The Role of Fecal Calprotectin in Pediatric Disease

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Abstract

Fecal calprotectin (FC) is a calcium- and zinc-binding protein of the S100 family expressed mainly by neutrophils protein that is released during inflammation. FC became an increasingly useful tool both for gastroenterologists and for general practitioners for distinguishing inflammatory bowel disease (IBD) from irritable bowel syndrome. Increasing evidences support the use of this biomarker for diagnosis, follow-up and evaluation of response to therapy of several pediatric gastrointestinal diseases, ranging from IBD to nonspecific colitis and necrotizing enterocolitis. This article summarizes the current literature on the use of fecal calprotectin in clinical practice.

**Keywords**: Fecal calprotectin, Inflammatory bowel disease (IBD), Pediatric gastrointestinal diseases
Introduction

Calprotectin constitutes up to 60% of the soluble proteins in the cytosols of human neutrophils. In addition, it is distributed in monocytes, macrophages, and epithelial cells.\textsuperscript{1-3}) Their release is activated through interaction of activated monocytes with endothelial cells that increase leukocyte recruitment, and through proinflammatory chemokines by which phagocytes further promote extravasation of leukocytes to the sites of inflammation.\textsuperscript{4}) When measured in feces, calprotectin correlates well with neutrophil infiltration of the intestinal mucosal surface and within the gut lumen are a hallmark of digestive inflammatory pathology.\textsuperscript{5}) This protein is able to bind calcium, zinc and manganese ions. Since these elements are of vital importance for bacterial growth, their chelation by calprotectin contribute to the alteration of the gut microbiota exerting an antimicrobial effect. Through the sequestration of zinc ions (Zn\textsuperscript{2+}), calprotectin is also able to inhibit many zinc-dependent enzymes such as matrix metalloprotease, inducing an antiproliferative effect as well as apoptosis in both normal and transformed human and animal cells.\textsuperscript{6}) These properties suggest a pivotal role of calprotectin in inflammatory processes through its effect on cells survival and growth. So, fecal calprotectin (FC) is useful as a non-invasive test reflecting various pathological processes occurring in the intestinal mucosa of pediatric patients.

In addition, its structure is very stable at room temperature of up to 7 days, and the resistance to bacterial degradation.\textsuperscript{7}) It makes calprotectin an ideal marker not only for hospitalized patients but for the outpatient management.

Numerous studies have been published showing evidence regarding FC usefulness for the detection and monitoring of several GI disorders, especially Inflammatory bowel disease.\textsuperscript{8-10}) Indeed, abdominal discomfort including pain, bloating and diarrhea is common in clinical practice. It often arises from functional gastrointestinal disorders but may indicate
inflammatory bowel disease (IBD). Differentiating patients with organic diseases from those with functional disorders (i.e. irritable bowel syndrome [IBS]) may be difficult. When measured in feces, FCP can be used to differentiate between non-organic and inflammatory intestinal disorders, especially to identify IBD. Also, it should be noted that elevated FC levels could be found not only in IBD but also in other GI pathological conditions including infective colitis, microscopic colitis, eosinophilic colitis, colorectal cancer and beyond intestinal disease.\textsuperscript{11-14}

This review aims to explain the role of calprotectin in a range of inflammatory bowel disease and other pathological conditions in Pediatric clinical practice.

1. Cut-off level of Fecal calprotectin in clinical practice.

Fecal calprotectin levels may vary with age. Currently, a FC level below 50 μg/g is considered normal older than 4 years.\textsuperscript{15} To obtain the mean FC level of children under 4 years of age, we studied FC levels in healthy children at four kindergartens for six months. Children were excluded if their parents reported signs of a cold, flu, or stomach or similar problems in the last two weeks. Additionally, children with a history of preterm birth, low or large birth weight, large or small weight for their age (< 3 percentile or > 97 percentile), and with positive results of the stool virus or bacterial PCR were excluded. According to a recent our study, newborn infants have high calprotectin levels that later decline and usually reach normal levels by the age of 4 years (Fig 1)\textsuperscript{16}. FC levels in a cohort of healthy children aged between 6 months and 4 years and found that the 95th percentile of FC values was 135 μg/g in 7-12 months group, 65 μg/g in 13-18 months group, 55 μg/g in 19-24 months group, 40 μg/g in 25-30 months group, 21 μg/g in 31-36 months group, and 12 μg/g in 37-48 months.
Also the FC level was especially very high and variable in the 0–6 months age category. In this period, FC levels were affected by feeding and delivery methods. FC values were higher in infants < 6 months old who were fed breast milk and born by NSVD.

1.1 Individual and environmental factors affecting fecal calprotectin levels

High FC levels was reported in individuals with increased Body Mass Index (BMI > 25). The diminished levels of Paneth cell, it plays a key role in the maintenance of the GI barrier, observed in obese subjects may partly explain the local intestinal inflammation. Dietary supplements such as zinc, vitamin D, and several probiotics can affect FC levels. Also, the use of non-steroidal anti-inflammatory drugs (NSAID) and proton pump inhibitors (PPI) have been associated with FC elevation. The distribution of calprotectin within a stool sample seems to be homogeneous and measurement of random aliquots give similar results. Day-to-day variability of fecal calprotectin showed also low.

2. The use of fecal calprotectin as a biomarker in Pediatric gastrointestinal disease

Monocytes and intestinal epithelial cells can be activated by different inflammatory triggers such as cytokines or bacterial products leading to the increased expression and secretion of calprotectin which can be detected in the stool. Also, calprotectin interact with the toll-like receptor 4 (TLR4) activating macrophages and endothelial cells and enhancing the expression of several adhesion molecules. In turn, TLR4 is activated also on neutrophils amplifying inflammation through the release of cytokines, reactive oxygen species (ROS) and calprotectin again. This process, which involves both phagocytic cells and epithelial cells, leads to neutrophils transmigration and mucosal damage.

2.1. Inflammatory Bowel Disease.

To optimize outcomes in patients with inflammatory bowel diseases (IBD), frequent
monitoring is aimed at evaluating treatment efficacy, severity of disease, and risk for potential complications. However, frequent endoscopic assessment of mucosal healing is invasive, time-consuming, costly, and associated with certain risks and discomfort especially in pediatric patients. So Fecal calprotectin (FC) has emerged as a new diagnostic tool to detect and monitor intestinal inflammation in children with IBD, as it is a simple, rapid, sensitive, specific, inexpensive, and non-invasive marker of inflammation. Elevated levels are observed in both Crohn’s disease and ulcerative colitis cases.\textsuperscript{24} The diagnostic precision of FC for IBD was higher in children than adults with better accuracy at a cutoff level of 100 μg/g versus 50 μg/g for endoscopically active disease (sensitivity of 81%, specificity of 69%).\textsuperscript{25} It is useful as a marker for assessing more accurately the severity mucosal inflammation as compared to other clinical and laboratory indices. FC cutoff of 250 μg/g had a sensitivity of 60% and a specificity of 80% in discriminating large CD ulcers from small ones.\textsuperscript{26} While treatment of active IBD with glucocorticoids and infliximab-a TNF-α antagonist has been shown to decrease FC concentrations and reach normal levels at 2 weeks in one third of pediatric patients with IBD, reflecting mucosal healing. FC concentrations fail to return to normal; this indicates continuing inflammatory activity though in a clinically silent disease.\textsuperscript{27-28} It can be used to predict relapse in teenagers with inflammatory bowel disease.\textsuperscript{29} Although for IBD in children, FC is plays an important role in the diagnosis, follow up, assessment of relapses and response to treatment, optimal testing time for monitoring of disease activity and response to treatment needs to be established. Also more efforts to confirmation for absolute cutoff value according to disease location in CD, disease extent in UC.

2.2. Acute infectious colitis.

Infectious diarrhea causes significantly higher FC concentrations than those displayed in irritable bowel syndrome (IBS) which are comparable with the values found in healthy
controls. FC levels correlate with the clinical severity of infectious diarrhea in children. A large study including 2383 consecutive patients with acute diarrhea (sensitivity and specificity of 83 and 87%), for fecal calprotectin to identify bacterial infection and showed better diagnostic accuracy than fecal lactoferrin and occult blood testing.\textsuperscript{30} Fecal calprotectin values were higher in acute bacterial compared with viral diarrhea both in adults and children.\textsuperscript{31} Although Children with Crohn’s disease also have higher FC values than children with IBS or infectious diarrhea, repeated test may be informative for with persistent diarrhea or digestive symptoms.

2.3. Necrotizing Enterocolitis.
In 2010 Thuigls et al. suggested that FC might be a useful diagnostic marker for NEC.\textsuperscript{32} Since then, several studies have investigated its efficacy. FC concentrations exceeding 350 $\mu$g/g are detected and followed by bowel perforation, bloody stool, and other clinical features of NEC representing signs of gastrointestinal injury.\textsuperscript{33} A significant correlation between FC and severity of NEC in preterm infants has also been reported.\textsuperscript{34} Add intestinal fatty acid-binding protein and FC seems to improve the diagnostic accuracy in suspected NEC infants early on in the disease. Also FC decreases as NEC resolves.\textsuperscript{33} However, the usefulness of FC as such a marker may be controversial since high inter-individual variations in Neonate, especially preterm infants and lack of data regarding specific cut-off values.

2.4. Microscopic colitis
Collagenous colitis is characterized by a primarily lymphocytic and plasma cellular inflammation of the lamina propria. The high levels of FC in active collagenous colitis (CC) patients can be explained by the fact that calprotectin is present not only in neutrophils but also in monocytes and macrophages;\textsuperscript{35} nevertheless, the use of FC as an inflammatory
marker for CC is not recommended for lack of universal evidence.

3. Fecal calprotectin: beyond intestinal organic diseases

There are few data on the potential clinical benefit of FC measurements in groups of individuals with chronic disease at increased risk of gastrointestinal inflammation outside IBD.

3.1 Juvenile Idiopathic Arthritis.

A recent study showed that FC may be used to evaluate the subclinical intestinal inflammation in children with juvenile idiopathic arthritis (JIA). Considering referral for further investigations only when FC remains significantly elevated in repeated measurements of FC, particularly in the absence of overt gastrointestinal symptoms. Further investigations are warranted to confirm the actual role of elevated FC in those children.

3.2 Atopic dermatitis

The gut immune system is an important regulator of immune-mediated diseases, such as allergies. Numerous investigators have shown that changes in the gut microbiota and intestinal inflammation have been associated with the development of allergic diseases. Altered gut microbiota induces epithelial damage resulting in increased intestinal inflammation, altered gut permeability and immunological balance, which affect the development of allergic diseases and subsequent development of atopic eczema. Recent reports showed elevated fecal calprotectin levels are associated with severity of atopic dermatitis in children. Also high fecal calprotectin levels at the age of 2 months had an increased risk of developing AD by age 6. These results suggest that a remarkable intestinal inflammation early in life is a risk factor for the development of allergic diseases later in life.
It means consists of microbiota colonization appeared to be an important regulator of the intestinal inflammation.


Fecal calprotectin is a reliable non-invasive tool for assessing intestinal inflammation and plays a relevant role in clinical practice. However, clinicians must be aware that FC levels could be affected by several non-pathological conditions especially younger age. Thus, the following key messages may be highlighted for Pediatric clinicians.

- A cut-off of 50 μg/g is widely accepted in children aged from 5 to 17 years but in those with less than 5 years, since age and diet could affect FC values, the laboratory results must always be interpreted according to the clinical context and in case of inconsistent results, FC measurement should be repeated.

- FC could be used to screen for inflammatory disease, and elevated results support the need in primary care to select the patients who need further investigation (colonoscopy).

- FC used to monitor the response to drug therapy in patients with chronic inflammatory conditions such as IBD. However more research is needed to elucidate the evidence-based clinical practice guidelines in tailoring and monitoring drug therapy in patients with IBD.

- Also, additional information is warranted in conditions such as microscopic colitis and other inflammatory diseases of the gastrointestinal tract associated with autoimmune, or allergy currently limited database.
Conclusion

The importance of FC in pediatric gastroenterology has increased over time. It provides higher sensitivity and specificity and better information in the evaluation of pediatric IBD than other tests and in the prediction clinical course of disease. Despite many possible functions of FC, its biological function related disease still remains unclear. Further studies are needed to elucidate the clinical relevance of FC in various pathological pediatric conditions and to establish whether FC has any implication beyond that of an inflammatory mediator.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References


20. Poullis A, Foster R, Mendall MA, Shreeve D, Wiener K. Proton pump inhibitors are associated with elevation of fecal calprotectin and may affect specificity. Eur J Gastroenterol


Figure 1. Diagram of FC levels in healthy children
TABLE 1. Fecal Calprotectin levels (μg/g )* in different age groups of healthy children.

<table>
<thead>
<tr>
<th>Age</th>
<th>7 - 12 months</th>
<th>13 – 18 months</th>
<th>19 – 24 months</th>
<th>25 – 30 months</th>
<th>31 – 36 months</th>
<th>37-48 months</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>No. subjects</td>
<td>46</td>
<td>39</td>
<td>39</td>
<td>49</td>
<td>30</td>
<td>31</td>
<td>234</td>
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<tr>
<td>Median</td>
<td>78.5</td>
<td>29</td>
<td>27</td>
<td>27</td>
<td>12.5</td>
<td>12</td>
<td>24.5</td>
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<tr>
<td>95% CI of median</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>45</td>
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<td>Upper limit</td>
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<td>55</td>
<td>40</td>
<td>21</td>
<td>12</td>
<td>35</td>
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<tr>
<td>Mean</td>
<td>145.91</td>
<td>101.79</td>
<td>48.79</td>
<td>41.41</td>
<td>23.43</td>
<td>22.97</td>
<td>68.5</td>
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<tr>
<td>Standard deviation</td>
<td>185.29</td>
<td>176.12</td>
<td>68.46</td>
<td>46.12</td>
<td>20.00</td>
<td>26.63</td>
<td>123.12</td>
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<tr>
<td>Standard error</td>
<td>27.32</td>
<td>23.20</td>
<td>10.96</td>
<td>6.59</td>
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<tr>
<td>Lower limit</td>
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<tr>
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<td>54.66</td>
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<td>12</td>
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<tr>
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<td>200</td>
<td>87</td>
<td>106</td>
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* A commercially available FEIA (Fluorescence Enzyme Immunoassay) (Green Cross Laboratories, Yongin-si, Gyeonggi-do, Korea) was used to measure quantitatively the concentration of FC. The lower detection limit was 12 μg/g, and higher detection limit was 2000 μg/g.
Figure 1. Diagram of FC levels in healthy children. Short lines are 95% percentiles, long lines are medians.