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Review Article

Calprotectin: Clinical Applications in Pediatrics

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As seen over the past 20 years, calprotectin has evolved as a novel, non-invasive biomarker of gastrointestinal (GI) inflammation. We present this review of calprotectin in pediatrics. This article will focus on studies using calprotectin concentrations from different body fluids to monitor inflammation in different disease states and conditions. The ultimate goal of our group is to lay down a foundation as we consider using calprotectin prospectively as a marker of intestinal inflammation that could lead to further testing and possibly a marker of preparedness for feeding. We surveyed all published studies in English of calprotectin in neonates, infants, children, and adolescents through February 2014. We will discuss calprotectin's basic properties and analysis such as characteristics, identification, presence in body fluids, and maturational development. In addition, calprotectin's use in inflammatory diseases exploring both GI and non-GI conditions will be evaluated and compared with other serum markers presently available. Finally, a summary of our findings and discussion of future work that could be undertaken in order to render calprotectin as a more useful monitoring tool to the medical research community will complete the review.

INDEX TERMS: biomarker, inflammation, leukocyte L1 antigen complex, pediatric

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INTRODUCTION

It is well-known that inflammation, either locally in the gastrointestinal (GI) tract or systemically, is a key factor that adversely affects nutrition and metabolic outcomes in patients. The quest for biomarkers depicting this complex process in the human body has been the interest of a wide variety of groups. Moreover, inflammation has often been identified as one of the root-causes of several chronic disease affecting patients nowadays. Having a biomarker that could reliably be detected in serum/plasma and/or urine could alleviate some of the difficulties that invasive procedures demand on follow-up for these patients. This would reduce part of the financial burden on our healthcare system today. Emerging studies are demonstrating the utility of calprotectin, increasing its clinical application in GI as well as other inflammatory diseases.

Currently in practice, there are other laboratory markers used to assess systemic inflammation such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Other less-commonly used protein markers that will be mentioned in this review are: lactoferrin, M_2 isoform of pyruvate kinase (M_2-PK), and intestinal-fatty acid binding protein (I-FABP). Lactoferrin is a 80-kDa iron-binding glycoprotein thought to be released by neutrophils, along with other host defense factors, and possess antimicrobial and anti-inflammatory properties.[1] M_2-PK is an important enzyme, present in all cells, whose one of its functions is to catalyze transphosphorylation as part of the last step of glycolysis.[2] Its role in innate immunity was observed as neutrophils with deficient PK activity led to ineffective intracellular killing and therefore susceptibility to infections.[3] I-FABP is 1 of 2 cytosolic fatty-acid binding proteins present in enterocytes, thought to be involved in fat transport across the digestive mucosa.[4] Recent evidence has shown that rises in serum levels of I-FABP as well as FABPs from other tissue sources could be reflective of tissue injury, depending on site of origin.[5] We speculate that calprotectin could provide greater sensitivity

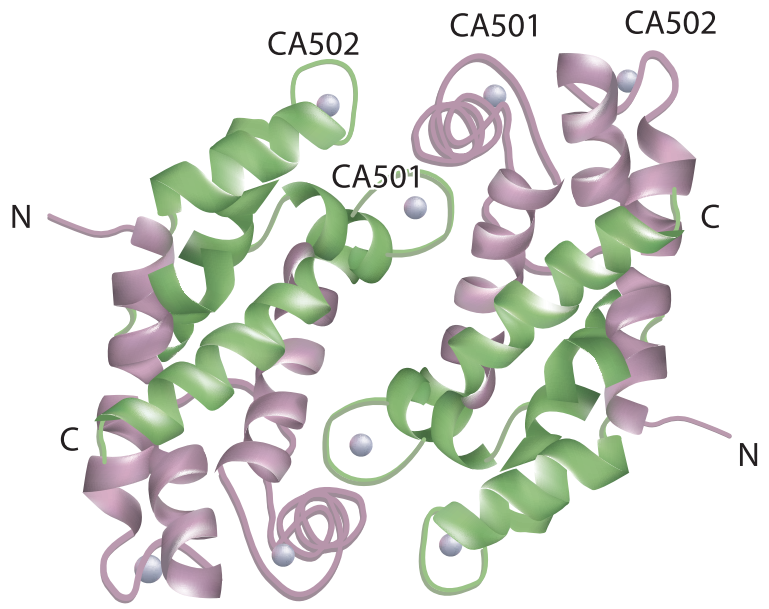

Figure 1. The heterotetramer structure of calprotectin.[2] S100A8 chains (upper left); S100A9 chains (upper right); CA501 and CA502 sites where Ca^{2+} ions (spheres) bind. C and N denote protein termini (with permission from *Elsevier*).

or specificity to distinguish inflammation, and be useful for practitioners as suggested by some studies herein.

BASIC PROPERTIES AND ANALYSIS

Identification and Presence in Body Fluids

Calprotectin is a 36-kDa protein, with 2 heavy and 1 light non-covalently linked chains that binds calcium and zinc (see Figure 1).[6,7] First isolated in the 1970s, calprotectin is found in the cytosolic fluid of neutrophils, monocytes, and macrophages.[8] Calprotectin has been referred to by several names: leukocyte derived (L1) protein,[9] MIF (Migration Inhibitory Factor) related protein 8 and 14 (MRP-8/14),[10] and cystic fibrosis antigen (CFA).[11] Wilkinson et al[12] determined the similarities between these aforementioned 3 entities and proposed the name calgranulin A and B due to its calcium-binding properties and its main source, granulocytes.[12] The structure was further categorized and found to belong to the S100 family of proteins,[13] S100A8 /S100A9 specifically. The S100 family of proteins contains several EF-hand (α-helix-loop-α-helix) calcium-binding proteins.[14] It has been shown that altered expression of these proteins plays key roles in neurodegenerative and inflammatory disorders.[15] These alterations can occur intracellularly as well as extracellularly, particularly for S100A8/S100A9 and a related protein, recently discovered, S100A12. The current name, calprotectin, was suggested following reports of its antimicrobial activity against Enterobacteriaceae in blood cultures and *Cryptococcus* spp. in cerebrospinal fluid (CSF) isolates.[16]

Calprotectin has been detected in multiple types of body fluids, though most of the literature reporting content in fecal samples. Initially, as seen in the earlier studies, values were reported in µg/mL. With the optimization of fecal analytical kits, most of the literature currently reports on µg/gram of feces. This presumably was instituted in order to off-set the dilutional effects that diarrhea could have in a sample that was being quantified based on volume. Clinicians are advised to take into account the units of values reported, particularly in feces, when surveying the literature. Reference ranges have also been determined in serum/plasma. Data from 533 blood donors showed values of 0.09 to 0.53 mg/L and 0.12 to 0.66 mg/L for females and males, respectively.[17] CSF calprotectin concentrations were 0.3 to 0.35 mg/L in human immunodeficiency virus (HIV)-infected patients with opportunistic infections compared to reference levels around 0.037 mg/L.[18] Saliva samples from 12 healthy adults yielded mean levels of 3.2 mg/L from parotid saliva, 22.0 mg/L from stimulated whole saliva, and 40.9 mg/L from mucosal transudate.[19] These findings highlighted the importance of sampling procedure and site of collection, and its likely role in host defense. Calprotectin concentrations in the urine of 7 patients with pyelonephritis was 1 mg/L compared to 0.024 mg/L in 63 healthy controls.[20] Meconium from 131 neonates had a mean ± SD calprotectin concentration of 145 ± 78.5 µg/g.[21] In patients with rheumatoid arthritis the median synovial fluid level was 18 mg/L compared to 0.9 mg/L in patients with osteoarthritis,[22] suggesting it as

Table 1. Pediatric Studies Analyzing Fecal Calprotectin by Age Group

Study	Age Group	Median FC (µg/g)	95% CI (µg/g)
Rugtveit et al[23]	6 wk	269.0	276.7-847.9
	3 mo	263.5	185.0-618.6
	6 mo	79.0	81.5-181.4
	1 yr	67.0	63.5-291.0
	2 yr	64.0	70.5-184.2
	5 yr	49.0	38.4-87.1
Hestvik et al[24]	0-3 mo	345	195-621
	3-6 mo	278	85-988
	6-12 mo	183	109-418
	1-4 yr	75*	53-119
	4-12 yr	28*	25-35
Fagerberg et al[25]	4-6 yr	28.2	NR
	7-10 yr	13.5	NR
	11-14 yr	9.9	NR
	15-17 yr	14.6	NR
Oord et al[26]	1-6 mo	538†	NR
	6 mo-3 yr	214†	NR
	3-4 yr	75†	NR

CI, confidence interval; FC, fecal calprotectin; NR, not reported; where reported
*$p < 0.0001$
†Based on 97.5 percentiles

a marker of local inflammation. Calprotectin has been isolated from almost every fluid possible in the human body as shown here and discussed in later sections. As the reader will encounter later on, investigators have mostly used fecal calprotectin (FC) concentrations to analyze and venture into other disease states, though clinical applications of calprotectin concentrations from other fluids have surfaced again. Research groups should still be cautious when assessing inflammatory status from raised calprotectin concentrations and determine if it depicts a generalized state or it only describes the local environment from which the sample was obtained.

Maturational Development

As more interest developed in calprotectin, researchers questioned if there were any age-dependent variations associated with its expression. Table 1 summarizes the studies that assessed FC concentrations in different pediatric age groups.[23-26] Differences have been attributed to developmental factors among subjects such as gestational and/or postnatal age or feeding patterns with either breastmilk or formula. Zopelli et al[27] associated FC values with postnatal and gestational age (GA) in 3 groups of premature infants. All groups experienced a decrease in FC levels in the first week of life; however, afterwards there was a statistically significant increase in those born between 26 and 32 weeks GA, compared to those born at less than 26 weeks GA who continued to decline. Likewise, a significant decrease was observed in FC concentrations of 52 preterm infants during the first week of life, which was followed by a significant increase over the next 7 weeks of life.[28] Similar studies in comparable preterm infant cohorts did not support those results.[29-31] Others thought differences, particularly in neonates and infants, may be explained by what was being fed to the gut. Savino et al[32] analyzed FC from 39 healthy term infants receiving breast milk exclusively from their mothers. Median FC (range) was 555.00 (122.50-2000.00) µg/g in breast-fed compared to 206.60 (31.20-797.60) µg/g, ($p < 0.001$) of 35 formula-fed infants. This was contradictory to data from another group of 69 healthy term infants

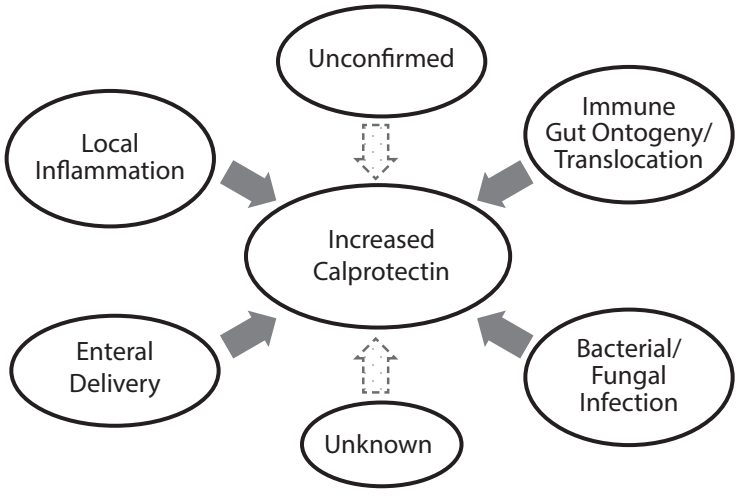

Figure 2. Factors possibly contributing to increased fecal expression of calprotectin in early infancy.

whose median FC was 167 µg/g.[33] There were no significant associations whether the infant was fed breast-milk, standard or prebiotic formula.[33] It begs the question "Can calprotectin be transmitted from mother to infant through breast milk?" To date no reports have been published of calprotectin being isolated from breast milk and/or colostrum. We hypothesize that there are many factors (Figure 2), yet to be fully elucidated, involved in the expression of calprotectin in the gut of early infants. If such factors create an effect, we should focus on investigating and determining new reference ranges for neonates/infants, premature and term, as well as older pediatric patients. Established references could help clinicians interpret calprotectin concentrations and be able to classify them as truly inflammatory, or just simply physiologic and part of ongoing development, much like bilirubin.

USE IN INFLAMMATORY DISEASES

A Marker of Inflammatory Bowel Disease

In the late 1990s to early 2000s, a few reports appeared in the literature, proposing the use of FC as a non-invasive marker of intestinal inflammation. Research focused initially in inflammatory bowel disease (IBD) and how calprotectin could be used to monitor disease activity. Up to that point inflammatory gut status could only be determined by histologic findings as a result of endoscopic procedures. Roseth et al[34] studied 62 patients with ulcerative colitis (UC) undergoing colonoscopy. They reported median fecal concentrations of 68 mg/L, compared to 11.5 mg/L in those with low disease activity, and 6 mg/L in controls ($p < 0.001$).[34] Similarly, Limburg et al[35] found FC concentrations to be significantly associated with colorectal inflammation. As analytical assays were optimized, the application of this protein was tested in populations such as children, in whom invasive procedures are increasingly challenging. Table 2 shows select studies utilizing FC analysis in pediatric populations with suspected or soon after diagnosis of IBD.[36-39] Table 3 lists pediatric studies in which FC was analyzed in patients with a long-standing diagnosis of IBD.[40-45] Most of these research groups adopted the manufacturer's recommended cut-offs for normal FC, between 50 and 100 µg/g. These studies revealed substantial differences in FC concentrations between those with versus those without inflammation at the time of assessment. Even more, some of the studies hinted at establishing differences for the 2 main IBD types: UC and Crohn's disease (CD). Children with UC may exhibit higher fecal concentrations of calprotectin than children with CD depending on disease severity.[46] Although there is conflicting evidence regarding which shows higher values given the different areas of involvement and invasiveness of the 2. A few investigators took a step forward to evaluate FC as a predictive marker of IBD relapse in children. Walkiewicz et al[47] examined 32 children with IBD, 11 of those with CD. A FC value above 400 µg/g in asymptomatic CD patients, predicted relapse within 9 months of collection in 89% of patients (95% confidence interval [CI] 51.8-99.7, $p = 0.03$) compared to no clinical relapse within 9 months when FC values were below 400 µg/g.[47] Similarly, van Rheenen[48] reported that teenagers with a FC above 500 µg/g had a 53% (10/19) risk of progressing to symptomatic relapse within 3 months, whereas a value below 500 µg/g only had a 12% (5/43) risk of symptomatic relapse. A retrospective analysis of 73 children with IBD found that a FC concentration of 275 µg/g had sensitivity and negative predictive value (NPV)

Table 2. Fecal Calprotectin Pediatric Studies on Suspected or at Diagnosis of Inflammatory Bowel Disease

Study (*n*)	Population	Assessment Standard	Results	Comments
Fagerberg et al[36] (n = 36)	Children with GI symptoms and suspected inflammation	Colonoscopy	Median FC: 349 (15.4-1860) µg/g for those with IBD (n = 22) and 16.5 (5-65) µg/g for those without (n = 14)	A target of < 50 µg/g had 95% sensitivity, 93% specificity, PPV 95% and NPV 93%
Quail et al[37] (n = 48)	33 children with CD, 5 with UC and 10 with unspecified IBD type	Standard criteria, and comparative laboratory markers: WBC, CRP, ESR, LFT(s) Hgb, albumin	Median FC (IQR) of 750 (235.8-1251) µg/g in 46/48 patients studied at diagnosis	Abnormal lab values in 32/45 (71.1%) for ESR, 19/38 (50.0%) for CRP, 12/45 (26.7%) for hypoalbuminemia, and 38/46 (82.6%) for Hgb
Diamanti et al[38] (n = 626)	68 UC patients, 49 with CD, 28 with normal mucosa, 52 with other IBD type and 429 controls	Rome and/or Porto criteria, plus colonoscopy	A conventional value of 100 µg/g had a sensitivity of 100% (95% CI: 97-100) and a specificity of 68% (95% CI: 56-78), with a LR of 3.1	A proposed value of 160 µg/g, produced a sensitivity of 100% (95% CI: 97-100) and a specificity of 80% (95% CI: 71-88) with a LR of 5
Henderson et al[39] (n = 190)	91 children with IBD and 99 non-IBD controls	Standard histological and radiological findings for diagnosis of IBD and lab markers such as WBC, CRP, ESR, Hgb, albumin and platelet count	Median FC (IQR) at diagnosis was 1265 (734-2024) µg/g for the IBD group compared with 65 (20-235) µg/g in controls ($p < 0.001$)	Area under the curve of the ROC for FC was significantly higher than ESR, CRP, WBC, Hgb and platelet count each ($p < 0.05$), but not for albumin ($p = 0.374$).

CD, Crohn's disease; CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Hgb, hemoglobin; IBD, inflammatory bowel disease; IQR, interquartile range; LFT, liver function tests; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; UC, ulcerative colitis; WBC, white blood count

of 97% and specificity and a positive predictive value (PPV) of 97%, with a likelihood ratio of 6.4 for predicting IBD relapse.[49] Here, we observe that investigators propose different cut-off values, as stated in several studies. The reason more than likely is that the stated value gave them the best sensitivity/specificity combination based on receiver-operator characteristic (ROC) curves. This can obviously create some confusion among clinicians as to which cut-off value they should use. In the meantime, clinicians would be advised to analyze intrapatient variability and have patients serve as their own controls to determine whether they are experiencing relapse or remission of their disease.

Additional studies have been published using FC in conjunction with other fecal protein markers, including other S100A proteins. Joishy et al[50] reported a median FC of 251 µg/g for the active IBD group compared to 35.4 µg/g for a GI-control group and 14.1 µg/g for a non-GI control group ($p < 0.001$). Similar significant differences were established between the groups when fecal lactoferrin was used.[50] M_2-pyruvate kinase (M_2-PK) is readily expressed in rapidly-dividing cells,[51] which could be a helpful monitoring tool during active IBD disease due to increased GI mucosal-cell turnover. Low M_2-PK expression could potentially mean flare-up resolution. Czub et al[52] reported abnormal M_2-PK levels in 49 of 75 UC patients and in 27 of 32 CD patients. Though in later studies, they were not able to demonstrate superiority in differentiating disease severity and remission compared with FC.[53] Turner et al[54] studied both of them used together in 101 children with UC. Their baseline fecal M_2-PK and FC

Table 3. Fecal Calprotectin Pediatric Studies on Proven Inflammatory Bowel Disease

Study (n)	Population	Assessment Standard	Results	Comments
Bunn et al[40] (n = 68)	16 children with UC, 21 with CD, and 31 healthy controls.	Modified Lloyd-Still (mLSS) and Green Childhood IBD activity score.	Median (range) FC: 11.5 (0.6-272.5) for UC and 14.0 (0.7-59.7) mg/L* for CD compared to controls 2.1 (0.5-6.3) mg/L* ($p < 0.001$)	FC correlated negatively with mLSS score ($r = -0.61$, $p < 0.001$) and positively with ESR ($r = 0.40$, $p = 0.01$).
Bunn et al[41] (n = 36)	Children with IBD	Colonoscopy and ^{99}Tc-labelled white cell scanning.	Median FC: 4.9 (0.1-272.5) µg/mL* for scoped children (n = 22); 9.1 (0.3-141.7) mg/L* for those who underwent ^{99}Tc scanning (n = 14).	FC correlated with macroscopic ($r = 0.75$, $p < 0.001$), histologic ($r = 0.85$, $p < 0.001$) inflammation, and ^{99}Tc scanning score ($r = 0.80$, $p = 0.001$)
Kohlo et al[42] (n = 57)	31 children with IBD, 13 with non-IBD disease, and 13 normal	Colonoscopy	FC was < 100 µg/g in 70% of normal or non-IBD patients. FC was > 100 µg/g in 13 of 15 patients with IBD, who later received steroids.	FC values decreased with clinical improvement in 7 children, normalizing in 4, and increasing back in 5 after discontinuation of steroids.
Fagerberg et al[43] (n = 39)	27 children with CD, 10 with UC, and 2 with indeterminate colitis.	Macroscopic and microscopic extent and severity scores from endoscopy.	Median (95% CI) FC: 392 (278-440) µg/g in patients with clinical IBD symptoms and 32.9 (9.4-237) µg/g in asymptomatic children.	FC correlated (Spearman) with macroscopic ($r = 0.65$) and microscopic ($r = 0.75$) combined scores.
Canani et al[44] (n = 58)	26 children with CD and 32 with UC.	Pediatric Crohn's Disease Activity Index (PCDAI) and Rachmilewitz Index for UC, as well as endoscopic and histologic scores.	Mean FC (95% CI): 138.2 (66.1-210.3) µg/g in remission vs 262.6 (230.8-294.4) µg/g active disease for UC; 100.7 (25.3-176.0) µg/g in remission vs 245.5 (217.4-273.6) µg/g active disease for CD.	Optimal FC cut-off to determine between active disease and remission was 143 µg/g, with a 83% (CI 71-95) probability of estimating it correctly.
Aomatsu et al[45] (n = 63)	17 patients with UC, 18 with CD, and 28 healthy controls	For UC: Matt's grading score and pediatric UC activity index (PUCAI). For CD: Simple endoscopic score for CD (SES-CD) and pediatric CD activity index (PCDAI).	In UC, patients with active disease had a median FC of 1562.5 µg/g vs 38.9 µg/g of patients in remission. In CD, patients with active disease had 2037.5 µg/g vs 172.5 µg/g of patients in remission.	Strong correlation for UC between FC levels and the sum of Matts' score ($r = 0.838$, $p < 0.01$); and for CD between FC levels and the SES-CD score ($r = 0.760$, $p < 0.01$).

CD, Crohn's disease; ESR, erythrocyte sedimentation rate; IBD, inflammatory bowel disease; UC, ulcerative colitis
*FC values are given in mg/L, others are in µg/g feces

were significantly lower in responders to steroid treatment compared to those who did not.[54] No differences were observed with fecal lactoferrin and fecal S100A12.[54] Investigators have also evaluated S100A12, which elicits transient neutrophil infiltration and delayed monocyte recruitment. This response occurs as a result of gene up-regulation by known inflammatory cytokine, tumor necrosis factor-alpha (TNF-α), involved in the pathogenesis of IBD.[55] Fecal S100A12 (median 55.2 µg/g) and FC of (median 1265 µg/g) in 31 children with IBD were higher compared to fecal S100A12 (median 1.1 µg/g) and FC (median 30.5 µg/g) in 30 children without IBD ($p < 0.0001$).[56]

In Other GI Diseases

Calprotectin became an acceptable marker of GI inflammation resulting from IBD. Investigators speculated about its use in detection of other acute and/or chronic inflammatory diseases. Limburg et al[35] examined 110 subjects with chronic diarrhea referred for colonoscopy and found a median FC of 378 µg/g for subjects with colorectal inflammation, which were later diagnosed with collagenous or eosinophilic colitis among others; as opposed to a median of 31 µg/g for those who did not ($p = 0.0001$).[35] Another study demonstrated that a FC cut-off of 50 µg/g had 64% sensitivity and 80% specificity with 70% positive and 74% NPVs in 70 adults; and 70% sensitivity, 93% specificity with 96% positive and 56% NPVs in 50 children (<18 years) with chronic diarrhea.[57] Calprotectin was also used to distinguish between children with constitutive enterocyte disorders, such as epithelial dysplasia (ED) and microvillus atrophy (MVA). These children had fecal concentrations consistent with reports in similar age cohorts.[23,24] Their values were either < 50 µg/g or undetectable, versus other immune-inflammatory disorders where the observed median (range) FC was 1145 (375-3095) µg/g at the onset of severe diarrhea, $p < 0.01$.[58] In celiac disease, untreated adults were shown to have comparable FC (45.02 ± 24.18 µg/g), while healthy controls had values of 36.51 ± 21.67 µg/g.[59] The application of calprotectin in celiac disease might be better suited for children and those receiving a specialized diet. There was a significant statistical difference in FC concentrations between newly diagnosed, untreated children and those receiving a gluten-free diet (GFD) for 1 year, while there was none between those receiving a GFD and healthy children.[60,61] Another disease in which FC was used to monitor the efficacy of a change in diet is cow's milk protein (CMP) allergy. Beser et al[62] evaluated 24 with Ig-E mediated and 8 with non-Ig-E mediated CMP allergic children. FC values were 392 ± 209 µg/g and 886 ± 278 µg/g before CMP elimination diet; and 218 ± 90 µg/g and 359 ± 288 µg/g after CMP elimination diet, respectively ($p = 0.001$ and $p = 0.025$). Chen et al[63] evaluated FC's diagnostic value in predicting bacterial from viral infectious diarrhea in 153 children. The median (range) FC level was higher in patients with *Salmonella* infection, 765 (252-1246) µg/g, or *Campylobacter* infection, 689 (307-1046) µg/g; compared to patients with rotavirus infection 89 (11-426) µg/g, norovirus infection 93 (25-405) µg/g, or adenovirus infection 95 (65-224) µg/g, $p < 0.05$. Even though there is some overlap with the ranges, it appears that FC expression would be higher as a result of bacterial compared to viral infections.

Particular interest was taken in the infant population as Campeotto et al[29] showed calprotectin could be used as an acute marker of intestinal distress. Such distress was defined as GI bleeding, diarrhea with liquid stools, and/or abdominal distention. They reported decreased FC levels a week before onset and following a GI episode.[29] Yang et al[31] detected differences in mean FC ± SD levels between infants classified as "not sick" and as "sick." They defined "sick" as those being evaluated for sepsis, requiring antibiotics or vasopressors, withholding enteral feeds, or requiring increased ventilator support. "Sick" infants showed higher FC concentrations 380.4 ± 246.3 µg/g, versus "not sick", 122.8 ± 98.9 µg/g, ($p < 0.001$).[31] These features could be useful for pediatric clinicians in determining temporal relations with disease causes at initial patient assessment. Calprotectin could also contribute to further understanding of disease severity or progression in other diseases that present with an inflammatory picture, such as necrotizing enterocolitis (NEC). Seven preterm infants with NEC showed higher mean ± SD FC at diagnosis 288.4 ± 49.1 mg/L compared to healthy matched controls, 98 ± 60.6 mg/L ($p = 0.0006$).[64] Thirty-one neonates with mild enteropathy displayed median (range) FCs of 393 (52-996) µg/g compared to those with severe enteropathy, 832 (168-4775) µg/g, including patients with NEC Stage IIb and III.[65] These results are consistent with those from

recent studies that observed higher FC concentrations in preterm infants with NEC compared to controls[66] and were linked with disease severity.[67] I-FABP may be another marker that correlates well with extent of disease and mucosal damage in NEC.[68,69] Recently, a combination of urinary I-FABP and FC provided a sensitivity of 94%, a specificity of 79%, a positive likelihood ratio of 4.48, and a negative likelihood ratio of 0.08, for diagnosing NEC in a cohort of neonates.[70] In that case, those investigators looked to improve I-FABP's ability to diagnose NEC by adding FC assessment, as I-FABP alone had not been definitive in previous analyses of their cohort.[71] FC was also analyzed to establish a possible relationship between gut inflammation and linear growth in 144 urban and rural Chinese infants. The median FC level was significantly higher in rural versus urban infants, 420.9 μg/g versus 140.1 μg/g ($p < 0.0001$), respectively, though there was wide variability observed in the ranges.[72] Those authors pointed out a significant inverse relationship: for an increase of 100 μg/g in FC, there was an associated decrease of 0.06 in length-for-age Z-score. This could hint to an explanation of those yet to be found relationships, proposed in Figure 2. Relationships between raised calprotectin concentrations expressed initially in the gut, and inflammation, either locally or systemically, could affect outcomes such as growth and/or recovery from disease. FC has not been a useful marker with other infant GI illnesses including infantile colic, transient lactose intolerance, constipation, functional gastrointestinal disorders (FGID), or with small intestine bacterial overgrowth (SIBO).[73-76]

Applications in Other Diseases

The use of calprotectin as a possible screening or monitoring tool has paved its way into other disease states. Early on, it was suggested as a marker of inflammation in patients with cystic fibrosis (CF) who had median concentrations of 0.727 mg/L compared to controls with median 1.832 mg/L, $p < 0.001$, though exhibiting considerable overlap.[77] More recently, calprotectin has been analyzed to monitor decreasing inflammation resulting from therapy after an acute exacerbation.[78] In preeclampsia, Braekke et al[79] examined 62 pregnant women reporting median maternal plasma concentrations were higher in the preeclampsia group compared with the control group (1.08 mg/L versus 0.55 mg/L, $p < 0.001$); others reported similar trends.[80] Forty-one children with different types of juvenile idiopathic arthritis (JIA), unrelated connective tissue disorders (CTDs), or otherwise healthy were evaluated to see if FC could be used to assess subclinical gut inflammation.[81] Median levels were highest in the children with arthritis, and lowest in the CTD controls.[81] In a cohort of 74 adults and children undergoing intestinal transplant, more variance was seen in FC during rejection than in non-rejection.[82] Serum calprotectin has been used in children with acute Kawasaki's disease as a monitoring tool to assess response to intravenous immunoglobulin (IVIG) therapy, and possibly lead to identification of those at risk of developing coronary injury.[83,84] The ability to monitor inflammation is key, whether before or after a major intervention. Plasma calprotectin revealed a hazard ratio of 1.26, relative risk of mortality with increasing levels, in patients that had suffered an ST segment elevation myocardial infarction (MI) and had undergone successful percutaneous coronary intervention followed up to a year.[85] Furthermore, one research group assessed serum and urine measurements of calprotectin in an obese population and its possible associations with insulin resistance and Type-II diabetes. Mean ± SD plasma calprotectin was significantly increased ($p < 0.0001$) in obese (131.4 ± 63.7 μg/L) compared with non-obese subjects (102.8 ± 71.7 μg/L).[82] Hestvik et al[86] determined reference values for children infected with HIV and undertaking highly active antiretroviral therapy for the first time. Median FC was 208 μg/g in infants 0 to 1 year, 171 μg/g among toddlers 1 to 4 years, and 62 μg/g for children 4 to 12 years, while also noticing that children with advanced disease and a low CD4 cell percentage had significantly higher median (range) FC concentrations, 203 (143-277) μg/g than those with a high CD4 cell percentage 99 (62-154) μg/g ($p < 0.05$). Muller et al[87] reported that low maximal responses in serum calprotectin during zidovudine therapy were associated with short survival in 51 HIV patients. They also noticed inverse relationships between serum calprotectin and CD4 counts above 50×10^6/L, showing similar trends to the fecal data. In a state of generalized inflammation caused by disease progression, perhaps calprotectin can be found in multiple body fluids.

Comparison to Other Serum/Plasma Markers

CRP and ESR are examples of other markers that alert clinicians of ongoing inflammatory processes in the body. Gray et al[88] analyzed serum and sputum samples during CF exacerbation and noticed that serum calprotectin predicted median time to exacerbation significantly better than CRP. In patients with rheumatic disease, calprotectin concentrations, but not CRP nor ESR, were significantly lower in those with no swollen joints compared to those with 1 or more swollen joints (2.614 mg/L versus 6.287 mg/L, $p < 0.001$).[89] Similarly, calprotectin was the first to normalize, compared to CRP and ESR, in patients with reactive arthritis.[90] In patients with JIA, it was shown to be a better diagnostic marker[91] and predictor of response to methotrexate therapy.[92] Terrin et al[93] evaluated serum calprotectin as a diagnostic marker for sepsis in neonates. Mean ± SD serum concentrations were significantly higher ($p < 0.001$) in 62 newborns with confirmed sepsis (3.1 ± 1.0 mg/L) than in either 29 non-infected subjects (1.1 ± 0.3 mg/L) or 110 healthy controls (0.91 ± 0.58 mg/L); while showing greater sensitivity, 89%, and specificity, 96%, than common laboratory markers, such as white blood cell count (WBC) and CRP. Other investigations have been reported in the literature evaluating calprotectin concentrations in patients with appendicitis,[94,95] congestive heart failure,[96] and others.[97-101] Results did not show significant clinical impact other than increased levels compared to control groups. We speculate that part of the reason why some of these studies have not shown greater merit or have not been followed up with additional investigations is that there has not been the same degree of development, in terms of performance of analytical kits, for serum/plasma and/or urine samples compared to fecal specimens.

SUMMARY AND FUTURE RESEARCH

In conclusion, calprotectin has been validated as a non-invasive marker of local GI inflammation in patients with IBD. Other protein markers discussed in this review could still be in their developmental phases or only available in large, research-driven facilities. Studies show potential for calprotectin to be used as a tool in other disease states that present with an inflammatory component. The majority of the medical community has relied on fecal samples to monitor this protein, though isolation from other body fluids appears feasible. Since there are perceived age-dependent variations in the expression of calprotectin, methods should be re-evaluated, as far as dilution technique and sample preparation, when handling pediatric as opposed to adult samples. Considering the manufacturer-reported stability of calprotectin in feces, it may serve better to utilize such sampling method for more stable patients or for those with routine follow-up. The utility of calprotectin in the clinical arena could be enhanced with development of assays that can reliably quantify it in serum/plasma or urine. This could prove extremely beneficial to clinicians when assessing more acutely-ill, hospitalized patients. Subsequent studies will be required to validate analytical methods extracting calprotectin from sources other than stool in order to show this protein can be an effective monitoring tool both in the in-patient and outpatient setting.

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Abbreviations CD, Crohn's disease; CF, cystic fibrosis; CFA, cystic fibrosis antigen; CI, confidence interval; CMP, cow's milk protein; CRP, C-reactive protein; CSF, cerebrospinal fluid; CTD, connective tissue disorder; ED, epithelial dysplasia; ESR, erythrocyte sedimentation rate; FC, fecal calprotectin; FGID, functional gastrointestinal disorders; GA, gestational age; GFD, gluten-free diet; GI, gastrointestinal; Hgb, hemoglobin; HIV, human immunodeficiency virus; IBD, inflammatory bowel disease; I-FABP, intestinal-fatty acid binding protein; IQR, interquartile range; IVIG, intravenous immunoglobulin; JIA, juvenile idiopathic arthritis; L1, leukocyte-derived protein; LFT, liver function tests; LR, likelihood ratio; M_2-PK , M_2-pyruvate kinase; MI, myocardial infarction; MRP 8/14, migration inhibitory factor related protein; MVA, microvillus atrophy; NEC, necrotizing enterocolitis; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver-operator characteristic; SIBO, small intestine bacterial overgrowth; TNF-α, tumor necrosis factor alpha; UC, ulcerative colitis; WBC, white blood count

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