed and somatic complaints composing the internalizing (i.e., emotional) broad-band scale; rule-breaking behavior and aggressive behavior composing the externalizing (i.e., behavioral) broad-band scale; and these two scales, together with the syndrome scales of social, thought and attention problems, compose the total problems scale.

Scientists from Chicago and Texas performed a cross-sectional study among 70 children with IBD and 157 healthy controls and their parents.(6) They categorized fatigue as general fatigue (e.g., “feeling tired”), sleep/rest fatigue (e.g., “feeling tired when waking up”) or cognitive fatigue (e.g., “attention problem”)(6) based on the PedsQL Multidimensional Fatigue Scale.(11) General fatigue and sleep/rest fatigue were more frequently observed in paediatric IBD patients than in healthy control subjects, even when their disease was in remission. Differences in cognitive fatigue were not observed.(6) A Canadian team from Toronto conducted in-depth interviews among 80 children and adolescents who were purposively selected for their variation in age and condition and found that children and teenagers with IBD commonly mentioned that “exhaustion” and “malaise” (“having no energy and being tired”) had large impacts on their lives.(13) A Finnish research team evaluated sleep problems and daytime tiredness in 160 adolescents by both a parent proxy-reported survey (CBCL) and a self-reported questionnaire (Youth Self Report, YSR).(17) Twenty-five percent of parents reported that their adolescent child had trouble sleeping. This was a significantly greater percentage than was found among the parents of healthy controls. Overall, parents of adolescents with IBD more commonly reported sleeping during the day and night and overtiredness than did parents of healthy controls. The self-reported questionnaire did not confirm the high prevalence of sleep-related problems among IBD patients when compared to healthy subjects (11% vs 16%).(16)

A Swiss research team evaluated 110 adolescents with IBD who were included in the national IBD Cohort Study.(7) They assessed fatigue as physical activity using the KIDSCREEN 27. Physical well-being (e.g., “feeling fit, being physical active, able to run”) was only moderately disturbed in IBD patients compared to healthy controls.(7)

A German research group conducted the only study that evaluated fatigue in an objective manner with a wearable device. They assessed physical activity using the SenseWear Pro2 armband (a portable motion sensor) and reported a trend towards a shorter duration of physical activity and significantly prolonged sleep duration in patients with mild IBD compared to controls, but there were no statistically significant differences.(8)

Biological factors related to fatigue

Disease activity

All included studies observed a positive correlation between disease activity and fatigue, but the scoring systems used to discriminate active disease from disease remission differed among the papers. The team from Chicago and Texas used the Paediatric Crohn's Disease Activity Index (PCDAI) and defined disease remission as a score < 10. They reported that children with active Crohn's disease had significantly more symptoms of general fatigue ("feeling tired") and sleep/rest fatigue ("feeling tired when waking up") than children and teenagers in remission.(6) The Finnish study among 160 children and teenagers used a visual analogue scale (VAS) to measure disease activity. Children with severe IBD (VAS scores above 3) had significantly more trouble falling asleep (41% vs 22%), felt significantly more overtired (80% vs 44%) and had significantly longer sleep duration than adolescents with less active disease (VAS score below 3). In that study, the results of the self-reported questionnaires and the parent reports were very similar when the adolescents had higher VAS scores, but this was less true in the parent-adolescent pairs with mild IBD symptoms.(10, 16)

Medication

The research team from Chicago and Texas evaluated the association between fatigue and medication and concluded that the use of mesalamine, thiopurine or anti-TNF were not predictors of fatigue as measured with the PedsQL Fatigue Scale.(6)

Psychobehavioral factors related to IBD

Family support

A group from Detroit found a significant association between fatigue and dysfunction in the family.(14) The researchers used the McMaster Family Assessment Device (18). They also evaluated two additional items created by the authors themselves, which assessed the frequency of IBD-related pain and IBD-related fatigue over the past 3 months. They found that maternal positive affect, including being attentive, active, and interested, was inversely related to fatigue but the association was not significant. Fathers were not included in the study because they almost never accompanied their children to the clinic,

and a considerable proportion of the adolescents did not have fathers living with them.(14)

Psychological variables

In another paper, the Detroit team assessed 56 adolescents with IBD (aged between 11 and 17 years) with the Revised Children's Manifest Anxiety Scale and found that adolescents with a negative affect (i.e., those who reported anxiety and depression) also experienced more pain and fatigue.(15) The group from Chicago and Texas used the Children's Depression Inventory and found that adolescents with primarily inactive IBD did not report more depressive symptoms than healthy controls (1.4 vs. 1.3%).(6)

Functional factors related to IBD

Disease type

The studies that used questionnaires to assess fatigue did not observe differences between CD and UC patients (8, 12, 16). The German research group that evaluated physical activity with a wearable device found that patients with CD tended towards taking fewer steps per day (8), and UC patients had a shorter duration of physical activity compared with healthy controls.(8)

DISCUSSION

Eight studies were included in this systematic review. These studies were selected for their focus on fatigue in adolescents with IBD.

Key findings

This review demonstrates that fatigue, exhaustion, diminished physical activity and trouble sleeping are more common in children and adolescents with IBD than in their healthy peers. Fatigue is likely to be a multifactorial phenomenon and includes biological factors (such as disease activity), psychobehavioral factors (such as anxiety, depression and family support) and functional factors (such as decreased functional capacity). The model depicted in Figure 2 addresses the various etiological factors and the connection

with the fatigue-related diagnostic tests mentioned in this paper. The model highlights the importance of the multifaceted nature of fatigue, and this fatigue model could act as a guide on which to base treatment interventions.

Biological factors

Fatigue is a common finding (6) in children and adolescents with IBD, and several studies have shown a positive relationship between the degree of disease activity and fatigue. Adolescents with active IBD experience more fatigue than their peers in disease remission, who, in turn, experience more fatigue than healthy controls. It is plausible that active disease impairs sleep quality due to nocturnal abdominal pain and diarrhoea. Inflammation and immune activation, together with the subsequent activation of glial cells and mitochondrial damage, likely account for the severe levels of intractable fatigue and disability seen in patients with autoimmune diseases.(19)

Adolescents in clinical remission are fatigued, but patients in deep remission were not assessed: deep remission could have an impact on less fatigue.

Reduced muscle mass (20) and anaemia (21-23), both of which are frequently observed in patients with IBD, even when their disease is in remission, may also have affect fatigue, but so far, these factors have not been investigated in the adolescent IBD population.

Psychobehavioral factors

The papers that sought correlations between psychobehavioral factors and fatigue showed conflicting results. One paper (14) linked anxiety, depression and lack of family support with IBD-related fatigue, while another paper failed to show that depression occurs more often in adolescents with IBD than in their healthy peers.(6) Sleep disorders can affect the feeling of being tired, as shown in 2 of the included papers. Sleep deprivation leads to more anxiety and depression and to an increase in somatic complaints and aggressive behavior.(13, 16, 17) Sleep itself was not often a research objective; only one study had sleeping problems as an outcome measurement (16), while a German study only reported a trend towards prolonged sleep duration in patients with mild IBD compared to healthy controls.(8)

Functional factors

Only one paper focused on functional capacity in relation to fatigue and used a wearable device to plot activity over time; this study did not find a significant difference between patients with IBD and healthy controls. It is rather surprising that only one paper looked at functional capacity in adolescents with IBD because it is a frequently used outcome measurement in other chronic diseases.(24-27)

Comparison with adult-oriented publications

In 2010, a systematic review (3) identified 10 papers about fatigue in patients with IBD and mentioned that the topic deserved more attention, as the prevalence of fatigue approached 50% in patients with IBD in remission and up to 86% in patients with active IBD. Cuzber-Dochan and colleagues published a systematic review in 2013 that included 28 papers on adults, and they concluded that the use of terminology regarding fatigue is inconsistent and that knowledge of the causes, severity and ways of measuring IBD fatigue is incomplete.(28) Three years later, the same research group repeated the literature search and identified a number of psychosocial and physical factors that could potentially be modified through targeted health interventions to improve fatigue in IBD. As in this study, they concluded that fatigue is multifactorial and is associated with active disease, poor sleep quality, anxiety and depression, but the complex interplay between these factors has yet to be deciphered.(29)

In studies among adolescents, disease activity and sleep quality are also related to fatigue, but the relationship with anxiety and depression is unclear. Approximately one-quarter of adolescents with IBD have somatic or cognitive symptoms of depression (30), and this is comparable with the prevalence observed in their healthy peers.

Depression among adult patients with IBD, on the other hand, is more common compared to among control subjects.(12, 31-33)

Previous studies described a poor to low degree of parent-adolescent agreement on psychosocial symptoms.(17, 30) Moreover, adolescents and parents report different symptoms. Therefore, to gain a comprehensive picture of the complaints in adolescents with IBD, both the adolescents and their parents need to be questioned.(34)

Methodological limitations of the review

The cross-sectional design of most included studies precludes the ability to draw conclusions concerning the causal relations between variables. Prospective observational cohort studies are needed to gain more insight into the direction and mechanism of the identified associations. If prospective cohort studies are conducted in ethnically and socioeconomically diverse groups of children and adolescents, causative factors of fatigue can be identified, and these could potentially lead to more efficacious ways of treating fatigue in adolescents with IBD.

Implications for clinical practice

Future research opportunities

The mechanism underlying fatigue in children and adolescents with IBD remains poorly understood. Fatigue is a subjective sensation and presents with a multitude of symptoms, which makes it difficult to describe, measure and quantify. Past studies have mainly focused on one aspect of fatigue. Future studies should explore fatigue manifestations at several levels simultaneously, including illness-related aspects (such as ongoing inflammation, disease activity, medication use and pain), physical functioning (health-related quality of life, sleep quality and disability), and psychobehavioral factors.

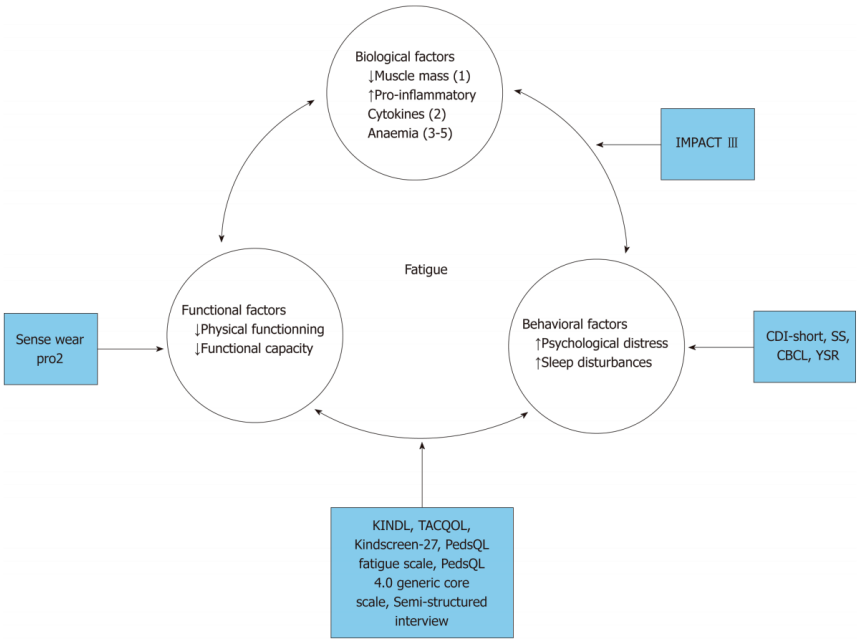

Figure 2 I Multidimensional fatigue model depicting the biological, psychobehavioral and functional factors that play roles in the etiology of fatigue. The fatigue-related diagnostic tests mentioned in the rectangles are also mentioned in this paper.

CONCLUSION

Fatigue is a common problem in children and teenagers with IBD, and it is significantly more prevalent among young patients with IBD than in the healthy control population. It is multidimensional and caused by both physical and psychosocial factors. The most predictive factor seems to be disease activity. Health care providers need to pay attention to this problem because it is associated with reduced quality of life, increased sleeping problems and increased anxiety. The multifactorial nature of fatigue necessitates multilevel testing.

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CHAPTER 6

THE RELATIONSHIP BETWEEN SELF-REPORTED FATIGUE IN CHILDREN WITH INFLAMMATORY BOWEL DISEASE AND HAEMOGLOBIN LEVELS AND DISEASE ACTIVITY: A MULTICENTRE CROSS-SECTIONAL STUDY

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ABSTRACT

OBJECTIVES AND STUDY: Inflammatory bowel diseases (IBD) are known to affect the patient's energy level. Although children and adolescents report fatigue as their most distressing symptom, even at times of disease remission, it has hardly been studied. We aimed to obtain a better understanding of the nature of fatigue in paediatric patients with IBD. We compared biological and functional parameters in fatigued and non-fatigued paediatric patients with IBD to assess possible (non-)correlations.

METHODS: We conducted a cross-sectional observational study of 106 children and adolescents with quiescent to moderately active IBD (defined as having Paediatric Ulcerative Colitis Activity Index (PUCAI) scores below 65 or Paediatric Crohn's Disease Activity Index (PCDAI) scores below 37.5). Participants were recruited from five tertiary care and six secondary care centers in Belgium and the Netherlands. Patients were considered fatigued when the PedsQL^{TM} fatigue was < -2.0 Z for age, while non-fatigued patients had scores above this cut-off point. We measured haemoglobin concentration, iron indicators, faecal calprotectin, six-minute walking distance (6MWD) and disease specific quality-of-life (IMPACT-III).

RESULTS: The study cohort's mean PedsQL^{TM} fatigue Z-score was -1.0. Twenty-three of 106 (22%) patients were fatigued. Fatigued and non-fatigued IBD patients were not significantly different in IBD disease phenotype. Fatigued IBD patients had a significant lower IMPACT-III score than non-fatigued patients (respectively 120 vs. 146, p<0.0001), and a larger proportion was not in clinical remission (26% vs. 63%, p=0.003). Mean haemoglobin Z-scores (-1.7 vs -1.5, p=0.589), ferritin concentration (14 vs 23 µg/L, p=0.206) and faecal calprotectin concentrations (414 vs 355 µg/g, p=0.928) were not significantly different between fatigued and non-fatigued IBD patients.

CONCLUSION: Our data indicate that neither haemoglobin levels nor faecal calprotectin levels are strongly correlated with the self-rating of fatigue. Further investigations are needed to identify practical treatment targets.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic illness of the gastrointestinal tract characterized by episodes of inflammation and remission and has significant impact on psychological and social functioning.(1-3) Fatigue is a common feature during active inflammation as well as during disease remission, which further decreases quality-of-life and hinders participation in daily activities.(5) Fatigue is defined as a 'subjectively overwhelming sense of tiredness, lack of energy, and feeling of exhaustion that decreases one's capacity for physical and mental activity'.(4) Though fatigue is considered a patient-relevant outcome measure, (5) studies on fatigue are limited.

We recently explored the paediatric literature about fatigue in IBD and concluded that biological, functional, as well as behavioral factors contribute to fatigue.(6) Several studies suggest that disease activity and anaemia in particular affect the physical functioning of paediatric IBD patients.(7-10)

Disease activity and fatigue

Persisting mucosal inflammation may go unnoticed when response to therapy is only monitored by clinical parameters such as the paediatric Crohn's disease activity index (PCDAI). The decision to escalate therapy may then be seriously delayed with consequently lower exercise capacity, quality of life and fatigue. Elevated faecal calprotectin levels correlate with endoscopic active disease and thus better reflect ongoing disease activity than clinical parameters.(11)

Anaemia and fatigue

Anaemia is a common systemic complication in IBD and significantly impacts on physical performance, quality of life, and absenteeism from school and extracurricular activities.(12-14) In adult IBD patients normalisation of haemoglobin levels improves general well-being, physical ability and quality of life.(15)

To improve our understanding of fatigue in paediatric IBD, we conducted a cross-sectional observational study to assess the relationship between biological and functional factors. We hypothesize that patients with IBD who have elevated faecal calprotectin levels (>250 ug/g) (16, 17), anaemia (Hb < -2SD) (18) or low iron stores (ferritin < 30 µg/L) (19) have a

significant lower exercise capacity, more fatigue and poorer quality of life than IBD-patients with normal parameters.

METHODS

Patients

Patients aged between 8 and 18 years were recruited at the outpatient clinics of five tertiary care centers and six large teaching hospitals in Belgium and the Netherlands. Patients were eligible for inclusion when they had a Paediatric Ulcerative Colitis Activity Index (PUCAI) score below 65 (20) or a Paediatric Crohn's Disease Activity Index (PCDAI) score below 37.5.(21) Patients were asked to complete two questionnaires, including the PedsQL^{TM} multidimensional fatigue score and the disease specific quality-of-life score IMPACT III, to provide a blood and stool sample, and to perform a 6-minute walking test(6MWT).

PedsQL^{TM} Multidimensional Fatigue Scale

The PedsQL^{TM} Multidimensional Fatigue Scale consists of a child and parent report and is a commonly used fatigue questionnaire with good reliability.(22) The scale comprises the General Fatigue Scale (GFS, 6 items), Sleep/Rest Fatigue Scale (S/RFS, 6 items), and Cognitive Fatigue Scale (CFS, 6 items).(22) The GFS contains questions regarding the subjective feeling of fatigue and the energy to execute activities, the S/RFS contains questions about the quantity and quality of sleep and rest and the CFS contains questions regarding attention and memory.(23) We used the Dutch version of the Child Self Report, which has been validated for children (8 to 12 years) and adolescents (12 to 18 years).(24) Several studies on fatigue have reported imperfect agreement between child self-reports and parent proxy reports (25) and the child and adolescent self-report questionnaires have a strict factorial invariance across gender and age subpopulations.(26) Participants were asked to rate how often a particular problem occurred in the past month, using a 5-point Likert scale. Scores were transformed on a scale from 0 to 100, in which higher scores indicated fewer symptoms of fatigue. PedsQL^{TM} scores were expressed as z-scores derived from published normative data.(11)

IMPACT-III questionnaire

The IMPACT-III questionnaire is a disease-specific quality-of-life questionnaire, that comprises 35 items in 6 domains: IBD-related symptoms (7 items), systemic symptoms (3), emotional functioning (7), social functioning (12), body image (3) and treatment/intervention-related concerns (3).(27) Each item is scored on a 5-point Likert scale, coded from 1 to 5 points. The maximum score is 175, higher scores indicate better quality-of-life. The Impact-III (NL) is a translated and modified version of the original Canadian Impact questionnaire (77) and has been validated for use in children of 8 years and older.(37)

Laboratory tests

Anaemia was defined as a haemoglobin (Hb) concentration more than two standard deviations below the mean of similarly aged children and adolescents from an iron supplemented USA reference population.(18) Hb Z-scores were calculated by subtracting the reference population mean (μ) from the individual score (x) and then dividing the difference by the reference population standard deviation (σ): $z = \frac{x-\mu}{\sigma}$.

Iron deficiency was defined as a ferritin concentration below 30 μg/L.(19) Iron deficiency anaemia was defined as a combination of Hb < -2 Z-scores in combination with ferritin concentration < 30 μg/L. Intestinal inflammation was assessed by measuring faecal calprotectin. We used three commonly used categories: <250 μg/g for disease remission, >500 μg/g for disease flare, and 250-500 μg/g for the indecisive range.(29)

6-minute walking test

Exercise capacity was assessed with the 6-minute walking test, and was expressed as the distance (in meters) a person can walk at a constant, uninterrupted pace in 6 minutes.(30) The 6-minute walking distance (6MWD) is age and sex dependent.(30, 31) Z-scores were calculated by subtracting the reference population mean (μ) from the individual score (x) and then dividing the difference by the reference population standard deviation (σ).(32)

Ethical considerations

This study was conducted according to the principles of the Declaration of Helsinki (59th version, October 2008). The independent Medical Ethical Committees of the Zuyderland Medical Center (Heerlen, the Netherlands) and the University of Antwerp (Antwerp, Belgium) approved the study (NL42995.096.12; BE17/50/56). Secondary approval was obtained from the boards of the other participating centers. All parents or legal guardians and participants aged 12–18 years gave informed consent.

Statistical analysis

Patients were considered fatigued when the PedsQL^{TM} fatigue was below -2.0 Z for age, while non-fatigued patients had scores above this cut-off point. Baseline characteristics were compared between fatigued and non-fatigued patients using a Chi-square test for categorical variables and an independent-samples t-test for continuous variables. In case of non-normal distribution, we used non-parametric alternatives (Fischer's exact test and Mann-Whitney test, respectively; median and IQR are reported). Distributions of PedsQL^{TM} and 6MWD Z-scores among IBD patients were compared with the healthy reference population using the Kolmogorov Smirnov test.

RESULTS

Baseline characteristics of the study population

Patients were recruited between June 2014 and January 2019. Of 124 patients who were assessed for eligibility, 18 were excluded for reasons listed in **Figure 1**. A total of 106 patients were included in the final analysis.

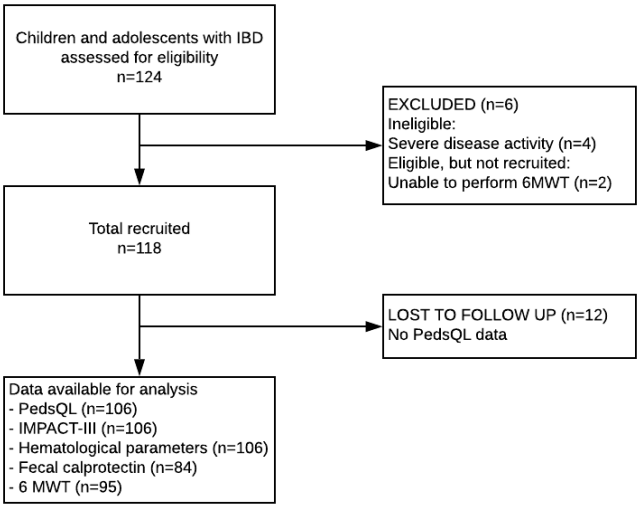

Figure 1 | Study flow diagram

Forty-five percent (48/106) of participants were female. Thirty-nine percent (41/106) were aged 8 to 12 years, and 61% (65/106) were aged 13 to 18 years. Twenty-four percent (25/106) of the participants had ulcerative colitis, 73% (77/106) had Crohn's disease, and 4% (4/106) had IBD-unclassified. Fifty-four percent (53/98) had disease activity scores indicating clinical remission, 45% (44/98) had mild disease activity and one participant had moderate disease activity.

Fatigue prevalence

The distribution of PedsQL^{TM} total fatigue scores in patients with IBD was significantly lower compared to healthy peers ($p<0.0001$). In patients aged 8 to 12 years the mean fatigue Z-score was -0.9 (SD 1.3). In patients aged 12 to 18 years the mean fatigue Z-score was -1.1 (SD 1.4). IBD patients scored lower than healthy peers on all dimensions of the PedsQL^{TM} fatigue scale. [Figure 2] Twenty-three patients (22%) had Z-scores below -2.0 and were considered fatigued.

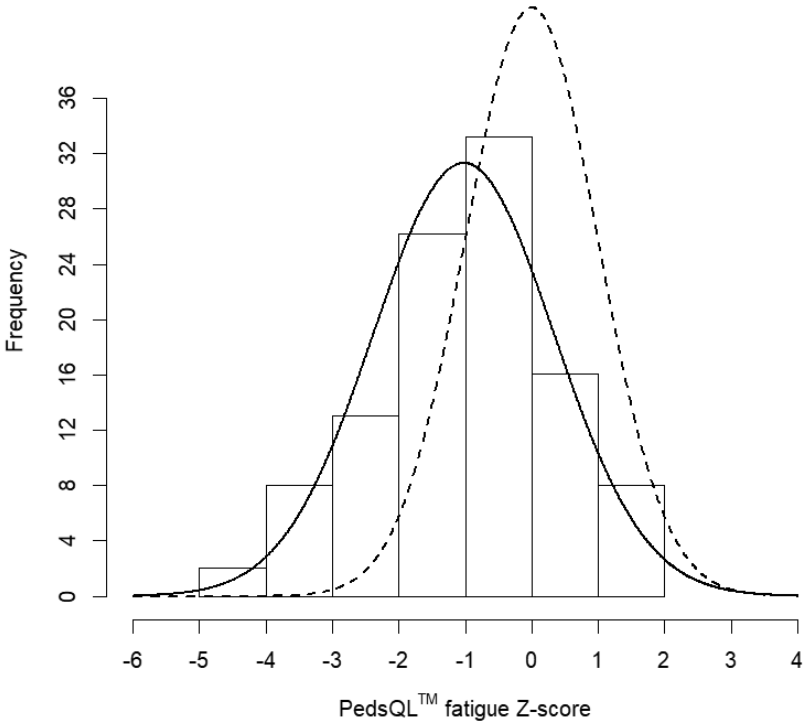

Figure 2 | Distribution of PedsQL™ fatigue Z-scores in IBD patients (solid line) vs. the healthy reference population (dashed line) for children

Table 1 shows that females were overrepresented in the fatigued category (p=0.008). Age distribution, disease phenotype and disease duration were not significantly different between fatigued and non-fatigued patients. A significant larger proportion of fatigued patients had higher disease activity scores compared to non-fatigued patients (74% vs. 37%, p=0.003). Fatigued and non-fatigued IBD patients did not differ in haemoglobin Z-score nor in ferritin concentration and had similar proportions with high faecal calprotectin values (>500 μg/g).

Table 1 | Demographic, clinical and laboratory characteristics of fatigued and non-fatigued patients

	Fatigued patients (n=23)	**Non-fatigued patients** (n=83)	**p-value**
Female gender	16 (70%)	32 (39%)	0.008
Age category			0.359
8-12 years	7 (30%)	34 (41%)	
13-18 years	16 (70%)	49 (59%)	
IBD phenotype			0.821
M Crohn	18 (78%)	59 (71%)	
CU	5 (22%)	20 (24%)	
IBDU	0 (0%)	4 (5%)	
Disease activity score		*	0.003
Remission	6 (26%)	47 (63%)	
Mild	17 (74%)	27 (36%)	
Moderate		1 (1%)	
Disease duration in years	2 (1-3)	1 (0-3) **	0.768
Mean haemoglobin Z-score (SD)	-1.7 (1.2)	-1.5 (1.7)	0.589
Number (proportion) of patients with anaemia (Hb < -2.0 SD)	12 (52%)	31 (37%)	0.200
Number (proportion) of patients with anaemia (HB<-1.5 SD)	13 (57%)	40 (48 %)	0.238
Mean ferritin (SD) in µg/l	14 (7-31)	23 (12-43) #	0.206
Number (proportion) of patients with iron deficiency anaemia	11 (50%)	24 (32.4%)	0.133
Faecal calprotectin in µg/g mean (SD)	1085.5 (1608.4)**	833.9 (1280.1)	
Faecal calprotectin (≥ 250 µg/g)	10 (53%)**	36 (55%)	0.832

*8 missing values,**4 missing values, #17 missing values, ##18 missing values

6-minute walking distance

The distribution of 6MWDs of patients with IBD children was significantly lower compared to age and sex matched healthy peers [**Figure 3**], but the mean 6MWD and 6MWD Z-scores between fatigued and non-fatigued patients were not significantly different (respectively 581 meters (-1.1 Z) and 594 meters (-1.0 Z))[**Table 2**].

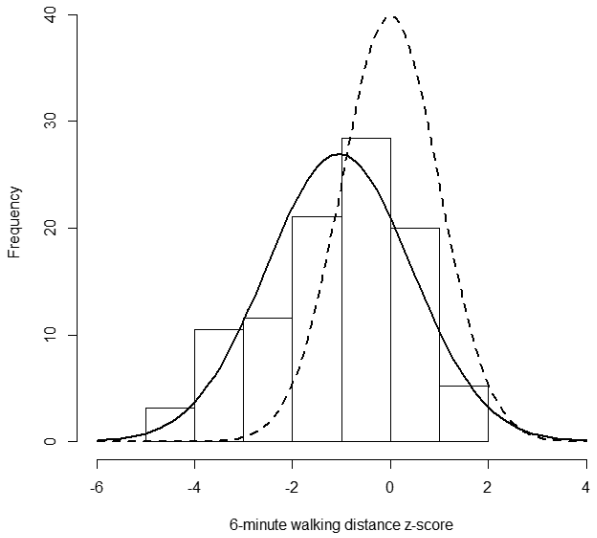

Figure 3 | Distribution of 6-minute walking distance Z-scores in IBD patients (solid line) vs. the healthy reference population (dashed line).

Table 2 | 6-minute walking distance in fatigued and non-fatigued participants**.**

	Fatigued patients (n=22)	**Non-fatigued patients (n=73)**	**p-value**
Mean 6MWD in m (SD)	581 (86)	594 (91)	0.533
Mean 6MWD Z-score (SD)	-1.1 (1.5)	-1.0 (1.5)	0.863

Quality-of-life

Fatigued patients had lower median quality-of-life scores compared to non-fatigued IBD patients (120 (IQR 115 to 127) vs. 146 (IQR 134 to 157); p<0.0001) [**Figure 4**].

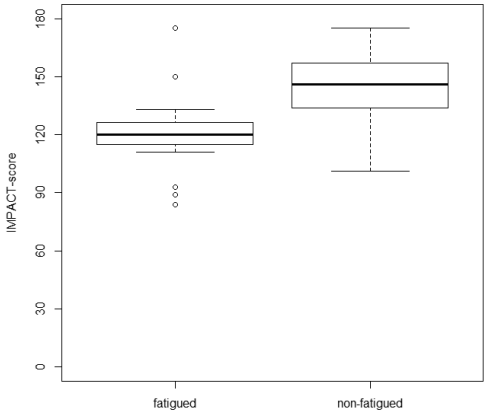

Figure 4 | IMPACT-III quality-of-life scores in fatigued and non-fatigued patients. Central boxes span 25th and 75th centiles (or the interquartile range). Horizontal line within box represents median.

DISCUSSION

Key findings

In this multicenter study we found that approximately a quarter of paediatric patients with quiescent to moderately active IBD qualified as fatigued. Interestingly, neither haemoglobin parameters nor faecal calprotectin nor the exercise capacity, expressed as the 6MWD, was significantly different between fatigued and non-fatigued IBD patients. Fatigued patients had a significantly lower quality-of-life scores and higher clinical activity indices, but otherwise this group did not differ in any respect from the non-fatigued patients.

The mean total Z-score of the Child Self Report PedsQL^{TM} Multidimensional Fatigue Scale in our study cohort (-1.0) was comparable with the total Z-score (-0.7) in a similarly aged American cohort of IBD patients described by Marcus and colleagues.(12) In this single center case-control study, IBD patients completed questionnaires about fatigue, quality-

of-life (IMPACT III) and depression (PedsQL Depression inventory), and were compared to healthy controls. This is the only other cohort in which fatigue was assessed with the PedsQL^{TM} fatigue scale. Like us, they concluded that adolescents with IBD are significantly more fatigued than healthy controls, even when clinical remission was reached.

The 6MWD as a proxy of exercise capacity has been used in many other chronic conditions, including congenital heart disease (33), cystic fibrosis (34) and juvenile idiopathic arthritis (35), but not yet in IBD. A German case-control study evaluated exercise capacity in adolescents with IBD with a wearable device (Sensewear Pro 2) to plot spontaneous physical activity over time.(36) Contrary to our observations, the exercise capacity was not different between patients with IBD and healthy controls. Werkstetter and colleagues included 39 IBD patients only, which may have been a too small sample size to detect a group difference.

Recently published adult studies on fatigue and IBD show results that are similar to ours.(37-39) These studies also suggest that fatigue negatively impacts on quality-of-life and is not associated with biological factors such as anaemia or persistent mucosal inflammation. Fatigue was rather associated with behavioural factors such as anxiety, depression and sleep disturbances.(37-39)

Methodological strengths and limitations

The cross-sectional design of this study does not allow to evaluate causality between fatigue and biological parameters. A prospective intervention study to evaluate whether iron supplementation reduces fatigue, improves exercise capacity and quality of life in paediatric patients, is currently underway.[toetsingonline NL42995.096.12) Also, the use of self-report questionnaires may have underestimated the prevalence of fatigue, as patients with low exercise capacity and anaemia may tend to adapt to a lower level of activity and accept this as is.

Implications for clinical practice and future research

The impact of fatigue on quality-of-life justifies a thorough evaluation of possible causal factors, including behavioural factors such as psychological distress and sleep disturbances. Cognitive Behavioural Therapy has recently been shown to be an effective

treatment against fatigue in another autoimmune inflammatory condition (systemic lupus erythematosus), and may also be of use in improving psychological distress-associated fatigue in IBD.(40) Further research is needed to identify practical treatment targets.

CONCLUSION

This study shows that fatigue is a rather common feature in children and adolescents with IBD, irrespective of the presence of mucosal inflammation and anaemia. Our findings confirm that fatigue negatively impacts on the quality-of-life. We found no association between self-reported fatigue and exercise capacity. In order to get a full picture of the possible causes of fatigue, we suggest that future studies measure functional as well as behavioral factors.

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CHAPTER 7

GENERAL DISCUSSION AND FUTURE PERSPECTIVES.

This dissertation addresses two particular knowledge gaps out of the many that exist in paediatric inflammatory bowel disease (IBD).

In the first part of this thesis, we evaluated diagnostic strategies to assess whether gastrointestinal complaints are due to IBD, for appropriate triage for endoscopic evaluation. In the second part, we quantified and characterised fatigue in IBD. In this chapter, we will discuss the main results and their clinical implications.

PART I - **Triage for endoscopy**

In our effort to develop an appropriate strategy whether or not endoscopy is indicated to evaluate IBD in a child with abdominal complaints, we first evaluated faecal calprotectin (FC) as an isolated triage test. In **Chapter 2** we described a cohort of 117 children with chronic diarrhoea and nonspecific abdominal pain. The treating physicians had to base their decision whether or not to perform endoscopy on the standard practice of that time: a combination of signs, symptoms and blood results. Without the knowledge of the FC result, 62% of the patients that were selected for endoscopy were diagnosed with IBD. If they would have based the selection for endoscopy on the combination of raised FC levels (i.e. >50 μg/g) and negative stool cultures, the yield of ileocolonoscopy towards diagnosing IBD would have improved to 78%, without missing any IBD patient. At the same time, FC levels below this cut-off point would have prevented a considerable proportion of patients being subjected to an endoscopic procedure that would not have led to the diagnosis of IBD, and, arguably, could then even have been labelled 'futile'. Even though adding FC results to the decision strategy improved the diagnostic yield compared to the standard diagnostic strategy of that time, still 22% of the patients would have been subjected to an IBD-negative ileocolonoscopy.

In **Chapter 3** we evaluated whether another faecal biomarker for mucosal inflammation, calgranulin-C, is better than FC in predicting IBD in children and teenagers. When predefined test thresholds were used (50 μg/g for FC and 0.75 μg/g for calgranulin-C), the diagnostic accuracy of calgranulin-C indeed appeared to be better. However, when receiver-operator characteristic (ROC) curves were used to identify the optimal test threshold for each test separately, what appeared to be 400 μg/g for FC and 0.75 μg/g for calgranulin-C, the superiority of calgranulin-C relative to FC disappeared. We therefore

concluded that the diagnostic accuracy of the calgranulin-C test was not superior to the FC test.

The cohort evaluated in Chapter 2 included patients with rectal blood loss and perianal disease. These red flag symptoms provide sufficient reasons for immediate endoscopic evaluation to obviate the need for additional diagnostic testing. Inclusion of these patients increases the pre-test probability and causes an overestimation of the discriminating power of FC relative to the practical situation, where a test seems particularly useful to discriminate between those with IBD and those with functional abdominal pain. Children and teenagers presenting with *non-bloody* diarrhoea and abdominal pain, in other words without red flag symptoms, are a spectrum of patients more commonly seen in general paediatric practice. These patients constitute the most challenging group to discriminate IBD from Irritable Bowel Syndrome (IBS) because the pre-test probability for IBD is low. Previously published meta-analyses pooled studies which included patients with red flag symptoms and may have exaggerated the diagnostic accuracy of FC to diagnose IBD.

We therefore set out to determine the optimal test strategy in patients without red flag symptoms **(Chapter 4).** This time we used a FC threshold of 250 µg/g, which was, according to new insights, (1) considered to be the optimal cut-off point to discriminate IBD from functional abdominal disorders.

We compared four diagnostic strategies to predict the need of endoscopy based on (A) symptoms alone, (B) symptoms + blood markers, (C) symptoms + faecal calprotectin, and (D) symptoms + blood markers + faecal calprotectin. Triaging with strategy C resulted in 20 of 100 patients undergoing endoscopy, and triaging with strategy D further limited this number to 14 of 100 patients. Eleven out of 14 had IBD and three did not have IBD. No IBD-affected child was missed.

Clinical Implications

Our search for the optimal diagnostic approach to triage paediatric patients with gastrointestinal complaints and absence of red flags for endoscopy culminated in a combination of meticulous history taking with measuring C-reactive protein in blood and calprotectin in stool **(Chapter 4)**. This strategy provides an easy and effective way to correctly selecting those who appeared to have IBD. Clinical practitioners can be

reassured that in patients with a low CRP (≤10 mg/L), normal haemoglobin and low FC (<250 µg/g), endoscopy can safely be avoided without missing a case of IBD. Effective therapeutic interventions in children with a negligible risk for IBD, e.g. gut-directed hypnotherapy, can be initiated without losing time on further diagnostics. Simultaneously, children with increased FC in combination with increased CRP, low haemoglobin, or both, who have a high risk for IBD, can have an endoscopic confirmation of this diagnosis sooner and consequently have an earlier start of appropriate treatment.

Omitting the diagnostic strategy that comprises the combination of CRP, haemoglobin and calprotectin in children with *non-bloody* diarrhoea and abdominal pain may cause considerable harm, such as linear growth impairment(2) and progressive bowel damage requiring surgery early after diagnosis.(3-5)

Tips for reliable faecal calprotectin results

The reliability of the diagnostic strategy strongly depends on biological, pre−analytical and analytical factors influencing the FC test. Stool samples are relatively easy to obtain, but there are several obstacles in the trajectory from stool collection to analysis that can affect the test result. First, it is advisable to use the first bowel movement of the day to catch the highest possible concentration of calprotectin.(6) The faeces sample must not come into contact with toilet water as it may contain bleaches and disinfectants that may degrade calprotectin. Secondly, medication that is commonly prescribed in patients with abdominal pain, including non-steroidal anti-inflammatory drugs (e.g. aspirin or ibuprofen) and proton pump inhibitors, can increase FC.(7, 8) Ideally, these medications should be discontinued a week before stool collection. Thirdly, recent publications have shown that the protein calprotectin may be less stable at room temperature than previously thought.(6, 9, 10) Protein degradation can be delayed when the filled stool container is refrigerated until delivery at the laboratory. Unrefrigerated stool samples of children with vague gastrointestinal complaints that arrive with a delay exceeding 48 hours and with a FC result between 50 and 250 µg/g, may falsely reassure doctors and patients because of degradation of initially increased FC levels and therefore require analysis of another fresh faecal sample.

Comparison of FC test accuracy per manufacturer

At present, most clinical practitioners have access to one or more faecal calprotectin tests, but these tests are neither standardized nor harmonized. We nevertheless feel that our findings can be extrapolated to settings with calprotectin tests from different manufacturers, as they fairly agree in the lower range (below 250 µg/g).(11) Above this cut-off point however, inter-assay variability is considerable. On the other hand, tests with a limited measuring range (say 50 to 300 µg/g) are considered unsuitable for triaging for endoscopy. In the absence of assay standardisation, more assay-specific cut-offs are needed.

Cost efficiency

Yang et al. performed a cost-effectiveness analysis comparing FC as triage for endoscopy with direct endoscopic evaluation alone in the United States.(12) They showed that cost-effectiveness of FC screening varied with the pre-test probability of IBD. Performing FC testing in all children was cost-effective when IBD prevalence was below 65%. The turning point, where direct endoscopic evaluation becomes more cost-effective is situated at an IBD prevalence of more than 80%.

We did not evaluate the cost-effectiveness of using a test combination of FC, CRP and haemoglobin. Since the publication of Yang et al. the price of a FC test has been reduced from €40 to €25, and the optimal cut-off point has increased from 50 to 250 µg/g. Furthermore, the cost of endoscopic evaluation with biopsies in day care has increased in recent years. These trends likely make the cost-effectiveness of our triage strategy, including FC analysis, even more favorable.

Applicability in the primary care setting

Our test-strategy was evaluated in second- and third-line care settings, but not in primary care. In primary care, where IBD prevalence is low, an isolated positive FC result is rarely indicative of IBD, but an FC result below 50 µg/g on the other hand, does rule out IBD.(13) The decision to refer children for endoscopy should therefore not be made at the general practitioner's level, but at the level of the paediatrician.

For this part of the thesis we conclude that the inclusion of the FC test in the triage for endoscopy allows to accurately select individuals with a high risk for IBD from a cohort of children with non-specific chronic intestinal complaints. Even in settings with high pre-test probability for IBD (i.e. prevalence > 70%), the optimal decision strategy based on symptoms, blood markers and faecal calprotectin continues to be beneficial. Paediatricians working at either secondary or tertiary care level can be reassured that this is a highly accurate and non-invasive approach to determine the likelihood of IBD.

PART II - **Quality of life beyond clinical remission: fatigue in paediatric IBD**

Children with IBD often experience fatigue and consider it one of the most burdensome symptoms. Fatigue is common at times of active inflammation, but a considerable proportion of the children also experiences fatigue when their IBD is in remission. The rates of fatigue in paediatric IBD are comparable to rates observed in paediatric oncology patients (50-75%).(14) IBD-related fatigue negatively impacts the quality-of-life and daily activities, including school attendance and sports participation. Despite its frequent occurrence, fatigue has only been addressed in paediatric IBD literature only scarcely and not in considerable detail.

In **Chapter 5** we systematically reviewed existing literature to identify factors contributing to fatigue. In the absence of randomised controlled trials, we selected cross-sectional or case-control studies reporting on fatigue in paediatric patients with IBD. The selected studies varied in the methodology to quantify or measure fatigue. Several studies used self-reporting surveys or a combination of parent-proxy reports and self-reports; only one tried to measure decline in activity with a portable pedometer. While working on the literature review it became clear that fatigue should be regarded as a multidimensional phenomenon, characterised by biological, psychobehavioral and functional factors (**table 1**).

Table 1 | Identification of factors contributing to IBD-associated fatigue. Adult studies printed in grey

Predictors of fatigue	Effect on fatigue	
	Aggravation	**Alleviation**
Biological factors		
Disease activity	Compared to patients with quiescent disease, adolescents with active disease have impaired physical wellbeing and more trouble sleeping (15) IBD adolescents are more tired in case of active disease (16)	Effective induction and maintenance therapy
Medication	Use of corticosteroids, thiopurines, and anti-TNF agents are associated with more fatigue (17-19)	Anti-inflammatory management. (20, 21)
Haematological factors	Iron deficiency anaemia(22)	iron supplements or intravenous iron therapy
Psychobehavioral factors		
Family support	Family dysfunction (23)	Maternal positive affect (23)
Psychological factors	Depression and anxiety (24)	Mindfulness and relaxation (25) Cognitive behavioural therapy (25, 26)
Functional factors		
Physical activity	Impairment in motor functioning(27) Decreased physical exercise (28)	Physical training reduces fatigue in postoperative IBD patients(29)

In **Chapter 6** we assessed the relationship between biological and functional factors and IBD-associated fatigue. We evaluated haemoglobin, iron status, calprotectin (as marker of intestinal inflammation), disease-specific quality-of-life (with the IMPACT-III questionnaire) and physical fitness (by 6 minute walking distance, 6MWD) in children with quiescent, mild or moderate IBD. Using the PedsQL^{TM} multidimensional fatigue scale, participating children with IBD were classified as fatigued or non-fatigued. We found no differences between the fatigued or non-fatigued groups in terms of haemoglobin concentration, faecal calprotectin, and ferritin concentration. The mean 6MWD in the cohort of paediatric IBD patients was 1 standard deviation below age-related healthy controls, but the mean 6MWD in the fatigued and non-fatigued IBD patients was not significantly different. The quality-of-life score was inversely related to fatigue: the more fatigued, the lower the quality-of-life score.

Future perspectives

Despite the high impact of fatigue in paediatric IBD there has been very limited evidence on successful pharmacological or non-pharmacological interventions, neither in paediatric nor in adults studies.(25) Future research needs to make use of validated measures of fatigue, and interventions should have a measurable effect on these fatigue scores.

One of the few ongoing RCTs focusing on IBD-associated fatigue in children is the POPEYE-study (EudraCT number: 2012-005644-26). In this study, we compare the effect of intravenous iron supplementation to oral supplementation on recovery of physical activity, anaemia, subclinical inflammation, quality of life and fatigue. The primary outcome is the proportion of patients per group that show a 15% increase in 6MWD four weeks after the initiation of iron treatment.

Non-pharmacological treatments also warrant further investigation in the paediatric IBD population. Physical activity, mindfulness, cognitive and behavioural therapy are some of the treatments to be invastigated, particularly in children and adolescents with cancer. Despite the scarce data in children, Robinson et al. underline the beneficial effect of physical activity interventions and relaxation or mindfulness exercise in the management of fatigue in children and adolescents with cancer.(14) Future research can show whether these beneficial effects can also be obtained in children with IBD.

In conclusion, this dissertation addressed the diagnostic strategy that best selects, out of a group of children with gastrointestinal complaints, those that are most likely to have IBD. Secondly, it provides an attempt to quantify and characterise fatigue in children with IBD.

With regard to the former point, we are confident in the quality of the optimal diagnostic strategy (with CRP, haemoglobin and faecal calprotectin). In the field of IBD-associated fatigue, however, it has become apparent that there is a lack of good quality studies. Measuring the efficacy of both pharmacological and non-pharmacological interventions for fatigue should be a research priority to improve the quality-of-life of children with IBD.

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Appendices

LEKENSAMENVATTING

Achtergrond

Inflammatoire darmziekten (in het Engels: Inflammatory Bowel Disease, of IBD) is een groep aandoeningen die gekenmerkt wordt door chronische ontsteking van het maagdarmkanaal, waarbij de ziekte van Crohn (ZvC) en colitis ulcerosa (CU) de meest voorkomende fenotypes zijn. De ontsteking bij de ZvC kan verspreid zijn in de hele maagdarm tractus met een voorkeur voor het meeste distale stuk van de dunne darm, het terminale ileum. CU heeft daarentegen een meer continu aspect van ontsteking in alleen de dikke darm. Zo'n 10% van de patiënten met IBD hebben manifestaties in de dikke darm met overlappende kenmerken van beide fenotypes die niet-geclassificeerde IBD (IBD-unclassified) worden genoemd.

De lichamelijke klachten van patiënten met IBD variëren naargelang de locatie van de ontsteking. Patiënten met de ZvC presenteren zich vaak met chronische buikpijn, gewichtsverlies en diarree. Bij patiënten met CU staat gewichtsverlies minder op de voorgrond. Bij hen is juist rectaal bloedverlies de meest voorkomende klacht. Ongeveer 10% van de patiënten bij wie IBD wordt vastgesteld is jonger dan 19 jaar. In vergelijking met volwassenen presenteren kinderen en jongeren zich vaker met een meer uitgebreide aantasting van het maagdarmkanaal, en hebben ze een groter risico op complicaties in het beloop van de ziekte.

IBD wordt gekenmerkt door afwisselende periodes van ontsteking (opvlamming) en ziekterust (remissie). De onvoorspelbaarheid van de opvlammingen, de frequente ziekenhuisopnames en het chronische gebruik van medicatie hebben een negatieve invloed op het psychosociaal functioneren van patiënten met IBD. Ze kunnen beperkingen ondervinden bij deelname aan sociale activiteiten, op school, en in hun functioneren op het werk.

Een definitieve genezing is vooralsnog niet mogelijk, maar in afwachting daarvan is het doel van pediatrische IBD-zorg gericht op het onder controle houden van ontstekingen en het beperken van de last van de ziekte.

In dit proefschrift worden twee kennishiaten binnen de pediatrisch IBD nader bestudeerd. Het eerste deel is gericht op een snelle en betrouwbare diagnostiek bij kinderen met klachten die kunnen wijzen op IBD, zodat het mogelijk wordt om de darmontstekingen in

een vroeg stadium aan te pakken zonder kinderen nodeloos te onderwerpen aan invasieve onderzoeken. In het tweede deel van dit proefschrift wordt het vóórkomen van vermoeidheid bij kinderen met IBD bestudeerd.

Deel 1: Selecteren voor endoscopie.

Voor het bevestigen van de diagnose IBD is een kijkonderzoek (endoscopie) nodig van het bovenste deel van het maagdarmkanaal (slokdarm, maag en twaalfvingerige darm) en het onderste deel van het maagdarmkanaal (endeldarm, dikke darm en het laatste deel van de dunne darm). Hierbij worden weefselstukjes (biopten) afgenomen voor microscopisch onderzoek. Deze test gebeurt doorgaans onder narcose en de patiënt heeft van tevoren een darmspoeling nodig. Aangezien slechts een fractie van de kinderen met maagdarmklachten IBD heeft, is het niet wenselijk om iedereen met klachten te onderwerpen aan dit ingrijpende en tijdrovende onderzoek. Het is voor de huisarts en kinderarts een uitdaging om de juiste kinderen te selecteren voor endoscopie. Een laboratoriumtest waarmee de kans op IBD voorspeld kan worden, biedt mogelijk uitkomst. Bij een ontstekingsproces komen verschillende eiwitten vrij, die gemeten worden in ontlasting, bloed of urine. Voor het screenen op IBD is het logisch om een ontstekingseiwit in de ontlasting te meten. Patiënten met een verhoogde hoeveelheid ontstekingseiwit ondergaan dan een endoscopie, en patiënten met een normale testuitslag hoeven niet aan het ingrijpende onderzoek te worden blootgesteld. Een goede triagetest moet aan een aantal voorwaarden voldoen: de test meet het bedoelde eiwit, de afname en de analyse van het materiaal moet eenvoudig zijn, en de test moet goedkoop en makkelijk beschikbaar zijn. De faecaal calprotectine (FC) test voldoet aan al deze voorwaarden. Het is een ontstekingseiwit dat vrijkomt bij iedere ontsteking waarbij witte bloedcellen betrokken zijn, dus niet alleen bij IBD, maar ook bij bijvoorbeeld darminfecties.

In **hoofdstuk 2** onderzoeken we wat het voor het diagnostisch selectieproces betekent wanneer FC als enige test gebruikt wordt. We beschrijven een cohort van 117 kinderen met chronische diarree en niet-specifieke buikpijn. De behandelende artsen baseerden hun besluit tot endoscopie op de standaardaanpak van dát moment: een combinatie van

klachten, kenmerken bij lichamelijk onderzoek en resultaten van bloedonderzoek. Daarnaast leverden alle deelnemende kinderen een ontlastingsmonster in voor een FC bepaling. Zonder kennis van het resultaat van de FC meting, kreeg 62% van de kinderen die een endoscopie ondergingen de diagnose IBD, en had 38% van de kinderen een negatieve endoscopie. Als de beslissing voor endoscopie zou hebben afgehangen van een verhoogde FC waarde (>50 µg/g) en negatieve ontlastingskweken, dan zou bij 78% van de kinderen die een endoscopie ondergingen de diagnose IBD gesteld zijn. De kinderen met een normale FC uitslag (≤50 µg/g) zouden geen endoscopie hebben ondergaan, en zouden ook in het eerste half jaar na de FC-test geen IBD ontwikkeld hebben. Er werden met andere woorden geen patiënten met IBD gemist. Ondanks het toevoegen van het FC-resultaat aan het diagnostische selectieproces zouden nog steeds 22% van de kinderen een negatieve endoscopie hebben. Of anders gezegd: de FC-test heeft een hoge sensitiviteit (aantal terecht positieve uitslagen) maar een matige specificiteit (aantal terecht negatieve uitslagen).

In **hoofdstuk 3** vergelijken we een ander ontstekingseiwit (Calgranuline-C, ook wel S100A12) met FC en evalueren we welke van de twee beter is in het voorspellen van IBD. Bij kinderen die zich presenteren met buikklachten die zouden kunnen passen bij IBD, bepalen we zowel het FC als het Calgranuline-C in de ontlasting. Wanneer ze op basis van de klachten, lichamelijk onderzoek, bloed- en stoelgangonderzoek een hoog risico hebben op IBD, worden ze verwezen voor een endoscopisch onderzoek van de darmen. De uitkomst (wel of geen IBD) vergelijken we met de uitslag van het stoelgangonderzoek. Zowel Calgranuline C als FC voorspellen zeer goed het risico op IBD en maken een correcte selectie van kinderen die een endoscopie moeten ondergaan. FC is een test die in veel laboratoria kan uitgevoerd worden. Deze beschikbaarheid vergemakkelijkt het gebruik in de klinische praktijk. Daarom verkiezen we deze test bij het vervolgonderzoek.

Het gebruik van een FC-test is pas zinvol wanneer een arts op basis van een gesprek en lichamelijk onderzoek alleen niet goed kan beslissen of de patiënt een endoscopie moet ondergaan of niet. Als de patiënt bloedverlies bij de stoelgang (rectaal bloedverlies) of huidafwijkingen rond de anus (peri-anale ziekte) heeft, dan zal de FC test niet zoveel meer bijdragen. Bij kinderen met deze alarmsymptomen (in het Engels “red flags”) is een endoscopisch onderzoek (ongeacht de FC uitslag) een logische beslissing. Bij kinderen met

vage buikpijnklachten is deze beslissing lastiger te maken en heeft het gebruik van een triagetest wel zin. Het gebruik van FC als triagetest is meer bepalend bij deze kinderen, omdat de a priori-kans op IBD klein is. Het is deze laatste groep die vaak gezien wordt in de algemene kinderartsenpraktijk en de huisartsenpraktijk.

In hoofdstuk 2 werden patiënten met alarmsymptomen meegenomen in de evaluatie van FC-bepaling in het diagnostisch selectieproces. Daardoor kan het aantal terecht positieve uitslagen hoger zijn en kan het onderscheidend vermogen van de FC test overschat zijn. Daarenboven heeft FC als triagetest een matige specificiteit, bij een afwijkend resultaat is de kans eerder gering dat het om IBD gaat.

In **hoofdstuk 4** verfijnen we de diagnostische selectiestrategie door een groot cohort van kinderen met buikpijnklachten zonder alarmsymptomen te bestuderen. We gebruiken een geoptimaliseerde grenswaarde voor FC en combineren dit met bloedresultaten. De resultaten van dit onderzoek tonen aan dat bij kinderen met een laag CRP, een normaal hemoglobine niveau en een laag FC, endoscopie kan vermeden worden zonder een geval van IBD te missen. Daarenboven heeft de groep kinderen met een verhoogde FC en verhoogd CRP of hemoglobine een sterke indicatie voor endoscopie, met een laag aantal onterecht gescopieerde kinderen. Anders gezegd, de sensitiviteit van de FC-test blijft hoog in deze groep kinderen en de specificiteit verbetert door het toevoegen van CRP of hemoglobine waarde in de diagnostische beslisboom.

Deel 2: Vermoeidheid bij IBD

De behandeling van een aandoening houdt niet op bij het verminderen of wegnemen van de klachten. Een hoge levenskwaliteit is een belangrijk doel om na te streven in de aanpak van een chronische ziekte. Vermoeidheid is één van de belangrijke klachten die deze levenskwaliteit negatief beïnvloedt. Kinderen met IBD hebben vaak last van vermoeidheid en beschouwen dit in tijden van remissie als één van de meest storende klachten.

Vermoeidheid heeft een negatief effect op het sociaal-emotioneel functioneren, aanwezigheid op school en deelname aan sportactiviteiten. In **hoofdstuk 5** bestuderen we de medische literatuur om te onderzoeken hoe vaak vermoeidheid voorkomt bij

kinderen met IBD, en welke factoren deze vermoeidheid beïnvloeden. De geselecteerde studies verschillen in de manier waarop vermoeidheid wordt bepaald of de ernst van de vermoeidheid wordt gemeten. De meeste studies gebruiken vragenlijsten voor kinderen of voor ouders, of een combinatie van beiden. Eén onderzoek gebruikt een draagbare stappenteller om fysieke activiteit te meten. Door het gebruik van deze verschillende methoden was het niet mogelijk om een betrouwbare prevalentie te berekenen. Uit deze studies blijkt wel dat vermoeidheid moet worden beschouwd als een multifactorieel probleem, waarbij zowel biologische, psychologische als functionele aspecten betrokken zijn.

In **hoofdstuk 6** onderzoeken we de relatie tussen biologische en functionele factoren en het effect op vermoeidheid. We evalueren het effect van bloedarmoede, van ontsteking en van het uithoudingsvermogen op het gevoel van vermoeidheid. Daarnaast werd ook de levenskwaliteit gemeten door een gevalideerde ziekte-specifieke vragenlijst af te nemen. Het uithoudingsvermogen werd gemeten door een 6-minuten wandeltest. De resultaten waren enigszins verrassend. In tegenstelling tot de verwachting hadden de vermoeide en niet-vermoeide groep kinderen een vergelijkbaar hemoglobinegehalte, en vergelijkbare concentratie van faecaal calprotectine en ferritine. Ook het resultaat van de 6-minuten wandeltest was vergelijkbaar tussen beiden groepen. Er was wel een sterke samenhang tussen vermoeidheid en levenskwaliteit: hoe ernstiger de vermoeidheid, hoe lager de gemeten levenskwaliteit.

Concluderend wordt in dit proefschrift overtuigend aangetoond dat het gebruik van de FC-test bijdraagt tot het waarschijnlijker maken van de diagnose IBD, en dat bij kinderen zonder alarmsymptomen de triage nog beter wordt als daar een CRP en een hemoglobinemeting aan worden toegevoegd. Het aandeel IBD-negatieve endoscopieën wordt door dit diagnostische selectieproces beduidend kleiner.

Ondanks de grote impact van vermoeidheid op het dagelijks leven van kinderen met IBD is er nog weinig bekend over oorzaken en effectieve behandelingen. Het onderzoeken van (niet-) medicamenteuze interventies zou daarom een onderzoeksprioriteit moeten worden om de levenskwaliteit bij kinderen met IBD te verbeteren.

Appendices

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Appendices

CURRICULUM VITAE

Els Van de Vijver werd op 29 maart 1977 geboren in Wilrijk, België. In 1995 behaalde ze haar diploma secundair onderwijs aan het Onze-Lieve-Vrouw-Instituut te Antwerpen, waarna ze geneeskunde ging studeren aan de Universiteit Antwerpen. Na het behalen in 2002 van haar artsen diploma met grootste onderscheiding, begon ze aan haar opleiding kindergeneeskunde. Ze liep stage op de afdeling kindergeneeskunde in achtereenvolgens het H. Hart ziekenhuis in Lier, het MUMC in Maastricht en het KPK ziekenhuis in Antwerpen en tenslotte haar laatste twee jaar in het Universitair Ziekenhuis Antwerpen onder leiding van Prof.dr J.Ramet. Van mei 2008 tot januari 2010 specialiseerde ze zich tot kinderarts maag-darm-leverziekten in het Beatrix kinderziekenhuis/UMCG in Groningen. Sinds mei 2010 werkt ze als senior staflid kinderarts maag-darm-leverziekten op dienst kindergeneeskunde van het Universitair Ziekenhuis Antwerpen.

Appendices

LIST OF PUBLICATIONS

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