Pediatric functional abdominal pain disorders

From diagnosis to management - a clinical approach

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CHAPTER 2

Clinical Evaluation of Inflammatory and Blood Parameters in the Workup of Pediatric Chronic Abdominal Pain

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ABSTRACT

Objective: To investigate the additional value of blood parameters (hemoglobin, C-reactive protein, erythrocyte sedimentation rate) to anti-tissue transglutaminase (anti-tTG), fecal calprotectin, and *Giardia lamblia* when discriminating a functional from an organic cause in the clinical evaluation of children with chronic abdominal pain.

Study design: This retrospective cohort study included patients (4-18 years of age) with abdominal pain for >2 months. Data on hemoglobin, C-reactive protein, erythrocyte sedimentation rate, anti-tTG, fecal calprotectin, alarm symptoms, and diagnosis were collected.

Results: We identified 853 patients, of whom 102 (12%) had an organic disorder. Sensitivity and the area under the curve of strategy 1 (fecal calprotectin, anti-tTG, *G lamblia*, blood parameters) were 90% (95% CI, 83-95) and 0.87 (95% CI, 0.81-0.93), respectively, compared with 88% (95% CI, 81-93) and 0.85 (95% CI, 0.79-0.91), respectively, for strategy 2 (fecal calprotectin, anti-tTG, *G lamblia*) (*P* = NS). In the presence of ≥1 alarm symptoms, the sensitivity of strategies 1 and 2 was 92% (95% CI, 83-96) and 92% (95% CI, 83-96), and the areas under the curve were 0.93 (95% CI, 0.89-0.98) and 0.90 (95% CI, 0.84-0.97) (*P* = NS).

Conclusions: To distinguish between a functional and an organic cause for chronic abdominal pain, hemoglobin, C-reactive protein, and erythrocyte sedimentation rate can be left out from the clinical evaluation as they might have no additional diagnostic yield. However, caution should be taken not to miss extraintestinal infections (2%).

INTRODUCTION

Chronic abdominal pain is a common problem in childhood and adolescence, accounting for approximately 3% of all office visits to healthcare professionals.[1,2] In the majority of cases, no structural or biochemical abnormalities are found, after which a diagnosis of a functional abdominal pain disorder (FAPD) can be established. FAPDs are defined according to the Rome IV criteria, and distinguish between four specific disorders: functional dyspepsia, irritable bowel syndrome, abdominal migraine, and functional abdominal pain-not otherwise specified.[3] The impact of FAPDs on the daily life of these patients is substantial, leading to a significantly lower quality of life, higher school absenteeism rates, and a higher risk of developing anxiety or depressive disorders compared with healthy school-aged children.[4–7]

The Rome IV criteria support symptom-based diagnoses instead of a diagnosis based on exclusion of an organic cause. This implies that the clinician is allowed to perform minimal selective testing to establish a positive diagnosis of a FAPD. According to the Dutch guideline, the first step in the diagnostic workup of FAPDs consists of a careful history taking and physical examination to identify possible alarm signals for organic causes.[8–10] Even when alarm signals are absent, most clinicians will order laboratory tests like hemoglobin (Hb), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR). Screening for celiac disease using anti-tissue transglutaminase (anti-tTG) antibodies is also recommended, because children presenting with irritable bowel syndrome have a 4 times higher risk of having celiac disease than children without irritable bowel syndrome.[11] Furthermore, fecal testing for *Giardia lamblia* is indicated when diarrhea is present alongside abdominal pain.[8]

In the last decade, the determination of fecal calprotectin as a noninvasive screening method for intestinal mucosal inflammation is increasingly being recognized. Fecal calprotectin is a calcium-binding protein, mainly found in neutrophil granulocytes and the epithelium.[12] Increased calprotectin levels in the feces reflect the migration of neutrophils into the gastrointestinal (GI) tract owing to an infection or an inflammatory process.[13] In a robust case-control study, it has been shown that fecal calprotectin is a highly useful biomarker to discriminate inflammatory bowel disease (IBD) from non-IBD in children presenting with suspected bowel inflammation who will subsequently undergo an endoscopy.[14]

In this patient group, it was demonstrated that fecal calprotectin performs better than all blood parameters, such as Hb, platelet count, white blood cell count, ESR, and CRP.[14] The use of fecal calprotectin as a biomarker in the clinical evaluation of FAPDs may be considered to distinguish between FAPDs and IBD.[8,9] Indeed, over the last few years, its use has increasingly become common practice for most Dutch pediatricians in the diagnostic workup of FAPDs. In accordance with the Dutch guideline for FAPDs,[9] fecal calprotectin is mostly performed in combination with blood parameters. This practice occurs despite the fact that fecal calprotectin has a greater specificity for intestinal inflammation compared with blood biomarkers such as ESR.[15,16] Furthermore, fecal calprotectin is a quick, cost-effective, and noninvasive tool.[17] In view of the high annual healthcare costs for children with FAPDs,[18] it is important to question whether fecal calprotectin should be used as an additive diagnostic tool or as a substitute for blood parameters. Moreover, testing for Hb, CRP, and ESR may provide results that are unrelated to the abdominal pain or have no clinical relevance, like a mildly elevated ESR. Repeating these tests to confirm the findings may result in delaying diagnosis and an increase in anxiety in patient and parents.

Therefore, the aim of this study was to evaluate the clinical value of a full screenings workup (Hb, CRP, ESR, celiac disease screening, fecal calprotectin, and *G lamblia*) compared with a limited workup without the most often used blood parameters (Hb, CRP, ESR) in discriminating a functional from an organic cause of chronic abdominal pain.

METHODS

Setting and Participants

All fecal calprotectin measurements carried oud between January 2012 and January 2017 in pediatric patients from the St. Antonius Hospital Nieuwegein and Maasstad Hospital Rotterdam were obtained retrospectively from the clinical biochemistry department laboratory records. In both hospitals, it was standard protocol at that time to screen for fecal calprotectin in all children presenting with chronic abdominal pain.

Thereafter, the clinical medical charts of these patients were collected. Patients were included if they were aged between 4 and 18 years and had chronic abdominal pain for >2 months at their initial presentation at the outpatient clinic. Patients

with incomplete medical charts, with a definite diagnosis of IBD before the first collection of fecal calprotectin, or patients who received a diagnosis of lactose intolerance were excluded. The study was exempted from ethical approval by the local institutional review board of the St. Antonius Hospital Nieuwegein.

Data Collection

Hospital charts of included patients were reviewed and data on fecal calprotectin, diagnosis, presence of alarm symptoms, serologic screening for celiac disease, blood parameters, and demographics were extracted. All blood, serologic, and fecal tests were performed within 3 months from the time of fecal calprotectin collection. Diagnostic criteria for alarm symptoms, blood markers, serologic markers and fecal markers are shown in **Supplemental Table 1**.

Fecal Calprotectin

Fecal calprotectin data obtained from the clinical laboratory included patient demographics, the unique patient identifier code, the sample date of fecal calprotectin, and the fecal calprotectin concentration in micrograms per gram of stool for all patients. Fecal calprotectin was measured by the immunoenzymatic EliA Calprotectin assay on a Phadia 250 instrument (Thermo Scientific, Waltham, Massachusetts) according to the manufacturer's instructions. Based on manufacturer's values and literature, a fecal calprotectin value of <50 µg/g reflected the normal reference range. Values between 51 and 250 µg/g were labeled as gray zone or possible GI inflammation, between 251 and 1000 µg/g as mildly elevated or GI inflammation, and >1000 µg/g as elevated or active GI inflammation.[19–21] If patients had repetitive fecal calprotectin measurements taken, only the first one was included in our study, because this one accounted for diagnosis, whereas the subsequent fecal calprotectin measurements were used for follow-up.

Clinical Diagnosis

The clinical diagnosis was retrieved from medical chart review. Patients were either diagnosed with a functional or organic disease by their treating pediatrician. The diagnosis was based on standard clinical, histologic, and radiologic criteria according to Dutch and European clinical guidelines.[9,22,23] In line with the Dutch guideline and with the Rome IV criteria, a diagnosis of functional abdominal

pain was based on symptoms, which could not be attributed to another medical condition after appropriate medical evaluation. Limited testing in the form of blood parameters, celiac disease screening, and fecal calprotectin was performed to exclude an organic cause for the chronic abdominal pain. Abdominal ultrasound examination, endoscopy, allergy testing, or liver and urine tests were only performed when there was a strong suspicion for an organic cause of the chronic abdominal pain following specific clinical signs. Diagnosis of a clinically relevant lactose intolerance was based on elimination and provocation. The diagnosis of scar pain was based on physical examination. In case of doubt, the diagnosis was checked by a third-party arbiter. The treating pediatricians were not blinded to the fecal calprotectin value or serologic or blood analyses during diagnosis.

Serological and Fecal Analysis

Chart review was performed for screening of celiac disease by history of elevated anti-tTG. Furthermore, in cases where diarrhea was present, stool screening for infection with *G lamblia* was performed.[9] Screening on other pathogens, such as *Dientamoeba fragilis* and *Blastocystis hominis*, is not suggested by the Dutch guideline owing to a lack of evidence for their pathogenicity in chronic abdominal pain.[24] Screening for *G lamblia* in the feces was performed via polymerase chain reaction. Charts were evaluated for laboratory confirmed *G lamblia* infection.

Blood Parameters

Blood results (taken within 3 months from the fecal calprotectin sample) were obtained to compare the clinical usefulness of fecal calprotectin and serologic markers with blood parameters, namely, Hb, CRP, and ESR.

Alarm Symptoms

Data on the presence of the following alarm symptoms were extracted from notes from patient medical records: family history of IBD (first-degree relative), celiac disease, peptic ulcer disease, or familial Mediterranean fever; persistent right upper or right lower quadrant pain; persistent vomiting; GI blood loss; arthritis; perirectal disease; involuntary weight loss; deceleration of linear growth; and unexplained fever.[3,9] The importance of an alarm sign for the final diagnosis was put in the context of history and physical examination through the clinical judgment of the treating physician.

Other Measurements

Age and hospital site were collected from the medical charts as well. Defecation pattern was classified as diarrhea, constipation, alternating, or not changed according to the Bristol Stool Chart or according to the clinical view of the treating pediatrician.[25] Abdominal regions were divided into right upper quadrant, both upper quadrants, whole abdomen, right lower quadrant, both lower quadrants, or inconclusive.

Outcomes

To assess the diagnostic value of testing for Hb, CRP, and ESR in the workup of children with chronic abdominal pain, we defined 2 different test strategies. Test strategy 1, the full workup, included a measurement of fecal calprotectin, anti-tTG, *G lamblia*, and blood parameters (Hb, CRP, and ESR). The second test strategy (limited workup) consisted of the measurements in test strategy 1, without the blood parameters. In addition, the diagnostic value of both test strategies was compared in both absence and presence of alarm symptoms. Moreover, the predictive value of individual alarm symptoms in differentiating between functional or organic disease was studied. Finally, the accuracy of fecal calprotectin in comparison with other blood parameters was obtained.

Statistical Analysis

First, patient characteristics were reported using means and SDs in case of continuous normally distributed data or percentage for categorical data. Data on the different diagnoses were reported descriptively. Differences in baseline measurements and alarm symptoms between children with a functional and organic disease were analyzed using independent *t* tests for continuous data and the χ^2 and Fisher exact tests for dichotomous data. The primary analyses focused on the clinical usefulness of test strategy 1 vs test strategy 2 to discriminate a functional from an organic cause in children with chronic abdominal pain. This was assessed using parameters of test reliability including sensitivity, specificity, positive predictive value, and negative predictive value with 95% CIs. In addition, the area under the curve (AUCs) for the 2 test strategies in general, and for the 2 test strategies in the absence and presence of alarm symptoms, were calculated. Furthermore, the accuracy of fecal calprotectin compared with other blood parameters was assessed as well by use of these described parameters of test reliability. The AUC values were classified as follows: poor accuracy, 0.6–0.7;

fair accuracy, 0.7-0.8; good accuracy, 0.8-0.9; and excellent accuracy, 0.9-1.0.[26] A *P* value of <.05 was used to define statistical significance. Statistical analysis was performed using IBM SPSS Statistics 25 (Chicago, Illinois) for Windows and MedCalc statistical software package for biomedical research (MedCalc Software, V.12.1.4, Ostend, Belgium).

RESULTS

Participants

During the study period, a total of 1769 charts were reviewed. Of these, 514 patients were excluded because they did not have abdominal pain for >2 months, 294 patients did not meet our age range criterion, 73 patients were lost to follow-up, 27 patients had lactose intolerance, 5 patients had no diagnosis, and 3 patients were excluded owing to urinary tract infection, vomiting, or scar pain. Finally, 853 patients were eligible and were included in the analysis (**Figure 1**). Children with an organic diagnosis for their abdominal pain (n = 102) were older and more frequently had alarm symptoms as well as positive laboratory markers, fecal calprotectin, and anti-tTG compared with children with a functional diagnosis (n = 751) (**Supplemental Table 2**).

Diagnoses

In 90 of 102 children with an organic disorder (88.2%), diagnosis was based on abnormal fecal calprotectin, a positive *G lamblia* stool test, and/or positive anti-tTG. These children were diagnosed with IBD (n = 48), celiac disease (n = 11), giardiasis (n = 11), infection with *Helicobacter pylori* (n = 8), gastroenteritis (n = 3), dientamoebiasis (n = 1), *Campylobacter* enteritis (n = 1), *Cryptosporidium enteritis* (n = 1), food allergy (n = 1), proctitis (n = 1), sinus pilonidalis (n = 1), ulcus duodeni (n = 1), viral enteritis (n = 1), and *Yersinia* enteritis (n = 1). The remaining 12 children had normal Hb and CRP levels. Two of the 12 remaining children had elevated ESR levels owing to extraintestinal infections (Epstein-Barr virus-hepatitis and salpingitis). In the other 10 children (9.8%), inflammatory parameters were not aberrant. These children were diagnosed with *Helicobacter pylori* (n = 5), food allergy (n = 2), anterior cutaneous nerve entrapment syndrome (n = 1), polycystic ovary syndrome (n = 1) and side effects owing to medication (n = 1).

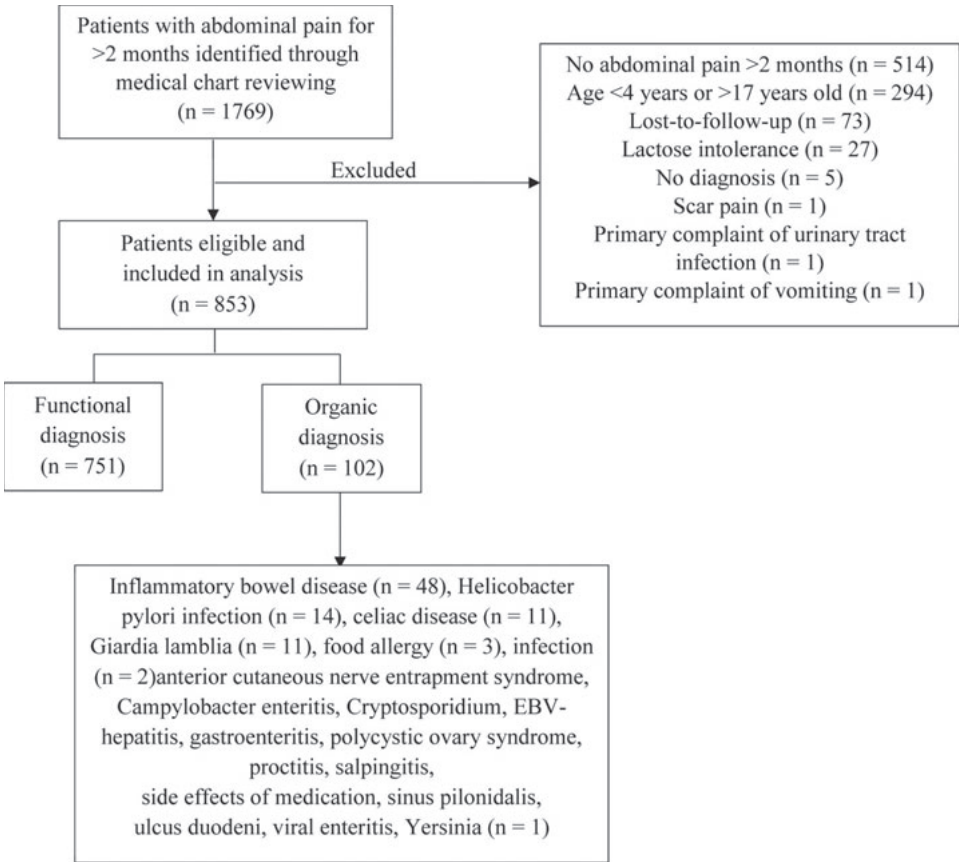

Figure 1. *Flow diagram showing the retrospective selection of study participants*
EBV, Epstein–Barr virus

Alarm Symptoms

Of the 853 patients, 344 (40%) had ≥1 documented alarm symptom (**Table 1**). Alarm symptoms were observed in 272 patients (36%) with functional disease and in 72 patients (71%) with organic disease ($P < .001$). Positive family history (IBD/celiac disease/familial Mediterranean fever) was the most common reported alarm symptom (13%), followed by chronic diarrhea (9%), GI blood loss (8%), weight loss (8%), and recurrent vomiting (5%).

Table 1. Alarm symptoms in patients with functional and organic causes for chronic abdominal pain

Symptoms	Total (n = 853)	Functional (n = 751)	Organic (n = 102)	*P* value*
None	509 (60)	479 (64)	30 (29)	<.001
Positive family history of IBD/celiac disease/FMF	107 (13)	92 (12)	15 (15)	.482
Chronic diarrhea	79 (9)	46 (6)	33 (32)	<.001
GI blood loss	71 (8)	35 (5)	36 (35)	<.001
Weight loss	66 (8)	48 (6)	18 (18)	<.001
Recurrent vomiting	45 (5)	35 (5)	10 (10)	.029
Pain right upper/lower region	27 (3)	23 (3)	4 (4)	.642
Joint pain	25 (3)	19 (3)	6 (6)	.106
Aphthous ulcer	22 (3)	17 (2)	5 (5)	.115
Fever	15 (2)	14 (2)	1 (1)	.524
Perianal complications	15 (2)	9 (1)	6 (6)	.001
Unexplained fever	14 (2)	12 (2)	2 (2)	.787
Impaired growth	10 (1)	6 (1)	4 (4)	.023
Erythema nodosum	4 (1)	2 (–)	1 (1)	.400
Hepatosplenomegaly	3 (–)	2 (–)	1 (1)	.318
Uveitis	1 (–)	1 (–)	–	1.000
Icterus	–	–	–	–

FMF = familial Mediterranean fever

*P values as determined with χ^2 test and Fisher's Exact test. Values are number (%).

Comparison of Full vs Limited Workup for Identifying Functional vs Organic Cause for Chronic Abdominal Pain

When applying test strategy 2, the limited workup, the 2 children with elevated ESR levels (diagnosed with Epstein-Barr virus-hepatitis and salpingitis) would not have been diagnosed owing to the exclusion of blood parameters from the diagnostic workup. The differences between test strategy 1 and test strategy 2 are highlighted in **Supplemental Figure 1**.

Comparing the 2 different strategies demonstrated that the limited workup (strategy 2) was accurate in distinguishing a functional from an organic cause for chronic abdominal pain (sensitivity, 88%; 95% CI, 81-93). Adding Hb, CRP, and ESR (strategy 1) resulted in a slightly increased accuracy (sensitivity, 90%; 95% CI, 83-95). However, this difference was not significant (**Table 2**). Furthermore, receiver operating characteristic analysis demonstrated an AUC of 0.87 (95% CI, 0.81-0.93) for strategy 1 and an AUC of 0.85 (95% CI, 0.79-0.91) for strategy 2, indicating that both strategies are accurate in correctly differentiating an organic from a functional cause for chronic abdominal pain.

Table 2. Accuracy of test strategy 1 vs test strategy 2, and of both strategies in the presence and absence of alarm symptoms

Criteria	Strategy		Alarm symptoms present		Alarm symptoms absent	
	Strategy 1*	Strategy 2†	Strategy 1*	Strategy 2†	Strategy 1*	Strategy 2†
Positive on criteria						
With outcome	92	90	66	66	26	24
Without outcome	97	97	6	6	4	6
Negative on criteria						
With outcome	10	12	4	6	6	6
Without outcome	654	654	26	24	66	66
Sensitivity	90 (83 – 95)	88 (81 – 93)	92 (83 – 96)	92 (83 – 96)	87 (70 – 95)	80 (63 – 90)
Specificity	87 (84 – 89)	87 (84 – 89)	87 (70 – 95)	80 (63 – 90)	92 (83 – 96)	92 (83 – 96)
PPV	49 (42 – 56)	48 (41 – 55)	94 (86 – 98)	92 (83 – 96)	81 (65 – 91)	80 (63 – 90)
NPV	98 (97 – 99)	98 (97 – 99)	81 (65 – 91)	80 (63 – 9)	94 (86 – 98)	92 (83 – 96)
AUC	0.87 (0.81 – 0.93)	0.85 (0.79 – 0.91)	0.93 (0.89 – 98)	0.90 (0.84 – 0.97)	0.87 (0.82 – 0.93)	0.83 (0.80 – 0.92)

NPV, negative predictive value; *PPV*, positive predictive value.
Values are number or number (95% CI).
*Fecal calprotectin, anti-tTG, *G lamblia*, and blood parameters.
†Fecal calprotectin, anti-tTG, and *G lamblia*.

In the case that no alarm symptoms were present (n = 30), strategy 1 yielded a sensitivity of 87% (95% CI, 70-95) compared with a sensitivity of 80% (95% CI, 63-96) for strategy 2 (*P* = NS). The AUC for strategy 1 in this case was 0.87 (95% CI, 0.82-0.93), which was slightly higher compared with the AUC for strategy 2 (0.86, 95% CI, 0.80-0.92) when no alarm symptoms were present (*P* = NS). If ≥1 alarm symptoms were present (n = 72), strategy 1 resulted in a sensitivity of 92% (95% CI, 83-96), compared with a sensitivity of 92% (95% CI, 83-96) for strategy 2 (*P* = NS). The AUCs for strategies 1 and 2 in the presence of ≥1 alarm symptoms were, respectively, 0.93 (95% CI, 0.89-0.98) and 0.90 (95% CI, 0.84-0.97).

Accuracy of Fecal Calprotectin Compared with Hb, CRP and ESR

Receiver operating characteristic analysis revealed a fair accuracy for fecal calprotectin (AUC, 0.83; 95% CI, 0.678-0.978; *P* < .001) for prediction of an organic cause vs a functional cause for abdominal pain, which was higher than that for Hb (AUC, 0.63; 95% CI, 0.46-0.81; *P* = .095), CRP (AUC, 0.53; 95% CI, 0.37-0.70; *P* = .68), and ESR (AUC, 0.64; 95% CI, 0.46-0.82; *P* = .081). The accuracy for fecal calprotectin at different cutoff levels and clinical blood parameters is shown in **Table 3**. Using the normal cutoff of >50 µg/g gives a sensitivity of 75% (95% CI, 65-82), and a specificity of 87% (95% CI, 84-89) for fecal calprotectin. This specificity increases steadily with increasing fecal calprotectin levels.

Table 3. Accuracy of fecal calprotectin and blood parameters

Parameters	Sensitivity	Specificity	PPV	NPV
Fecal calprotectin				
Cutoff (µg/g)				
>50	75 (65 – 82)	87 (84 – 89)	44 (37 – 51)	96 (94 – 97)
>250	48 (39 – 58)	99 (97 – 99)	82 (70 – 89)	93 (91 – 95)
>1000	38 (29 – 48)	100 (99 – 100)	98 (87 – 100)	92 (90 – 94)
Blood parameters				
Hb	18 (11 – 26)	99 (98 – 100)	77 (57 – 90)	89 (87 – 91)
CRP	67 (57 – 76)	94 (91 – 96)	79 (68 – 86)	90 (86 – 93)
ESR	64 (53 – 73)	78 (74 – 81)	29 (23 – 36)	94 (91 – 95)

NPV, negative predictive value; *PPV*, positive predictive value.
Values are percent (95% CI).

DISCUSSION

We demonstrated that a limited diagnostic workup with fecal calprotectin, celiac disease screening, and testing for *G lamblia* in the case of diarrhea, is equally accurate in terms of sensitivity and AUC, compared with a more extensive workup when blood parameters are added. Furthermore, the accuracy of both workups further improved in the presence of ≥1 alarm symptoms. Finally, as expected, alarm symptoms were significantly more present in children with an organic cause for the chronic abdominal pain.

Fecal calprotectin is increasingly being used as a diagnostic marker in the workup of FAPDs in adults.[27,28] However, in children with chronic abdominal pain it is not yet known whether fecal calprotectin should be used as an additive biomarker or if it can replace certain biomarkers such as Hb, CRP, and ESR. Our results are in line with a prospective cohort study in children, which demonstrated that the addition of fecal calprotectin to alarm symptoms outperformed the addition of CRP to alarm symptoms (AUC, 0.97 and 0.88, respectively) in reducing the referrals for further diagnostic workup of suspected IBD.[29] Nevertheless, this AUC was higher (0.97) compared with the AUC we found (0.85) for our fecal calprotectin test strategy (the limited screenings workup). This finding might be explained by the fact that the prospective cohort study focused on ruling out IBD specifically, whereas our study focused on ruling out organic disease in general.[29] It might be expected that fecal calprotectin has a higher diagnostic accuracy for IBD in particular compared with organic disease in general, which might explain the lower AUC in our study. Moreover, with respect to our own study, the AUC of the limited screenings workup was somewhat lower than the AUC of the full screenings workup (0.85 vs 0.87, respectively). This finding might be explained by the fact that AUC values of receiver operating characteristic analysis increase with the addition of more parameters.[30]

The use of fecal calprotectin in addition to Hb, CRP, and ESR in the clinical evaluation of FAPDs does not seem surprising because fecal calprotectin is a direct and sensitive marker of intestinal inflammation.[31,32] At certain levels, it may detect inflammatory activity that is insufficient to cause an increase in ESR and CRP. Moreover, fecal calprotectin levels seem to be unaffected by nonintestinal conditions.[33,34] As reflected in our study by higher sensitivity, specificity, positive predictive value, and negative predictive value for fecal calprotectin compared with CRP and ESR, it should be considered to use fecal calprotectin when distinguishing between patients with organic and functional intestinal disease.

The elimination of Hb, CRP, and ESR in the clinical evaluation of pediatric FAPDs could be important for clinical practice, because it can decreases costs, which are already substantial for children with FAPDs.[18] In 2010, the average costs per child with a FAPD in the United States were $6104.30.[35] Moreover, a study in adults in the UK found that fecal calprotectin testing saved £100 compared with CRP and ESR testing, taking into consideration all costs in primary and secondary care.[36] In accordance with other studies, a poor accuracy for ESR and CRP in differentiating an organic from a functional cause in patients with chronic abdominal pain was found.[37,38] The clinical usefulness of these markers is therefore questioned in patients with chronic abdominal pain. Leaving these markers out of the clinical evaluation in patients presenting with chronic abdominal pain will enhance daily clinical practice as the chance of patients with false-positive or false-negative diagnoses based on ESR or CRP, and subsequent additional diagnostic tests, will decrease. This finding is in concordance with the new Rome IV criteria, which stipulate that diagnosis of a functional GI disorder (FGID) only by excluding organic disease is becoming outdated.[3] The revised Rome IV criteria underlined this concept by removing the phrase "no evidence for organic disease" in all definitions and replacing it with "after appropriate medical evaluation the symptoms cannot be attributed to another medical condition." This implies that the clinician will now be able to perform selective testing to support a positive diagnosis of an FGID. Our finding that Hb, CRP, and ESR might be left out of the clinical evaluation is in line with this concept. In addition, selective testing enhances the development of a time- and cost-effective management strategy as well. However, caution should be taken for patients with extraintestinal manifestations such as Epstein-Barr virus-hepatitis and salpingitis, because these diagnoses might be missed by leaving out CRP and ESR from the clinical evaluation.

In total, 6 alarm symptoms were significantly more present in children with an organic disease compared with children with a FGID. The most common alarm symptoms were a positive family history (IBD/celiac disease/familial Mediterranean fever), chronic diarrhea, GI blood loss, weight loss, and recurrent vomiting. These findings are in line with a study by El-Chammas et al that found that patients with Crohn's disease were significantly more likely to experience hematochezia and weight loss.[10] In our study, some alarm symptoms were present in the functional group. For GI blood loss, it was found that this symptom was often present in children with severe constipation. Fever was often only present for a short period, in contrast with chronic abdominal pain, and was therefore considered unrelated

to the abdominal pain. In the 2 children with erythema nodosum, a diagnosis of IBD was excluded because their abdominal pain was unrelated to the skin problem. Finally, the hepatosplenomegaly in the 2 patients disappeared spontaneously, thereby excluding an organic disease as well. Based on our findings, the absence of the alarm symptoms a positive family history, chronic diarrhea, GI blood loss, weight loss, and recurrent vomiting seem to be helpful in confirming an FGID diagnosis. Interestingly and in contrast with the Rome guidelines, we found no difference in persistent right upper or right lower quadrant pain, between functional and organic disease, suggesting that this factor could be omitted from the list of alarm symptoms, although a trend was seen for a difference in right upper quadrant pain.

A strength of our study is the large sample size. Second, we included pediatric patients with chronic abdominal pain for ≥2 months using a strict definition, exemplified by the exclusion of patients with lactose intolerance, because it remains debatable if lactose intolerance is a significant cause of abdominal pain or that the decrease in pain after a lactose-free diet is a placebo effect.[39] A limitation of our study is the fact that no information on medication use was collected. It is, for example, well-known that the use of nonsteroidal anti-inflammatory drugs can increase fecal calprotectin levels.[40,41] Increased fecal calprotectin levels, therefore, might have reflected medication use instead of the presence of an organic disease. Therefore, the accuracy of fecal calprotectin may have been underestimated. Moreover, there was no standardization of the timing of fecal sampling for fecal calprotectin measurement. Fecal calprotectin concentrations are fluctuating during the day and this can induce clinically significantly variable numbers.[42] In addition, a time frame of 3 months between fecal calprotectin screening and blood test screening might be large. However, the long-lasting and stable character of chronic abdominal pain complaints, which often exist for >1 year, decreased the need for a smaller time frame. Last, the retrospective nature of this study is subject to publication bias and other biases.

In conclusion, this study showed that Hb, CRP, and ESR can be left out in the clinical evaluation of chronic abdominal pain in children. The combination of fecal calprotectin, celiac disease and *G lamblia* screening has a high sensitivity and specificity and is therefore accurate enough to discriminate between an organic and a functional cause for chronic abdominal pain, even when ≥1 alarm symptoms are present. However, before our proposed strategy can be adopted in routine care, these findings should ideally be duplicated in a large prospective cohort study.

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SUPPLEMENTARY FILES

Supplementary material of chapter 2 is available online

Supplemental Table 1. Diagnostic criteria for alarm symptoms, blood markers, serologic markers, and fecal markers

Supplemental Table 2. Baseline characteristics of study patients

Supplemental Figure 1. Different strategies for identifying functional vs organic cause for chronic abdominal pain.