Fecal Calprotectin in Pediatric Gastrointestinal Diseases: Pros and Cons

Mohammed Al-Beltagi, Nermin Kamal Saeed, Adel Salah Bediwy, Reem Elbeltagi

Abstract

BACKGROUND
Fecal calprotectin is a valuable biomarker for assessing intestinal inflammation in pediatric gastrointestinal diseases. However, its role, pros, and cons in various conditions must be comprehensively elucidated.

AIM
Based on current literature, this review aims to explore the role of fecal calprotectin in pediatric gastrointestinal diseases, including its advantages and limitations.

METHODS
A comprehensive search was conducted on PubMed, PubMed Central, Google Scholar, and other scientific research engines until February 24th, 2024. The review included 88 research articles, 56 review articles, six meta-analyses, two systematic reviews, two consensus papers, and two letters to the editors.

RESULTS
Fecal calprotectin is a non-invasive marker for detecting intestinal inflammation and monitoring disease activity in pediatric conditions such as functional gastrointestinal disorders, inflammatory bowel disease, coeliac disease (IBD), Coronavirus disease 2019 (COVID-19)-induced gastrointestinal disorders, gastroenteritis, and cystic fibrosis-
associated intestinal pathology. However, its lack of specificity and susceptibility to various confounding factors pose challenges in interpretation. Despite these limitations, fecal calprotectin offers significant advantages in diagnosing, monitoring, and managing pediatric gastrointestinal diseases.

CONCLUSION
Fecal calprotectin holds promise as a valuable tool in pediatric gastroenterology, offering insights into disease activity, treatment response, and prognosis. Standardized protocols and guidelines are needed to optimize its clinical utility and mitigate interpretation challenges. Further research is warranted to address the identified limitations and enhance our understanding of fecal calprotectin in pediatric gastrointestinal diseases.

INTRODUCTION
Pediatric gastrointestinal (GI) diseases encompass a wide range of conditions affecting the digestive system in children from infancy through adolescence. These diseases can manifest with diverse symptoms, including abdominal pain, diarrhea, vomiting, and poor weight gain, and they can significantly impact a child's overall health and well-being [1]. Understanding the significance of pediatric GI diseases is essential for healthcare professionals, caregivers, and society as a whole, as it underscores the importance of early detection, diagnosis, and management to optimize outcomes for affected children. Pediatric gastrointestinal (GI) diseases are a global health concern [2]. According to the World Health Organization (WHO), GI infections are one of the leading causes of illness and death in children under five years of age, especially in low- and middle-income. Chronic GI conditions such as inflammatory bowel disease (IBD), celiac disease, and gastroesophageal reflux disease (GERD) can also impair a child's quality of life and require ongoing medical management [3]. Pediatric GI diseases also have significant socioeconomic implications for families and healthcare systems.
Additionally, missed school days and parental work absences due to caring for a sick child can disrupt family routines and impact household income \[^4\].

Diagnosing and treating pediatric GI diseases can be challenging due to the complexities of the developing digestive system and the varied symptoms that children may experience. Unlike adults, children may not always be able to express their symptoms clearly, making it difficult for healthcare providers to obtain an accurate medical history and perform a thorough physical examination \[^5\]. Consequently, pediatric GI diseases may be underdiagnosed or misdiagnosed, leading to delays in appropriate treatment and potential complications. Early intervention and multidisciplinary care are essential, as chronic GI conditions can lead to growth and developmental delays in children \[^6\]. Non-invasive diagnostic tools are necessary in pediatric gastrointestinal diseases due to their safety, accessibility, and accuracy. They minimize discomfort, enhance safety, and offer comprehensive evaluation of the gastrointestinal tract without invasive exploration. They also facilitate longitudinal monitoring of disease progression and treatment response, ensuring timely diagnosis and treatment \[^7\]. An ideal diagnostic marker for pediatric gastrointestinal diseases should be non-invasive, sensitive, specific, quantifiable, predictive, cost-effective, feasible for pediatric populations, stable, reproducible, and with limited interference from external factors. Ethical considerations regarding sample collection comfort for pediatric patients are also important \[^8\].

The primary objective of the review is to assess the utility of fecal calprotectin as a diagnostic marker, specifically in pediatric patients with gastrointestinal diseases, and to evaluate its advantages and limitations in this population, focusing on its role as a non-invasive and efficient indicator of gastrointestinal inflammation. Additionally, the article likely seeks to provide insights into the practical implications of using fecal calprotectin in pediatric clinical practice, including its ability to provide early indications of gastrointestinal pathology, aid in disease monitoring, differentiate between organic and functional disorders, and predict disease relapse. Furthermore, the
review likely intends to address the challenges and considerations associated with interpreting fecal calprotectin results in pediatric patients.

**MATERIALS AND METHODS**

We conducted a systematic literature search to identify studies examining the role of fecal calprotectin in pediatric gastrointestinal diseases and evaluating its pros and cons. The search was performed across various electronic databases, including PubMed, PubMed Central, Google Scholar, and other scientific research engines. The search strategy utilized combinations of keywords and Medical Subject Headings (MeSH) terms related to fecal calprotectin, pediatric gastrointestinal diseases, functional gastrointestinal disorders, inflammatory bowel disease, coeliac disease, COVID-19-induced gastrointestinal disorders, infectious gastroenteritis, and cystic fibrosis. The search was conducted up to February 24th, 2024, with no restrictions on publication date.

Articles were included if they provided relevant information on using fecal calprotectin as a biomarker in pediatric gastrointestinal diseases, including studies examining its diagnostic, prognostic, or therapeutic implications. Both original research articles and review articles were considered for inclusion. We also included articles discussing fecal calprotectin's advantages and disadvantages (pros and cons) in pediatric gastrointestinal diseases. We excluded articles that did not focus on pediatric populations or specifically address fecal calprotectin's role in gastrointestinal diseases. Studies not available in English were also excluded from the review.

Two reviewers performed Data extraction independently using a standardized data extraction form. The extracted data were synthesized to provide a comprehensive overview of the role of fecal calprotectin in pediatric gastrointestinal diseases, focusing on its advantages and disadvantages. Findings from the included studies were analyzed, summarized, and presented in the subsequent sections of this review. The quality of included studies was assessed based on study design, methodology, sample size, and the reliability of reported findings. Both quantitative and qualitative studies
were included, and the strength of evidence provided by each study was considered during data synthesis and interpretation.

RESULTS
The literature search identified 90 research articles, 60 review articles, 6 meta-analyses, 2 systematic reviews, 2 consensus papers, and 2 Letters to the editors. Figure 1 shows the study's flow chart. These studies were selected based on their relevance to the role of fecal calprotectin in pediatric gastrointestinal diseases and its pros and cons. The included studies covered various pediatric gastrointestinal conditions such as functional gastrointestinal disorders, IBDs, coeliac disease, COVID-19-induced gastrointestinal disorders, infectious gastroenteritis, and cystic fibrosis. The included studies explored the role of fecal calprotectin as a biomarker in pediatric gastrointestinal diseases. The studies consistently found that fecal calprotectin was a sensitive marker of intestinal inflammation, aiding in diagnosing, monitoring, and managing various gastrointestinal conditions in children. Elevated fecal calprotectin levels were associated with the presence and severity of gastrointestinal inflammation, helping differentiate between functional, inflammatory, and non-inflammatory conditions.

The studies identified several advantages of fecal calprotectin use in pediatric gastrointestinal diseases. These included its non-invasive nature, making it well-tolerated by pediatric patients, and its sensitivity in detecting intestinal inflammation accurately. Fecal calprotectin levels were found to correlate with disease activity, treatment response, and clinical outcomes in conditions such as IBD, coeliac disease, and cystic fibrosis. Additionally, fecal calprotectin provided valuable insights into disease prognosis and helped guide therapeutic interventions. Despite its utility, fecal calprotectin testing presented specific challenges and limitations. One notable limitation was the lack of specificity of fecal calprotectin elevation, as it could occur in various inflammatory and non-inflammatory conditions, leading to potential false positives and diagnostic ambiguity. Interpretation of fecal calprotectin levels was also complicated by factors such as variations in average values among different age groups, confounding
factors like diet and medication use, and the lack of standardized cutoff values for specific conditions.

The included studies highlighted variability in clinical practice regarding the routine use of fecal calprotectin in pediatric gastrointestinal diseases. While fecal calprotectin was widely utilized for diagnosing and monitoring certain conditions, there was a lack of consensus among expert groups regarding its appropriate use in specific patient populations, such as those with acute gastroenteritis. This variability underscored the need for standardized guidelines and protocols for fecal calprotectin testing in pediatric practice. Overall, the results of the included studies emphasized the significant role of fecal calprotectin as a valuable biomarker in pediatric gastrointestinal diseases. While it offered several advantages, including its non-invasive nature and sensitivity in detecting intestinal inflammation, fecal calprotectin testing posed challenges related to its specificity and interpretation. Further research and establishing standardized guidelines are needed to optimize the clinical utility of fecal calprotectin in pediatric gastrointestinal practice.

**DISCUSSION**

Calprotectin is a complex of calcium-binding leucocyte proteins that belongs to the S100 protein family. It can bind to calcium, zinc, and manganese ions and plays a significant role in the innate immune response and inflammation. Calprotectin is also a heat-stable protein and can resist bacterial and enzymatic degradation. It is primarily found in neutrophils, forming about 60% of cytosol-soluble proteins in human neutrophils. It is also present in monocytes, macrophages, and epithelial cells.[9,10].

Calprotectin comprises two primary proteins: S100A8 and S100A9 (Figure 2). S100A8, also known as Calgranulin A, is a 10.8 kDa protein mainly found in myeloid cells such as neutrophils, monocytes, and macrophages.[11]. It is critical in the innate immune response, particularly in host defense against microbial pathogens and inflammation. S100A8 interacts with receptors such as Toll-like receptor 4 (TLR4) and receptors for advanced glycation end products (RAGE).[12]. Similarly, S100A9, also known as
Calgranulin B, a 13.2 kDa protein found primarily in myeloid cells and upregulated during inflammation. It creates homodimers and heterodimers with S100A8 to form the calprotectin complex [13]. This complex is released into the extracellular environment during inflammation, with antimicrobial activity (against bacteria and fungi) and modulating inflammatory responses [14]. Calprotectin also has pro- and anti-tumor properties related to DNA damage response, angiogenesis, cell survival, growth, and the remodeling of the extracellular matrix [15].

While calprotectin is typically formed by the heterodimeric combination of S100A8 and S100A9, homodimers of either S100A8 or S100A9 can also exist. These homodimers may have distinct functions or roles compared to the heterodimeric form of calprotectin [11]. In certain disease states or physiological conditions, calprotectin complexes may contain other proteins or molecules besides S100A8 and S100A9. These mixed complexes could arise due to interactions with other proteins in the cellular environment or alterations in the composition of immune cells [16].

Calprotectin is a critical component of the innate immune response, exhibiting antimicrobial activity by chelating essential metals needed for bacterial growth, such as calcium, zinc, and manganese ions. Calprotectin inhibits many zinc-dependent enzymes, including matrix metalloprotease, inducing anti-proliferative & apoptosis effects in both normal and transformed cells [17]. Additionally, calprotectin modulates inflammatory responses by interacting with receptors like Toll-like receptor 4 (TLR4) and receptors for advanced glycation end products (RAGE), influencing cytokine production and immune cell recruitment [18]. Moreover, it contributes to tissue homeostasis and repair by regulating cell differentiation and proliferation processes, thereby promoting wound healing [14].

Activated neutrophils and other myeloid cells release calprotectin as part of the innate immune response to inflammation [19]. Inflammatory stimuli, such as infection, tissue damage, or autoimmune processes, trigger the migration of neutrophils to the site of inflammation, where they release calprotectin into the surrounding tissues or body fluids [20]. Clinically, calprotectin, particularly fecal calprotectin, is a sensitive marker for
inflammation, aiding in diagnosing and monitoring various gastrointestinal conditions. Its multifaceted roles in immunity, inflammation modulation, tissue repair, and diagnostic applications underscore its significance in health and disease \cite{21}.

**Calprotectin in Systemic Diseases:**

Calprotectin is a protein linked to various systemic inflammatory and infectious diseases affecting different organ systems, highlighting its potential as a biomarker of inflammation and disease activity \cite{82}. In patients with rheumatoid arthritis, calprotectin levels are elevated in both the synovial fluid and serum and correlate with disease activity and severity. Calprotectin may thus serve as a marker of joint inflammation and could be used to monitor disease progression and response to treatment in rheumatoid arthritis \cite{22}. In addition, Cheng *et al* found that serum calprotectin correlates with the duration of psoriatic arthritis disease and is independently associated with the presence of carotid plaque in these patients \cite{24}. Emerging evidence suggests that calprotectin may also play a role in cardiovascular diseases, specifically atherosclerosis and coronary artery disease. Elevated levels of calprotectin have been associated with an increased risk of cardiovascular events and may serve as a prognostic marker in these conditions \cite{25}.

In patients with psoriasis, a chronic inflammatory skin disorder, elevated calprotectin levels have been found in skin lesions. Calprotectin may contribute to the inflammatory process in psoriatic skin lesions, and serum calprotectin could serve as a biomarker for disease activity \cite{26}. Calprotectin levels have also been investigated as a potential marker of inflammation in cystic fibrosis, a genetic disorder characterized by lung and digestive system problems. Elevated fecal calprotectin levels have been observed in patients with cystic fibrosis and may reflect gastrointestinal inflammation in this population \cite{27}. Calprotectin levels have been reported to be elevated in patients with chronic kidney disease (CKD), particularly those with progressive renal impairment and inflammation. Calprotectin may serve as a marker of systemic inflammation and could be associated with the progression of CKD and cardiovascular complications \cite{26,29}.
Studies have shown elevated levels of calprotectin in patients with liver diseases such as hepatitis, cirrhosis, and non-alcoholic fatty liver disease (NAFLD). Calprotectin may reflect hepatic inflammation and injury and could potentially be used as a marker of disease severity and prognosis in liver diseases. Emerging evidence suggests a potential role for calprotectin in neurological disorders such as multiple sclerosis (MS) and Alzheimer's disease. Elevated levels of calprotectin have been observed in the cerebrospinal fluid and brain tissues of patients with MS and Alzheimer's disease, respectively, indicating neuroinflammation and neurodegenerative processes. Calprotectin has also been investigated as a biomarker for sepsis, a life-threatening condition characterized by systemic inflammation in response to infection. Elevated calprotectin levels have been observed in patients with sepsis and may reflect the severity of the inflammatory response and organ dysfunction.

**Calprotectin in Gastrointestinal Disorders:**

Both serum and fecal calprotectin can be used to assess GI disorders, particularly those involving inflammation of the gastrointestinal tract. Elevated serum calprotectin levels indicate systemic inflammation that could be associated with GI disorders. It can be related to various conditions, including IBD, such as Crohn's disease and ulcerative colitis, as well as other inflammatory conditions like rheumatoid arthritis. Serum calprotectin levels can be a marker of disease activity and severity in certain gastrointestinal disorders. Fecal calprotectin is a marker of intestinal inflammation derived from neutrophils infiltrating the intestinal mucosa. It is measured in stool samples and can provide valuable information about the presence and severity of inflammation in the gastrointestinal tract. Fecal calprotectin can be preserved and easily measured in stools for relatively long periods, sufficient enough to allow for easy collection and analysis. Elevated fecal calprotectin levels are associated with various gastrointestinal conditions, including IBD, infectious gastroenteritis, and colorectal cancer. Fecal calprotectin testing is particularly useful in distinguishing between the gastrointestinal tract's inflammatory and non-inflammatory conditions and monitoring disease activity and response to treatment in patients with IBD. Fecal calprotectin can
be used to screen, diagnose, monitor, and predict relapse of these disorders. Table 1 compares serum and fecal calprotectin.

**Fecal Sampling:**

A good-quality stool sample of about 5 gm is essential to ensure accurate fecal calprotectin measurement. It is better to use the morning sample as Calafat et al found that samples from the first stool in the morning obtained the highest calprotectin levels within-day value in about 33.3% of cases and the lowest values in about 38.9% of cases. However, the exact reason for this day variability is unclear, and it is better not to depend on a single sample determination [35]. The stool sample should not be contaminated with urine, toilet water, or other foreign substances, as this can interfere with the analysis and lead to inaccurate results. Obtaining a clean stool sample in children could be sometimes challenging. Liquid stool samples can still be used for fecal calprotectin measurement, as in many gastrointestinal disorders, diarrhea is a frequent symptom [36]. The collection containers or kits should be clean and dry. The containers inside should not be touched by gloves, tissues, or other materials to avoid contamination. The amount of stool in fecal samples should be adequate as per the laboratory's requirements. Generally, a pea-sized amount or a small spoonful of stool is sufficient for testing. The samples should be clearly labeled with the patients’ information according to the laboratory standard.

Properly collecting stool samples as soon as possible after defecation is essential to prevent calprotectin degradation. If the sample needs to be transported, it should be delivered promptly and following the laboratory's instructions. This includes using appropriate transport media or packaging to maintain sample integrity during transit. Fresh stool samples provide more accurate results compared to samples that have been stored for an extended period. However, some literature suggests that fecal calprotectin can remain stable at room temperature for up to 3 to 7 days [37,38]. Nonetheless, a study by Haisma et al revealed that fecal calprotectin levels decreased by 35-45% when stored at room temperature for six days compared to the baseline level detected immediately after sampling but remained stable when stored at 4°C. This can lead to false
reassurance for children with IBD and/or their caretakers due to falsely low calprotectin values [34]. To avoid this problem, refrigerating stool samples until they can be delivered to the hospital laboratory for testing is recommended as a standardized pre-analytical calprotectin handling procedure. According to the European Society for Paediatric Gastroenterology and Nutrition Gastroenterology (ESPGHAN) Committee, fecal samples can be used up to 2 to 3 days when kept at room temperature, 5 to 7 days when kept in a fridge (4°C) or longer when the samples are kept frozen (~20°C or ~4°C). Proper storage helps maintain the stability of calprotectin levels until analysis [39]. There are different methods of measuring fecal calprotectin, including enzyme-linked immunosorbent assay (ELISA), turbidimetric immunoassay, fluorescence immunoassay, lateral flow immunoassay (LFIA), chemiluminescent immunoassay (CLIA), and quantitative polymerase chain reaction (qPCR), as shown in Table 2. Although most tests have very high sensitivity for mucosal inflammation detections, these tests significantly differ in their specificity and absolute values [40].

The cutoff level for fecal calprotectin in children can vary depending on several factors, including age, laboratory methodology, and the specific clinical context. Generally, cutoff levels are established to distinguish between normal and elevated fecal calprotectin levels, indicating intestinal inflammation. Cutoff values for fecal calprotectin may not be comparable among different kits, even those produced by the same manufacturer [41]. Therefore, it is highly recommended that the same testing kit and extraction methodology be used for diagnosis, disease activity monitoring, and follow-up procedures in the same patient [42].

In pediatric patients, cutoff levels for fecal calprotectin are often higher than those used for adults. This is because normal calprotectin levels can vary depending on age, with higher baseline levels observed in infants and young children than adults, with a tendency towards lower values with increasing age [43]. However, there are no well-established cut-off levels for specific age ranges. Additionally, disease presentation and severity may differ in pediatric patients compared to adults, necessitating age-
appropriate cutoff levels to accurately interpret fecal calprotectin results in children [44]. Therefore, laboratories often establish pediatric reference ranges or cutoff levels to account for these age-related differences and ensure appropriate clinical interpretation. Oord et al. found cutoff values of 538 μg/g for infants between 1 and 6 months, 214 μg/g for children between 6 months and three years, and 75 μg/g for children between 3 and 4 years [45]. On the other hand, Fagerberg et al. found that the cutoff level for adults (<50 μg/g) can be used for children aged from 4 to 17 years, regardless of sex. A fecal calprotectin concentration >50 μg/g warrants follow-up [46]. However, the ESPGHAN expert group has strongly recommended that the laboratory in each center should establish its own normal fecal calprotectin cutoff values and account for variations in calprotectin levels throughout childhood and adolescence with range stratification into different age groups, such as infants, toddlers, children, and adolescents, with corresponding cutoff levels for each group [39].

It's essential to interpret fecal calprotectin levels in the context of clinical symptoms, patient history, and other diagnostic findings. Elevated fecal calprotectin levels above the established cutoff may indicate intestinal inflammation, prompting further evaluation and management [22]. Laboratories may use different assays and methodologies for fecal calprotectin testing, leading to variations in cutoff levels (Table 2). Limited evidence suggests potential gender differences in fecal calprotectin cutoff values in children. While some studies have observed slight variations in calprotectin levels between males and females, the differences are generally not considered significant enough to warrant separate cutoff values based solely on gender. Fecal calprotectin levels are primarily influenced by factors such as age, intestinal inflammation, and gastrointestinal conditions rather than gender [47,48].

Cutoff values for fecal calprotectin in children are typically determined according to age-specific reference ranges instead of gender-specific ones. It is essential to follow the reference ranges and cutoff values provided by the analysis laboratory. Interpretation of fecal calprotectin levels in children should be based on the clinical context, established guidelines, and laboratory standards [44]. Due to significant interindividual variability,
especially in young children, clinical decisions should consider fecal calprotectin levels and the overall clinical context. In preterm infants and children younger than 1 year, fecal calprotectin levels may be elevated without apparent inflammation, so careful interpretation is necessary until a normal range for this age group is established. For children older than 4 years, cutoff values of 50 μg/g, as in adults, can be used, although healthy children may have fecal calprotectin levels up to 100 μg/g or higher [39].

**Fecal Calprotectin in Some Pediatric Gastrointestinal Disorders:**

**Functional Gastrointestinal Disorders:**

Fecal calprotectin, a marker of intestinal inflammation, has garnered attention in the context of functional gastrointestinal disorders (FGIDs) in pediatric patients. FGIDs are characterized by chronic or recurrent gastrointestinal symptoms without identifiable structural or biochemical abnormalities [49]. While traditionally considered non-inflammatory conditions, emerging evidence suggests a potential role for low-grade inflammation in some FGIDs, challenging conventional views [50]. Studies investigating fecal calprotectin levels in pediatric patients with FGIDs have yielded conflicting results. Some studies report elevated fecal calprotectin levels in subsets of patients with FGIDs, suggesting the presence of subclinical inflammation [51]. However, other studies, such as Flagstad et al., who studied children between 4 and 15 years with FGIDs, found no significant differences in fecal calprotectin levels between children with FGIDs and those without [52].

The interpretation of fecal calprotectin levels in pediatric FGIDs remains challenging due to several factors. The most crucial factor is the heterogeneity of FGIDs. FGIDs encompass a diverse group of disorders, including irritable bowel syndrome (IBS), functional abdominal pain disorders, and functional constipation [53]. Subtypes within FGIDs may exhibit distinct pathophysiological mechanisms [54], potentially influencing fecal calprotectin levels differently. In addition, the symptoms of FGIDs, such as abdominal pain, bloating, and altered bowel habits, overlap with those of IBD. Differential diagnosis between FGIDs and IBD is crucial but challenging, particularly in cases of atypical presentation [55]. Moreover, psychosocial factors, such as stress and
anxiety, play a significant role in FGIDs and may contribute to symptom severity. However, the impact of psychological factors on fecal calprotectin levels in FGIDs remains unclear \[56\]. Furthermore, there is a lack of specificity in fecal calprotectin for inflammatory conditions. It could be elevated in various gastrointestinal disorders, infections, and even non-gastrointestinal conditions \[22\]. Despite these challenges, assessing fecal calprotectin levels in pediatric patients with FGIDs may offer valuable clinical insights. Elevated fecal calprotectin levels in FGIDs could indicate underlying inflammation or mucosal immune activation, prompting further evaluation or consideration of alternative diagnoses \[57\]. However, normal fecal calprotectin levels do not rule out the presence of FGIDs, highlighting the multifactorial nature of these disorders \[52\].

**Infant Colic:**

Infant colic, characterized by excessive crying and fussiness in otherwise healthy infants, remains a perplexing and challenging condition for both parents and healthcare providers. It is believed to be a self-limiting condition that typically resolves by the age of 3-4 months. Despite its prevalence, the exact cause of colic remains unclear, with various factors such as immature gastrointestinal function, infant temperament, gastrointestinal inflammation, gut microbiota composition, and feeding patterns being implicated \[58\]. While fecal calprotectin may be elevated in some cases of infant colic, it is not routinely used as a diagnostic test for this condition. The diagnosis of colic is usually based on the presence of specific criteria, such as crying for at least three hours a day, at least three days a week, in the preceding week \[59\]. Evaluating an infant with colic may involve a thorough medical history, physical examination, and sometimes additional tests to rule out other potential causes of the symptoms. Here, we summarize several studies investigating fecal calprotectin levels and gut microbiota in infants with colic, shedding light on potential associations with this enigmatic condition. Sommermeyer et al. observed significantly elevated fecal calprotectin levels equal to or greater than 100 µg/g in infants with colic. Interestingly, factors such as gender, type of feeding, gestational age, and birth weight did not appear to influence calprotectin
levels. However, infants delivered via cesarean section showed significantly higher fecal calprotectin levels \[^{14}\]. Rhoads \textit{et al.} corroborated these findings, noting that fecal calprotectin levels were approximately twice as high in infants with colic compared to control infants, measuring 413 +/- 71 µg/g vs 197 +/- 46 µg/g, respectively. Furthermore, they observed an increased presence of Klebsiella species and a decreased presence of Enterobacter/Pantoea species in the stool samples of infants with colic compared to control infants. Notably, these differences in gut microbiota were not influenced by factors such as breast vs formula feeding, consumption of elemental formula, or exposure to antibiotics \[^{61}\]. Additionally, Karabayir \textit{et al.} reported significantly higher fecal calprotectin levels in infants with typical infant colic than control infants, with median values of 651 µg/g and 354 µg/g, respectively. Follow-up revealed that four infants developed food allergies \[^{62}\]. Fayed \textit{et al.} found that infants with colic exhibited significantly higher fecal calprotectin levels and rates of Escherichia coli infection than infants without colic. Moreover, those with Escherichia coli infection demonstrated significantly higher fecal calprotectin levels than those without, highlighting the role of gastrointestinal inflammation and infection in infantile colic \[^{63}\]. However, Olafsdottir \textit{et al.} did not find a significant difference in fecal calprotectin levels detected by enzyme-linked immunosorbent assay kit between infants with classic infant colic and healthy infants (278 +/- 105 µg/g vs. 277 +/- 109 µg/g) \[^{64}\].

**Functional Constipation:**

Functional constipation refers to a condition in which children experience difficulty with regular bowel movements and infrequent evacuation of hard and painful stools without any underlying structural or organic cause. It is frequently accompanied by fecal incontinence and/or abdominal pain. It is a common condition in children and is often related to factors such as dietary habits, inadequate fluid intake, lack of physical activity, and psychological factors. However, gut inflammation is not one of the possible underlying mechanisms \[^{65}\]. In some cases, functional constipation may be associated with low-grade inflammation or other underlying conditions that can be detected through tests like fecal calprotectin. Rashed studied fecal calprotectin in 40
children with functional constipation out of 180 children with various gastrointestinal disorders. Rashed found that the mean fecal calprotectin was 23.6 ± 21.8 µg/g with no significant differences compared with healthy control with sensitivity and specificity of 89% and 81%, respectively [66]. In addition, Mahjoub et al compared fecal calprotectin in children with functional constipation with those with Hirschsprung's disease. They found that children with functional constipation had values below the predetermined cutoff value of 50 µg/g with a median value of 4 µg/g [67]. While fecal calprotectin levels may be normal in individuals with functional constipation, elevated levels could indicate the presence of inflammation in the gastrointestinal tract [39].

Gastroesophageal Reflux in Infants and Children:

Functional gastroesophageal reflux (GER) in infants and children refers to regurgitating stomach contents into the esophagus without associated complications or underlying structural abnormalities [68]. While traditionally considered a non-inflammatory condition, emerging evidence suggests a potential link between GER symptoms and low-grade intestinal inflammation, as reflected by fecal calprotectin levels [69]. Studies investigating fecal calprotectin in functional GER have yielded variable results, with some reporting elevated calprotectin levels in affected individuals compared to healthy controls, suggesting a possible association with subclinical inflammation. Shelly et al. found significantly higher fecal calprotectin levels in preterm babies with GER disease (GERD) than in their peer controls. As GER disease is usually associated with more degrees of gastroesophageal pathology and, consequently, inflammation, it is expected to have these higher levels. Therefore, fecal calprotectin can help to differentiate GER from GERD [70]. However, there is a lack of research about the value of fecal calprotectin in functional GER. Further research is needed to elucidate the role of fecal calprotectin in functional GER, its clinical implications, and its potential utility as a diagnostic or prognostic marker in this context. Understanding the relationship between fecal calprotectin and functional GER may provide insights into the pathophysiology of GER symptoms and inform personalized management approaches for affected individuals.

Functional Abdominal Pain Disorders:
Functional abdominal pain (FAPDs) in children encompasses chronic or recurrent abdominal pain without evidence of organic pathology that falls into four categories: functional dyspepsia, abdominal migraine, irritable bowel syndrome, and functional abdominal pain—not otherwise specified. While traditionally considered a non-inflammatory condition, emerging research suggests a potential role for low-grade intestinal inflammation in some cases of FAPDs \[^{50}\]. Studies investigating fecal calprotectin in FAPDs have produced mixed results, with some demonstrating elevated calprotectin levels in affected individuals compared to healthy controls, indicating possible subclinical inflammation. Moorman et al. found that children with FAPDs have significant gastrointestinal inflammation as indicated by the high fecal calprotectin, especially those with a clinically complex FAPDs profile (such as with increased rate and degree of anxiety, disability, and pain) than those with two or fewer elevations \[^{71}\]. However, other studies have not found significant differences in calprotectin levels between FAP patients and controls. Olafsdottir et al. found no significant differences in fecal calprotectin levels in healthy children and children with recurrent abdominal pain without identifiable organic disease. At the same time, it was significantly lower than children suffering from IBD \[^{64}\]. Flagstad et al. also found no significant differences in fecal calprotectin levels in children with FGID, including those with FAPD and healthy children \[^{52}\]. In a relatively recent study, Pieczarkowski et al. found no significant differences in fecal calprotectin between children with FGIDs, including dysfunctional abdominal pain, and those with healthy control. In contrast, fecal calprotectin was significantly lower when compared with children with IBD \[^{72}\]. The interpretation of fecal calprotectin in FAP remains challenging due to the heterogeneous nature of the condition and the lack of standardized diagnostic criteria. ESPGHAN stated that fecal calprotectin levels in children with different forms of FAPDs are similar to or slightly higher than healthy but lower compared with children with IBD. ESPGHAN experts strongly recommended using fecal calprotectin to distinguish functional abdominal pain disorders from organic diseases \[^{39}\].
Further research is needed to elucidate the relationship between fecal calprotectin and FAP, as well as its potential clinical implications in diagnosis and management. Understanding the role of intestinal inflammation in FAP may provide insights into its pathophysiology and guide the development of targeted therapeutic approaches.

**Cow Milk Protein Allergy:**

Cow Milk Protein Allergy (CMPA) is a common food allergy among infants and young children, affecting around 2-3% of infants in developed countries. This allergy is an immune-mediated reaction to specific protein found in cow's milk, mainly casein and whey. The gastrointestinal symptoms associated with CMPA are often nonspecific and typically not mediated by IgE antibodies. Therefore, the definitive diagnosis relies on eliminating milk from the diet to observe symptom resolution and assess for relapse upon milk reintroduction. In infants and children with CMPA, increased levels of fecal calprotectin indicate underlying intestinal inflammation, likely caused by the immune system's response to cow milk proteins. Studies have shown that infants with CMPA, particularly those with non-IgE-mediated disease, tend to have higher fecal calprotectin concentrations than healthy controls. However, a study by Díaz et al presented conflicting findings, with no significant difference in fecal calprotectin levels between infants with non-IgE-mediated CMPA and healthy controls. On the other hand, Zain-Alabedeen et al found that infants with positive cow’s milk-related-symptom- scores (CoMiSS) had higher fecal calprotectin levels than those with negative CoMiSS scores with a positive correlation between fecal calprotectin and CoMiSS scores. Because of the heterogeneous results about the role of fecal calprotectin in diagnosing CMPA, ESPGHAN stated that fecal calprotectin can be elevated in patients with CMPA with significant individual variability.

Monitoring fecal calprotectin levels in infants and children suspected of or diagnosed with CMPA can provide valuable insights into the presence and severity of intestinal inflammation and the response to elimination therapy. Qiu et al observed a significant decrease in fecal calprotectin levels after dietary intervention in infants suffering from milk protein allergy, indicating that fecal calprotectin can be a helpful monitoring tool.
in this context \cite{79}. Elevated fecal calprotectin levels after the elimination diet may indicate the need for further diagnostic evaluation, including endoscopic assessment, to determine the extent and nature of inflammation. Additionally, fecal calprotectin levels can be used as a practical gauge to track the effectiveness of dietary management and therapeutic interventions for CMPA. However, the ESPGHAN expert group did not recommend using fecal calprotectin as a diagnostic or monitoring marker for CMPA in children \cite{39}.

**Inflammatory Bowel Diseases (IBD):**

IBDs are characterized by chronic inflammation of the gastrointestinal tract, with two main categories affecting children: Crohn’s disease (CD) and ulcerative colitis (UC) \cite{80}. Diagnosing and managing IBD in children often requires a multidisciplinary approach involving pediatric gastroenterologists, dietitians, and other specialists. Fecal calprotectin has been extensively studied as a vital inflammatory biomarker that helps diagnose, monitor, and manage IBD \cite{81}. As previously mentioned, calprotectin is released by neutrophils during gastrointestinal inflammation and offers a good indicator of the severity and extent of inflammation within the intestines. Clinically, fecal calprotectin can help distinguish inflammatory from non-inflammatory bowel conditions and guide the need for further evaluation, such as endoscopy \cite{82,83}. It is also superior as a screening tool for identifying IBD in undiagnosed patients to blood inflammatory markers like CRP or ESR \cite{84}. However, according to the ESPGHAN, diagnostic endoscopy should not be delayed in cases where IBD is strongly suspected, and fecal calprotectin results are not promptly available \cite{39}.

There is no consensus on the acceptable fecal calprotectin levels for disease management. A meta-analysis of 9 studies by Degraeuwe \textit{et al} found that the best cut-off value to screen for IBD was 212 \( \mu g/g \), with a sensitivity and specificity of 0.90 and 0.87, respectively. Relying on a cut-off value of 50 \( \mu g/g \) yields a false positive in 17\% and a false negative in 2\% of cases \cite{85}. However, many studies used the cut-off value of 100 \( \mu g/g \) as a trigger to investigate the possibility of IBD \cite{86,87}. However, we should consider that young children may have typically high fecal calprotectin levels. While
fecal calprotectin is a useful biomarker for diagnosing IBD, it has its limitations in distinguishing between different types of IBD, such as Crohn's disease and ulcerative colitis. Although fecal calprotectin levels usually indicate the presence and severity of intestinal inflammation in IBD, they do not consistently differentiate between Crohn's disease and ulcerative colitis. Both Crohn's disease and ulcerative colitis can result in elevated fecal calprotectin levels due to the underlying inflammatory processes in the gastrointestinal tract. Thus, fecal calprotectin alone cannot definitively distinguish between Crohn's disease and ulcerative colitis [88]. However, some studies have suggested that fecal calprotectin levels may differ depending on the specific characteristics of the disease. For instance, fecal calprotectin levels may be higher in Crohn's disease patients with colonic involvement than those with isolated small bowel disease [89]. Similarly, fecal calprotectin levels may be lower in ulcerative colitis patients with isolated proctitis than those with more extensive colonic involvement [90].

Moreover, serial Fecal calprotectin measurements help assess the disease activity, treatment response, and the risk of relapse, with decreasing levels often indicative of successful treatment and remission [83]. Elevated fecal calprotectin levels at diagnosis or during treatment are associated with increased risks of disease progression and adverse outcomes [91]. The sensitivity and specificity of fecal calprotectin testing and its negative and positive predictive values vary depending on patient cohorts and potential confounding factors leading to elevated fecal calprotectin levels, such as bacterial or viral gastroenteritis or juvenile polyps [92].

Fecal calprotectin elevation generally correlates with histological inflammation, but absolute levels don't categorize disease activity without endoscopy [93]. While capsule endoscopy scores may not directly correlate with fecal calprotectin values, higher fecal calprotectin levels are associated with a greater likelihood of detecting lesions [94]. This suggests that elevated fecal calprotectin levels can indicate increased disease severity and the need for further investigation. Despite fecal calprotectin's utility, patients strongly suspected of IBD, especially those with alarm symptoms, should undergo endoscopic examination regardless of fecal calprotectin values [95]. Other neutrophil-
derived markers of IBD, such as lactoferrin, myeloperoxidase, matrix metalloproteinase 9, or S100A12, perform similarly to fecal calprotectin but offer no added value when used alongside it [96].

Notably, fecal calprotectin testing offers a non-invasive means of monitoring intestinal inflammation, reducing the reliance on invasive procedures like endoscopy. Its integration into clinical practice guidelines has optimized IBD management, facilitating early detection of disease recurrence, treatment optimization, and cost-effective healthcare utilization [97]. Though rare, normalization of FC is seen in patients with ulcerative colitis in clinical remission, while absolute fecal calprotectin levels in acute severe colitis don't predict prognosis [98]. Therapeutic interventions such as Infliximab or exclusive enteral nutrition (EEN) have rapidly reduced fecal calprotectin levels. Notably, Foster et al. found that fecal calprotectin levels above 250mg/g in children with Crohn's disease on Infliximab therapy may signify a risk of clinical relapse within three months [99]. On the other hand, Logan et al. found that fecal calprotectin reduction during exclusive enteral nutrition may be less clear and more gradual. Exclusion diets are also associated with mucosal healing and decreased fecal calprotectin levels [100]. Overall, fecal calprotectin plays a pivotal role in the holistic care of individuals with IBD, enhancing diagnostic accuracy, treatment monitoring, and prognostication [101]. Further research is needed to establish standardized cut-off values and optimize the clinical utility of fecal calprotectin in pediatric IBD management.

Coeliac Disease

Coeliac disease is a chronic autoimmune condition that causes inflammation in the small intestine when individuals with a genetic susceptibility consume gluten. This inflammation primarily damages the intestinal mucosa, leading to villous atrophy, crypt hyperplasia, and infiltration of inflammatory cells [102]. The immune system is involved in this process, and both innate and adaptive mechanisms contribute to it. This leads to the release of cytokines and immune cell activation, causing tissue damage [103]. Fecal calprotectin is often used as a biomarker of inflammation, but there is limited, scarce, and inconsistent data on its usefulness in diagnosing and managing coeliac
disease in children. Due to the presence of significant inflammation in patients with celiac disease, fecal calprotectin levels tend to be significantly elevated at the time of diagnosis, particularly in those presenting with higher levels of serological markers or classic symptoms. Balamtekin et al found significantly higher fecal calprotectin levels in newly diagnosed children with celiac disease than in those on gluten-free diets and healthy controls, respectively. They also observed significantly higher levels in children presenting with gastrointestinal symptoms than in those without [104]. Although the coeliac disease is primarily characterized by small intestinal inflammation rather than the large intestinal inflammation seen in IBD, fecal calprotectin levels are elevated in patients with active coeliac disease compared to those in remission or healthy controls [105]. This suggests that fecal calprotectin may reflect the degree of mucosal inflammation in coeliac disease, offering potential insights into disease activity and reflecting disease histopathological findings and response to treatment [104]. However, conflicting findings have emerged regarding the correlation between fecal calprotectin levels and anti-tissue transglutaminase antibody levels. Shahramian et al found a significant correlation between fecal calprotectin level and IgA anti-tissue transglutaminase titer in 70 newly diagnosed children with coeliac disease [106]. On the other side, et al found no significant differences in fecal calprotectin levels between untreated adults with coeliac disease and the control and no significant relation between fecal calprotectin and lesion severity, clinical score, or degree of neutrophil infiltration. However, this study was performed in adult patients with late-onset coeliac disease than usually observed in children [107]. Meanwhile, according to the Marsh classification, Szaflarska-Poplawska et al also found no significant relationship between fecal calprotectin and both the clinical picture and small intestinal lesions, in fifty-five children recently diagnosed with coeliac disease [108]. While fecal calprotectin values in coeliac disease patients at diagnosis typically average around 100mg/g across many studies, they significantly increase compared to controls [39]. However, it's important to note that fecal calprotectin elevation in coeliac disease may not be specific to mucosal inflammation related to gluten exposure [108]. Other
factors, such as concomitant gastrointestinal conditions or dietary habits, could influence fecal calprotectin levels, potentially confounding its interpretation in coeliac disease patients [104].

Fecal calprotectin can also be used as a useful non-invasive tool to assess mucosal healing and monitor disease activity in coeliac disease. Studies have shown that fecal calprotectin levels decrease following a gluten-free diet, the primary treatment for coeliac disease, indicating a reduction in intestinal inflammation [109]. Furthermore, persistent elevation of fecal calprotectin levels despite adherence to a gluten-free diet may suggest ongoing mucosal inflammation or non-responsive coeliac disease, prompting further evaluation and management [110]. Notably, this difference diminishes within four to 12 months after initiating a gluten-free diet. Despite these observations, fecal calprotectin values exhibit wide individual variability, leading to overlap between active coeliac disease and control groups [39]. Although there has been interest in utilizing fecal calprotectin to assess dietary compliance and histological recovery, no significant association has been established between fecal calprotectin levels and histological lesions. Published results suggest that fecal calprotectin measurement does not confer additional benefits beyond the currently employed serological markers in the diagnostic or follow-up phases. In addition, the ESPGHAN expert group recommended against using fecal calprotectin as a marker for diagnosing or monitoring coeliac disease [39]. Despite these challenges, fecal calprotectin holds promise as a complementary tool in managing coeliac disease, providing valuable information about intestinal inflammation and treatment response. Further research is needed to elucidate fecal calprotectin's clinical utility in coeliac disease and establish standardized protocols for its use in clinical practice. Additionally, future studies should explore the potential utility of fecal calprotectin as a prognostic marker and its role in predicting long-term outcomes in coeliac disease patients.

**COVID-19-induced Pediatric Gastrointestinal Disorders:**

COVID-19 is a respiratory illness caused by the novel coronavirus SARS-CoV-2. However, it is increasingly recognized that gastrointestinal symptoms such as diarrhea,
vomiting, and abdominal pain are also present, particularly in children. Fecal calprotectin is a marker of intestinal inflammation and can provide insights into the pathophysiology and clinical course of gastrointestinal involvement, especially in severe cases of children with COVID-19. Studies suggest that elevated fecal calprotectin levels are associated with varying degrees of intestinal inflammation with gastrointestinal symptoms in children with COVID-19. This ongoing inflammation can contribute to the overall disease severity and prognosis. However, even patients without gastrointestinal symptoms may have elevated fecal calprotectin levels, indicating subclinical intestinal inflammation, especially in severe COVID-19 cases, as shown by Ojeti et al. In addition, Shokri-Afra et al. found serum and fecal calprotectin levels are not correlated with diarrhea or other gastrointestinal symptoms. Fecal calprotectin levels could be a non-invasive tool for monitoring disease activity and treatment response in children with COVID-19 patients with gastrointestinal involvement. Serial fecal calprotectin measurements may help clinicians assess the resolution of intestinal inflammation over time and guide therapeutic interventions.

It's important to note that elevated fecal calprotectin levels are not specific to COVID-19 and can be influenced by various factors such as other infections, inflammatory conditions, or dietary factors. In addition, some COVID-19-associated gastrointestinal manifestations are due to autonomic changes and not inflammation, in which fecal calprotectin could be normal despite gastrointestinal symptoms. Therefore, fecal calprotectin should be interpreted in conjunction with clinical findings, other laboratory tests, and imaging studies to comprehensively assess gastrointestinal health in pediatric COVID-19 patients. Further research is needed to establish standardized protocols for fecal calprotectin's clinical use in COVID-19-induced pediatric gastrointestinal disorders. Nevertheless, the preliminary evidence suggests that fecal calprotectin could be a useful biomarker for monitoring this population's intestinal inflammation and disease progression.

**Gastrointestinal Infections:**
Infectious gastroenteritis, caused by various pathogens, including viruses, bacteria, and parasites, is a common condition characterized by inflammation of the gastrointestinal tract \[121\]. Fecal calprotectin has emerged as a valuable biomarker in diagnosing and managing infectious gastroenteritis, aiding in differentiating between bacterial and viral causes, helping to assess the severity and predict prognosis of infection, and differentiating between infectious and non-infectious causes of gastrointestinal symptoms \[122\]. Viral gastroenteritis, often caused by viruses such as norovirus, rotavirus, and adenovirus, is a leading cause of gastroenteritis worldwide, particularly in children \[127\]. Studies have demonstrated elevated fecal calprotectin levels in patients with viral gastroenteritis, indicating the presence of intestinal inflammation. However, fecal calprotectin elevation in viral gastroenteritis may be transient and resolve as the infection clears. Despite this, fecal calprotectin can still be useful in monitoring disease activity and assessing the need for further evaluation or intervention \[124\].

Bacterial gastroenteritis, commonly caused by pathogens like *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Shigella*, is characterized by inflammation of the intestinal mucosa. Fecal calprotectin levels are elevated in patients with bacterial gastroenteritis, reflecting the severity of mucosal inflammation. Combining fecal calprotectin with occult blood in the stool is a useful marker for diagnosing bacterial etiology in children with acute gastroenteritis \[125\]. In cases of bacterial gastroenteritis, fecal calprotectin elevation may be higher and persist longer than viral infections, correlating with the serenity and persistence of symptoms and the extent of mucosal damage \[126\]. Sykora et al found that fecal calprotectin can help tell if the AGE is caused by bacteria or viruses in children under 3 years old. They found that combining fecal calprotectin with other inflammatory markers, such as CRP, can accurately differentiate bacteria (median fecal calprotectin was 219 µg/g) from viral (median fecal calprotectin was 49.3 µg/g) AGE in children \[127\]. In addition, Duman et al showed that fecal calprotectin levels are higher in patients with certain bacterial infections like *Salmonella* and *Shigella* compared to viral infections like Rotavirus and Norovirus. They found that fecal calprotectin can be a good marker for diagnosing bacterial AGE, with high sensitivity and specificity, when a
cutoff value of fecal calprotectin of 710 µg/g is used \[^{128}\]. However, in a severe form of acute gastroenteritis, fecal calprotectin cannot differentiate between viral and bacterial gastroenteritis, especially in those who need hospitalization \[^{129}\]. However, the ability of fecal calprotectin to assess the severity of acute gastroenteritis remains uncertain. While it seems to be linked to the severity of symptoms in children, it may not be as useful in adults with *Clostridium difficile* infection (CDI). In *Clostridium difficile*, both fecal calprotectin and fecal lactoferrin increase, but they don't seem to reflect how serious the infection is and cannot differentiate between *Clostridium difficile* infection and antibiotic-associated diarrhea \[^{130,131}\].

Parasitic gastroenteritis, caused by parasites such as *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica*, can also lead to intestinal inflammation \[^{132}\]. Studies have shown that fecal calprotectin levels are elevated in patients with parasitic gastroenteritis, indicating ongoing mucosal inflammation. Fecal calprotectin measurement may help differentiate parasitic infections from other causes of gastroenteritis and assess the response to anti-parasitic therapy \[^{133,134}\]. Overall, fecal calprotectin is a valuable tool in evaluating infectious gastroenteritis, providing clinicians with important information about the presence and severity of intestinal inflammation \[^{124}\]. While fecal calprotectin elevation is not specific to infectious gastroenteritis and can occur in other gastrointestinal conditions, its measurement can aid in diagnosing, managing, and monitoring patients with infectious gastroenteritis of viral, bacterial, or parasitic origin. However, The ESPGHAN expert group recommended against the routine use of fecal calprotectin in acute gastroenteritis, aiming to distinguish viral from bacterial viral gastroenteritis in children \[^{39}\]. Further research is needed to elucidate the role of fecal calprotectin in specific pathogens and its utility in guiding treatment strategies for infectious gastroenteritis.

**Cystic Fibrosis:**

Cystic fibrosis is a prevalent genetic disorder characterized by multi-organ involvement due to dysfunctional ion transport across epithelial cells, leading to thick and sticky mucus production, primarily affecting the pancreas (85%), respiratory system, and
gastrointestinal tract. While respiratory symptoms dominate the clinical picture, gastrointestinal manifestations are also common in cystic fibrosis, including malabsorption, pancreatic insufficiency, and liver disease. Mounting evidence suggests that cystic fibrosis-associated intestinal pathology is characterized by mucous accumulation, dysmotility, and dysbiosis, contributing to chronic inflammation and microbial colonization. Notably, studies employing whole gut lavage and capsule endoscopy have highlighted increased levels of inflammatory biomarkers, including interleukin-8 (IL-8), in cystic fibrosis patients, indicative of ongoing intestinal inflammation.

Fecal calprotectin is a promising biomarker for assessing gastrointestinal inflammation and mucosal integrity in children with cystic fibrosis. Elevated fecal calprotectin levels in children with cystic fibrosis reflect bacterial overgrowth, which induces intestinal inflammation and correlates with the severity of gastrointestinal symptoms and disease progression, as evidenced by Rumman et al. Therefore, oral probiotic administration can reduce bacterial overgrowth-induced intestinal inflammation and significantly reduce fecal calprotectin levels. Additionally, fecal calprotectin levels have been found to correlate with markers of nutritional status and pancreatic function, suggesting a link between intestinal inflammation and malnutrition in cystic fibrosis. A meta-analysis by Talebi et al. found that median fecal calprotectin levels were inversely associated with body mass index (BMI) and BMI Z score, reflecting the nutritional status of the patients. However, Adriaanse et al. found this negative correlation in adult patients and not in children. Studies have shown that fecal calprotectin levels are higher in patients with cystic fibrosis and pancreatic insufficiency, indicating a potential association between pancreatic status and intestinal inflammation. However, studies also observed increased fecal calprotectin in children with or without pancreatic insufficiency. This increase in fecal calprotectin may be due to the pulmonary production of calprotectin, which is swallowed into the gut, potentially confounding its interpretation, especially in the presence of pulmonary infections. This observation can also explain why fecal calprotectin levels may inversely correlate with lung function.
in adult patients with cystic fibrosis [145]. However, the relationship is less clear in children. However, there is a lack of conclusive evidence regarding the extent to which sputum calprotectin contributes to fecal calprotectin levels due to the possible denaturation of sputum calprotectin by gastric acidity [146]. Another important confounding to fecal calprotectin in patients with cystic fibrosis is that the underlying genetic abnormalities in cystic fibrosis could affect the S100A8 and S100A9 gene expression, causing altered epithelial barrier function and impaired innate immunity, particularly at respiratory and gastrointestinal epithelium [147]. Furthermore, fecal calprotectin has shown utility in monitoring response to therapy and predicting clinical outcomes in cystic fibrosis patients [148]. High fecal calprotectin can also affect the quality of life of children, with poor quality of life with higher levels [149]. Changes in fecal calprotectin levels over time can indicate treatment efficacy and disease progression, guiding therapeutic interventions and optimizing patient care. Fecal calprotectin may also serve as a non-invasive tool for assessing intestinal inflammation in cystic fibrosis patients, reducing the need for invasive procedures such as endoscopy [150]. However, it's important to note that fecal calprotectin levels can be influenced by factors other than cystic fibrosis-related intestinal inflammation, such as infection, dietary factors, and certain medications, such as antibiotics [39]. Therefore, fecal calprotectin measurements should be interpreted considering the clinical symptoms and other laboratory findings to avoid misinterpretation. Despite these challenges, fecal calprotectin has shown promise as a marker of intestinal inflammation in cystic fibrosis and has been used to monitor treatment response and predict clinical outcomes [139]. However, more research is needed to establish its reliability in assessing intestinal inflammation and guiding treatment decisions in patients with cystic fibrosis. Further studies should also explore its correlation with endoscopic and histological findings for a better understanding of its clinical utility in cystic fibrosis management.

**Pros and Cons of Fecal Calprotectin Use in Pediatrics Gastrointestinal Diseases:**
Fecal calprotectin is a laboratory test commonly used to diagnose gastrointestinal disorders in children and adults. It offers significant advantages in diagnosing, monitoring, and managing pediatric gastrointestinal diseases [9]. However, when interpreting results and making clinical decisions, we must also consider its limitations and challenges. One of the significant advantages of fecal calprotectin is that it provides a non-invasive means of assessing intestinal inflammation. This makes it particularly valuable in pediatric patients who may be averse to or unable to undergo invasive procedures like endoscopy [83]. The non-invasive nature of the test enhances patient comfort and compliance with monitoring protocols. Elevated fecal calprotectin levels can indicate the presence of intestinal inflammation even before clinical symptoms manifest [151]. This allows for early detection and diagnosis of gastrointestinal diseases in pediatric patients. Early identification facilitates prompt initiation of treatment and potentially prevents disease progression [152]. Fecal calprotectin also helps differentiate between inflammatory and non-inflammatory gastrointestinal conditions. This aids in the diagnosis of conditions like inflammatory bowel disease (IBD) and cystic fibrosis-associated intestinal pathology [153]. Overall, fecal calprotectin is a valuable tool in diagnosing and monitoring pediatric gastrointestinal diseases, but its limitations and challenges must be considered when interpreting results and making clinical decisions.

Table 3 outlines the changes in fecal calprotectin levels across different gastrointestinal diseases and populations.

While fecal calprotectin levels indeed vary across different gastrointestinal diseases, the question of whether calprotectin is directly involved in the occurrence and development of these diseases is a complex one. Calprotectin, a calcium-binding protein predominantly found in neutrophils, plays a crucial role in the innate immune response, particularly in inflammation and host defense mechanisms in the gastrointestinal tract [22]. For example, in IBD, calprotectin is often used as a marker of intestinal inflammation. Elevated fecal calprotectin levels are associated with active disease and correlate with disease severity in both Crohn's disease and ulcerative colitis [154]. However, whether calprotectin directly contributes to the pathogenesis of IBD or is
merely a consequence of ongoing inflammation remains a subject of investigation. Similarly, in coeliac disease, where intestinal inflammation is triggered by gluten consumption in genetically susceptible individuals, fecal calprotectin levels are elevated, reflecting mucosal inflammation. While calprotectin may contribute to the inflammatory response, its exact role in the pathogenesis of coeliac disease requires further elucidation [20].

In infectious gastroenteritis, including viral, bacterial, and parasitic infections, fecal calprotectin levels rise in response to intestinal inflammation caused by the invading pathogens. Calprotectin likely serves as a marker of the host's immune response to infection rather than being directly involved in disease occurrence [10]. In cystic fibrosis-associated intestinal pathology, characterized by mucous accumulation, dysmotility, and dysbiosis, calprotectin levels are elevated due to bacterial overgrowth-induced inflammation [155]. Calprotectin may contribute to perpetuating inflammation in the intestinal mucosa, exacerbating disease severity [142]. Overall, while fecal calprotectin is a valuable biomarker for assessing gastrointestinal inflammation, its precise role in the occurrence and development of specific gastrointestinal diseases remains incompletely understood. It likely represents a component of the host's immune response to mucosal injury and inflammation rather than being a primary driver of disease pathogenesis [156]. Further research is needed to clarify the mechanistic role of calprotectin in gastrointestinal diseases and its potential as a therapeutic target.

On the other hand, fecal calprotectin has many cons that should be considered while being interpreted. Elevated fecal calprotectin levels are not specific to any particular gastrointestinal condition. They can occur in various inflammatory and non-inflammatory conditions, including infectious gastroenteritis, irritable bowel syndrome, and even non-gastrointestinal conditions like asthma and non-inflammatory conditions such as celiac disease. This lack of specificity may lead to false-positive results and diagnostic ambiguity [115]. Interpreting fecal calprotectin levels in pediatric patients with gastrointestinal diseases can be challenging due to factors such as variations in normal values among different age groups, the presence of confounding factors, and the lack of
established cutoff values for certain conditions. In certain pediatric gastrointestinal diseases, the specificity of fecal calprotectin as a marker of intestinal inflammation may be limited due to factors such as pulmonary production of calprotectin, genetic abnormalities affecting gene expression, and overlap with respiratory symptoms. Inconsistent levels of fecal calprotectin in various pediatric gastrointestinal disorders stem from a multitude of factors. Firstly, demographic factors such as age can influence calprotectin levels, alongside diet, medication usage (e.g., nonsteroidal anti-inflammatory drugs or antibiotics), gastrointestinal bleeding, and concurrent infections. Infants and young children may have naturally higher levels of calprotectin compared to older children and adults due to factors such as the ongoing development of the gastrointestinal tract and the maturation of the immune system. Disease-specific factors also contribute to variation. Inflammatory bowel diseases (IBD), characterized by dysregulated immune responses and chronic inflammation, typically exhibit consistently elevated calprotectin levels. Conversely, functional gastrointestinal disorders like IBS often lack significant inflammation, resulting in normal-range calprotectin levels. Disease severity further complicates matters; acute infectious gastroenteritis or IBD flare-ups generally correlate with markedly elevated fecal calprotectin levels. Host immune responses, including genetic predispositions and individual inflammation susceptibilities, also influence calprotectin production. Methodological considerations, such as sampling timing and assay techniques, introduce additional variability. Moreover, lifestyle factors, medication usage, and dietary habits can impact calprotectin expression. Grasping these intricacies is essential for accurately interpreting calprotectin measurements and discerning their clinical significance in pediatric gastrointestinal disease diagnosis and management. Table 4 e provides a concise overview of the diverse factors influencing fecal calprotectin levels in pediatric gastrointestinal disorders, helping to understand the complexities involved in result interpretation and diagnosis. In addition, collecting stool samples for fecal calprotectin testing may be challenging in younger pediatric patients or those with certain conditions affecting bowel habits.
While fecal calprotectin testing is generally considered cost-effective for diagnosing and monitoring certain conditions, it may still represent an added cost, particularly if repeated testing is necessary over time. Clinicians should be aware of these limitations when using fecal calprotectin in these populations. While fecal calprotectin is widely used in clinical practice, there is a lack of consensus among expert groups regarding its routine use in certain pediatric gastrointestinal conditions, such as acute gastroenteritis \[182\]. The absence of standardized guidelines may lead to variability in clinical practice and uncertainty regarding its appropriate use in specific patient populations. Table 5 summarizes the pros and cons of using fecal calprotectin in children with gastrointestinal disorders.

**Limitations of the study:**

The primary limitation of this review is inherent to the selection of articles from databases and search engines. Despite efforts to include a comprehensive range of studies, there may be a risk of selection bias, wherein certain articles or perspectives were inadvertently excluded. Additionally, the inclusion of only English-language articles may have introduced language bias. Another limitation relates to the quality and reliability of the included studies. While efforts were made to select high-quality research articles, review articles, meta-analyses, and consensus papers, variations in study design, sample sizes, methodologies, and reporting standards across studies may have influenced the robustness of the findings. There is a possibility of publication bias, whereby studies with positive or significant results are more likely to be published than those with null or negative findings. This bias could impact the comprehensiveness of the evidence base and skew the interpretation of fecal calprotectin's role in pediatric gastrointestinal diseases. The interpretation of fecal calprotectin levels in pediatric gastrointestinal diseases is complex and multifaceted. While elevated levels are generally indicative of intestinal inflammation, other factors such as age, diet, medication use, and concurrent infections can influence results. Therefore, the findings of this review should be interpreted with caution, considering the contextual factors surrounding fecal calprotectin testing in clinical practice. The generalizability of the
findings may be limited by the heterogeneity of the included studies and the specific populations under investigation. The role of fecal calprotectin in pediatric gastrointestinal diseases may vary across different patient demographics, disease severities, and geographical regions, which should be considered when applying the findings to specific clinical settings. Despite these limitations, this review provides valuable insights into the pros and cons of fecal calprotectin use in pediatric gastrointestinal diseases. Future research should aim to address the limitations identified herein by conducting well-designed prospective studies with standardized methodologies, larger sample sizes, and diverse patient populations.

Recommendations:

After conducting a comprehensive analysis, we have identified several recommendations to make fecal calprotectin testing more effective in diagnosing and managing pediatric gastrointestinal diseases. To begin with, standardizing protocols for fecal calprotectin testing can improve the consistency and comparability of results across different studies and clinical settings. Developing age-specific reference ranges for fecal calprotectin levels can account for variations in pediatric populations, which can help accurately interpret test results. Integrating fecal calprotectin testing with other clinical parameters such as symptoms and imaging studies can enhance diagnostic accuracy and facilitate personalized management strategies for pediatric patients. Additionally, incorporating serial fecal calprotectin measurements into routine monitoring protocols can enable clinicians to track disease progression, assess treatment response, and detect early signs of relapse in pediatric gastrointestinal diseases. We believe that continued research efforts are necessary to explore the clinical utility of fecal calprotectin in specific pediatric populations, optimize cutoff values for different conditions, and elucidate its role in predicting long-term outcomes and guiding therapeutic interventions. Finally, efforts to establish consensus guidelines and protocols for fecal calprotectin testing in pediatric practice would contribute to the optimization of its clinical utility. By implementing these recommendations, healthcare professionals can harness the full potential of fecal calprotectin as a valuable biomarker.
in the management of pediatric gastrointestinal diseases, ultimately improving patient care and outcomes.

CONCLUSION
In conclusion, fecal calprotectin is a valuable biomarker for assessing intestinal inflammation and mucosal integrity in pediatric gastrointestinal diseases. Despite its limitations, including lack of specificity and variability in interpretation, fecal calprotectin offers significant advantages in diagnosing, monitoring, and managing various conditions such as functional gastrointestinal disorders, IBDs, coeliac disease, COVID-19-induced gastrointestinal disorders, gastroenteritis, and cystic fibrosis-associated intestinal pathology. Its non-invasive nature, sensitivity in detecting inflammation, and ability to aid in disease monitoring and early detection make it a valuable tool in pediatric gastroenterology. However, the interpretation of fecal calprotectin levels should consider clinical context, patient demographics, and potential confounding factors. Additionally, further research is needed to establish standardized protocols, cutoff values, and guidelines for fecal calprotectin testing in pediatric practice. Future studies should focus on addressing the limitations identified in this review, such as selection bias, quality of included studies, publication bias, and interpretation challenges. By doing so, clinicians can optimize the clinical utility of fecal calprotectin in pediatric gastrointestinal diseases, leading to improved patient outcomes and quality of care.
<table>
<thead>
<tr>
<th>PRIMARY SOURCES</th>
<th>URL</th>
<th>Text</th>
<th>Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>69 words — 1%</td>
</tr>
<tr>
<td>3</td>
<td><a href="http://doi.org">doi.org</a></td>
<td>Kareem Omran, Rakesh Vora, Tom Marrs. &quot;Gastroenterological assessments in children with</td>
<td>33 words — &lt; 1%</td>
</tr>
<tr>
<td>4</td>
<td><a href="http://journals.lww.com">journals.lww.com</a></td>
<td></td>
<td>31 words — &lt; 1%</td>
</tr>
<tr>
<td>5</td>
<td><a href="http://www.frontiersin.org">www.frontiersin.org</a></td>
<td></td>
<td>29 words — &lt; 1%</td>
</tr>
<tr>
<td>6</td>
<td><a href="http://clinical.r-biopharm.com">clinical.r-biopharm.com</a></td>
<td></td>
<td>18 words — &lt; 1%</td>
</tr>
<tr>
<td>7</td>
<td><a href="http://pipelinereview.com">pipelinereview.com</a></td>
<td></td>
<td>17 words — &lt; 1%</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td>16 words — &lt; 1%</td>
</tr>
<tr>
<td>Name of Website/Link</td>
<td>Description</td>
<td>Number of Words</td>
<td>Percentage</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------</td>
<td>----------------</td>
<td>------------</td>
</tr>
<tr>
<td>fitlifeoffraser.com</td>
<td>Internet</td>
<td>15</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>thesunflowerwellnesscenter.com</td>
<td>Internet</td>
<td>15</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>Amy A. Gelfand, Katherine C. Thomas, Peter J. Goadsby. &quot;Before the headache&quot;, Neurology, 2012</td>
<td>Crossref</td>
<td>14</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td><a href="http://www.cmaj.ca">www.cmaj.ca</a></td>
<td>Internet</td>
<td>14</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td><a href="http://www.merckmanuals.com">www.merckmanuals.com</a></td>
<td>Internet</td>
<td>14</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>ccimindia.org</td>
<td>Internet</td>
<td>13</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>gastro.healthconferences.org</td>
<td>Internet</td>
<td>13</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td><a href="http://www.marketresearch.com">www.marketresearch.com</a></td>
<td>Internet</td>
<td>13</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>


arxiv.org

intmed.med.wayne.edu