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

Koninckx, Carmen Ribes

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Koninckx , C R , Donat , E , Benninga , M A , Broekaert , I J , Gottrand , F , Kolho , K-L , Lionetti , P , Miele , E , Orel , R , Papadopoulou , A , Pienar , C , Schappi , M G , Wilschanski , M & Thapar , N 2021 , ' The Use of Fecal Calprotectin Testing in Paediatric Disorders : A Position Paper of the European Society for Paediatric Gastroenterology and Nutrition Gastroenterology Committee ' , Journal of Pediatric Gastroenterology and Nutrition , vol. 72 , no. 4 , pp. 617-640 . https://doi.org/10.1097/MPG.0000000000003046

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Calprotectin in Pediatric gastrointestinal disorders. A position paper of the ESPGHAN Gastroenterology Committee

Authors GIC + invited contributors

Ribes Koninckx C. Department of Pediatric Gastroenterology, hepatology and Nutrition. La Fe University Hospital Valencia, Spain

Donat Aliaga , Ester Department of Pediatric Gastroenterology, hepatology and Nutrition. La Fe University Hospital Valencia, Spain

Benninga M.A. Department of Pediatric Gastroenterology and Nutrition, Emma Children's Hospital, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands
Broekaert, Ilse Julia, University Children's Hospital Cologne, Faculty of Medicine, University of Cologne, Germany

Papadopoulou Alexandra, Division of Gastroenterology and Hepatology, First Department of Pediatrics, University of Athens, Children's hospital «Agia Sofia», Athens, Greece

Gottrand Frederic, MD, PhD, Department of Pediatric Gastroenterology, Hepatology and Nutrition, CHU Lille, Univ. Lille, France
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Kolho Kaija-Leena, MD, PhD, Children´s Hospital, University of Helsinki, Helsinki, Finland and Tampere University, Tampere, Finland…

Miele Erasmo, Department of Translational Medical Science, Section of Pediatrics, University of Naples ''Federico II'', Naples, Italy

Orel Rok, Department of Gastroenterology, Hepatology and Nutrition, University Children's Hospital; Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Schäppi M.G. Paediatric Center, Clinique des Grangettes and Centre Médical Universitaire, Geneva, Switzerland
….

Thapar N BM PhD. UCL Great Ormond Street Institute of Child Health and Department of Gastroenterology, Great Ormond Street Hospital, London, UK; Queensland Children's Hospital, Brisbane, Australia

Abbreviations:

AA: Acute appendicitis
AD: atopic dermatitis
CD: Coeliac Disease
CDI Clostridium difficile infection
CMP: Cow's Milk protein
CMPA: Cow's Milk protein Allergy
CrD: Crohn´s disease
EEN: exclusive enteral nutrition
FC fecal calprotectin
GER(D): gastroesophageal reflux (disease)
GoR: grade of recommendation
IBD: inflammatory bowel disease
LoE: level of evidence
OR: Odds Ratio
PPI: proton pump inhibitor
RCT: randomized controlled trial
SIBO: Small intestinal bacterial overgrowth
UC: ulcerative colitis

Abstract

Objectives: To review the evidence of the value of fecal Calprotectin in different gastrointestinal disorders in children.

Methods: A literature search was conducted in the PubMed and Cochrane databases, end date being 31 August 2019. Subtopics were identified and each assigned to individual authors.

Results: A total of 30 recommendations were voted on using the nominal voting technique. Recommendations are given related to sampling, measurement methods and results interpretation.

Conclusions:

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. 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 (Fagerhol,1980). Variably named 'L1 protein', MRP-8/14, calgranulin and cystic fibrosis antigen (Sorg C 1992, Fagerhol MK,1996) it is present in tissues and fluids and especially abundant in neutrophils and monocytes (Dale, 1985). Calprotectin has numerous biological functions such as antimicrobial and antifungal activity (Steinbakk, 1990). It is resistant to enzymatic degradation and therefore preserved and easily measured in stools, a property which underlies its clinical utility.

In inflammatory diseases of the intestinal tract defined by mucosal neutrophil aggregation (Johne 1997) the activation and death of these cells releases high amounts of calprotectin into the intestinal lumen, which is then excreted in the feces (Hanai 2004). High fecal calprotectin (FC) levels are described in children and adults, in diverse pathological conditions such as: Crohn´s disease (CrD), ulcerative colitis (UC), cystic fibrosis, rheumatoid arthritis, bacterial infections, and gastric cancer (Johne 1997, Roseth AG 1993). FC levels correlate well with Indium white cell scans and also gut permeability measured by other means [Tibble J 9]. FC is considered a useful marker for intestinal inflammation, especially as it is non-invasive, stable, simple, easy to perform, rapid and reproducible. Despite this, little is known about the determinants of levels in normal subjects, and contradictory data on sensitivity and specificity are reported for different conditions.

The normal range for FC is considered <50 μg/g of feces by many manufactures of the test kits; however, although the size of the reference cohort is seldom reported, it is apparent that there is a great variability within healthy populations and specific effects from factors such as age. In children calprotectin levels are overall higher, especially below 5 years of age (Roca et al, 2017). This could be

related to increased permeability of the intestinal mucosa and differences in intestinal flora at early ages (Li F, Early Hum Dev 2014).

The aim of this paper is to review the evidence of the value of fecal calprotectin in different gastrointestinal disorders in children. Statements expressing the efficacy for diagnosis or follow up in different conditions have been formulated and agreed by consensus to support general conclusions and recommendations.

Methods

The literature search was conducted by 2 of the authors (CR and ED) in the PubMed and Cochrane databases, until 31 August 2019, firstly looking for all publications relating to fecal calprotectin OR calprotectin, followed by searching specifically for Calprotectin in the context of specific conditions namely: Coeliac disease, IBD, Functional Gastrointestinal Disorders, 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 basic science exclusively 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 pediatric populations or those including a mix of adults and children. Where no pediatric data were available adult studies were included. The final number of publications selected was 171 (Fig 1).

Papers from the total pool were classified according to subtopics and assigned to individual authors who were then provided with the abstracts related to his/her specific topic. Authors then completed the search for their specific question, as a

Kommentoinut [CRK1]: There were some papers were FC was only tangentially named in the manuscript but its efficiency was not really analyzed or discussed .
perhaps this wording is more appropriate

Kommentoinut [TN2]: May need to define what this means

result of which an additional 10 papers were added, taking the final number of included papers to 181 (Fig 1) .

Kommentoinut [CRK3]: including the Reflux guidelines

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 (Rachel Rosen, JPGN 2018;66: 516–554) recommendations were classified according to the quality of available evidence including the methodology and outcomes assessed.

We categorized the grade of the recommendation (GoR) as:
Strong: if there were adequately powered, prospective studies supporting the conclusions.
Moderate: if there were large retrospective studies or small prospective studies supporting the evidence.
Weak: if there were only retrospective studies or expert opinion supporting the results.

Strong or **Moderate** recommendations are formulated as '*the ESPGHAN expert group recommends to or not to* .....…(Strong / Moderate Recommendation)'.
Weak recommendations are formulated as '*the ESPGHAN expert group suggests to or not to .....…).*'(weak Recommendation)

The 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. Consensus was reached for all recommendations.

Kommentoinut [CRK4]: To be assessed after the Voting

Fecal Calprotectin measurement and Reference values

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

1. Collection and Handling of Stool Samples

In general, FC tests are performed on 50 mg to 100 mg of stool sample. Given that FC is reported to be evenly distributed in faeces (Roseth A, 1992), homogenization of the stool to be sampled from, or indeed the sample, is not thought necessary.

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 (Calafat et al 2015; Kristensen et al 2016).

The levels of FC seem to be influenced by stool consistency, but there are contradictory results reported by several authors (Anders Lasson, 2014; Shulman, 2008). For young infants the fact that the concentration of FC may be increased by 30% by the absorption of water in the diaper must be taken into account (E. Olafsdottir, 2002). If bowel cleansing is ongoing, the FC values show large variation hampering the interpretation of results. Thus, samples during bowel cleansing and even a few days after a lower bowel endoscopy should not be used (Kolho et al, 2012). It has been suggested, that both menstrual and nasal bleeding influence FC levels, by contaminating the stool sample (Fagerberg, JPGN 37, 468–472 (2003). However from a previous study in adults it was calculated that a blood loss ≥ 100 ml is needed to increase FC levels above the reference value (Roseth 1993); no similar studies in pediatric population have been found.

Once collected, FC appears stable at room temperature for up to seven days (Roseth et al 1997, Ton H, 2000) suggesting samples can even be sent to the laboratory by ordinary mail. Most analysis methods, however, recommend to keep fecal samples for up to 2-3 days at room temperature, 5-7 days in a fridge (4ºC) or frozen (-20ºC or -4ºC) for long term storage (3 months)(Ton H, Brandsnes, Dale S, et al. Clin Chim Acta 2000). 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 (Ton H, Brandsnes, Dale S, et al.. Clin Chim Acta 2000).

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 levels are affected by bowel cleansing.

1.c The absorption of water into the diapers/nappies may significantly increase FC concentrations in diaper collected feces**.**

Recommendation 1

The ESPGHAN expert group recommends

1(a) To collect samples for FC at any time of the day directly from the stool without any prior processing. (GoR: Moderate)

1(b) To keep the samples for FC measurement for a maximum of 3 days at room temperature prior to processing or alternatively 7 days if refrigerated immediately and up to 3 months if frozen. (GoR : Moderate)

1(c) NOT to obtain samples for FC measurement during or following bowel cleansing and/or lower endoscopy. (GoR : Moderate)

1(d) NOT to obtain samples for FC measurement from diapers/nappies. (GoR : Moderate)

2 Calprotectin Measurement

The first method for quantifying FC in stools was developed by Roseth in 1992 (Roseth AG, 1992). Improved and validated Enzyme-linked immunosorbent assays (ELISA) methods were developed later using better extraction techniques and measuring FC in mg/kg (Ton H, 2000). 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 (Sherwood R, 2015;

Radillo, 2016). The different tests are very sensitive for detecting mucosal inflammation, but major differences exist between specificity and absolute values.

Diverse extraction devices and methods have been tested and impact upon the stability of fecal extracts. Regarding rapid home tests, it has been shown that results from laboratory-performed extraction and patient-performed extraction correlate significantly (Kristensen V Lauritzen 2015; Kristensen V-Lauritzen, 2016). Bourdillon has shown that absolute FC concentrations measured by different kits, even produced by the same manufacturer, may not be comparable (G. Bourdillon The Routine Use of Fecal Calprotectin in Clinical Pediatric Practice: Almost there or Still Issues to Address ? Am J Gastroentrol 2013, 108 (11) : 1811-1813) . As a result it is highly advised to use the same extraction methodology and test kit for follow-up and disease activity monitoring in the same patient overtime (Bourdillon 2013, Prell, 2014).

Statement 2

There is considerable variability in extraction methodology and 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. (GoR: Strong)

3 Reference values

In early studies, the median stool FC concentration in healthy adults was 2μg/L with a suggested cut-off for a positive test of 10 μg/L. In newer assays, the suggested upper limit of normal has been increased by a factor of five, to 50μg/g

(Fagerberg et al., 2003). However, the test appears to have better diagnostic precision for IBD at a cut-off of 100 μg/g than at 50 μg/g (Konikoff & Denson, 2006). Several factors including age, gender, diet, microbiota and certain drugs may influence FC levels.

Recommendation 3.

The ESPGHAN expert group recommends

that the centres using FC as screening test for the presence of mucosal inflammation, should validate first locally, the generally suggested cut-off value and the normal range. (GoR: Strong)

3.1 Effect of Age and gender

Levels of FC are not related to gender. (Fagerberg et al., 2003, Roca et al, 2017). It is well recognized, that in a general population FC values are higher in children than in adults the most relevant results from the literature search being summarized 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 (Joshi 2010) . Various age grouping have been evaluated by different authors most studies suggesting to consider under 4 and above 4 years of age (Davidson & Lock 2017), In a pediatric cohort of > 7000 fecal samples obtained in clinical practice, there were no significant differences in. median FC values in children older than 1 year of age (Kolho et al, unpublished).

Davidson et al (Davidson & Lock 2017) assessed FC in 3 age range groups, with a median FC of 77 μg/g for children aged 1-3.9 years, 62μg/g for 4-17.9 years; and 61 μg/g for those older than 18 years. No significant differences were found between the 4-17.9 and >18 years age groups. Joshi et al evaluating pediatric 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 aged 10-59 years. (Joshi et al., 2010). In a study in South Korea the median FC concentration in samples from 234 healthy children aged between 6 months and 4 years was 245 μg/g (range 12-1033 μg/g, mean 68.5 μg/g, SD 123μg/g) (Song et al., 2017). These children were further analyzed by dividing them into six age groups showing a trend towards negative

muotoili: ruotsi (Ruotsi)

Kommentoinut [KK5]: as this is an important for clinical practice I attached a confidental picture of our data

Kommentoinut [CRK6]: Added by KK To be discussed

muotoili: englanti (Yhdysvallat)

muotoili: englanti (Yhdysvallat)

correlation between age and the FC concentration (the upper limit of 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 Chinese study (Li et al., 2015), studying 173 healthy children aged 1 to 18 months, also found a downward trend 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 analyze a bigger group of 274 children aged 1 to 4 years and found a median FC concentration of 83.19 μg/g (range 4.58 to 702.50 μg/g) (Zhu et al., 2016).

In more recent work Roca et al (Roca et al., 2017), developed a useful nomogram that was based on the results of a regression analyses. A total of 174 healthy children aged 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 95th percentile for FC in each group, were 910, 286 and 54 μg/g respectively. A high interdindividual variability was observed in infats below 1 year of age (Roca 2017) . Oord T (Oord T & Hornung, 2014) 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 - 3 years) and 75 μg/g mg/kg (3 - 4 years). Fagerberg et al (Fagerberg et al., 2003) had earlier suggested that the cut-off level for adults as recommended by the manufacturer' (<50μg/g) could also be used for children aged 4 years and older; they found in their study group of 117 children, that the median FC concentration was 13.6 μg/g.

FC levels depend on gestational and postnatal age and extreme preterm infants have particularly low FC levels (Zoppelli et al., 2012). Before the age of one-year and in premature babies, the levels may be significantly higher compared to healthy children, but there is a downward trend with increasing age (Josefsson et al, 2007; Oord and Hornung, 2014; Li et al, 2015; Peura et al, 2018). The FC levels observed in healthy preterm infants are higher than those reported for adults and children *(Kapel et al., 2010; Josefsson et al., 2007;* Campeotto et al., 2007; Yang et al., 2008; Nissen et al., 2004); means from 98-122 μg/g (SD

Kommentoinut [CRK7]: figures of FC for preterms have been added

between 68 -98μg/g) and medians from 150-253 μg/g (range <15 -1867) have been reported in preterms, age of the different study populations ranging from minimum 3-18 days to 1-8 weeks (Kapel 2010) . For full term newborns younger than 3 months, means from 145-277 μg/g (SD between 46 -109μg/g) and median from 167 - 269 μg/g (range 31 - 2880) have been published. Due to the wide variety in population size as well as in age range in the retrieved studies, no definite cut off can be established in preterms nor in infants younger than 1 year.

Statement 4

In infants younger than one year of age FC may be elevated without any known cause for inflammation.

Recommendation 4

The ESPGHAN expert group recommends

4(a)To take patient age into account when interpreting FC levels as a marker of intestinal inflammation. (GoR: Strong)

4(b) NOT to use a cut off level of < 50μg/g (as recommended in adults) for children younger than 1 years of age. (GoR: Strong)

4(c) To be cautious when using a cut off level of < 50μg/g (as recommended in adults) for children 1 to 4 years of age. (GoR: Strong)

Kommentoinut [TN8]: Can we recommend a different cut off for this age group?

Also should we recommend what cut off for abnormality we should use in the over 4 year old – are we suggesting 50 ….or 100?

Overall this section is very important and we need to provide clear consensus for each age group (although may do so for each disease área)

Kommentoinut [CRK9]: The issue is right now we can NOT recommend a cut off valid for **all methods** on the market and for all populations . If you look at our paper M Roca 2017, we propose a Normograme , but this is probably only valid for the used method

3.2 Effect of Geographical area and socio-economic status

In a rural population in Guatemala (Soto-Méndez et al., 2015; Soto-Méndez et al., 2017) median FC levels in children aged 2 to 7 years was 58 mg/g. In Uganda (Hestvik et al., 2011) 472 healthy children were evaluated with FC concentrations being 249 mg/kg in 0-1 year, 75 mg/kg in 1-4 years and 28 mg/kg in 4-12 years (n = 159). The authors concluded that FC concentrations amongst healthy children, living in rural areas or low-income countries, are comparable to those in

healthy children living in high-income countries. Liu JR (Liu et al., 2012), however, found significantly elevated FC in infants in children from rural China.

3.3 Effect of Diet

There are several studies that refer to the relationship between diet and FC, mainly comparing FC levels in breastfed infants with levels in those receiving mixed-feeding or only formula. Most authors found that FC was significantly higher in the exclusively breastfed group (Dorosko et al., 2008; Lee et al., 2017; Li et al., 2014; Savino et al., 2010) although others state that FC levels are not influenced by breastfeeding (Selimoğlu et al., 2012) nor by the type of formula (standard versus prebiotic supplemented formula) (Campeoto et al, 2004). In preterm infants Rouge found a positive correlation beween FC levels and the volume (ml/Kg/d) of enteral feeding needed .(Rougé et al., 2010).

In adults aged 50-70 years Poulis (Poulis A, 2004) observed a 10 % decrease in FC levels per daily portions of fiber consumed; he found an inverse relationship between FC fiber (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 pediatric 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
Not to follow any specific diet before sampling for FC (GoR: Weak)

Kommentoinut [TN10]: This section needs to be re-written as slightly confusing

Kommentoinut [CRK11]: DONE

Kommentoinut [CRK12]: No specified about the bulk of stools

3.4. Effect of concomitant Drugs

The effect on drugs on FC levels have been mainly studied in the adult population. Relevant findings are summarized in table 3. Adult patients taking 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 ulcerative colitis (UC) or CrD (Tibble 1999, NM, Davies1995) (Tibble 1). Of 90 patients (median age 9.1 years) with juvenile idiopathic arthritis, 40% complained about abdominal pain with one-third of these showing elevated FC values (>100 μg/g). For most of them FC values declined along with the discontinuation or reduction of NSAIDs. (K Aalto et al.2017). Although FC has been shown to significantly increase in adults after exposure to both ibuprofen (Goldstein *et al*.3) and celecoxib (Maiden 2005 *et al*.4)[3] the increase was much lower in the latter and some specific Cox-2 inhibitors show no increasing effect at all (Hawkey2008, Goldstein JL, Shah et 2001) (Table 2). The effect of NSAIDs on FC may be seen as early as a few days after the initiation of these medications (Davies 1995). FC has therefore been used in several publications as a marker of NSAID enteropathy. Accordingly it is recommended that patients on NSAID, who are assessed for a non-drug related inflammatory condition, cease taking the drug for 3 weeks before collecting a sample for FC measurement .

Kommentoinut [CRK13]: It is probably clearer now

Low-dose acetyl salicylic acid (ASA) (100mg/d, 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 (E SMECUOL et al 2009). The use of proton pump inhibitors (PPIs) has also been shown to be associated with higher levels of FC in an adult population not related to the presence of dyspepsia (Poulis 2003, Cohen , 2016). However, a recent prospective study in 51 children aged 3-18 years with gastrointestinal symptoms of whom 37 were treated by PPIs, failed to show evidence of intestinal inflammation related to PPI use through FC measurement (Kim, 2019).

Statement 7

Mildly elevated FC levels might be found in patients using any drug having an inflammatory effect on the GI tract.

Recommendation 7

Recommendation 7

The ESPGHAN expert group recommends

To be careful when interpreting mild elevated FC levels whilst a patient is on ASA, NSAIDS and/or PPI's (GoR: Moderate).

3.5. Effect of the microbiome

Levels of FC appear to associate with microbiome profile (Kolho et al, 2015; Quince et al, 2015)[1], with studies describing subsets of taxa most likely to be associated to the level of inflammation and, in turn, to levels of FC. In the study by Quince et al, 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. Regarding to the therapeutic response, the similarity and diversity of the microbiota to the controls increased in the anti-TNFα responder group. In contrast, after receiving exclusive enteral nutrition (EEN) the degree of dysbiosis increased but the operational units (OTUs) that correlated with FC (either positively or negatively) decreased (Quince et al, 2015)[51]. Thus, FC is a valuable surrogate marker of inflammation when exploring the role of microbiome in IBD.

Kommentoinut [CRK14]: Please paolo and Kaija can you respond to **next comment by Rok:**

The findings of different studies of microbiota in IBD were different. I do not believe that we can say that FC level reflects inflammation but very difficult that it reflects microbiota changes. Although microbiota is involved in IBD, it is quite possible that in a child with similar microbiota composition but without IBD would be normal (microbiota icomposition s definitely not the one and only cause of IBD).

Kommentoinut [CRK15]: Please paolo and Kaija can you respond **to next comment by Rok:**

I do not believe zhat changes in microbiota during enteral nutrition can be called dysbiosis. Disbiosis means bad (unhealthy) composition of bacteria, which is hardly true if these changes result in decreasing the inflammation (reduction in diversity does not necessarily means something bad).

3.6. Other factors

There is very scarce published information on the effect of environmental factors on FC with little reference to the pediatric population (Mendall, 2016). In a healthy general population of 300 adults, age range 50-70 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 CRP. The authors conclude FC levels are associated with lifestyle risk factors for colorectal cancer (Poulis A 2004).

FC levels in specific GI diseases

1 Inflammatory Bowel Disease (IBD)

1.1. FC in screening for IBD

FC is reported to be a better screening tool for the presence of IBD in undiagnosed patients than blood inflammatory markers such as C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR) (Levine et al, 2014).. FC could thus help to select those children in whom IBD needs to be evaluated (Holtaman 2016, Sridhar M 2019)

The reported sensitivity and specificity of the FC test, in line with negative and positive predictive values, depends on the studied patient cohort, including the number of patients with causes other than IBD that could result in elevated FC values (e.g. bacterial or viral gastroenteritis (Chen et al, 2012) or juvenile polyp (Olafsdottir et al, 2016; Zijlstra et al, 2016). A thorough review (Degrauwe 2015)[9], found the best cut-off value in IBD screening for abnormality to be 212µg/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 (for review e.g. Henderson et al, 2014[10]; Sipponen and Kolho, 2015[11]). 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 in suspicion of IBD the cut-off should vary according to age.

To date, there is only a single published study on the cost-effectiveness of FC measurement to identify adults and children who require endoscopy examination compared to direct endoscopy evaluation alone to assess presence of inflammation (IBD). A pre-test probability of IBD less than 65% made FC screening cost-effective but where it is more than 78% it is cost ineffective given the delay in reaching the diagnosis in evident cases while waiting for the results of FC measurement (Yang et al, 2014).

Kommentoinut [KK16]: No data or studies that have assessed cut-offs for IBD in different age groups

Kommentoinut [TN17]: NEED TO PROVIDE CLARITY FOR EACH AGRE GROUP REGARDING THE VALUE

Kommentoinut [CRK18]: TO Kaija

ROK comment
I do not understand, what this sentence wants to say!

Kaija, do you think you can modify the writing so as to make it clearer ??

FC reflects the mucosal influx of inflammatory cells (mostly neutrophils and monocytes). Therefore, disease activity is the most important denominator of the observed levels. Disease extent as such does not necessarily correlate with levels, i.e. 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 (Kolho et al, 2006).

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 pediatric IBD, UC and CrD patients were both included and there were no major differences in the performance of FC between the patient groups (Bunn et al, 2001a; Bunn et al 2001b). A multicenter study on pediatric CrD showed good performance of the test in pediatric patients with Crohn´s colitis or terminal ileal disease (Shaoul et al, 2012), confirming the previous findings of comparable levels in UC and CrD (e.g. Aomatsu et al, 2011). In CrD without colonic involvement, elevated levels of FC may indicate active disease in the small intestine (Kolho et al, 2006; Shaoul et al, 2012; Klang E et al, 2017; Aggrawal et al, 2017). Isolated upper gastrointestinal CrD is rare, and we did not find any pediatric reports on the performance of FC in such cases. Based on adult series, the levels of FC may be low in patients with CrD limited to the ileocecal valve and showing non-inflammatory behavior (Sipponen et al Alim Phar Ther 2008). 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 (Bunn et al, 2001a; Bunn et al 2001b; Fagerberg et al, 2007; Canani RB, et al, 2008; Diamanti et al, 2008; Hradsky et al, 2014) but the absolute levels, as such, cannot be used to categorize disease activity (e.g. high, moderate or low unless endoscopy is performed. In UC, only few are in complete remission according to FC (Sipponen and Kolho 2010; see below). Although normalization 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, i.e. 500 (van Rheenen 2012) or 800 ug/g (Kolho and Turner, 2013). In acute severe colitis, the absolute level is not associated with prognosis and cannot be used to anticipate therapeutic response (Turner et al,

Kommentoinut [CRK19]:
ROK comment
What this sentence means?
Probably this sentence can be revritten in a more clear way.

2010). In pediatric patients on infliximab therapy, a cut-off of > 250 µg/g is associated with risk of clinical relapse within three months (Foster et al WJG 2019).

Other neutrophil-derived markers of IBD such as e.g. 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 (Turner et al, 2010; Kolho et al 2014; Kopylov et al, 2014).

Statement 8.

8(a) FC is a better indicator of the possibility of IBD than blood inflammatory markers. Where IBD is strongly suspected a FC test is not needed for screening as endoscopy is mandatory.

8 (b) The degree of elevation in the levels of FC cannot be used for differential diagnosis of IBD from non-IBD causes of inflammation or to differentiate ulcerative colitis from Crohn´s disease or to ascertain the extent of disease.

8 (c) In acute severe colitis, the level of FC is not associated with therapy outcome.

Recommendation 8

The ESPGHAN expert group recommends

8 (a) To consider FC measurement if differential diagnosis of IBD from functional GI diseases is needed, but not in the evident IBD, in which endoscopy is mandatory. (GoR: Strong)

8 (b) Not to use FC as a prognostic marker in acute severe colitis.

(GoR: Strong)

1.2. Fecal calprotectin in the follow-up of IBD patients

Kommentoinut [CRK20]:
Rok comment
This is based on one study. So it would be better to say: “Foster et al found that in pediatric patients on infliximab therapy FC> 250 µg/g was associated with increased risk for clinical relapse within three months.”

Kommentoinut [MOU21]: The above paragraph is not just about outcome

Kommentoinut [CRK22]:
ROK suggests
I believe that there is a need for another twostatements:
In some cases (eg. Ulcerative proctitis) FC levels can be normal even with localized active inflammation.
There is not enough data about FC levels in CrD patients with isolated upper GI involvement (esophagus, stomach duodenum).

Kommentoinut [CRK23]: Please paolo and Kajia . review and correct this R
DONE

Kommentoinut [MOU24]: NOTHING IN THE TEXT TALKS ABOUT THIS
DONE

Kommentoinut [CRK25]:
Kaija , Paolo I Made some changes Just to homogenize the format and wording of the Recommendations . Do you agree ¿

Kommentoinut [CRK26]: Similar Recmmendation in FGID
Propose to modify this one into:

Not to use FC in the differential diagnosis of evident IBD , in which endoscopy is mandatory.

FC has provided an additional tool for monitoring pediatric IBD patients. Both in adults and children FC levels have been shown to correlate with disease activity as defined by clinical parameters, endoscopic findings and histology (Fagerberg et al, 2007; Canani RB, et al, 2008, Schoepfer AM,. Inflamm Bowel Dis 2009; Tibble JA, Gastroenterology 2000, Ashorn S, Honkanen T, Kolho KL, et al.. Inflamm Bowel Dis 2009;[31, 33, 37-39]) However, more recently, Melmed did not find any correlation between capsule endoscopic scores and biochemical inflammatory parameters including FC (Melmed 2019). However, if no findings in endoscopy, the frequency of detecting lesions in capsule endoscopy increases along increasing FC values as shown in adults (FC < 50 µg/g 10%, FC 50-100 µg/g 25%; FC >100 µg/g 62%; Egea-Valenzuela et al, 2016). With a rapid-onset therapeutic agent, the FC levels may drop back into the normal range within two weeks (Kolho et al, 2006; Hämäläinen et al, 2011), although with exclusive enteral nutrition (EEN) the decline may be less clear and occur over a longer period of six to eight weeks of therapy (Gerasimidis et al 2011, Grogan et al, 2012; Frivolt et al, 2014; Levine & Turner et al, 2014; Copova 2018). However, the reduction of FC values during EEN. is lost rapidly after food re-introduction (Gerasimidis et al 2011; Logan et al APT 2019 Sept). Intriguingly, partial enteral nutrition with exclusion diet (Levine et al, Gastroenterology 2019) or a personalized exclusion diet (Svolos et al Gastroenterology 2019) 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 (Kolho and Sipponen 2010; Turner et al, 2018 (Ruemmele et al, 2014; Zubin and Peter, 2015). A recent report on magnetic resonance imaging enterography-based follow up of pediatric patients with CrD reported a FC cut-off below 300 ug/g to identify children with mucosal healing but the FC cut-off below 100 µg/g used in screening for IBD to identify children with deep healing (Weinstein-Nakar et al 2018). The reports on FC values in predicting a disease flare (in conservatively treated disease) show values ranging from 400 to 800 ug/g in pediatric CrD and UC (Walkiewicz et al, 2008; van Rheenen 2012; Kolho and Turner, 2013) but as low as 300 ug/g in adult UC and CrD (Theede K et al, 2016; Ferreiro-Iglesias et al, 2018). However, FC levels start to rise before clinical or endoscopic relapse as shown in a group of adult IBD patients with FC–based, endoscopy confirmed remission concluding that FC may be used for identifying patients requiring close follow-up in clinical practice (Molander et al, 2015).

Kommentoinut [CRK27]: Please Kaija , could you check if these are the rigth references

muotoili: Fontti: Helvetica, 11 pt, (Aasialainen) japani, (Muu) ruotsi (Ruotsi), Ei oikeinkirjoituksen tai kieliopin tarkistusta

Kommentoinut [MOU28]: Such as?

Kommentoinut [CRK29]: Kaija, Paolo, could you answer this ¿

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 may be individual.

Recommendation 9

The ESPGHAN expert group suggest recommends

To include routine FC measurement at least once a year in the laboratory investigations of IBD patients during follow-up, including those in remission. (GoR: Weak)

1.3. Fecal calprotectin in the follow-up of Crohn´s disease patients after intestinal resection

FC measurement has also been reported to provide a non-invasive means to follow up pediatric IBD patients after intestinal surgery (Pakarinen et al, 2010; Hukkinen et al, 2016; Amil Dias et al, 2017). 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 (Amil Dias et al, 2017).

Statement 10

FC measurement may help establishing the right timing for performing a follow up endoscopy after intestinal resection

Recommendation 10

The ESPGHAN expert group recommends

To measure FC at least twice during the first year after intestinal resection, to identify an early relapse requiring endoscopic evaluation. (GoR: Moderate)

1.4. Fecal calprotectin in post-colectomy patients

In post-colectomy patients with J-pouches, FC levels increase in the presence of pouchitis. Interestingly, in patients with recurrent pouchitis, the levels of FC were higher in a cross-sectional study of 32 pediatric patients after restorative proctocolectomy compared to patients with no history of pouchitis or reporting a single episode (Pakarinen et al 2010). 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 in adults (Schoepfer et al, 2015).

Kommentoinut [CRK30]:
ROK coment
Why “interestingly”? It’s “logically”.

Statement 11.

FC 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. When FC levels are low, the likelihood of inflammation is low.

Kommentoinut [CRK31]:
ROK comment
This word needs prior explanation, because it is not well-known.

Recommendation 11.

The ESPGHAN expert group recommends

To use FC levels after colectomy to detect pouchitis and inflammation at the anastomosis (GoR: Moderate)

1.5. Fecal calprotectin in perianal disease

The data on FC in perianal disease is sparse (Hukkinen et al 2014). Seton drainage in combination with infliximab therapy is used with the same indications as in grown-ups (Amil Dias et al, 2017). FC values decline along healing when elevated at management initiation and increase in relapse (Hukkinen et al, 2014).

Kommentoinut [CRK32]:
ROK comment
Something is missing!!!

Statement 12

In perianal disease, FC may be used in the follow-up of therapeutic response accordingly as most patients exhibit elevated values at therapy initiation.

Recommendation 12

The ESPGHAN expert group recommends

To use FC also in the follow up of perianal disease. (GoR: Moderate)

1.6. Fecal calprotectin, e-Health and home-monitoring in IBD

Kommentoinut [CRK33]: This heading has been removed

2 Functional Gastrointestinal Disorders

The revised Rome IV criteria provide symptom-based guidelines by which functional gastrointestinal disorders (FGIDs) can be diagnosed in children from 0 to 18 years of age. (Benninga & Faure, 2016; Hyams JS, 2016) FGIDs include a variable combination of often age dependent, chronic, or recurrent presumed or definite gastrointestinal symptoms, such as infant colic, regurgitation, abdominal pain, diarrhea and constipation not explained by structural or biochemical abnormalities.

Kommentoinut [CRK34]:
ROK comment
What are presumed symptoms? Symptoms are allways subjective and reported by patient/family, while signs are objective (can be observed by observer) Eg.: pain is a symptom, while stunted growth is a sign.

2.1 Infant colic

In infant colic as defined by the Rome IV criteria (Childhood Functional gastrointestinal Disorders: Neonate/Toddler Benninga MA, Faure C, Gastroenterology. 2016) the underlying pathophysiological mechanisms are not well understood although neurogenic, gastrointestinal, microbial, and psychosocial factors have been proposed (Benninga & Faure, 2016) Another possible candidate mechanism is gut inflammation, although, inconsistent data exist regarding this. FC was measured by an ELISA kit in spot stool samples of 76 infants diagnosed with infant colic and compared with 27 healthy infants (Olafsdottir E, 2002). 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

Kommentoinut [CRK35]: Marc do you agre with skiping the definition ¿?

with age (p = 0.04). In contrast other studies have shown elevated concentrations of FC in infants with colic. (Rhoads,2009; Sung 2013, Rhoads et al J Pediatr 2018;203:55-61) Roads et al. 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 to control infants (413 ±71 vs 197 ± 46 µg/g, P = .042). Rhoads,2009; [4] Sung et al. reported that FC levels after 1 month òf treatment with either *Lactobacillus reuteri* (treatment group n=31) or placebo (n=34) were similar. However, infants in both groups with at least a 50% reduction in duration of crying or fussing at 1 month had significantly lower FC levels than non-responders (responders n=50, non-responders n=52, mean difference 96.6 mg/kg, 95% confidence interval 5.1 to 188.1, p=0.04) (Sung 2013) . 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 14

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

Recommendation 14

The ESPGHAN expert group recommends

NOT to use FC in babies with infantile colic. (GoR: Moderate)

2.2 Functional abdominal pain

Since 2016 the Rome IV criteria recognizes 4 different abdominal pain related Funtional gastrointestinal disorders (FGIDs); functional dyspepsia, irritable bowel syndrome, abdominal migraine and functional abdominal pain not otherwise specified. (Hyams 2016) The etiology and pathogenesis of these FGIDs 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. (KorterinK 2015)

Differentiation between abdominal pain-related FGIDs and IBD is sometimes difficult, because symptoms can be non-specific and frequently overlap. Olafsdottir et al. compared 19 children with recurrent abdominal pain as defined by Apley and Naish (mean age 11.5 y) with 17 children with IBD (mean age 11.1 y) of whom 10 had CrD and 7 UC as well as with 24 healthy children (mean age 5.3 y) Olafsdottir E, 2002; Apley J 1958. Children with IBD had FC levels (293 ± 218 mg/kg) 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. (Flagstad 2010, Pieczarkowaki 2016) Eighty-three of 126 Norwegian children with the 4 different abdominal pain-related FGIDs (66%) had FC concentrations below the detection limit of 16 μg/g while nine (7%) had levels between 50 and 100 mg⁄kg (five IBS, two functional abdominal pain and two functional dyspepsia), and three had levels above 100 μg/g (one aerophagia, one functional abdominal pain, one functional constipation). Flagstad 2010[9] There were no significant differences in median concentrations between the different abdominal pain-related FGIDs. In the Polish study in all 22 healthy controls and all 33 patients with FGIDs, FC levels were below 100 μg/g, whereas in most patients with IBD the FC were markedly above the cutoff value (median level 1191.5 μg/g; 25–75 percentile range: 265.2 μg/g–1684.9 μg/g). (Pieczarkowaki 2016 [10]) 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 μg/g vs 43.2 ± 39.4 μg/g (P<0.01).(Shulman RJ 2008)11 Moreover they showed that FC levels correlated with pain-related interference of daily activities (P=.01).

Di Nardo et al. 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.(di Nardo, 2014) In addition they showed that this close spatial association was related to intensity and frequency of abdominal pain. However, and in contrast with the study by Shulman et al., 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.(di Nardo, 2014)

Lastly, Diederen et al. investigated the prevalence of IBS-type symptoms in children with IBD in clinical remission (Diederen K, 2016).[13] A total of 184 patients (92 female; 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 <250 μg/g, this cut-off being predictive of endoscopic disease activity (Bremner, 2005).14 No difference in FC or CRP was found between patients in clinical remission with or without IBS-type symptoms (FC: IBS+ median 58 μg/g, IBS-221μg/g, P = 0.12; CRP: IBS+ median 1.4 mg/L, IBS-1.1 mg/L, P = 0.63). Based on these findings the authors suggested that persistent symptoms in children with IBD in remission appear to be unrelated to ongoing inflammation. Interestingly, IBD patients with IBS-type symptoms were less frequently using IBD-related medication, compared to those without IBS-type symptoms which may imply that anti-inflammatory treatment may prevent or reduce IBS-type symptoms.

Statement 15

15 (.a) FC levels in children with abdominal pain-related FGIDs are similar to healthy controls..

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

Recommendation 15

The ESPGHAN expert group recommends

that FC may be considered as a tool to differentiate functional abdominal pain disorders from organic diseases. (GoR; Strong)

Kommentoinut [CRK36]:
ROK comment
Isn't it too broad definition. Are you sure that for example eosinophilic gastroenteritis (it's definitely an organic disease) results in elevated FC level?

2.3 Constipation

Constipation is characterized by infrequent evacuation of hard and painful stools, frequently accompanied by fecal incontinence and/or abdominal pain. In

more than 95% of constipated children, no organic cause can be found and affected children and are defined, according to the Roma IV criteria as functional constipation. Benninga & fare, 2016; Hyams JS, 2016)[2] Although the exact pathophysiological mechanisms underlying functional constipation are unknown, gut inflammation has not been associated with functional constipation.

FC levels were measured in stool samples from 100 children aged 5–17 years.(Molsi 2015) No differences in FC levels were found between healthy controls (median 15.6μg/g, range 15.6–39μg/g, , n=7, p < 0.0001) and children with constipation (median 15.6μg/g, range 15.6– 63.1μg/g, n=31, p <0.0001). Children with IBD had significantly higher FC (median 336.6μg/g, range 22.4–1596, n=43) compared to these two groups. Of the children with constipation, 3/31 (9.7%) had FC ≥ 50 μg/g, but none of these children had a FC ≥ 200 μg/g. These data were confirmed by another study including 76 children, age 1-120 months, suspected of Hirschsprung's disease.(Mahjoub 2013) In the 19 patients diagnosed with Hirschsprung's disease, the median FC concentration was 20μg/g (under 0.5 to 106.0 μ/g), whereas in the 57 children with functional constipation, the median was 4 gμ/g (under 0.5 to 110.8 μg/g).

Kommentoinut [CRK37]:
ROK comment
That is so logically, that is stupid to mention (in the chapter about constipation).

Marc do we need this sentence ??

Statement 16.

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

Kommentoinut [KK38]: there were 9% with raised values in one of the studies referenced above

Kommentoinut [CRK39]: please Marc , your comment to KK -

Recommendation16.

The ESPGHAN expert group recommends

NOT to measure FC in children with functional constipation. (GoR: Moderate)

3. COW´S MILK PROTEIN ALLERGY (CMPA) / Food Allergy

In the developed countries, an estimated 5%–10% of children suffer from food allergy, mainly caused by allergen specific IgE (Osborne 2011, Gupta 2011). ~~C~~cow's-milk protein allergy (CMPA) being ~~is~~ the most common food allergy in infants and children younger than 3 years (Sicherer 2011, Rona 2007). 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 (Koletzko 2012).
FC has received attention due to its altered levels in children with food allergy. (Waligora-Dupriet, 2011)

In 2011, Waligora-Dupriet et al (Waligora-Dupriet, 2011) reported that the concentrations of FC in infants with food allergy were 2-fold higher compared with healthy controls (7- Waligora-Dupriet, 2011). Later, Beser et al (7 Besse OF, 2014) evaluated in a randomized 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 to 39 healthy controls (mean age 11.5 months). Infants with non-IgE CMPA had higher FC concentrations compared to healthy controls (886±278 μg/g vs 296±94 μg/g respectively; p<0.001), as well as to infants with IgE-mediated CMPA both at baseline (886±278 μg/g vs 392±209 ìg/g respectively; p=0.025) and after the elimination diet (359±288 μg/g vs 218±90 ìg/g, respectively, p=0.001). In contrast, a very recent study (Diaz et al 2018) in a small group of 17 infants with non-IgE –mediated CMPA aged up to 2 years, showed that FC levels in these patients were not different compared to 10 age-matched healthy controls: median (range) FC were 47.25μg/g (28.80–106.10) vs 68.40μg/g (30.38–76.73) respectively, p=1.00.
Merras-Salmio et al (Merras-Salmio, 2014) assessed FC during elimination diet and after double blind placebo controlled food challenge (DBPCFC), in 55 Finnish infants and young children of a median age of 8 months with gastrointestinal symptoms attributed to CMPA (such as excessive crying or fussiness, vomiting or loose stools). Only 32% of the patients (median age 8.4 months) had positive DBPCFC's. The authors reported that FC levels were higher in the challenge positive group (n = 18) than in the negative (n = 37), with respective geometric

Kommentoinut [CRK40]:

Are there any epidemiologic data showing that IgE mediated food allergies are more frequent that non-IgE mediated

Kommentoinut [CRK41]: Alexandra , I suggest to delete this part of the sentence ; otherwise the reading is a bit difficult and we should give more details about IgE and non IgE mediated ....
Do you agree ?

Kommentoinut [CRK42]: somehow contradictory with above paragraph

means during cow's milk free diet of 55 µg/g (95% confidence interval 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) *vs* 60µg/g (30-122) respectively; p=0.5995, while 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 (Baldassarre, 2010) 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 hematochezia 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 to healthy controls (157.5 +/- 149.13 *versus* 93.72 +/- 36.65 µg/g, p = 0.03). Interestingly, a significant decrease in FC was also observed in the control group.

Winberg et al (Winberg, 2016) assessed FC and eosinophil-derived neurotoxin levels at diagnosis and after a 3-session DBPCFC in 12year old children from a population-based cohort, reporting complete avoidance of milk, egg, cod or wheat, due to perceived hypersensitivity to these foods, manifested as eczema, urticaria, vomiting, diarrhea or flatulence. Six of the above patients had a positive and 6, a negative DBPCFC. Both at baseline and post challenge, the FC levels in children with a positive DBPCFC tended to be higher compared to children with a negative DBPCFC although the difference did not reach statistical significance: median FC (µg/g stool) at baseline was 25.8 vs 16.45 respectively, p = 0.150 while, after challenge 24.10 vs 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 (10- Winberg, 2016).

Seo et al compared fecal calprotectin levels in children with atopic dermatitis (AD) according to the severity of the disease (11- Seo , 2017). Fifty-five (84.6%) children had mild-to-moderate AD with Scoring Atopic Dermatitis (SCORAD) index < 40, while 10 (15.4%), had severe AD (SCORAD ≥ 40). The geometric mean (range of 1 SD) fecal calprotectin levels in severe (SCORAD 53.0 ± 11.8) AD was significantly higher than that in mild-to-moderate (SCORAD 19.6 ± 9.9) AD: 66.7μg/g (13.5-330.3) vs 29.4μg/g (10.1-85.6); p=0.044 (11- Seo , 2017).

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 (OrivuoriL, 2015, Kukkonen 2010). Orivuori et al (12-OrivuoriL, 2015) 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 to 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 to the non-farmers' children [FC 181.81 (101.38/308.09) vs 156.27 (76.86/261.30) respectively; p= 0.003] ; in the children with one or more siblings [FC 165.97 (87.02/326.82) and 185.89 (109.92/329.46), respectively], when compared to the children without siblings [FC 140.01 (77.26/229.03); p< 0.001] ; and in breastfed children (exclusively and partially breastfed) when compared to non-breastfed children [FC 180.27 (106.03/307.97) and 149.57 (81.88/318.07), respectively vs 104.93 (63.78/192.14); p< 0.001]. Because the distribution of FC levels was skewed, the authors evaluated the importance of high FC levels (above the 90th percentile) that indicated high degree intestinal inflammation. The infants with FC levels at 2 months >90th 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 to infants (n=80) who had FC levels <90th percentile (FC ranging from 39.2 to 490.3 μg/g). Only thirty-nine of 75 (52%) children who had FC above the 90th percentile were from a farming environment indicating that

Kommentoinut [CRK43]: Alexandra do you want to answer ROK comment
That are really very interesting results! Factors like living in a farm, having a sibling and beeing breast-fed are regarded more protective than a risk factors for allergy development. Therefore, you can expect either lower FC in these groups or low-grade intestinal inflammation in this early age (see later cyted Finish study) somehow protexts to allergy development (for example by inducing a tolerance)? However, the next sentence tells that infants with higher FC were at an increased risk for atopic diseases.

high levels of FC were not explained by the farming environment (OrivuoriL, 2015). In the same study, only the very high levels of FC in the whole cohort, above the 90th 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 randomized DBPC allergy-prevention trial in 237 infants in Finland (Kukkonen K, Kuitunen M, Haahtela T, Korpela R, Poussa T, Savilahti E. High intestinal IgA associates with reduced risk of IgE-associated allergic diseases. Pediatr Allergy Immunol.2010), using a combination of four probiotic strains pre-natally and during the first 6 months from birth, reported that high FC concentrations at the age of 6 months were associated with a tendency to reduce the risk of having any allergic disease up to the age of 2 years [odds ratio (OR: 0.52)] and with a significant reduction of the risk for any IgE-associated atopic diseases (OR: 0.49). The mean FC concentrations at 3 months of age was 180 μg/g (95% CI 154-212μg/g) in infants who developed allergic diseases up to the age of 2 years compared to 152μg/g (95% CI 12729--18343μg/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) compared to 36 μg/g (95% CI 127-183) respectively, p=0.357. The authors reported a significant correlation between fecal IgA and fecal inflammation markers (alpha1-AT, TNF-alpha as well as FC; p < 0.001) concluding that minimal intestinal inflammation indicated a reduced risk for IgE-associated allergic diseases.

Kommentoinut [CRK44]:
ROK comment
There must be something wrong with these numbers (no statistical difference between the groups) and conclusions

Kommentoinut [CRK45]: Please Alexandra , this needs rewriting !!

Statement 17

FC levels show considerable variability in children with atopic diseases making difficult to draw definitive conclusions regarding the efficacy of this test in diagnosis or management., Further studies in the field are needed.

Kommentoinut [CRK46]: suggest to delete this part of the Statement .

Recommendation 17

The ESPGHAN expert group recommends

Not to use FC either as diagnostic tool or as prognostic marker of atopic disease in children (GoR: Moderate)

4. Celiac disease

Limited data is available about FC in children with Celiac Disease (CD). The published literature in this area is scarce and heterogeneity in the detection methods used makes conclusions difficult to obtain.

FC values are significantly higher in CD patients at diagnosis especially in those with higher levels of serological markers or classical symptoms (Biskou 2016, Balamtekin 2012), however, no correlation was found either with histological findings or with anti-transglutaminase antibody levels (Biskou 2016). Although FC values were not very high (on average around 100 µg/ g) 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 (Canani 2008, manguso F 2004, Biskou 2016, Rajani S 2016). 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 (*Balamtekin et al.; 2012*). 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 18 FC is elevated in CD patients at diagnosis, but the individual variability is high .

Recommendation 18

The ESPGHAN expert group recommends

Not to use FC as marker for the diagnosis or monitoring of CD. (GoR: Moderate)

5. Cystic Fibrosis

CF is the most common cause of exocrine pancreatic insufficiency in children and is treated with pancreatic enzyme replacement therapy (PERT). In Cystic Fibrosis (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 steatorrhea 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 colonization in the intestine (Norkina 2004). Whole gut lavage of CF patients has shown increased immunoglobulins and inflammatory biomarkers such as IL-8 in the stool as compared to controls (Smith RL, 2000). 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 Enteropathy" (Werlin 2010). Nonetheless, CF patients generally do not experience the symptoms associated with intestinal inflammation as those observed in IBD.

Kommentoinut [CRK47]: I suggest rewriting : **do not experience symptoms as severe as those observed in IBD** or **symptoms experienced by CF children are less severe tnan those observed in IBD**

FC has been determined in several studies in CF. Bruzesse et al. showed FC to be elevated in 27 of 30 children with CF (4 Bruzzese E 2004). In 10 patients, FC normalized after treatment with Lactobacillus GG suggesting that bacterial overgrowth is a possible etiology of CF enteropathy. Werlin et al showed CF enteropathy in both PI and PS patients but FC was markedly raised only in the PI patients (Werlin 2010). In a subsequent study (Rumman N, 2014) FC was found to be equivalently elevated in PI and PS patients supporting the concept of a generalized CF enteropathy unrelated to exocrine pancreatic status. In an Australian study, however, Dhaliwal et al. reported increased FC in PI patients but examined only 6 children with PS (Dhaliwal 2015). 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). However, after 4 years of age there was an increase in FC in both PI and PS indicating generalized CF enteropathy (Garg M 2017). However, Ellemunter et al reported in 171 patients in a longitudinal study over a median observation period of 7 years increased FC in PI >PS but unlike the Australian study did not find reduced levels in the younger age group (Ellemunter H).

In another study Adriaanse et al. reported FC levels were elevated in 40 CF patients (93%) with higher values in PI compared to PS. Interestingly, FC correlated positively with age ($r = .321$, $p<0.05$) while no association was found with gender ($p = 0.67$). FC levels correlated inversely with lung function in CF patients (FEV1 $r = -.428$, $p<0.05$) . Dividing the study population into children and adults revealed a significant inverse correlation between FC and lung function in adult CF patients (FEV1 $r = -.484$, $p<0.05$; FVC $r = -.304$, $p = .207$; FEV1/VC $r = -.509$, $p<0.05$, $n = 19$), while no correlation was found in children (9 Adriaanse MPM , 2015). This was not confirmed ?by other studies (Rumman N, 2014, Ellemunter H)

Since it is thought that intestinal inflammation influences nutritional status negatively, the relationship between FC level and nutritional status was assessed. In CF children, weight-for height Z-score was positively correlated with FC ($r = .531$, $p<0.05$, $n = 23$), whereas no significant relation was found between BMI and FC in adult CF patients ($r = -.346$, $p = .147$). Linear regression showed that CFRD (Cystic Fibrosis Related Diabetes), proton pump inhibitors (PPI) use and PI were associated with elevated FC in CF patients (Adriaanse 2015).

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 feces. 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. To date FC has

Kommentoinut [MOU48]: ? claRIFY

Kommentoinut [CRK49]: Please clarify . in adults, children , both??

Kommentoinut [CRK50]:
ROK comment
Does positive corelation means higher the Z score (better nurished or fat?) higher the FC level?

not been utilised in published trials. A single case report has described improvement of intestinal histopathology changes on ivacaftor (Safe M 2016). There is not 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, multicenter 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.

Kommentoinut [CRK51]:
ROK comment
What has this with calprotectin?

CRK not really with FC

Kommentoinut [CRK52]: I suggest to move this paragraph here and not in Recommendation

Statement 19

FC may be considered a marker of intestinal inflammation in CF but there is not enough evidence on 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.

Kommentoinut [MOU53]: SUGGEST WE RE-WRITE THE RECOMMENDATIONS – 'LARGER MC STUDIES ARE NEEDED' IS PERHAPS TOO VAGUE AS NEEDED IN ALL ASPECTS.

THE 2ND RECOMMENDATION PERHAPS COULD BE '….COULD BE USED AS A MARKER…' OR 'SHOULD NOT BE USED……' DON'T NEED TO QUALIFY THE RECOMMENDATION AS ALREADY DONE SO IN TEXT

Recommendation 19

The ESPGHAN expert group recommends

Caution when interpreting individual FC values as a marker of enteropathy in CF (GoR:)

Kommentoinut [CRK54]: Michael , I suggest a recommendation more like suggested above

6. Infectious Gastroenteritis : Viral, Bacterial, 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 diarrhea 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 C reactive protein (CRP), they observed an overall diagnostic accuracy up to 94% in discriminating

between bacterial and viral AGE (Sykora J, 2010). 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 to 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% if the threshold was taken as 710 mg/L (Duman M, 2015). By contrast, in children with AGE needing hospitalization, 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 (Czub E, 2014). Higher concentrations of FC in Rotavirus AGE in the present study can be partially explained by the context of the study, given that only hospitalized patients were recruited and these can be assumed to have greater gastrointestinal inflammation compared to patients managed in primary care . These findings are consistent with those obtained by Shastri et al. in a large cohort of hospitalized adult patients (Shastri YM; 2008).

The role of FC as biomarker of AGE severity is controversial. Indeed, FC seems to be correlated with clinical severity (e.g., Vesikari score) of AGE, providing information for disease management in children, although seemingly not in Clostridium difficile infection (CDI) in adults (Chen CC, 2012). Both FC and fecal lactoferrin increase during CDI, especially in those with detectable toxin in feces, and distinguish between CDI cases and antibiotic-associated diarrhea (Swale A 2014, 7-Barbut F, 2017). Altough lactoferrin but not FC levels seem to be associated with disease severity, both parameters show high inter-individual variability. Thus, FC and lactoferrin seem unlikely to be useful as biomarkers of complicated CDI disease (Swale A 2014,).

Only one study evaluated the role of FC in parasitic gastroenteritis. In this 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 (Bustinduy AL 2013).

Statement 20

FC is significantly higher in bacterial in comparison to viral and no detectable pathogen AGE. **However as AGE management guidelines do not recommend to perform microbiological studies routinely in non-hospitalized children, FC measurement in AGE in clinical setting has a low the utility**

Recommendation 20

The ESPGHAN expert group recommends

Not ~~T~~to routinely use FC in AGE to distinguish bacterial from viral gastroenteritis in children. (GoR: Moderate)

Kommentoinut [CRK55]:
ROK comment
AGE management guidelines even do not reccomend to perform microbiology routinely in non-hospitalized children (because the result has no direct treatment consequences). Why to recomend something vaguely confirmed to discriminate between viral and bacterial (high levels in severe rotavirus AGE) and also having no therapeutic consequences. If we want to use antibiotics for severe cases or special risk groups in the case of bacterial infection, we need to know which bacteria caused it (and have antibiotic sensitivity of it to prescribe optimal effective therapy).

7 Appendicitis

Acute appendicitis (AA) may be missed at initial clinical examination in 28% to 57% of children aged 12 years or younger and in nearly 100% of children under 2 years old (Callahan MJ, 2002, Rothrock SG, 2000). Given these concerns, especially in the pediatric 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 (Allister L, 2011). Since 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 while no reaction was seen in uninflamed appendix. (Ambe PC, 2016). The accumulation of calprotectin-carrying cells in AA supports the study of FC as a new diagnostic tool in patients with suspected appendicitis.

Recently, favorable 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 non-appendicitis, and it was also higher in perforated appendicitis compared to nonperforated appendicitis. In the same study, at a cutoff value of

159 ng/mL, plasma calprotectin provided a sensitivity of 100 % and a specificity of 27 % to identify children at risk for AA (Kharbanda AB, 2012). Nevertheless, a more recent study determined the serum calprotectin levels at the cutoff value of 670 ng/ml as 73.3 % sensitive and 100 % specific (Sarsu SB, 2017). 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 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), 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 CT scans by a large percentage (Huckins DS, 2013). These findings are comparable with the data of two more recent studies, in these 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 to 99.9% and 95.9-100%), and NPV of 98.6 and 100% (95% CI, 91.2 to 99.9 and 89.9 - 100%) for ruling out the disease, respectively (Gonzalez del Castillo 2016, Benito J, 2016). 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 to those with AA. Equally, higher levels of FC in patients with AA compared to patients without clinical diagnosis of either AA or AGE were found. the control group 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 (Ambe PC, 2016). However, Sarsu et al reported that in the differential diagnosis of uncomplicated and complicated AA in children, the most accurate parameter was fecal 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(Sarsu SB, 2017).

Statements 21

21 (a) Circulating and fecal levels of calprotectin are increased in acute appendicitis.

21b) For the diagnosis of AA, the use of serum and FC has not been demonstrated to be more effective than classical inflammatory markers.

21(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 21

The ESPGHAN expert group recommends

Not to use FC, either alone or in combination with other inflammatory biomarkers, in screening children with abdominal pain for the presence of acute appendicitis.(GoR: Moderate)

8. Helicobacter Pylori Infection

We found only two studies addressing FC concentrations in connection with Helicobacter pylori (HP) infection in children. In the first study Hestvik et al. measured FC concentrations in 302 apparently healthy children aged 0 and 12 years in Uganda, also testing their feces for HP with a rapid monoclonal antigen test as well as enteropathogens and parasites (Hestvik E, 2011). Since 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 HP positive (78 children, mean concentration 34 mg/kg, 95% confidence interval 25-46) and HP negative (81, mean concentration 26mg/kg, 95% confidence interval 22-34) was not significant ($p = 0.12$). FC concentrations in children infected with HP were within the normal range. In contrast with the Ugandan study referring to symptom-free children, the second study by Sykora et al. concentrated on children with abdominal pain-related FGIDs according to the pediatric Rome III criteria (Sykora J, 2016). 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 HP infected children (7.8

g/g, 95% confidence interval 7.8-8.4) including those with gastritis, and controls (9.1 μg/g, 95% confidence interval 7.8-11.3).

Statement 22

HP infection either asymptomatic or symptomatic does not affect FC concentration.

Recommendation 22

The ESPGHAN expert group recommends

Not to use FC measurement for determination of Helicobacter pylori infection or to screen for diseases related to HP infection. (GoR: Moderate)

9. Malnutrition~~:~~

Obesity

Obesity is associated with a chronic low-grade inflammation, which originates and resides mainly in the adipose tissue. Elevated BMI, has been shown to be associated with increased gut permeability through a variety of mechanisms, including altered bowel flora, the effects of circulating inflammatory cells and markers or through direct effects of dietary fats on local cytokine production [Teixeira TFS, et al. 2012]. It has recently also been reported that a distinct obesity related microbial profile was associated with elevated FC levels ([Verdam FJ, ~~Fuentes S, de Jonge C, et al. Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. Obesity.~~ 2013)

Spagnuolo et al measured FC in 34 obese children (Spagnulo, 2010). 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.

muotoili: Fontti: Ei Kursivoitu

Kommentoinut [CRK56]:
ROK comment
probably cytokines not markers

Kommentoinut [CRK57]: Please Corina , check if we need the 2 references of only one of them. Also please check Rok comment
THANKS !!

Kommentoinut [CRK58]: Perhaps we need to explain worsening

Statement 23

FC may be mildly elevated in obese children.

Recommendation 23

The ESPGHAN expert group recommends

Not to use FC as a routine measurement in obese children . (GoR:

Kommentoinut [MOU59]: SUSPETC NEED MORE SPECIFIC RECOMMENDATION HERE. WHAT ABOUT MALNUTRITION?

Kommentoinut [CRK60]: Please Corina: see Nikhils comment . We also need you to grade the Rec

Kommentoinut [TN61]: Meaning it should be used on clinical suspicion of other conditions e.g. IBD

Undernutrition

Severe acute malnutrition (SAM) in children is frequently associated with intestinal pathology and diarrhea. A randomized controlled trial that included 95 Malawi children aged 9-23 months showed that FC is markedly increased in SAM: 547 (744) µg/g stool (Bartels et al, 2019). 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.

Kommentoinut [CRK62]: is this SD ??

In an observational study, Versloot et al followed 47 Malawi children aged 8 to 59 months. They assessed stool pathogens and FC at admission and after clinical stabilization. FC was high in most children at admission and was higher in those still harboring an infection (mostly parasitic) after clinical stabilization: 383 µg/g (149-903 µg/g) vs 140 µg/g (71-300 µg/g). After clinical stabilization, 40% of children had FC levels above age-specific cut-offs.

Statement 24

FC is elevated in children with SAM due to multiple factors and might remain high after different therapies addressing these factors.

Kommentoinut [CRK63]: and also after nutritional intervention mild nutritional improvement

Please Corina could yoy review the ST

Recommendation 24

The ESPGHAN expert group recommends

Not using FC for establishing therapeutic efficacy in SAM. (GoR :)

10. . Other conditions

10. 1 Necrotizing Enterocolitis

Necrotizing 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 due to mucosal damage (Pergialiotis, 2016). 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 to healthy infants of the same gestational age (Caroll D, 2003, Houston JF, 2015). In a group of 206 very low weight infants (<1500g), 12 to 48 hours before the onset of disease, FC levels were ~~found to be~~ significantly elevated in infants who went on to suffer moderate NEC (Zoppelli L, 2012). The median FC levels in infants with NEC were between 241.1 and 792 ug/g stool (Houston JF, 2015), and at a cut-off level of 286.2 ug/g stool the sensitivity was 0.86 and the specificity was 0.93 (Thuijls G, 2010). 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 (Josefsoson S, 2007). FC levels were not statistically different in mild or severe NEC, and in fulminant NEC FC levels were unusually low (Zoppelli L, 2012). Furthermore, there seemed to be no correlation between FC levels in moderate NEC and the need for surgery (Zoppelli L, 2012). Interestingly, in focal intestinal perforations FC levels remained normal (Josefsoson S, 2007). The disadvantage of FC for predicting severe NEC is its high inter-individual and intra-individual variability (Pergialiotis, 2016). In addition, in 50% of infants with NEC stool samples could not be obtained (Thuijls G, 2010).

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 (3- Zoppelli L, 2012). On follow-up after NEC surgery, FC showed to be a good marker to monitor improvement of intestinal inflammation (Josefsoson S, 2007).

Kommentoinut [TN64]: need to check tense in this to keep in line with the rest of the manuscript. NEED TO CLARIFY WHETHER FC IS USEFUL OR NOT – A BIT CONFUSING. ALSO IS THIS LARGELY BASED ON A SINGLE PAPER – MAY NEED TO ADD SOME DETAIL OF THE STUDY – HOW MANY? HOW PREM?

Kommentoinut [TN65]: Is this correct?

Kommentoinut [CRK66]:
ROK comment
What were the values in non-NEC group? Tell me very little in this age group without saying what was normal.

Statement 25

A rise in FC concentration may indicate a risk of developing NEC and may thus prevent or attenuate NEC and improve follow-up of patients with NEC.

Kommentoinut [CRK67]: Ilse , do you think we can avoid repeating 3 times NEC ?

Recommendation 25

The ESPGHAN expert group recommends/suggests

To use FC as a non-invasive screening tool to alert to the risk of developing NEC . (GoR:

Kommentoinut [CRK68]: could you please garde the Recommendation

10.2. Juvenile polyps

Colonic juvenile polyps (CJP) have a prevalence in the pediatric population that ranges between 0.08% and 3.7%. They are most frequently diagnosed in boys, between 3 and 10 years of age (1Olafsdottir, 2016). CJP usually present with atypical symptoms such as abdominal discomfort and painless rectal blood loss. CJP are nonadenomatous structures characterized by high vascularity, ulcerations, and the presence of many neutrophils. 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 (Josefsoson S, 2007, Pauley-Hunter, 2015; Teitelbaum, 2010).

Until recently, only sporadic case reports/series have been published on children with CJP and elevated FC. (Pauley-Hunter, 2015; Teitelbaum, 2010)) Agardh 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) (1 Olafsdottir, 2016).).

Kommentoinut [CRK69]:
ROK comment
Are there any data for polyposis syndromes? To the best of my knowledge, it can be slightly elevated (in compare with healthy controls) but of no practical use (still in the normal range in majority of patients)

Statement 26

26 (a) JP are associated with increased FC, although normal FC does not exclude this diagnosis.

26 (b) FC as screening test cannot differentiate CJP from IBD ~~CJP should be added to the differential diagnosis of patients with an elevated FC, although on it own FC cannot reliably distinguish between CJP and IBD.~~

Kommentoinut [CRK70]: Do you agree ¿?

Recommendation 26

The ESPGHAN expert group recommends

Not to use FC in children with a suspicion of CJP.

10.3. Short bowel Syndrome

Short bowel syndrome (SBS) is defined as a spectrum of diarrhea and malabsorption with associated complications (ie bloodstream infections (BSI) and small bowel bacterial overgrowth (SBBO) due to insufficient bowel length mainly resulting from massive small bowel resection due to necrotizing enterocolitis (NEC) or congenital gastrointestinal malformations (e.g. gastroschisis, intestinal atresia). SBBO and use of parenteral nutrition (PN) are considered as inducers of systemic or local inflammation concomitant with gut barrier dysfunction (Cole CR, 2010). In a study of 10 children with SBS due to 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 SBBO (breath test) had higher FC levels (median 394 µg/g; range 144-786 µg/g) compared with children with SBS without SBBO (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 (ranges : 76–400) versus 24 µg/g (ranges 11–157) in the non-dilated group) and lower citrulline levels and more intestinal bloodstream infections and liver anomalies (Hukkinene M, 2017).

Statement 27

FC might be a useful marker of small intestinal ~~bowel~~ bacterial overgrowth in SBS children.

Recommendation 27

The ESPGHAN expert group recommends

Not to use FC routinely in SBS children

10. 4. Small intestinal bacterial overgrowth.

There was no difference in FC levels in 58 children affected by small intestinal bacterial overgrowth (SIBO), as diagnosed by lactulose breath test (LBT) compared to 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) ([3]-Fundaro C, 2011).

Statement 28~~7~~

There are no differences in FC levels between previously healthy children with SIBO, compared to a control population.

Recommendation 28~~7~~

The ESPGHAN expert group recommends

~~n~~Not to use FC measurement for the diagnosis of SIBO in previously healthy children (GoR:)

Kommentoinut [CRK71]: Please ILSE can you answer !!

Kommentoinut [CRK72]:
Alexandra comment : The statement needs to 'summarize the evidence'

She proposes to modify the St
Increased FC concentrations may be indicative of small bowel bacterial overgrowth in SBS children.

Do you AGREE ??

Kommentoinut [CRK73]:
ROK cmment
I agree with Alexandra, however, there is a problem. Even in children without small bowel bacterial overgrowth the FC levers were higher than normal values. Put even more doubt into practical value of FC measurement as they found no elevation in SIBo in non-short gut patients (next chapter

Kommentoinut [CRK74]: Yes ,no new found

Kommentoinut [GF75]: No recent paper in puubmed

Kommentoinut [TN76]: SIBO OR SBBO – NEED TO BE CONSISTENT

Kommentoinut [CRK77]: Do you agree to keep SIBO ¿

Kommentoinut [FG78]: My point here is to differentaite SIBO due to secondary conditions (enteroptahies, short bowel… from SIBO occuing in previously healthy children as in the study by Fundaro (primary SIBO where ther is no need of dosing fecal calprotectin
Suggest to leave this . agree to reword if you think this is unclear?

Kommentoinut [CRK79]: Perhaps you can explain this more in detail in the textrt and then the Statement and Rec will become clear .

Kommentoinut [CRK80]: I suggest to delete ***in previously.....***

Kommentoinut [CRK81]: Comment by Alexandra : This part may cause confusion becuase it partly contradicts to the previous chapter. I would suggest to omit this.
Please Frederic , your view

Kommentoinut [MOU82]: DO WE MEAN THAT IF THEY HAVE AN ILLNESS E.G. CIPO WE CAN MEASURE IT?

Kommentoinut [CRK83]: Frederic. Do you suggest to add a new chapter on GVHD ¿?
No objection from my side

Broglie et al 2018: Fecal calprotectin and serum albumin as markers of gastrointestinal graft versus host disease Hematol Oncol Stem Cell Ther (2018)11, 169–174

10.5. Autism

By the use of two independent markers of intestinal inflammation, i.e. rectal NO and FC there was no inflammation found in a group of 24 consecutive children with autism (aged 3-13 years), except in 2 cases with Clostridium difficile infection and severe constipation ([4]- Fernell E, 200). On the contrary, FC was elevated in 24.4% of patients with autism and in 11.6% of their relatives; it was not, however, correlated with abnormal intestinal permeability (IPT) (de Magistris L, 2010). On analyzing disaccharidase activity, intestinal inflammation, and permeability in 61 children with autism and 50 nonautistic individuals with gastrointestinal symptoms, no differences were found in FC (111.10 µg/g +/-21.82 in autism versus 125.57_µg/g +/-27.36 in controls)(Kushak, 2016).

Statement 29

FC identifies children with and without autism who have intestinal inflammation, but high FC levels are not more commonly found in children with autism.

Recommendation 29

the ESPGHAN expert group recommends

Not to use FC measurement in children with autism except if there are symptoms suggesting intestinal inflammation.

10. 6. 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 fecal occult blood, gastric wall thickness and duodenal wall thickness- compared to those without intestinal implication (median 124.2 (430.7) µg/g versus 16.57 (17.8) µg/g, respectively p

Kommentoinut [CRK84]: Yes , No new papers found

Kommentoinut [GF85]: No new significant paper in pubmed

Kommentoinut [GF86]: I cheked that there are no new serie in this área in pubmedFinbalyy found a new paper!!!

Kommentoinut [CRK87]: are numbers in brackets SD??

=0.01). The median FC (241.0) µg/g in the children with mild gastrointestinal involvement was lower than the group with more severe involvement (392 (524.6) µg/g; p= =.02). [7]The FC concentration was a better indicator for the evaluation of gastrointestinal involvement than the faecal occult blood test (Kanik A, 2015).

In a recent study of 40 children with Henoch-Schönlein purpura compared to 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 in being well correlated to remission and relapse (Clin Rheumatol. 2018 Jun;37(6):1667-1673. Clinical significance of fecal calprotectin for the early diagnosis of abdominal type of Henoch-Schonlein purpura in children.Teng X(1), Gao C(2), Sun M(1), Wu J(3)).

Kommentoinut [TN88]: PLEASE CHECK THAT THIS CORRECT

Statement 30

FC is useful ~~may be a reliable marker~~ for identifying children with gastro-intestinal involvement during Henoch-Schönlein purpura

Kommentoinut [CRK89]: Frederic , do you agree ¿

Kommentoinut [FG90]: I did not read any comment in these 2 papers about this question. Unfortunaltly not Access to the eur J gastroenterol hepatol from my home and could not look again. Will check tomorrow at office

Recommendation 30

The ESPGHAN expert group recommends

To use FC measurement to identify gastro-intestinal involvement in children with Henoch-Schönlein purpura in the abscence of overt bleeding. .

Kommentoinut [TN91]: AGAIN WHAT ABOUT THE PRESENCE PR ABSENCE OF BLEEDING

Kommentoinut [MOU92]: SHOULD WE QUALIFY AND SAY – IN THE ABSENCE OF OVERT GI BLEEDING

. General Conclusions:

Authors: Nikhil , ALL

Summary of Recommendations for the use of FC in pediatric gastrointestinal conditions