The Use of Fecal Calprotectin Testing in Paediatric Disorders: A Position Paper of the European Society for Paediatric Gastroenterology and Nutrition Gastroenterology Committee

*Carmen Ribes Koninckx, *Ester Donat, †Marc A. Benninga, ‡Ilse J. Broekaert, §Frederic Gottrand, ||Kaija-Leena Kolho, ¶Paolo Lionetti, #Erasmo Miele, **Rok Orel, ††Alexandra Papadopoulou, ‡‡Corina Pienar, §§Michela G. Schäppi, ||||Michael Wilschanski, and ¶¶##Nikhil Thapar

ABSTRACT

Objectives: The aim of the study was to review the evidence regarding the clinical use and value of faecal calprotectin (FC) measurements in different gastrointestinal disorders in children.

Methods: A literature search was conducted in the PubMed, MEDLINE, EMBASE, and Cochrane databases until October 31, 2019. Subtopics were identified and each assigned to individual authors.

Results: A total of 28 recommendations were voted on using the nominal voting technique. Recommendations are given related to sampling, measurement methods, and results interpretation. The 14 authors anonymously voted on each recommendation using a 9-point scale (1 strongly disagree to 9 fully agree). Consensus was considered achieved if at least 75% of the authors voted 6, 7, 8, or 9.

Conclusions: Consensus was reached for all recommendations. Limitations for the use of FC in clinical practice include variability in extraction methodology, performance of test kits as well as the need to establish local reference ranges because of the influence of individual factors, such as age, diet, microbiota, and drugs. The main utility of FC measurement at present is in the diagnosis and monitoring of inflammatory bowel disease (IBD) as well as to differentiate it from functional gastrointestinal disorders (FAPDs). FC, however, has neither utility in the diagnosis of infantile colic nor to differentiate between functional and organic constipation. A rise in FC concentration, may alert to the risk of developing necrotizing enterocolitis and help identifying gastrointestinal involvement in children with Henoch-Schönlein purpura. FC measurement is of little value in Cow's Milk Protein Allergy, coeliac disease (CD), and cystic fibrosis. FC does neither help to distinguish bacterial from viral acute gastroenteritis (AGE), nor to diagnose *Helicobacter Pylori* infection, small intestinal bacterial overgrowth (SIBO), acute appendicitis (AA), or intestinal polyps.

Key Words: Crohn disease, fecal calprotectin, inflammation, inflammatory bowel disease, intestine, position paper, ulcerative colitis

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What Is Known

- Limitations to the interpretation of faecal calprotectin results include variability in extraction methodology, performance of test kits, and the need to establish local reference ranges.
- The main utility of faecal calprotectin measurement at present is in the screening and monitoring of inflammatory bowel disease.

What Is New?

- Although faecal calprotectin may be considered as a tool to differentiate functional gastrointestinal disorders from organic diseases, it has not proven its value in this respect apart from identifying possible inflammatory bowel disease within these common clinical presentations.
- A rise in faecal calprotectin concentration over serial readings may alert to the risk of developing necrotizing enterocolitis.
- Other than inflammatory bowel disease, the applicability of faecal calprotectin measurement in gastrointestinal inflammatory and immune-mediated conditions remains to be defined.

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From the *Department of Paediatric Gastroenterology, Hepatology and Nutrition, La Fe University Hospital Valencia, Spain, the †Department of Paediatric Gastroenterology and Nutrition, Emma Children's Hospital, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands, the ‡Department of Paediatrics, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany, the §Department of Paediatric Gastroenterology, Hepatology and Nutrition, CHU Lille, University Lille, France, the ||Children's Hospital, University of Helsinki, Helsinki, Finland and Tampere University, Tampere, Finland, the ¶Department NEUROFARBA, University of Florence - Meyer Children's Hospital, Florence, the #Department of Translational Medical Science, Section of Pediatrics, University of Naples ''Federico II'', Naples, Italy, the **Department of Gastroenterology, Hepatology and Nutrition, University Children's Hospital, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, the ††Division of Gastroenterology and Hepatology, First Department of Paediatrics, University of Athens, Children's hospital «Agia Sofia», Athens, Greece, the ‡‡Department of Paediatrics, ''Victor Babes'' University of Medicine and Pharmacy, Timisoara, Romania, the §§Paediatric Centre, Clinique des Grangettes and Centre Médical Universitaire, Geneva, Switzerland, the ||||Paediatric Gastroenterology Hadassah Hebrew University Medical Centre, Jerusalem, Israel, the ¶¶Neurogastroenterology and Motility, UCL Great Ormond Street Institute of Child Health and Department of Gastroenterology, Great Ormond Street Hospital, London, UK, and the ##Gastroenterology, Hepatology and Liver Transplant, Queensland Children's Hospital, Brisbane, Australia.

INTRODUCTION

Since its first description in 1980 by Fagerhol, calprotectin has seen unprecedented use in clinical practice across a wide variety of conditions ranging from inflammatory bowel disease (IBD) to functional gastrointestinal disorders (FAPDs).

Calprotectin is a 36 kDa calcium and zinc-binding heterocomplex protein with 2 heavy and 1 light chain proteins belonging to the S100 protein family (S100 A8/A9) with regulatory functions in inflammatory reactions (1). Variably named ''L1 protein,'' MRP-8/14, calgranulin, and cystic fibrosis antigen (2,3), it is present in tissues and fluids and especially abundant in neutrophils and monocytes (4). Calprotectin has numerous biological functions, such as antimicrobial and antifungal activity (5). It is relatively resistant to enzymatic degradation, and therefore, preserved and easily measured in stools for periods of time sufficient to allow for collection and analysis, a property, which underlies its clinical utility (6,7).

In inflammatory diseases of the intestinal tract defined by mucosal neutrophil aggregation (8), the activation and death of these cells releases high amounts of calprotectin into the intestinal lumen, which is then excreted in the faeces (9). High fecal calprotectin (FC) levels are described in children and adults, in diverse pathological conditions, such as: Crohn disease (CrD), ulcerative colitis (UC), cystic fibrosis, rheumatoid arthritis, bacterial infections, and gastric cancer (8,10). FC levels correlate well with Indium white cell scans and also gut permeability measured by other means (11). FC is considered a useful marker for intestinal inflammation, especially as it is stable and its measurement is noninvasive, simple, easy to perform, rapid, and reproducible. Despite this, little is known about the determinants of levels in normal subjects, especially children, and contradictory data on sensitivity and specificity are reported for different conditions. A comprehensive review by van Rheenen et al (12), for example, found that for suspected IFD, screening with FC in children has a similar sensitivity as in adults but a lower specificity.

The aim of this article is to review the evidence of the value of FC in different gastrointestinal disorders in children and formulate, wherever possible, consensus-based recommendations for its use.

METHODS

Composition of the Author Group

The members of the European Society for Paediatric Gastroenterology and Nutrition (ESPGHAN) Gastroenterology Committee are representative of the majority of ESPGHAN working groups dealing with the main gastrointestinal disease groups being addressed in this article. All authors and/or working group representatives are involved with the utility of FC in clinical practice. Some noncommittee members, deemed to be more expert in the FC field (and with recent publications on the subject), were asked to join the authorship to reinforce the expertise. The final authorship acknowledges that outside this author group, there are many other experts we were not able to include.

Search Strategy

The literature search was conducted by 2 of the authors (CR and ED) in the PubMed and Cochrane databases, until August 31, 2019, firstly looking for all publications relating to FC OR calprotectin, followed by searching specifically for Calprotectin in the context of specific conditions namely: Celiac disease, Inflammatory Bowel Disease (IBD), FAPDs, Cow's milk protein allergy (CMPA), Food Allergy, Cystic Fibrosis, Infectious Gastroenteritis, Parasitosis, Appendicitis, *Helicobacter Pylori*, Malnutrition, Obesity, Necrotizing Enterocolitis, Polyposis, Autism, Small Intestinal Bowel Overgrowth, Hirschsprung disease, Henoch-Schönlein purpura, and Short bowel syndrome. An initial list of 425 titles was retrieved, the oldest dating from the year 1984.

A review and preliminary sorting of selected articles was then performed looking into their titles and abstracts and those articles that did not specifically address the topic were discarded. A further detailed review of content then removed studies addressing exclusively basic science without clinical application and/or those not related to gastrointestinal conditions. Publications not written in English were not considered. Particular focus was given to studies of paediatric populations or those including a mix of adults and children. Wherever no paediatric data were available, adult studies were included. The final number of publications selected was 172 (Fig. 1).

Articles from the total pool were classified according to subtopics based on a set of specific questions formulated and agreed upon by the authors. Individual authors were assigned topics and then provided with the abstracts related to these. Authors then completed further literature searches; firstly, by extending the search to October 31, 2019 on their specific topic(s), as a result of which 30 articles were added, and secondly, by searching for any additional articles through the EMBASE database, adding a further 5 articles. These took the final number of included articles to 207 (Fig. 1).

Formulation of Recommendations

Validated methods for determining the strength of the recommendation are available only for questions related to therapy. In keeping with other ESPGHAN guidelines (13), recommendations were classified according to the quality of available evidence including the methodology and outcomes assessed.

We categorised the grade of the recommendation (GoR) as:

1. Strong: if there were adequately powered, prospective studies supporting the conclusions.
2. Moderate: if there were large retrospective studies or small prospective studies supporting the evidence.
3. Weak: if there were only retrospective studies or expert opinion supporting the results.
4. Strong or Moderate recommendations are formulated as 'the ESPGHAN expert group *recommends* to or not to. . .' (strong/moderate recommendation).
5. Weak recommendations are formulated as 'the ESPGHAN expert group *suggests* to or not to . . .' (weak recommendation).

The 14 authors anonymously voted on each recommendation using a 9-point scale (1 strongly disagree to 9 fully agree). Consensus was considered achieved if at least 75% of the authors voted 6, 7, 8, or 9. Percentage of authors voting 6 or above, that is,

Address correspondence and reprint requests to Professor Nikhil Thapar, BM, FRCPCH, FRACP, PhD, Gastroenterology, Hepatology and Liver Transplant, Queensland Children's Hospital, Brisbane, Australia (e-mail: Nikhil.Thapar@health.qld.gov.au).

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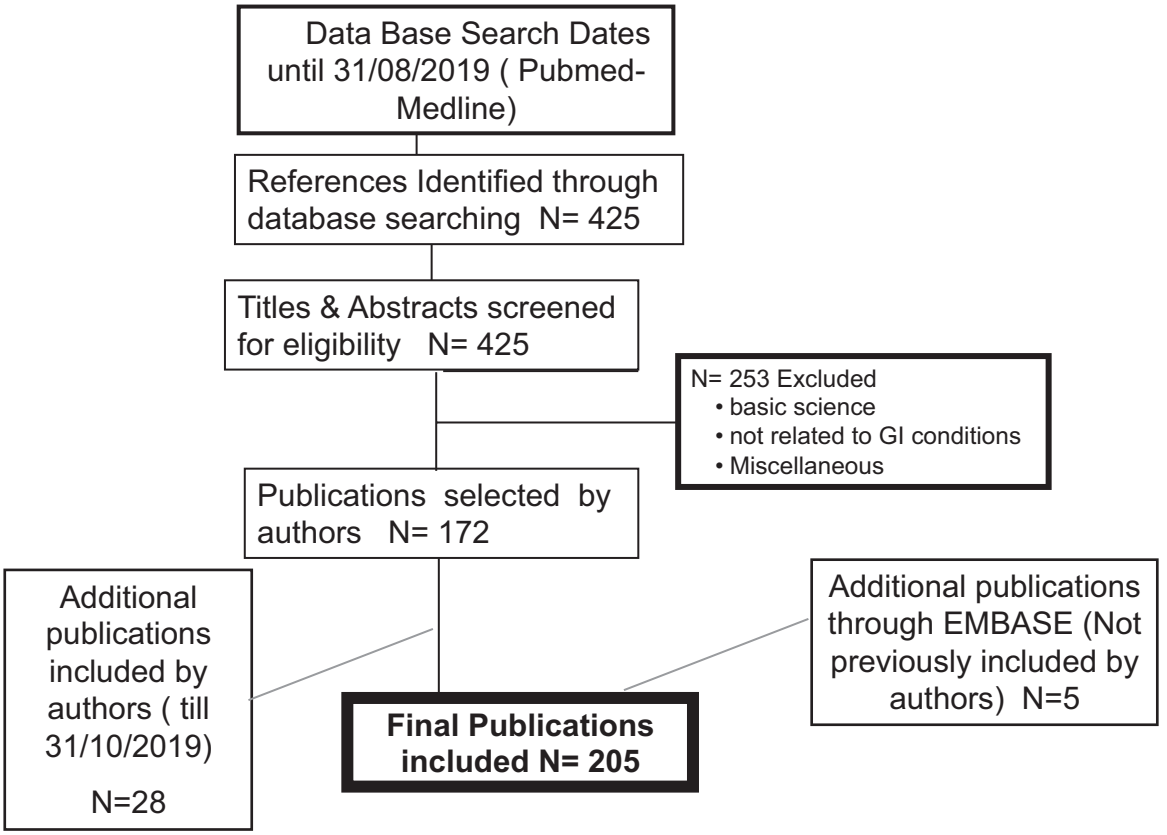

FIGURE 1. Literature search and sorting.

percentage of agreement, as well as the mean score (MS) for each recommendation are specified; the number of abstentions if any are quoted. A total of 28 recommendations were voted upon.

FECAL CALPROTECTIN MEASUREMENT AND REFERENCE VALUES

FC measurement is dependent on the collection of appropriate stool samples and analysis using validated tools.

Collection and Handling of Stool Samples

In general, FC tests are performed on 50 to 100 mg samples of stool. Given that FC is reported to be evenly distributed in faeces (14), homogenisation of the stool to be sampled from, or indeed the sample, is not thought necessary (6). Although there are suggestions that the sample should be taken in the morning, there appears not to be a specific reason to restrict the use of samples taken at other times of the day (15,16). Once collected, a body of literature does suggest that FC appears stable at room temperature between 3 and 7 days (6,17,18) suggesting samples can even be sent to the laboratory by ordinary mail. Haisma et al (7), however, found that FC concentrations decline from the first day on in stool samples at room temperature and remain stable at 4°C. Overall, most analysis methods, recommend to keep faecal samples for up to 2 to 3 days at room temperature, 5 to 7 days in a fridge (4°C) or frozen (−20°C or −4°C) for long-term storage (18). As for the extracts, different storage conditions are recommended by the different manufacturers ranging from storage at room temperature for a few days to keeping samples in the fridge or frozen should immediate analysis not be possible (18).

The levels of FC seem to be influenced by stool consistency, presumably relating to water content but there are contradictory results reported by several authors (6,19). For young infants, the fact that the concentration of FC may artificially increase by 30% by the absorption of water in the diaper must be taken into account (20). FC values also show large variation during bowel cleansing and for a few days after a lower bowel endoscopy, hampering the interpretation of results (21).

It has been suggested, that menstrual or nasal bleeding as well as anal fissures and haemorrhoids influence FC levels, by contaminating the stool sample (22). From a previous study in adults, however, it was calculated that a blood loss ≥100 mL is needed to increase FC levels above the reference value (10); no similar studies in paediatric population were identified.

Statement 1

1(a) There is no evidence to suggest that FC samples are best taken at a specific time of the day.

1(b) FC concentrations in diaper collected faeces may be artificially elevated by the absorption of water into the diapers/nappies.

1© FC levels are affected by bowel cleansing.

1(d) FC concentrations may be elevated because of the presence of blood in stool samples also for reasons other than intestinal inflammation, like haemorrhoids, anal fissures, or menstruation.

Recommendation 1
The ESPGHAN expert group recommends to
1(a) Collect samples for FC at any time of the day directly from the stool without any prior processing. (GoR: Moderate)
Agreement: 100% MS: 8.9
1(b) NOT obtain samples for FC measurement from diapers/nappies especially when stool consistency is loose. (GoR: Moderate)
Agreement 92.9% MS 7.7
1(c) NOT obtain samples for FC measurement during or following bowel cleansing and/or lower endoscopy. (GoR: Moderate)
Agreement 100% MS 8.7
1(d) NOT keep the samples for FC measurement for more than 3 days at room temperature before processing or for more than 7 days if refrigerated immediately. (GoR: Moderate)
Agreement 100% MS 8.7

Calprotectin Measurement

The first method for quantifying FC in stools was developed by Roseth in 1992 (14). Improved and validated enzyme-linked immunosorbent assays (ELISA) methods were developed later using better extraction techniques and measuring FC in mg/kg (18). Such ELISA methodology is now commonly used for FC measurement, with several commercial kits from different companies available on the market (Table 1).

In some studies, semiquantitative and qualitative point-of-care assays have been evaluated and the results are comparable to ELISA testing especially at low levels (23,31,32).

The different tests are very sensitive for detecting mucosal inflammation but major differences exist in specificity and absolute values. Diverse extraction devices and methods have been tested and these have an impact upon the stability of faecal extracts. Regarding rapid home tests, it has been shown that results from laboratory-performed extraction and patient-performed extraction correlate significantly (33,34). Bourdillon et al (35) has shown that absolute FC concentrations measured by different kits, even produced by the same manufacturer, may not be comparable. As a consequence, for results to be comparable, it is highly advised to use the same extraction methodology and test kit for follow-up and disease activity monitoring in the same patient over time (35,36).

Statement 2
There is considerable variability in extraction methodology and performance of test kits even from the same manufacturer.

Recommendation 2
The ESPGHAN expert group recommends to use the same extraction methodology and test kit for the measurement of FC for the purposes of diagnosis and assessing disease activity in an individual patient over time. (GoR: Strong)
Agreement 92.9% MS 8.6. Abstentions:1

Reference Values

In early studies, the median stool FC concentration in healthy adults was 2 μg/L of faecal homogenate with a suggested cut-off for a positive test of 10 μg/L (14). In newer assays, results are expressed per gram of faeces and the suggested upper limit of normal has been increased by a factor of 5 to 50 μg/g (22). Currently, the normal range for FC is considered <50 μg/g of faeces by many manufactures of the test kits (Table 1); however, the size of the reference cohort is seldom reported, and it is apparent that there is a great variability within healthy populations. Additionally the test appears to have better diagnostic precision for IBD at a cut-off of 100 μg/g than at 50 μg/g (37). Several factors including age, gender, diet, microbiota, and certain drugs may influence FC levels.

Recommendation 3
The ESPGHAN expert group recommends that the laboratory in each centre establish its own normal values for FC. (GoR: Strong)
Agreement 100% MS: 8.6

Effect of Age and Gender

It is well recognised, that in a general population, FC values are higher in children than in adults. The most relevant results from the literature are summarised in Table 2. Although there is a tendency towards lower values with increasing age, there are no well-established cut off levels for specific age ranges (38). Published studies by different authors have considered a wide spectrum of age ranges and performed diverse statistical analysis; however, the majority of studies established a difference considering children under 4 and above 4 years of age (43).

Davidson and Lock (43) assessed FC in healthy children across 3 age range groups, with a median FC of 77 μg/g for children ages 1 to 3.9 years, 62 μg/g for 4 to 17.9 years; and 61 μg/g for those older than 18 years. No significant differences were found between the 4 and 17.9 years and >18 years age groups. Joshi et al (38) evaluating paediatric and adult healthy volunteers found a median FC of 34 μg/g in the 2 to 9 years age group and 22 μg/g in those ages 10 to 59 years. In a study in South Korea, the median FC concentration in samples from 234 healthy children ages between 6 months and 4 years was 245 μg/g (range 12–1033 μg/g, mean 68.5 μg/g, SD 123 μg/g) (42). These children were further analysed by dividing them into 6 age groups showing a trend towards negative correlation between age and the FC concentration (the upper limit of 95% confidence interval (95% CI) of median FC values was 135 μg/g in the 7–12 months group and 12 μg/g in the 37–48 months group).

A study (40) in 173 healthy Chinese children ages 1 to 18 months, also found a downward trend in FC values with increasing age but greater than normal levels in healthy adults with a median FC concentration of 174.3 μg/g (range: 6.0–1097.7 μg/g). The same authors went on to analyse a bigger group of 274 children ages 1 to 4 years and found a median FC concentration of 83.19 μg/g (range 4.58 to 702.50 μg/g) (41).

TABLE 1. Commercial available methods for FC measurement

Assay (manufacturer)	Method	Extraction method	Measurement range, μg/g	Cut off level (μg/g)	Efficacy	Comments
Calprotectin EliA Phadia/ Thermo- Fisher (Uppsala, Sweden)	EFIA	Phadia Laboratory System	15 to ≥3000	>50 positive	Sensitivity 97.7% Specificity 89.8% PPV 0.96 NPV 0.95 LR+ 9.58 LR− 0.03 *	
EliA Calprotectin 2 Phadia/Thermo- Fisher (Uppsala, Sweden)	EFIA	Phadia Laboratory System	3.8 to ≥6000	>50 positive	Sensitivity 98% Specificity 75,9% PPV 0.829 NPV 0.969 *	
Calfast Eurospital (Trieste, Italy)	Semi-quantitative IC test (automated reading)	Eurospital	50 to 300	<70 negative 70 to 100 borderline >100 positive	Sensitivity 86.4% specificity 86.6% (23) NPV: 99% *	
Calprest Eurospital (Trieste, Italy)	ELISA	Eurospital	15 to 500	<50 negative 50 to 100 borderline >100 positive	Sensitivity 95% Specificity 93% NPV 98% *	The average value of calprotectin in healthy adults is approximately 25 μg/g
Calprotectin ELISA (APL) CALP0100 Calpro AS (Lysaker, Norway)	ELISA	Patented Faecal Extraction Buffer	Up to 1250	>50 positive	NA	
Calprotectin ELISA (HRP) CALP0300 Calpro AS (Lysaker, Norway)	ELISA	Patented Faecal Extraction Buffer	Up to 1250	>50 positive	NA	
Calprolab ELISA (APL) CALP0170 Calpro AS (Lysaker, Norway)	ELISA	Calpro EasyExtract CAL0510	25 to 2500	>50 positive	Sensitivity 83% Specificity 89% PPV 83% NPV 89% LR+ 7.9 LR− 0.19 (24)	Satisfactory correlation and agreement has been found between samples analysed in CAL0100 and CALP0170
Calprolab ELISA (HRP) CALP0270 Calpro AS (Lysaker, Norway)	ELISA	Calpro EasyExtract	25 to 2500	>50 positive	Sensitivity 78.2% Specificity 74.8% PPV 0.538 NPV 0.902 LR+ 2.9 LR− 0.29 (25)	CalproLab Elisa tests (ALP/HPR) has a higher dynamic range than the Calprotectin Elisa tests (ALP/HPR)
CalproSmart, Calpro AS (Lysaker, Norway)	Semi-quantitative IC test	Calpro faecal extraction buffer	70 to 1500	<200 (Mild disease IBD, green) 200 to 500 (moderate IBD activity, yellow) >500 (severe IBD activity, red)	Sensitivity: 82% Specificity: 85% PPV: 47% NPV: 97% (25)	Intraassay and interassay coefficients of variation of the CalproSmart test were 4.42% and 12.49%, respectively. (26) Optimal cut off at 150. (26) Smartphone application
BÜHLMANN fCAL ELISA Bühlmann Laboratories AG (Schonenbuch, Switzerland)	ELISA	Calex	10 to 600 30 to 1800	<50 negative 50 to 200 result uncertain >200 positive	Sensitivity: 79% Specificity: 87% (27)	
Quantum Blue fCAL Buhlmann Laboratories AG (Schonenbuch, Switzerland)	Quantitative IC test	Calex	30 to 300 100 to 1800 30 to 1000	50 for adults (can be used for children ages from 4 to 17 years)	Sensitivity 83% Specificity 68% PPV 0.63 NPV 0.87 LR+ 2.7 LR− 0.24 (24)	Automated reading
BÜHLMANN fCAL turbo Buhlmann Laboratories AG (Schonenbuch, Switzerland)	PETIA	Calex	20 to 8000	50 for adults (can be used for children ages from 4 to 17 years)	NA	
BÜHLMANN IB*Doc* Buhlmann Laboratories AG (Schonenbuch, Switzerland)	Semi-quantitative IC test	Calex	30 to 1000	>50 positive	NA	Home testing application CalApp app (test cassette reader)
CALcheck Blue Buhlmann Laboratories AG (Schonenbuch, Switzerland)	Semi-qualitative IC test	NA	NA	>50 positive	Overall agreement to ELISA: 97.6%; PPV: 92.6%; NPV: 100% *	CALcheck Blue has high correlation to quantitative Calprotectin ELISA and Quantum Blue tests *

Assay (manufacturer)	Method	Extraction method	Measurement range, μg/g	Cut off level (μg/g)	Efficacy	Comments
Certest Calprotectin Certest Biotec (Zaragoza, Spain)	Semiquantitative IC test	Certest	50 to 200	<50 negative >50 to 100 positive	Sensitivity 83% Specificity 84% PPV 0.77 NPV 0.89 LR+ 5.3 LR− 0.20 (24)	
Certest calprotectin 50/200 Certest Biotec (Zaragoza, Spain)	Semi to quantitative IC test	Certest	NA	Two cut offs: >50 positive >200 positive	Sensitivity: >94% Specificity: >93% PPV: >94% NPV: >93%*	
Calprotectin Turbilatex Certest Biotec (Zaragoza, Spain)	Turbidimetric assay (quantitative)	NA	20 to 1500	>50 positive	Sensitivity 94% Specificity >99%*	
Prevent ID CalDetect 50/200 Preventis GmbH (Bensheim, Germany)	Semiquantitative IC test	PreventID CalDetect test device	NA	Two different cut-offs (50 and 200) >50 positive >200 High positive	Sensitivity: 96% Specificity: 53% PPV: 0.44 NPV: 0.97 (28)	
Prevent ID Cal Screen Preventis GmbH (Bensheim, Germany)	Semi-quantitative IC test	PreventID CalDetect test device	NA	>50 positive	Currently in review*	
PreventID QuantOn Cal Preventis GmbH (Bensheim, Germany)	Quantitative IC test	PreventID CalDetect test device	Up to 2000	>50 positive	NA	App Home test
Biohit Calprotectin ELISA BIOHIT HealthCare Helsinki, Finland	ELISA	BIOHIT Extraction	2 to 2500	>50 positive	NA	
IDK Calprotectin ELISA (K 6927) Immundiagnostik (Bensheim, Germany)	ELISA	IDK Extract	Up to 2100	<50 negative 50 to 100 borderline >100 positive	NA	
IDK Calprotectin ELISA (K 6967) Immundiagnostik (Bensheim, Germany)	ELISA	IDK Extract	NA	<50 negative 50 to 100 borderline >100 positive	NA	1-point-calibration
RIDASCREEN Calprotectin R-Biopharm (Darmstadt, Germany)	ELISA	RIDA TUBE Calprotectin	19.5 to 800	>50 positive (can be used for children ages from 4 to 17 years)	Sensitivity: 74% Specificity: 84% (27) Sensitivity: 88% Specificity: 78% PPV: 79% NPV: 87% Ref (29)	
Calprotectina test Francisco Soria Melguizo, S.A. (Madrid, Spain)	Qualitative IC test	Francisco Soria Melguizo	NA	>50 positive	>94% and 93% correlation in sensitivity and specificity, respectively, compared with other market immunoassays*	
FC ELISA kit (HK382) Hycult Biotech (Uden, The Netherlands)	ELISA	Fecal extraction buffer Or Roche Fecal Sample Preparation kit	16 to 625 (range I; 50xsample solution) 48 to 1875 (range II; 150× sample solution)	>50 positive	NA	
LIAISON Calprotectin DiaSorin S.p.A. (Saluggia, Italy)	CLIA	LIAISON® Calprotectin Stool Extraction Device	5 to 800	>50 positive	Sensitivity: 87.5% Specificity: 66.6 PPV 0.5 NPV 0.93 (30)	
EpiTuub Rapid Test System Epitope Diagnostics, Inc (San Diego, USA)	Qualitative IC test	NA	NA	>50 positive	NA	Distributed mainly in US
EDI Quantitative Fecal Calprotectin ELISA (KT-849) Epitope Diagnostics, Inc (San Diego, USA)	ELISA	KT-843 Calprotectin Sample Collection Kit	0 - 2000	>43.2 positive	NA	Distributed mainly in US

CLIA = chemiluminescent immunoassay; EFIA = enzyme fluoroimmuno assay; FC = fecal calprotectin; PETIA = particle-enhanced turbidimetric immunoassay.
*Manufacturer data; NA: not applicable; IC test: immunochromatography rapid test.

In a more recent work, Roca et al (44) developed a useful nomogram that was based on the results of a regression analysis. A total of 174 healthy children ages 0 to 12 years were divided into 3 age groups: from 0 to 12 months, 1 to 4 years, and 4 to 12 years. Cut off levels established for the 3 different groups based on the lower value of [9]5th percentile for FC in each group, were 910, 286, and 54 μg/g respectively. A high interindividual variability was observed in infants below 1 year of age (44). Oord and Hornung (39) also established cut off levels for FC based on the 97.5% percentiles of FC in different age groups: 538 μg/g (1–6 months), 214 μg/g (6 months to 3 years), and 75 μg/g (3–4 years). Fagerberg et al (22) had earlier suggested that the cut-off level for adults as recommended by the manufacturer (<50 μg/g) could also be used for children ages 4 years and older; they found in their study group

TABLE 2. Reference values of FC in healthy children according to age

Reference	No. of subjects	Age	Median FC (μg/g)	97.5 percentile (μg/g)	Significative differences
Fagerberg et al (22)	117	4 to 6 y	28	NS	NS
		7 to 10 y	13.6		
		11 to 14 y	9.9		
		15 to 17 y	14.9		
Joshi et al (38)	132	2 to 9 y	34	166	$P = 0.004$, between 2 and 9 y and the other age groups
		10 to 59 y	22	51	
		>60 y	27	112	
Oord and Hornung (39)	75	1 to 6 mo	192	538	NS
		6 mo-3 y	47	214	
		3 to 4 y	36	75	
Li et al (40)	288	1 to 3 mo	375.2	962	$P < 0.001$ between 1 and 3 vs 3 to 6 m, and 1 to 6 vs 6 to 18 m
		3 to 6 mo	219.7	621	
		6 to 9 mo	123.5	362	
		9 to 12 mo	109.5	398	
		12 to 18 mo	177.9	501	
Zhu et al (41)	274	1 to 2 y	96.14	447.73*	$P = 0.016$ among the 3 age groups
		2 to 3 y	81.48	368.04*	
		3 to 4 y	65.36	379.33*	
Song et al (42)	234	7 to 12 mo	78.5	135	$P < 0.01$ among the different age groups
		13 to 18 mo	29	65	
		19 to 24 mo	27	55	
		25 to 30 mo	27	40	
		31 to 36 mo	12.5	21	
		37 to 42 mo	12	21	
Davidson and Lock (43)	8676	1 to 3.9 y	49	77	$P < 0.005$
		4 to 17.9 y	40	62	between 1 and 3.9 y and the other age groups
		>18 y	56	61	
Roca et al (44)	174	0 to 12 mo	NS	910*	$P < 0.001$ between the older group (>4 to 12 y) and the other groups
		1- < 4 y		286*	
		>4 to 12 y		54*	
Peura et al (45)	84 (246 evaluations across time)	0 mo	67	233	
		6 mo	31	615	
		12 mo	19	136	
		24 mo	17	57	

FC = fecal calprotectin; NS = not stated.
*Data corresponding to 95 percentile.

of 117 children, ages 4 to 17 years, that the median FC concentration was 13.6 μg/g. Peura et al (45) studied FC levels in 84 individuals across 4 different time points during the first 2 years of life; they confirm that the high values at neonatal age decrease fast and apparently stabilises at 1 year of age.

FC levels depend on gestational and postnatal age. Although extreme preterm infants have particularly low FC levels (46), premature babies and term infants before the age of 1 year have significantly higher levels compared with healthy children, through a downward trend with increasing age (40,39,45,47). The FC levels observed in healthy preterm infants were higher than those reported for adults and children (47–51); means from 98 to 122 μg/g (SD between 68 and 98 μg/g) and medians from 150 to 253 μg/g (range <15 to 1867 μg/g) have been reported in preterm infants (age 3–18 days to maximum 1–8 weeks) (48). For full-term newborns younger than 3 months, mean FC levels from 145 to 277 μg/g (SD between 46 and 109 μg/g) and medians from 167 to 269 μg/g (range 31 to 2880 μg/g) have been reported in a comprehensive literature review (48). Because of the wide variety in population size as well as in age range in the retrieved studies, no definite cut off can be established neither in preterm infants nor in infants younger than 1 year of age.

Levels of FC do not appear to be related to gender (22,44).

Statement 4

With regards to the screening of patients for the presence of disease

4(a) Due to high interindividual variability especially at young ages, any decision for clinical intervention should be based not only on FC levels but also additionally on the global clinical context.

4(b) In preterm and infants younger than 1 year of age, FC may be elevated without any known cause for inflammation and until a normal range for this age group is firmly established, FC levels should be interpreted with particular caution.

4(c) In children older than 4 years of age cut off values of 50 mg/g, as in adults, can be used, although healthy children may have FC levels up to 100 μg/g or even higher.

Recommendation 4
The ESPGHAN expert group recommends for the screening of paediatric patients for evidence of a GI disease to
4(a) Always pay equal emphasis to the clinical context as to the absolute FC values (GoR: Strong)
Agreement 100% MS 9
4(b) Not use a cut off level <100 μg/g for children younger than 4 years of age because of the wide variability in FC values detected in healthy children in this age range. (GoR: Strong)
Agreement 92.9% MS 8.3
4(c) Exert caution when using a cut off level of less than 50 μg/g (as recommended in adults) for children >4 years of age as reference values at this age range have not been irrefutably established. (GoR: Strong)
Agreement 92.9% MS 8.3

Effect of Geographical Area and Socioeconomic Status

In a rural population in Guatemala (52,53), the median FC level in children ages 2 to 7 years was 58 mg/g. In Uganda (54), 472 healthy children were evaluated with median FC concentrations of 249 mg/kg in 0 to 1-year-old, 75 mg/kg in 1 to 4-year-olds, and 28 mg/kg in 4 to 12-year-olds (n = 159). The authors concluded that FC concentrations amongst healthy children, living in rural areas or low-income countries, were comparable with those in healthy children living in high-income countries. Liu (55), however, found a significantly higher FC in healthy infants and children from rural China, as compared with urban ones (median FC, respectively, 420.9 and 140.1 μg/g, $P < 0.0001$).

Statement 5
5(a) Living in an urban or rural area seems to have no impact on FC levels.
5(b) FC concentrations are likely not related to socioeconomic status.

Recommendation 5
The ESPGHAN expert group recommends to NOT take into consideration geographical area or socioeconomic status when interpreting FC results. (GoR: Moderate)
Agreement 85.7% MS 7.9 Abstentions: 1

Effect of Diet

There are several studies that refer to the relationship between diet and FC, mainly comparing FC levels in breastfed infants with those receiving mixed-feeding or only formula. Most authors found that FC was significantly higher in the exclusively breastfed group (56–59), although others stated that FC levels appear neither to be influenced by breast-feeding (60) nor by the type of formula (standard vs prebiotic supplemented formula) (61). In preterm infants, a positive correlation was found between FC levels and the volume ($mL \cdot kg^{-1} day^{-1}$) of enteral feeding needed (62).

In adults ages 50 to 70 years, Poullis et al (63) observed a 10% decrease in FC levels per daily portion of fibre consumed; they found an inverse relationship between FC and fibre ($P = 0.02$) or vegetable intake ($P = 0.04$) but no relation to the proportion of fruits or fats consumed in the diet. No data in paediatric populations are reported.

Statement 6.
There is contradictory evidence on the effect of breast milk and diet on FC levels.

Recommendation 6
The ESPGHAN expert group suggests to NOT advise a specific diet before sampling for FC. (GoR: Weak)
Agreement 92.9% MS 8.3

Effect of Concomitant Drugs

The effect on drugs on FC levels has been mainly studied in the adult population. Relevant findings are summarised in Table 3. Adult patients taking nonsteroidal anti-inflammatory drugs (NSAIDs) have higher FC levels apparently related to drug-induced enteropathy at different levels of the GI tract even though concentrations were considerably lower than in patients with active UC or CrD (66,67,72,73) (Table 1). Of 90 patients (median age 9.1 years) with juvenile idiopathic arthritis, 40% complained about abdominal pain with only one-third of these showing elevated FC values (>100 μg/g). For most of them, FC values declined with the discontinuation or reduction of NSAIDs (74). Although FC has been shown to significantly increase in adults after exposure to both ibuprofen (65) and celecoxib (75), the increase was much lower in the latter and some specific Cox-2 inhibitors show no increasing effect at all (64,65) (Table 2). The effect of NSAIDs on FC may be seen as early as a few days after the initiation of these medications (73). FC has, therefore, been used in several publications as a marker of NSAID enteropathy. Accordingly, it is recommended that patients on a NSAID, who are assessed for a nondrug-related inflammatory condition, cease taking the drug for 3 weeks before collecting a sample for FC measurement.

Low-dose acetyl salicylic acid (ASA) (100 mg/day, for 14 days) also appears to induce a significant increase in mean FC in healthy adult volunteers suggesting the development of intestinal inflammation; however, there was no strict correlation between FC levels and mucosal abnormalities of the GI tract (68). The use of proton pump inhibitors (PPIs) has also been shown to be associated with higher levels of FC in an adult population but not related to the presence of dyspepsia (69,70). A recent prospective study in 51 children ages 3 to 18 years with gastrointestinal symptoms of whom 37 were treated by PPIs, however, failed to show evidence of intestinal inflammation related to PPI use through FC measurement (71).

Statement 7
Elevated FC levels might be found in patients using any drug having an inflammatory effect on the gastrointestinal tract.

TABLE 3. Effect of drugs on FC levels

Drug	Effect on FC levels	Observations	References
Lumiracoxib (NSAID)	No effect	No differences with placebo	(64)
Naproxen plus Omeprazole (NSAID)	Increase		(64)
Celecoxib (NSAID)	No effect	No differences with placebo	(65)
Ibuprofen plus Omeprazole (NSAID)	Increase	No correlation between levels and small bowel mucosal breaks	(65)
Diclofenac (NSAID)	Increase	Adults	(66)
Naproxen (NSAID)	Increase		(67)
Nimesulide (NSAID)	No effect	Due to inhibition of COX- 2	(67)
Aspirin (ASA)	Increase	Low-dose ASA might induce enteropathy and thus FC increase,	(68)
Proton pump inhibitors	Increase (adults)		(69,70)
	No effect (3–18 y)		(71)

ASA = acetyl salicylic acid; FC = fecal calprotectin; NSAID = nonsteroidal anti-inflammatory drug.

Recommendation 7
The ESPGHAN expert group recommends to exert caution when interpreting mildly elevated FC levels whilst a patient is on nonsteroidal anti-inflammatory drugs, ASA, and/or PPIs. (GoR: Moderate)
Agreement 92.9% MS 8.4 Abstentions: 1

Other Factors

Levels of FC appear to associate with the microbiome profile (76,77), with studies describing subsets of taxa most likely to reflect the level of inflammation, and in turn, to levels of FC. In the study by Quince et al (77), the gradient of increasing intestinal inflammation amongst IBD children was associated with reduced microbial diversity, abundance of butyrate producers and relative abundance of Gram-positive bacteria. Thus, FC appears to be a valuable surrogate marker of inflammation when exploring the role of microbiome in IBD.

There is very scarce published information on the effect of environmental factors on FC and little reference to the paediatric population (78). In a healthy general population of 300 adults, age range 50 to 70 years, a significant positive relationship between FC and increasing age ($P = 0.002$), physical inactivity ($P = 0.01$), and obesity ($P = 0.04$), was observed, although the latter was attenuated by controlling for serum C-reactive protein (CRP). The authors conclude FC levels were associated with lifestyle risk factors for colorectal cancer (63).

FECAL CALPROTECTIN LEVELS IN SPECIFIC GASTROINTESTINAL DISEASES

Inflammatory Bowel Disease

Fecal Calprotectin as a Screening tool for Inflammatory Bowel Disease

FC is reported to be a better screening tool for the presence of IBD in undiagnosed patients than blood inflammatory markers, such as CRP or erythrocyte sedimentation rate (ESR) (79). FC could, thus help to select those children in whom IBD needs to be evaluated (80,81).

The reported sensitivity and specificity of the FC test, in line with negative-predictive and positive-predictive values (NPV and PPV), depends on the studied patient cohort, including the number of patients with causes other than IBD that could result in elevated FC values [eg, bacterial or viral gastroenteritis (82); juvenile polyp (83,84)]. A thorough review (85), found the best cut-off value in IBD screening for abnormality to be 212 μg/g corresponding with a sensitivity of 0.90 (95% CI 0.87–0.93) and specificity of 0.87 (95% CI 0.81–0.88). In several reports, the cut-off for a raised value is 100 μg/g (86,87). As discussed earlier, there is some evidence that younger children may have a wide normal range of levels of FC; however, there is no strong evidence indicating that where IBD is suspected that the cut-off should vary according to age. There are no data or studies that have assessed cut-offs for IBD in different age groups.

To date, there is only a single published study on the cost-effectiveness of FC measurement to identify adults and children who require endoscopic examination compared with direct endoscopy evaluation alone to assess the presence of inflammation (88). A pretest probability for IBD less than 65% made FC screening cost-effective in children but if it was more than 78%, FC measurement was calculated to be cost-ineffective, given the delay in reaching the diagnosis in evident cases while waiting for the results of FC measurement (88).

FC reflects the mucosal influx of inflammatory cells (mostly neutrophils and monocytes), and therefore, disease activity appears to be the most important denominator of the observed levels. Disease extent, however, does not necessarily correlate with levels, that is, whether the disease is pancolitis or left-sided colitis cannot be differentiated based on FC values alone. In isolated proctitis, the levels may be within normal range (89).

There has been some debate on whether the performance of FC as a screening method differs between UC (or IBDU) and CrD. In the first reports on FC in paediatric IBD, UC and CrD patients were both included, and there were no major differences in the performance of FC between the patient groups (90,91). A multicentre study on paediatric CrD showed good performance of the test in paediatric patients with Crohn colitis or terminal ileal disease (92), confirming the previous findings of comparable levels in UC and CrD (93). In CrD without colonic involvement, elevated levels of FC may indicate active disease in the small intestine (89,92,94,95). Isolated upper gastrointestinal CrD is rare, and we did not find any paediatric reports on the performance of FC in such cases. On the basis of adult series, the levels of FC may be low in patients with CrD limited to the ileocecal valve and showing noninflammatory behaviour (96). In children, such a disease subtype is rare.

It is generally accepted that elevated values of FC correlate with intestinal inflammation at the histological level (90,91,97–100) but the absolute levels, as such, cannot be used to categorize disease activity (eg, high, moderate, or low) unless endoscopy is performed. In UC, only few patients reach complete remission according to FC when on maintenance therapy (101). Although normalisation of FC is rare, most patients with UC in clinical

remission; however, have FC levels lower than the suggested cut-offs for an increased risk of a relapse, that is, 500 (102) or 800 μg/g (103). In acute severe colitis, the absolute level is not associated with prognosis and cannot be used to anticipate therapeutic response (104). In a single-centre study in paediatric patients on Infliximab therapy, a cut-off of more than 250 μg/g was associated with risk of clinical relapse within 3 months (105).

Albeit accepting the above, patients strongly suspected to have IBD, especially those presenting with alarm symptoms or signs, such as bleeding, iron deficiency anaemia, weight loss, and fever, should undergo endoscopic examination regardless of FC value (87).

Other neutrophil-derived markers of IBD, such as, for example, myeloperoxidase, lactoferrin, S100A12, or matrix metalloproteinase 9 (MMP-9) perform in a similar manner to FC but provide no additional value when used in combination with FC (104,106,107).

Statement 8.
8(a) FC is a better indicator of the possibility of IBD than serum inflammatory markers and should be obtained to decide whether an endoscopic examination is warranted. Wherever IBD is strongly suspected and the screening FC result not available in a timely fashion the diagnostic endoscopy should not be delayed, however.
8(b) The degree of elevation in the concentration of FC cannot be used for the differential diagnosis of IBD from non-IBD causes of inflammation or to differentiate UC from CrD or to ascertain the extent of disease.
8(c) There is not enough data about FC levels in CrD patients with isolated upper GI involvement.
8(d) In acute severe colitis, the level of FC is not associated with therapy outcome.

Recommendation 8
The ESPGHAN expert group recommends to
8(a) Perform a diagnostic endoscopy where IBD is strongly suspected and not to wait for FC results prior if these cannot be obtained in a timely fashion, to avoid any delay in confirming the diagnosis. (GoR: Strong)
Agreement 100% MS 8.7
8(b) NOT use FC as a prognostic marker in acute severe colitis. (GoR: Strong) **Agreement 100%** MS 8.7

Fecal Calprotectin in the Follow-up of Inflammatory Bowel Disease Patients

FC has provided an additional tool for monitoring paediatric IBD patients. In both adults and in children, FC levels have been shown to correlate with disease activity as defined by clinical parameters, endoscopic findings, and histology (86,87,108–110).

In the European Crohn's and Colitis Organisation (ECCO)/ESPGHAN guideline for management of CrD, it is stated that FC is particularly useful for children as a way of monitoring resolution or recurrence of intestinal inflammation, although the specific cut-off value for management decisions has not been defined (111). According to the updated (2020) ECCO/ESPGHAN CrD guidelines (112), the closer the calprotectin value gets to 50 μg/g, the higher the likelihood for complete endoscopic healing (113). The UC guideline (114), however, proposes the need for an endoscopic evaluation in those patients in clinical remission and with a FC concentration >250 μg/g, as this FC value predicts mucosal inflammation more accurately.

More recently, no correlation was found between capsule endoscopic scores and biochemical inflammatory parameters including FC (115). Nevertheless, from adult studies, if no findings in endoscopy are reported, the frequency of detecting lesions when performing capsule endoscopy correlates with increasing FC values (FC < 50 μg/g, 10%; FC 50–100 μg/g, 25%; FC >100 μg/g, 62%) (116). With a rapid-onset therapeutic agent, such as Infliximab, the FC levels may drop back into the normal range within 2 weeks (89,117), although with exclusive enteral nutrition (EEN), the decline may be less clear and occur over a longer period of 6 to 8 weeks of therapy (79,118–121). The reduction of FC values during EEN; however, is lost rapidly after food re-introduction (118,122). Intriguingly, partial enteral nutrition with exclusion diet (123) or a personalised exclusion diet (124) may also result in mucosal healing and decline in FC. In most patients, regardless of the given therapy, the FC values remain somewhat elevated indicating ongoing inflammatory activity at the tissue level (histological disease activity). There is no consensus on what level is acceptable for patient outcome unless the FC level is within the normal range (101,111,114,125). A recent report on magnetic resonance imaging enterography (MRE)-based follow-up of paediatric patients with CrD reported a FC cut-off below 300 μg/g to identify children with mucosal healing (assessed by endoscopy) and below 100 μg/g to screen for IBD to identify children with deep healing (assessed by endoscopy with MRE) (126). The reports on FC values in predicting a disease flare (in conservatively treated disease) show values ranging from 400 to 800 μg/g in paediatric CrD and UC (102,103,127) but as low as 300 μg/g in adult UC and CrD (128,129). FC levels, however, start to rise before clinical or endoscopic relapse as shown in a group of adult IBD patients with FC-based, endoscopy-confirmed remission (130). The authors conclude that FC can be used for identifying patients requiring close follow-up in clinical practice (130).

Statement 9
9(a) In IBD patients in clinical remission with minor or absent clinical symptoms, FC may be used to confirm remission.
9(b) Upon follow-up in the presence of repeatedly high FC levels, endoscopy should be considered.
9(c) The optimal interval for FC follow-up is not determined and should be decided upon on an individual basis.

Recommendation 9
The ESPGHAN expert group suggests to
9(a) Include FC measurement in the laboratory investigations of IBD patients at least every

6 months during follow-up, even in remission, unless clinical picture suggests a relapse, where earlier investigation may be indicated. (GoR: Weak)
Agreement 100% MS 8.2
9(b) Consider endoscopic evaluation in IBD patients in clinical remission with a FC >300 μg/g as this cut off level accurately predicts mucosal inflammation. (GoR: Weak)
Agreement 92.9% MS 8 Abstentions: 1

Fecal Calprotectin in the Follow-up of Crohn Disease Patients After Intestinal Resection

FC measurement has also been reported to provide a noninvasive means to follow up paediatric IBD patients after intestinal surgery (131–133). Thus, monitoring of FC may aid in timing of the follow-up endoscopy. If a patient is doing well and the FC level is normal, the endoscopy may be postponed or conversely, in a symptomatic patient with an elevated FC level, there is an immediate need for an endoscopic evaluation (133).

Statement 10
FC measurement may help establish the right timing to perform a follow-up endoscopy after intestinal resection.

Recommendation 10
The ESPGHAN expert group recommends to measure FC repeatedly after intestinal resection as it is superior to CRP to detect early asymptomatic relapse requiring endoscopic evaluation. (GoR: Moderate)
Agreement 100% MS 8.4

Fecal Calprotectin in Postcolectomy Patients

In postcolectomy patients with J-pouches, FC levels increase in the presence of pouchitis. Also, in patients with recurrent pouchitis, the levels of FC were higher in a cross-sectional study of 32 paediatric patients after restorative proctocolectomy compared with patients with no history of pouchitis or reporting a single episode (131). The FC values decline accordingly along attenuation of inflammation in a pouch. Serial measurement of FC is an approved strategy to detect pouchitis and inflammation in the stapler line (cuffitis) in adults (134). ECCO/ESPGHAN UC guidelines for UC management (114) recommend that FC may be used to assess pouch inflammation to minimize repeated pouchoscopies in recurrent pouchitis and to monitor response to treatment. Calprotectin >300 μg/g is suggestive of pouchitis, although lower levels do not preclude it.

Statement 11FC levels can be used to assess the possibility of pouch inflammation and response to treatment in endoscopic assessed pouchitis and most likely also for the presence of cuffitis.

Recommendation 11.
The ESPGHAN expert group recommends to use FC levels after colectomy to screen for pouchitis and inflammation at the anastomosis. (GoR: Moderate)
Agreement 100% MS 8.1

Fecal Calprotectin in Perianal Disease

The data on FC in perianal disease is sparse (135). Seton drainage in combination with infliximab therapy is used with the same indications as in adults (133). FC values decline along with mucosal healing when elevated at management initiation and increase in relapse (135). For evaluating the response in routine practice, however, clinical assessment in combination with MRI or anal sonography is recommended (126).

Functional Gastrointestinal Disorders

The revised Rome IV criteria provide symptom-based guidelines by which FAPDs can be diagnosed in children from 0 to 18 years of age (136,137). FAPDs include a variable combination of, often age-dependent, chronic, or recurrent gastrointestinal symptoms, such as infant colic, regurgitation, abdominal pain, diarrhoea, and constipation not explained by obvious structural or biochemical abnormalities.

Infant Colic

In infant colic as defined by the Rome IV criteria, the underlying pathophysiological mechanisms are not well understood although neurogenic, gastrointestinal, microbial, and psychosocial factors have been proposed (136). A possible candidate mechanism is gut inflammation, although there is inconsistent data. FC was measured by an ELISA kit in spot stool samples of 76 infants diagnosed with infant colic and compared with 27 healthy infants (20). In this study, the mean FC concentration in infants with colic was not different from that in healthy infants (278 ± 105 vs 277 ± 109 mg/kg, $P = 0.97$). Furthermore, the FC level was similar in boys and girls and fell significantly with increasing age ($P = 0.04$). In contrast, other studies have shown elevated concentrations of FC in infants with colic (138–140). Rhoads et al (138) found elevated amounts of *Klebsiella* spp. associated with low-grade gut inflammation (not assessed by endoscopy) as demonstrated by FC levels, which were 2-fold higher in infants with colic compared with control infants (413 ± 71 vs 197 ± 46 μg/g, $P = 0.042$). The authors reported that FC levels after 1 month of treatment with either *Lactobacillus reuteri* (treatment group, n = 31) or placebo (n = 34) were similar. Infants in both groups with at least a 50% reduction in duration of crying or fussing at 1 month, however, had significantly lower FC levels than nonresponders (responders, n = 50; nonresponders, n = 52, mean difference 96.6 mg/kg, 95% CI 5.1—188.1, $P = 0.04$) (140). It, perhaps, remains to be established whether gut microbiota alterations in colicky infants cause gut inflammation, or whether dysbiosis is a result of intestinal inflammation.

Statement 12
Inconsistent data exist regarding the correlation between FC levels and infant colic.

Recommendation 12
The ESPGHAN expert group recommends to NOT use FC in babies with infantile colic. (GoR: Moderate)
Agreement 92.9% MS 8.9 Abstentions: 1

Functional Abdominal Pain

Since 2016, the Rome IV criteria recognizes 4 different FAPDs: functional dyspepsia, irritable bowel syndrome, abdominal migraine, and functional abdominal pain—not otherwise specified (137). The aetiology and pathogenesis of these FAPDs are still largely unknown, but a growing body of evidence suggests a disordered brain-gut communication with evidence for a role of visceral hypersensitivity, altered conscious awareness of gastrointestinal sensory input, gastrointestinal microbiota, and gastrointestinal dysmotility (141).

Differentiation between FAPDs and IBD is sometimes difficult as symptoms can be nonspecific and frequently overlap. Olafsdottir et al (20) compared 19 children with recurrent abdominal pain as defined by Apley and Naish (142) (mean age 11.5 years) with 17 children with IBD (mean age 11.1 years) of whom 10 had CrD and 7 UC as well as with 24 healthy children (mean age 5.3 years). Children with IBD had FC levels (293 ± 218 μg/g) significantly higher than healthy children (40 ± 28 μg/g, $P < 0.0001$) or children with recurrent abdominal pain without identified organic disease (18 ± 24 μg/g, $P < 0.0001$). Studies from Norway and Poland confirmed these findings (143,144). Eighty-three of 126 Norwegian children with 4 different FAPDs (66%) had FC concentrations below the detection limit of 16 μg/g whereas 9 (7%) had levels between 50 and 100 μg/g (5 IBS, 2 functional abdominal pain, and 2 functional dyspepsia), and 3 had levels above 100 μg/g (1 aerophagia, 1 functional abdominal pain, 1 functional constipation) (143). There was no significant differences in median concentrations between the different FAPDs. In a Polish study, all 22 healthy controls and 33 patients with FAPDs had FC levels below 100 μg/g, whereas in most patients with IBD, the FC were markedly above the cut-off value (median level 1191.5 μg/g; 25–75 percentile range: 265.2–1684.9 μg/g) (144). In contrast to these, an American study showed that FC levels in 93 children with IBS/FAP, were significantly higher compared with healthy controls; 65.5 ± 75.4 versus 43.2 ± 39.4 μg/g ($P < 0.01$) (19). Moreover, they showed that FC levels correlated with pain-related interference of daily activities ($P = 0.01$).

Di Nardo et al (145) demonstrated that mast cell-nerve interactions, suggesting low-grade immune activation and neuroimmune interactions within the colonic mucosa resulting in hypersensitivity in IBS patients were increased in the ileocolonic mucosa of children with IBS compared with controls. In addition, they showed that this close spatial association was related to intensity and frequency of abdominal pain. In contrast with the study by Shulman et al, however, they neither found differences in FC concentration in children with IBS compared with controls nor statistical correlation between FC and abdominal pain or stool pattern (145).

Lastly, Diederen et al (146) investigated the prevalence of IBS-type symptoms in children with IBD in clinical remission. A total of 184 patients (92 girls; mean age: 14.5 years) with IBD of whom 123 had CrD and 61 UC were included. Respectively, 71.5% and 60.7% of the children with CrD and UC, were in clinical remission. The prevalence of IBS-type symptoms in this latter group was 6.4% (95% CI 2.5%–11.1%; CD 4.5%; UC: 10.8%). Biochemical remission was defined as FC level less than 250 μg/g, this cut-off being predictive of endoscopic disease activity (147). No difference in FC or CRP was found between patients in clinical remission with or without IBS-type symptoms (FC: IBS symptoms median 58 μg/g, no IBS symptoms median 221 μg/g, $P = 0.12$; for CRP: median levels were 1.4 and 1.1 mg/L, respectively, $P = 0.63$). On the basis of these findings, the authors suggested that persistent symptoms in children with IBD in remission appear to be unrelated to on-going inflammation. Interestingly, IBD patients with IBS-type symptoms were less frequently using IBD-related medication, compared with those without IBS-type symptoms, which may imply that anti-inflammatory treatment may prevent or reduce IBS-type symptoms.

Statement 13
15 (a) FC levels in children with FAPDs are similar to healthy controls.
15 (b) FC levels in children with IBS symptoms are slightly higher than in healthy controls but lower compared with children with IBD.

Recommendation 13
The ESPGHAN expert group recommends to use FC as a tool to differentiate functional abdominal pain disorders from organic diseases. (GoR; Strong)
Agreement 92.9% Mean 8.3 Abstentions: 1

Functional Constipation

Constipation is characterised by infrequent evacuation of hard and painful stools, frequently accompanied by faecal incontinence and/or abdominal pain. In more than 95% of constipated children, no organic cause can be found, and affected children are defined, according to the Roma IV criteria as functional constipation (136,137). Although the exact pathophysiological mechanisms underlying functional constipation are unknown, gut inflammation has not been associated.

FC levels were measured in stool samples from 100 children ages 5 to 17 years (109). No differences in FC levels were found between healthy controls (median 15.6 μg/g, range 15.6–39 μg/g, $n = 7$, $P < 0.0001$) and children with constipation (median 15.6 μg/g, range 15.6– 63.1 μg/g, $n = 31$, $P < 0.0001$). Of the children with constipation, 3/31 (9.7%) had FC $\geq$50 μg/g, but none of these children had a FC $\geq$200 μg/g. These data were confirmed by another study including 76 children, ages 1 to 120 months, suspected of Hirschsprung disease (148). In the 19 patients diagnosed with Hirschsprung disease, the median FC concentration was 20 μg/g (under 0.5—–106.0 μg/g), whereas in the 57 children with functional constipation, the median was 4 gμ/g (under 0.5–110.8 μg/g).

Statement 14
FC levels in most children with functional constipation are not different from controls.

Recommendation 14
The ESPGHAN expert group recommends to NOT measure FC in children with constipation as it cannot differentiate between functional and the majority of organic causes (eg, Hirschsprung disease). (GoR: Moderate)**Agreement 87.7%** MS 8.3 Abstentions: 1

Cow's Milk Protein Allergy/Food Allergy

In developed countries, an estimated 5% to 10% of children are reported to suffer from food allergy (149,150) with CMPA being the most common food allergy in infants and children younger than 3 years (151,152). The gastrointestinal manifestations of CMPA are nonspecific and most commonly not mediated via IgE, and therefore, the only definitive way to confirm the diagnosis is by elimination of allergens in the diet to see if symptoms resolve and assess for relapse after reintroduction of the food allergen (153). FC has received attention because of its altered levels in children with food allergy. In 2011, Waligora-Dupriet et al (154) reported that the concentrations of FC in infants with food allergy were 2-fold higher compared with healthy controls (154). Later, Beşer et al (155) evaluated, in a randomised controlled study, FC concentrations in 32 infants with newly diagnosed CMPA: 24 infants with IgE-mediated disease (mean age 12.5 months) and 8 infants with non-IgE-mediated disease (mean age 2.87 months) and compared them with 39 healthy controls (mean age 11.5 months). Infants with non-IgE CMPA had higher FC concentrations compared with healthy controls (886 ± 278 vs 296 ± 94 μg/g, respectively; $P < 0.001$), as well as to infants with IgE-mediated CMPA both at baseline (886 ± 278 vs 392 ± 209 μg/g, respectively; $P = 0.025$) and after the elimination diet (359 ± 288 vs 218 ± 90 μg/g, respectively, $P = 0.001$). In contrast, a very recent study (156) in a small group of 17 infants with non-IgE-mediated CMPA ages up to 2 years, showed that FC levels in these patients were not different compared with 10 age-matched healthy controls: median (range) FC were 47.25 μg/g (28.80–106.10) versus 68.40 μg/g (30.38–76.73), respectively, $P = 1.00$.

Merras-Salmio et al (157) assessed FC during elimination diets and after double-blind placebo-controlled food challenges (DBPCFC), in 55 Finnish infants and young children with a median age of 8 months and gastrointestinal symptoms attributed to CMPA (such as excessive crying, fussiness, vomiting, or loose stools). Only 32% of the patients (median age 8.4 months) had positive DBPCFCs. The authors reported that FC levels were higher in the challenge-positive group ($n = 18$) than in the negative group ($n = 37$), with respective geometric means during cow's milk-free diet of 55 μg/g (95% CI 38–81) and 29 (24–36) μg/g, respectively; $P = 0.0039$. In children with gastrointestinal symptoms suggestive of non-IgE CMP allergy, there were no differences in FC concentrations measured during CMP-free diet and DBPCFC: median (range) FC concentrations were 52 μg/g (33–86) versus 60 μg/g (30–122), respectively; $P = 0.5995$, whereas in healthy controls, median (range) of FC were 25 μg/g (13–50). The fact that the difference between the groups was small, the within-group variation in both patient groups was high, no increase in FC levels was found after DBPCFC and that most FC values still remained within the normal range, makes it difficult to draw conclusions from this study and raises the need for future studies in this patient group.

Baldassarre et al (158) assessed FC in 30 infants with suspected CMPA before and after 4 weeks of CMP elimination diet compared with that of healthy controls. FC in infants with haematochezia was significantly higher than in healthy controls (mean ± SD 325.89 ± 152.31 vs 131.97 ± 37.98 μg/g stool, $P < 0.0001$). After 4 weeks of elimination diet, a 50% decrease in FC was observed but the levels still remained significantly higher compared with healthy controls (157.5 ± 149.13 vs 93.72 ± 36.65 μg/g, $P = 0.03$). Interestingly, a significant decrease in FC was also observed in the control group.

Another study in 29 breastfed infants (mean age 4 months) with CMP allergy manifesting with bloody stools reported that, despite clinical improvement in the infants, FC levels showed a nonsignificant decline 2 and 6 weeks following the initiation of CMP-free diet by the lactating mothers: median (SD) of FC levels (μg/g) at baseline, 2 and 6 weeks following the initiation of CMP-free diet were 209.1 (SD: 387.9), 189.5 (SD: 382.4), and 125.2 (SD: 105.4), respectively ($P = 0.741$ and $P = 0.284$, respectively). The authors concluded that FC was not a good indicator of clinical response to CMP-free diet in infants with CMP allergy (159).

Winberg et al (160) assessed FC and eosinophil-derived neurotoxin levels at diagnosis and after a 3-session DBPCFC in 12-year-old children from a population-based cohort, reporting complete avoidance of milk, egg, cod or wheat, because of perceived hypersensitivity to these foods, manifested as eczema, urticaria, vomiting, diarrhoea, or flatulence. Six of the above patients had a positive and 6, a negative DBPCFC. Both at baseline and postchallenge, the FC levels in children with a positive DBPCFC tended to be higher compared with children with a negative DBPCFC, although the difference did not reach statistical significance: median FC (μg/g stool) at baseline was 25.8 versus 16.45, respectively, $P = 0.150$ while, after challenge 24.10 versus 8.82, respectively, $P = 0.078$. The limitation of the study was that the study groups were small and heterogeneous with regards to the type of food allergy, challenge food, and the serving order of active and placebo substances during the DBPCFC series (160).

Seo et al (161) compared FC levels in children with atopic dermatitis (AD) according to the severity of the disease. Fifty-five (84.6%) children had mild-to-moderate AD with Scoring Atopic Dermatitis (SCORAD) index less than 40, whereas 10 (15.4%) had severe AD (SCORAD ≥40). The geometric mean (range of 1 SD) FC levels in severe (SCORAD 53.0 ± 11.8) AD were significantly higher than that in mild-to-moderate (SCORAD 19.6 ± 9.9) AD: 66.7 μg/g (13.5–330.3) versus 29.4 μg/g (10.1–85.6); $P = 0.044$ (161).

Furthermore, several studies have evaluated the possible association of early-age FC levels as an indicator of early gut inflammation, to the later development of allergic diseases (162,163). Orivuori et al (162) measured FC at the age of 2 months in 758 infants participating in the PASTURE study (a substantial prospective birth cohort study conducted in rural areas in Austria, Finland, France, Germany, and Switzerland). Pregnant women, who worked or lived on family-run farms where livestock were kept, were recruited during the third trimester of pregnancy and compared with a reference group consisting of women from the same rural areas not living on a farm. Data of environmental factors, doctor-diagnosed AD, and asthma were collected by questionnaire. Increased concentrations of FC (expressed as median values with interquartile ranges) were reported in children from farming environments when compared with the nonfarmers' children (FC 181.81 μg/g [101.38/308.09] vs 156.27 μg/g [76.86/261.30], respectively; $P = 0.003$); in the children with 1 or more siblings (FC 165.97 μg/g [87.02/326.82] and 185.89 μg/g [109.92/329.46], respectively), when compared with the children without siblings (FC 140.01 μg/g [77.26/229.03]; $P < 0.001$); and in breastfed children (exclusively and partially breastfed) when compared with nonbreastfed children (FC 180.27 μg/g [106.03/307.97] and

149.57 μg/g [81.88/318.07], respectively vs 104.93 μg/g [63.78/192.14]; $P < 0.001$). As the distribution of FC levels was skewed, the authors evaluated the importance of high FC levels (above the ^{9}0th percentile) that indicated high-degree intestinal inflammation. The infants with FC levels at 2 months $^{>9}$0th percentile (n = 75, FC ranging from 517.6 to 1542.0 μg/g) had an increased risk of developing AD and asthma/asthmatic bronchitis by the age of 6 years (OR: 2.02 [1.06–3.85] and 2.41 [1.25–4.64]), compared with infants (n = 80) who had FC levels $^{<9}$0th percentile (FC ranging from 39.2 to 490.3 μg/g). Only 39 of 75 (52%) children who had FC above the ^{9}0th percentile were from a farming environment indicating that high levels of FC were not explained by the farming environment (162). In the same study, only the very high levels of FC in the whole cohort, above the ^{9}0th percentile, were associated with asthma and AD later on in life. It should be noted, however that no linear association between the levels of FC and the allergic diseases was found. The authors suggested that early changes in the gut immune system had long-term effects on the development of allergic diseases.

In contrast, a randomised DBPC allergy-prevention trial in 237 infants in Finland (157) using a combination of 4 probiotic strains prenatally and during the first 6 months from birth, reported that high FC concentrations (>51 μg/g) at the age of 6 months were associated with a significant reduction of the risk for any IgE-associated atopic diseases (unadjusted OR: 0.49) as well as a tendency to reduce the risk of having any allergic disease up to the age of 2 years (unadjusted OR: 0.52; 95% CI 0.26–1.04, $P = 0.066$). The mean FC concentrations, however, between the 2 groups at 3 and 6 months of age were comparable: at 3 months of age, mean FC was 180 μg/g (95% CI 154–212 μg/g) in infants who developed allergic diseases up to the age of 2 years compared with 152 μg/g (95% CI 127–183 μg/g) $P = 0.357$ in those who did not. At 6 months of age, mean FC concentrations were 31 μg/g (95% CI 25–38 μg/g) compared with 36 μg/g (95% CI 127–183 μg/g), respectively, $P = 0.357$.

Statement 15
FC levels show considerable variability in children with atopic diseases making it difficult to draw definitive conclusions regarding the efficacy of this test in diagnosis or management of atopic conditions.

Recommendation 15
The ESPGHAN expert group recommends to NOT use FC either as diagnostic tool or as prognostic marker of CMPA in children. (GoR: Moderate)
Agreement 92.9% MS 8.4 Abstentions: 1

Coeliac Disease

Limited data is available about the use of FC in children with coeliac disease (CD). The published literature in this area is scarce and heterogeneity in the detection methods used makes difficult to reach conclusions.

FC values are significantly higher in CD patients at diagnosis especially in those with higher levels of serological markers or classical symptoms (164,165); however, no correlation was found either with histological findings (164). Contradictory results have been reported related to the correlation between FC and with antitransglutaminase antibody levels (164,166). Although FC values were only around 100 μg/g on average in all reviewed studies, FC was significantly elevated in CD patients at diagnosis when compared with controls. Four to 12 months after commencing a gluten-free diet, this difference disappeared (97,164,167). Overall values of FC show a wide individual variability with an overlap between active CD and controls.

Potential use of FC to assess dietary compliance and histological recovery has been evaluated but no association between FC level and the histological lesion has been found (165). According to published results, there is no added benefit of FC measurement either at diagnosis or for follow-up over and above currently employed serological markers.

Statement 16
FC is elevated in CD patients at diagnosis, but individual variability is high.

Recommendation 16
The ESPGHAN expert group recommends to NOT use FC as a marker for the diagnosis or monitoring of CD. (GoR: Moderate)
Agreement 92.9% MS 8.8 Abstentions: 1

Cystic Fibrosis

Cystic fibrosis (CF) is the most common cause of exocrine pancreatic insufficiency in children and is treated with pancreatic enzyme replacement therapy (PERT). In CF, 85% of patients are pancreatic insufficient (PI) and 15% pancreatic sufficient (PS).

Despite adequate PERT, many subjects with CF continue to suffer from gastrointestinal symptoms including steatorrhoea and abdominal pain. It has been established that the intestine is abnormal in CF. Murine models have shown abnormal mucous accumulation, predisposing to gut dysmotility and abnormal microbial colonisation in the intestine (168). Whole gut lavage of CF patients has shown increased immunoglobulins and inflammatory biomarkers, such as IL-8 in the stool as compared with controls (169). Capsule endoscopy findings in CF have shown a variety of inflammatory changes including mucosal breaks and mucosal ulceration giving rise to the coining of the term ''CF Enteropa''y'' (170). Nonetheless, gastrointestinal symptoms in CF patients are generally less severe than those observed in IBD.

FC levels have been determined in several studies in CF. Bruzzese et al (171) showed FC to be elevated in 27 of 30 children with CF. In 10 patients, FC normalised after treatment with Lactobacillus GG suggesting that bacterial overgrowth is a possible aetiology of CF enteropathy. Werlin et al (170) showed CF enteropathy in both PI and PS patients but FC was markedly raised only in the PI patients. In a subsequent study (172), FC was found to be equivalently elevated in PI and PS patients supporting the concept of a generalised CF enteropathy unrelated to exocrine pancreatic status. In an Australian study, however, Dhaliwal et al (173) reported increased FC in PI patients but examined only 6 children with PS. In a subsequent larger study from the same group, Garg et al reported age-related variations in FC. For the first years of life,

FC levels were lower than controls and only began to increase after 4 years of age. This low value did not occur in PS patients (n = 9, 16 samples). After 4 years of age, however, there was an increase in FC in both PI and PS indicating generalised CF enteropathy (174). Ellemunter et al (175), however, reported in 171 patients in a longitudinal study over a median observation period of 7 years increased FC in PI compared with PS but unlike the Australian study did not find reduced levels in the younger age group.

In another study, Adriaanse et al reported FC levels were elevated in 40 CF patients (93%) with higher values in PI compared with PS patients. FC correlated positively with age ($r = 0.321$, $P < 0.05$) whereas no association was found with gender ($P = 0.67$). Although FC levels correlated inversely with lung function in CF patients (FEV1 $r = -0.428$, $P < 0.05$), after dividing the study population into children and adults a significant inverse correlation (higher FC in worse lung function) between FC and lung function was found only in adult CF patients (FEV1, $r = -0.484$, $P < 0.05$; FVC, $r = -0.304$, $P = 0.207$; FEV1/VC $r = -0.509$, $P < 0.05$, n = 19); no correlation was found in children (176). The relationship between FC level and nutritional status was assessed, the latter being considered to be negatively influenced by intestinal inflammation. In CF children, weight-for height *z*-score was positively (higher FC in children with better *z*-score) correlated with FC ($r = 0.531$, $P < 0.05$, n = 23), whereas in adult CF patients, no significant relation was found between BMI and FC ($r = -0.346$, $P = 0.147$); thus the correlation of FC with nutritional parameters is not conclusive. Linear regression showed that CFRD (CF-related diabetes), PPI use, and PI were associated with elevated FC in CF patients (176). The correlation between FC and intestinal inflammation was not confirmed by other studies (172,175).

Calprotectin is not only produced by neutrophils in the intestine but also by those in the lung, and expectorated sputum containing calprotectin may be swallowed, and subsequently detected in the faeces (172). There is, however, little evidence clarifying how, or to what degree, sputum calprotectin contributes to calprotectin recovered in faeces. For example, it is possible that swallowed sputum-derived calprotectin may be denatured to some extent within the acid environment of the stomach and limit its detection in faeces. This might make it difficult to make a clear distinction between intestinal and pulmonary inflammation by using FC. Even with this potential drawback, FC could be a practical parameter to monitor intestinal inflammation in trials with modulators and potentiators. In a recent published trial, treatment with Ivacaftor, a CFTR potentiator, improved pulmonary and nutritional status, and in 16 patients FC was significantly decreased following treatment (154.4 [102.1–284.2] vs 87.5 [19.5–190.2] mg/kg, $P = 0.03$) (177).

There is neither enough evidence of a correlation between FC and endoscopic or, histological lesions, nor has any relationship between FC and clinical symptoms associated to enteropathy been demonstrated. Larger, multicentre prospective studies may help determine if serial FC measurement is clinically relevant as a marker of intestinal inflammation in CF or whether it can be used as a marker of recovery/improvement of CF enteropathy in treatment trials.

Statement 17

FC may be considered a marker of intestinal inflammation in CF but there is not enough evidence of a correlation between FC and enteropathy; more studies are required to verify the status of FC in PS patients and age-related values as well as the contribution of confounding factors, such as lung calprotectin on FC levels.

Recommendation 17

The ESPGHAN expert group recommends to be cautious when interpreting individual FC values as a marker of enteropathy in CF. (GoR: moderate)

Agreement 92.9% MS 8.3 Abstentions: 1

Infectious Gastroenteritis: Viral, Bacterial, and Parasitic

In acute gastroenteritis (AGE), distinguishing between bacterial and nonbacterial causes is relevant to gauge the most appropriate management. In the literature, there is little data comparing acute infectious diarrhoea in children (viral or bacterial) and FC values as a function of the various pathogens and severity of the acute illness course. Sykora et al suggested that FC facilitates early discrimination between bacterial and viral causes of AGE in children before the age of 3 years. In particular, by combining FC with CRP, they observed an overall diagnostic accuracy up to 94% in discriminating between bacterial and viral AGE (178). Similarly, Duman et al showed that FC levels are significantly higher in patients with positive stool microscopic examination especially in proven bacterial gastroenteritis, such as *Salmonella* and *Shigella* infections, compared with patients with Rotavirus, Adenovirus, and Norovirus infections. In the diagnosis of bacterial AGE, they found that the area under the ROC curve for FC was 0.867 (95% CI 0.763–0.971), sensitivity was 88.9%, and specificity was 76.0% when the threshold was taken as 710 mg/L (179). By contrast, in children with AGE needing hospitalisation, no significant differences were found in the performance of FC (or pyruvate kinase isoform M2, an enzyme present in leukocytes) between children with AGE caused by Rotavirus and those with *Salmonella enteritidis* (180). Higher concentrations of FC in Rotavirus AGE in the present study can be partially explained by the context of the study, given that only hospitalised patients were recruited, and these can be assumed to have greater gastrointestinal inflammation compared with patients managed in primary care. These findings are consistent with those obtained by Shastri et al (181) in a large cohort of hospitalised adult patients.

The role of FC as biomarker of AGE severity is controversial. Indeed, FC seems to be correlated with clinical severity (eg, Vesikari score) of AGE, providing information for disease management in children, although seemingly not in *Clostridium difficile* infection (CDI) in adults (82). Both FC and faecal lactoferrin increase during CDI, especially in those with detectable toxin in faeces, and distinguish between CDI cases and antibiotic-associated diarrhoea (182,183). Although lactoferrin but not FC levels seem to be associated with disease severity, both parameters show high interindividual variability. Thus, FC and lactoferrin seem unlikely to be useful as biomarkers of complicated CDI disease (182).

Publications evaluating the role of FC in parasitic gastroenteritis are scarce. In 1 study, FC levels were significantly associated with active schistosomiasis as detected by eggs in stool with a significant decrease in test positivity after praziquantel treatment (184). Sorokman et al (185) found significantly higher FC levels in

90 symptomatic children ages 6 to18 years with *Giardia duodenalis* infection, as compared with 110 children negative for the parasite.

Statement 18
FC is significantly higher in bacterial population in comparison to viral and no detectable pathogen in acute gastroenteritis. As acute gastroenteritis management guidelines do not, however, recommend performing microbiological studies routinely in nonhospitalised children, FC measurement in gastroenteritis in clinical setting has a low utility.

Recommendation 18
The ESPGHAN expert group recommends to NOT use FC in acute gastroenteritis to distinguish bacterial from viral gastroenteritis in children. (GoR: Moderate)
Agreement 100% MS 8.5

Appendicitis

Acute appendicitis (AA) may be missed at initial clinical examination in 28% to 57% of children ages 12 years or younger and in nearly 100% of children under 2 years old (186,187). Given these concerns, especially in the paediatric age group, a noninvasive and cheap screening tool would be extremely useful. Various markers that are products of the inflammatory reactions have recently been proposed, including procalcitonin, interleukin 6, interleukin 8, haptoglobin, granulocyte colony-stimulating factor, lactoferrin, and calprotectin but their role in diagnosing AA is still controversial (188). As AA primarily begins at the level of the mucosa, it is plausible that FC could have a diagnostic value in patients with suspected AA. This hypothesis was tested in a qualitative analysis using calprotectin-specific antibodies in the vermiform appendix. Strong immunostaining was recorded in specimens from patients with AA whereas no reaction was seen in uninflamed appendix (189,190). The accumulation of calprotectin-carrying cells in AA supports the study of FC as a new diagnostic tool in patients with suspected appendicitis.

Recently, favourable test performance characteristics of serum calprotectin for diagnosing appendicitis have been described. In children, Kharbanda et al reported that median plasma calprotectin levels were higher in appendicitis versus nonappendicitis, and it was also higher in perforated appendicitis compared with no perforated appendicitis. In the same study, at a cut-off value of 159 ng/mL, plasma calprotectin provided a sensitivity of 100% and a specificity of 27% to identify children at risk for AA (191). Nevertheless, a more recent study determined the serum calprotectin levels at the cut-off value of 670 ng/mL as 73.3% sensitivity and 100% specificity (192). Considering these data, the use of calprotectin alone for the diagnosis of AA has not been demonstrated to be more effective than classical inflammatory markers. For this, combinations of biomarkers, such as CRP, serum calprotectin, serum amyloid A-1, and white blood cells (WBC) have been proposed to improve the diagnostic accuracy of distinguishing AA from other causes of abdominal pain. A study in children suggested that a panel of biomarkers, including WBC, CRP, and serum calprotectin yielded a sensitivity of 96.5% (95% CI 92–99), a NPV of 96.9% (95% CI 93–99) with a specificity of 43.2% (95% CI 38–48). With these results, the authors affirm that the introduction of this panel of laboratory tests into the diagnostic process may reduce the use of abdominal computed tomography (CT) scans by a large percentage (193). These findings are comparable with the data of 2 more recent studies, in the APPY1 test, a biomarker panel including a mathematical combination of 3 biomarkers (WBC, CRP, and serum calprotectin) demonstrated a sensitivity of 99.1% and 100% (95% CI 94.4–99.9 and 95.9–100), and NPV of 98.6% and 100% (95% CI 91.2–99.9 and 89.9–100) for ruling out the disease, respectively (194,195).

Data on the role of FC in the diagnosis of AA are limited. The results of a recent study in the general population showed higher FC values in patients with infectious conditions compared with those with AA. Equally, higher levels of FC in patients with AA compared with patients without clinical diagnosis of either AA or AGE were found. ROC curve showed a close to 80% specificity and sensitivity of FC for AA at a cut-off value of 51 μg/g, AUC = 0.7 (189,190). Sarsu et al, however, reported that in the differential diagnosis of uncomplicated and complicated AA in children, the most accurate parameter was faecal lactoferrin with an AUC of 0.977. Whereas for FC, an AUC of 0.951 in complicated AA but an AUC of 0.669 in uncomplicated AA was found (192).

Statement 19
20(a) Serum and faecal levels of calprotectin are increased in AA.
20(b) For the diagnosis of AA, the use of serum and FC has not been demonstrated to be more effective than classical inflammatory markers.
20(c) Combinations of inflammatory biomarkers, including serum and FC, provided a good sensitivity but a low specificity in identifying children at risk for AA.

Recommendation 19
The ESPGHAN expert group recommends to NOT use FC, either alone or in combination with other inflammatory biomarkers, in screening children with abdominal pain for the presence of AA. (GoR: Moderate)
Agreement 100% MS 8.8

Helicobacter Pylori Infection

Only 2 studies addressing FC concentrations with *H pylori* infection in children were identified. In the first study, Hestvik et al (54) measured FC concentrations in 302 apparently healthy children ages 0 to 12 years in Uganda, and tested their faeces for *H pylori* with a rapid monoclonal antigen test as well as for enteropathogens and parasites. As FC concentrations were higher in children under the age of 4 years, only values in children above this age were used for the analysis of the influence of different demographic factors and pathogens. The difference between FC concentrations in *H*

pylori-positive (78 children, mean concentration 34 mg/kg, 95% CI 25–46 mg/kg) and *H pylori*-negative children (81, mean concentration 26 mg/kg, 95% CI 22–34 mg/kg) was not significant ($P = 0.12$). FC concentrations in children infected with *H pylori* were within the normal range. In contrast with the Ugandan study in symptom-free children, the second study by Sykora et al (196) was performed in children with abdominal pain-related FAPDs according to the paediatric Rome III criteria. They enrolled 56 children with abdominal pain (27 with functional dyspepsia) and the same number of healthy controls. The median FC concentrations were similar in *H pylori*-infected children (7.8 μg/g, 95% CI 7.8–8.4) including those with gastritis, and controls (9.1 μg/g, 95% CI 7.8–11.3).

Statement 20
HP infection either asymptomatic or associated with symptoms does not affect FC concentration.

Recommendation 20
The ESPGHAN expert group recommends to NOT use FC measurement for screening or follow up of *H pylori* infection or *H pylori*-related diseases. (GoR: Moderate) **Agreement 92.9%** MS 8.8 Abstentions: 1

Malnutrition

Obesity

Obesity is associated with a chronic low-grade inflammation, which originates and resides mainly in the adipose tissue. Elevated BMI, is associated with increased gut permeability through a variety of mechanisms, including altered bowel flora, the effects of circulating inflammatory cells and cytokines, or through direct effects of dietary fats on local cytokine production (197).

Spagnuolo et al measured FC in 34 obese children (198). They found increased FC in 16 (47%). Values ranged from 15 to 270 μg/g (mean value of 77 ± 68 μg/g), indicating a mild increase over normal. Individual values exceeded 100 μg/g in 12 patients (35%). A significant correlation was detected between FC and worsening obesity. It has recently also been reported that a distinct obesity-related microbial profile was associated with elevated FC levels (199).

Statement 21
FC may be mildly elevated in obese children.

Recommendation 21
The ESPGHAN expert group recommends to NOT use FC as a routine measurement in obese children if no other clinical condition relevant to FC measurement is suspected. (GoR Moderate) **Agreement 92.9%** MS 8.76 Abstentions: 1

Undernutrition

Severe acute malnutrition (SAM) in children is frequently associated with intestinal pathology and diarrhoea. A randomised controlled trial including 95 Malawi children ages 9 to 23 months showed that FC is markedly increased in SAM: mean 547 (SD 744) μg/g stools (200). Despite a moderate clinical improvement, FC remained high after the children were administered standard WHO feeds or elemental and polymeric feeds for up to 14 days. In an observational study, Versloot et al (201) followed 47 Malawi children ages 8 to 59 months. They assessed stool pathogens and FC at admission and after clinical stabilisation. FC was high in most children at admission and was higher in those still harbouring an infection (mostly parasitic) after clinical stabilisation: 383 μg/g (149–903 μg/g) versus 140 μg/g (71–300 μg/g). After clinical stabilisation, 40% of children had FC levels above age-specific cut-offs.

Statement 22
FC is elevated in children with SAM because of multiple factors and might remain high after different therapies addressing these factors, including after mild nutritional improvement.

Recommendation 22
The ESPGHAN expert group recommends to Not use FC for establishing therapeutic efficacy in SAM. (GoR: Moderate)
Agreement 100% MS 8.7

OTHER CONDITIONS

Necrotising Enterocolitis

Necrotising enterocolitis (NEC) is a severe inflammatory disease of the gut predominantly seen in preterm infants. During its early phase, FC is secreted into the intestinal lumen because of mucosal damage (202). To date, there is no rapid, noninvasive test to confirm NEC in its earliest stages, as imaging may be nonspecific.

Preterm infants with NEC have shown a significant transient early rise in FC compared with healthy infants of the same gestational age (203,204). When compared with gestational age-matched healthy infants, very low weight infants (<1500 g) who went on to suffer moderate NEC had significantly elevated FC levels 12 to 48 hours before the onset of clinical signs (46). The median FC levels in infants with NEC were between 210 and 402.2 μg/g stool versus 79.6 till 180 μg/g stool in healthy infants (204), and at a cut-off level of 286.2 μg/g stool, the sensitivity was 0.86 and the specificity was 0.93 (205). With regards to collection it should be noted, however, that the concentration of FC may be increased by 30% by the absorption of water in the diaper (47). FC levels were not statistically different in mild or severe NEC, and in fulminant NEC, FC levels were unusually low (46). Furthermore, there seemed to be no correlation between FC levels in moderate NEC and the need for surgery (46). Interestingly, in focal intestinal perforations, FC levels remained normal (47). The disadvantage of FC for predicting severe NEC is its high interindividual and

intraindividual variability (202). In addition, in 50% of infants with NEC, stool samples could not be obtained (205).

In summary, a rise in FC at the onset of abdominal distension appeared indicative of NEC and serial FC measurements seemed to be a useful screening tool for assessing risks and benefits of stopping enteral feeds (46). FC results in this context should be made available as rapidly as possible in order to allow timely and informed critical decisions to be made. On follow-up after NEC surgery, FC showed to be a good marker to monitor improvement of intestinal inflammation (47).

Statement 23
A sustained elevation or rising FC on serial measurements may indicate a risk of developing necrotising enterocolitis, and may thus be useful to predict it; successive FC measurement may also be useful for monitoring patient follow-up.

Recommendation 23
The ESPGHAN expert group recommends to consider using serial FC measurements as a noninvasive screening tool to alert to the risk of developing NEC. (GoR: moderate)
Agreement 100% MS 7.7

Intestinal Polyps

Colonic juvenile polyps (CJP) have a prevalence in the paediatric population that ranges between 0.08% and 3.7%. They are most frequently diagnosed in boys, between 3 and 10 years of age (84). CJP usually present with atypical symptoms, such as abdominal discomfort and painless rectal blood loss.

CJP are nonadenomatous structures characterised by high vascularity, ulceration, and the presence of tissue neutrophilia. Exfoliation of these latter cells into the stool may lead to increased FC. In keeping with this one would expect FC to return to normal once polyps are removed (47,206,207).

Until recently, only sporadic case reports/series have been published on children with CJP and elevated FC (206,207). Olafsdottir et al looked retrospectively at clinical data and endoscopy results of 266 children. They found CJP in 12 (4.5%). FC levels in these children (844; range 28–2287 mg/kg) and children with active IBD (962; range <20–7780 mg/kg) were similar ($P = 0.6299$), and higher than in children with normal colonoscopies (130; range <20–2443 mg/kg, $P < 0.0001$). Three months after polypectomy, FC (measured in 9/12 children) had decreased to 49 mg/kg (range <20–281, $P < 0.0078$) (84).

Statement 24
25 (a) Juvenile polyps are associated with increased FC, although normal FC does not exclude this diagnosis.
25 (b) FC as a screening test cannot differentiate CJPs from IBD.

Recommendation 24
The ESPGHAN expert group suggests to NOT use FC as a screening tool in children with a suspicion of intestinal polyps. (GoR;Weak)
Agreement 85.7% MS 8.4 Abstentions: 2

Short Bowel Syndrome

Short bowel syndrome (SBS) is defined as a spectrum of diarrhoea and malabsorption with associated complications (ie, bloodstream infections and small intestinal bacterial overgrowth [SIBO]) because of insufficient bowel length mainly resulting from massive small bowel resection because of NEC or congenital gastrointestinal malformations (eg, gastroschisis, intestinal atresia). SIBO and use of parenteral nutrition (PN) are considered as inducers of systemic or local inflammation concomitant with gut barrier dysfunction (208). In a study of 10 children with SBS because of NEC on parenteral nutrition (mean age 7.2 months), FC levels were significantly higher (median 309 μg/g; range: 205–786 μg/g) compared with healthy age-matched controls (median 61 μg/g; range: 45–214 μg/g). When further subdivided, children with SBS diagnosed with SIBO (breath test) had higher FC levels (median 394 μg/g; range 144–786 μg/g) compared with children with SBS without SIBO (median 154 μg/g; range 20–461 μg/g). FC levels did not have any significant correlations with the length of the remnant small intestine, blood cytokine levels or quantity of enteral feeds. In addition, a recent study of 50 children with SBS on parenteral nutrition showed that small bowel dilation was associated with higher FC (median 194 μg/g [range 76–400] vs 24 μg/g [range 11–157]) in the nondilated group and lower citrulline levels and more intestinal bloodstream infections and liver anomalies (137).

Statement 25
FC might be elevated in SBS children with SIBO.

Recommendation 25
The ESPGHAN expert group suggests to NOT use FC routinely in SBS children. (GoR Weak)
Agreement 100% MS 8.5

Small Intestinal Bacterial Overgrowth

There was no difference in FC levels in 58 children without previous intestinal disorders, or chronic diseases (eg, respiratory or urinary tract infections, or chronic autoimmune diseases, or intestinal surgery) affected by SIBO, as diagnosed by lactulose breath test compared with a control population of 60 healthy children (median 36.0 mg/kg, mean 43.0 ± 31.6 mg/kg and median 29.5 mg/kg mean 35.7 ± 20.7 mg/kg, respectively) (209).

Statement 26
There are no differences in FC levels between children with SIBO, compared with a control population.

Recommendation 26
The ESPGHAN expert group suggests to NOT use FC measurement for the diagnosis of SIBO in previously healthy children. (GoR: Weak)
Agreement 92.9% MS 8.3 Abstentions: 1

Autism

Using 2 independent markers of intestinal inflammation, that is, rectal NO and FC, there was no apparent inflammation found in a group of 24 consecutive children with autism (ages 3–13 years), except in 2 cases with CDI and severe constipation (210). In contrast, FC was found to be elevated in 24.4% of patients with autism and in 11.6% of their relatives, with a mean value of FC in these patients (159.7 ± 74.0 μg/g) indicating a mild degree of intestinal inflammation, although it did not correlate with abnormal intestinal permeability (IPT) (211). In a more recent study evaluating intestinal inflammation using FC in 61 children with autism and 50 nonautistic individuals with gastrointestinal symptoms, no differences were found in FC levels (111.10 ± 21.82 μg/g in autism vs 125.57 ± 27.36 μg/g in controls) (212).

Statement 27
Baseline higher FC levels are not more commonly found in children with autism.

Recommendation 27
The ESPGHAN expert group recommends to NOT use FC measurement in children with autism unless they have symptoms suggestive of conditions relevant to FC levels. (GoR: Moderate)
Agreement 92.9% MS 8.8 Abstentions: 1

Henoch-Schönlein Purpura

In 66 children with Henoch-Schönlein purpura (mean age, 7.5 ± 2.9 years) FC assessed during the first 3 days of disease onset was significantly higher in those with intestinal involvement—assessed by the presence of faecal occult blood (only found in 28% of them), gastric wall thickness and duodenal wall thickness (median 124.2 [interquartile ranges 430.7] μg/g vs 16.57 [interquartile ranges 17.8] μg/g in those without obvious intestinal involvement; $P = 0.01$). The median FC (50.3 [interquartile ranges 241.0 μg/g]) in the children with mild gastrointestinal involvement was lower than the group with more severe involvement [392 (interquartile ranges 524.6) μg/g; $P = 0.02$]. The FC concentration was a better indicator for the evaluation of gastrointestinal involvement than the faecal occult blood test (213). In a recent study of 40 children with Henoch-Schönlein purpura compared with 40 controls, FC >264.5 μg/g displayed a 93.1 sensitivity and 87.5% specificity for early diagnosis of intestinal involvement and also showed good performance for the follow-up (214). None of these authors discussed an influence of intestinal bleeding, which is frequent in these patients, on the FC levels.

Statement 28
FC is useful for identifying gastrointestinal involvement in children with Henoch-Schönlein purpura.

Recommendation 28
The ESPGHAN expert group suggests to consider using FC measurement to identify gastrointestinal involvement in children with Henoch-Schönlein purpura in the absence of overt bleeding. (GoR: Weak)
Agreement 100% MS 7.7

CONCLUSIONS

FC measurement has seen an unprecedented rise in utility as a marker of gastrointestinal disease, specifically inflammation. There are, however, many considerations and limitations, which need to be defined and acknowledged before FC measurement is used to influence clinical management. This article has attempted to clarify these and provide the best evidence-based recommendations or, wherever not possible, expert consensus, for the use of FC. From the outset, the authors stress that the use of FC should always be alongside and in support of good clinical judgement.

Limitations include variability in extraction methodology, performance of test kits, and the need to establish local reference ranges. The latter are hampered by significant variation at and across the paediatric age range and the influence of individual factors, such as age, diet, microbiota and drugs, accepting that much still needs to be elucidated in all these areas. There is no question that the main utility of FC measurement at present is in the diagnosis and monitoring of IBD, even over and above that of established serum inflammatory markers. It, however, lacks the finesse to differentiate UC from CrD or to ascertain the extent of the disease or therapy outcome. Measuring FC repeatedly may be useful in IBD patients with minor or absent clinical symptoms to not only confirm remission but also to suspect relapse and to consider re-evaluation or change of management.

With regards to other GI inflammatory and immune-mediated conditions, however, the value of FC measurement remains questionable. There appears to be little value in using FC as a diagnostic tool or prognostic marker of CMPA, or for the diagnosis or monitoring of CD. In children with cystic fibrosis, there is not enough evidence on a correlation between FC and enteropathy. A rise in FC concentration, however, may alert to the risk of developing necrotizing enterocolitis and in children with Henoch-Schönlein purpura and in the absence of overt bleeding, FC measurement helps identifying gastrointestinal involvement.

FC does not help to distinguish bacterial from viral AGE, is not useful to detect *H Pylori* infection or related disease or for the diagnosis of SIBO. It is no more effective than classical inflammatory markers in screening for AA. It cannot be considered as a screening tool in children with a suspicion of intestinal polyps.

Importantly, although FC may be considered as a tool to differentiate FAPDs from so-called organic diseases, it has not proved its value apart from identifying possible IBD within these common clinical presentations. This may relate to the fact that in conditions, such as the functional abdominal pain disorders, there is increasing evidence of a low-grade inflammatory process and much

still remains to be done in terms of phenotyping and classifying them. FC has neither utility in babies with infantile colic, nor to differentiate between functional and organic constipation.

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