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Review article: Faecal biomarkers for assessing small intestinal damage in coeliac disease and environmental enteropathy

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Summary

Background: In coeliac disease and environmental enteropathy, dietary gluten and enteric infections cause reversible inflammation and morphological changes to the small intestinal mucosa that can be detected in biopsy samples obtained by endoscopy. However, there is a clear need for non-invasive biomarkers. Constant shedding of mucosal material into the bowel lumen and faeces, together with easy availability of stool, makes it an interesting sample matrix.

Aims: To conduct a systematic literature search and summarize the existing evidence for host mucosa-derived faecal biomarkers in evaluating small intestinal damage.

Methods: We searched for studies on PubMed (MEDLINE) until 1 March 2024.

Results: We identified 494 studies and included 35 original case–control and cohort studies. These assessed host mucosal transcripts and 14 other markers aiming specifically to reflect inflammation and cell-mediated, innate and gluten-induced immune responses. In coeliac disease, faecal calprotectin and anti-gliadin, tissue transglutaminase, endomysium and deamidated gliadin peptide antibodies were the most studied but with inconsistent results. Single studies reported positive findings about microRNA transcripts, β-defensin-2, lipocalin-2, zonulin-related proteins and angiotensin-converting enzyme. In environmental enteropathy, a non-significant association was reported between calprotectin and urine lactulose/mannitol ratio; there were conflicting results for neopterin, myeloperoxidase and host transcripts. Single studies reported a positive association for lactoferrin, and a negative association for regenerating islet-derived protein 1. Studies comparing faecal markers against small intestinal biopsy findings were not identified in environmental enteropathy.

Conclusions: Further studies are needed to determine reliable faecal markers as a proxy for small intestinal mucosal damage.

The Handling Editor for this article was Professor Jason Tye-Din, and this uncommissioned review was accepted for publication after full peer-review.

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1 | INTRODUCTION

The small intestine is crucial in the digestion and absorption of nutrients, in protecting against external pathogens and in the development of tolerance of food antigens. Its structural and/or functional impairment has significant negative effects on overall health, and in children also on growth and development causing morbidity and mortality.[1] The two most common but significantly underdiagnosed conditions affecting the small intestine are coeliac disease and environmental enteropathy. Coeliac disease affects approximately 1%–3% of the population worldwide, although most of the patients are undiagnosed due to various and in some cases subclinical features.[2,3] Environmental enteropathy is estimated to affect up to 40% of children, especially those living in low- and middle-income countries and is likely also common in adults.[1,4–6] However, exact prevalence figures are lacking due to the frequently asymptomatic nature of the condition, poor access to health care and lack of specific diagnostic criteria.[1]

In coeliac disease, the immune reaction to dietary gluten, found in wheat, rye and barley, drives duodenal enteropathy and gastrointestinal as well as systemic disease manifestations in a subset of genetically predisposed people.[2] Strict and life-long gluten-free diet results in mucosal healing and symptom alleviation in most patients. Coeliac disease pathogenesis is fairly well understood, although the initial triggering factor(s) remains uncertain. Diagnosis is based on the detection of histological damage in duodenal biopsies or, in selected children, on high levels of circulating transglutaminase-2 antibodies (TGA) together with positive endomysium autoantibodies (EmA).[7–10] However, although serology correlates with the severity of duodenal damage at diagnosis,[11–13] its sensitivity to milder changes and persistent mucosal damage during gluten-free diet is insufficient, and it is of limited use at individual level to reflect duodenal damage.[14,15] Reliable, non-invasive markers for small bowel mucosal damage are called for in coeliac disease follow-up but also as endpoints in drug studies.[16,17]

Environmental enteropathy is also known as tropical enteropathy, subclinical malabsorption and environmental enteric dysfunction.[1] These various names reflect not only the discovery and pathogenesis of this condition but also the lack of a single unambiguous definition. Environmental enteropathy is a condition frequently manifesting with subtle gastrointestinal symptoms attributed to be caused by recurrent exposure to faecal pathogens in surroundings with poor hygiene.[1,6] In children, it may present with stunted growth, malnutrition and poor response to oral vaccinations, whereas in adults, the condition is typically asymptomatic or presents with weight loss, altered stool consistency and frequency. The more severe form of environmental enteropathy overlaps with the condition historically described as 'tropical sprue'.[18] Histological findings of small intestinal biopsies in environmental enteropathy have reportedly included reversible enteropathy reminiscent of coeliac disease, but the diagnosis of environmental enteropathy is in practice rarely set based on the histology.[18,19] Instead, for example, dual sugar absorption tests, such as the lactulose–mannitol ratio or lactulose permeability measured by urine excretion, have been used as the gold standard for measuring malabsorption, permeability and disease activity.[1,20] However, these methods often entail significant practical challenges affecting their reliability and comparability between studies.[21] Various other biomarkers have also been studied and interest has been expressed in finding practical non-invasive markers through which to define and follow-up the condition.[22]

Intestinal changes in coeliac disease and environmental enteropathy are reflected in proteins and other biochemical compounds originating in the mucosa[23] and given the constant shedding of mucosal material into the intestinal lumen, these could be measurable in faeces. As stool is a non-invasive and accessible sample matrix, also in low-income settings, the possibilities for using faecal biomarkers as a proxy for small intestinal damage would be an attractive approach globally. This review summarizes the existing evidence on host mucosa-derived faecal biomarkers in evaluating small intestinal damage in coeliac disease and environmental enteropathy.

1.1 | Small intestinal damage in coeliac disease and environmental enteropathy

While the underlying drivers of enteropathy in coeliac disease and environmental enteropathy are different, the conditions have much in common (Figure 1). However, what is known so far about histological damage in environmental enteropathy is based on a relatively small number of studies mostly of symptomatic patients.[19,24–26] The small intestinal mucosal inflammation and villous blunting characteristic for both conditions ranges in severity from a pre-disease form characterized by increased lymphocyte infiltration with normal architecture all the way to complete villous atrophy, crypt hyperplasia and pronounced immune cell infiltration.[18] The intraepithelial lymphocytes in coeliac disease and environmental enteropathy are similar in terms of expression of at least CD3, CD8 and $\gamma\delta$ surface receptors.[19,25,27–29] In coeliac disease, $CD8^+$ $\alpha\beta$ cells are considered to drive the intestinal damage by inducing enterocyte apoptosis,[30,31] whereas $\gamma\delta$ T cells may have an inflammatory Th1 phenotype.[32] In environmental enteropathy, no similar mechanistic insight on different intraepithelial lymphocyte subpopulations is available.

Both disease entities present with lamina propria inflammation characterized by T and B lymphocytes and plasma cells.[19,25–27,33] In coeliac disease, gluten reactive $CD4^+$ T cells are activated by human leukocyte antigen (HLA) DQ2 or DQ8 restricted presentation of deamidated gliadin peptides (DGPs) generated from partially digested oligopeptides deamidated by the transglutaminase-2 (TG2) enzyme. T cells contribute to the generation of an inflammatory milieu by secreting cytokines such as interferon-γ (IFN-γ) and interleukin (IL) -21.[2] (Figure 1). Furthermore, they are believed to interact with gluten- and TG2-specific B cells enabling their differentiation into immunoglobulin (Ig) A class TG2 and DGP secreting plasma cells, which are by far the most numerous immune cell population in the coeliac disease mucosa.[27,34,35] No such

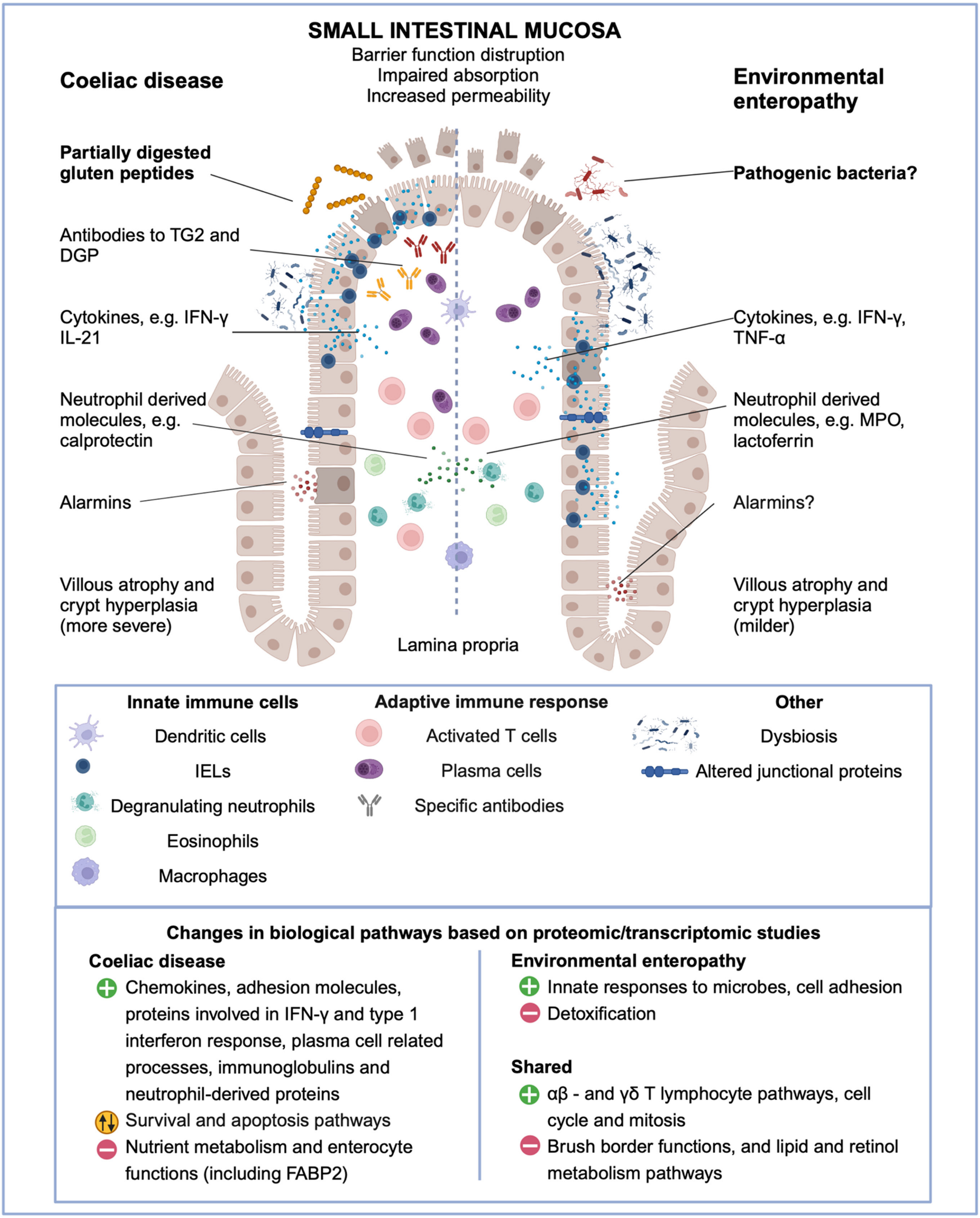

FIGURE 1 Pathogenetic processes in the small intestinal mucosa of patients with untreated coeliac disease and environmental enteropathy. Mucosal inflammation and villous atrophy, increased density of IELs and inflammatory cytokines as well as impaired nutrient absorption and increased intestinal permeability are features common to both conditions. Epithelial regeneration and enterocyte shedding also occur continuously in healthy intestinal mucosa. Possible changes in biological pathways are based on evidence from proteomic and transcriptomic studies 'plus' indicating upregulated and 'minus' downregulated pathways. DGP, deamidated gliadin peptide; FABP2, fatty acid-binding protein 2; IEL, intra-epithelial lymphocyte; IFN-γ, interferon-gamma; IL, interleukin; MPO, myeloperoxidase; TG2, transglutaminase-2; TNF-α, tumour necrosis factor alpha.

detailed knowledge about the molecular mechanisms is available in environmental enteropathy, although the lesion seems to be characterized by inflammatory cytokines, such as tumour necrosis factor α and IFN-γ.[19,26] Due to differing aetiologies, the initial pathogenetic steps are likely to differ from those in coeliac disease despite similarities at the level of small bowel mucosal cell types and pro-inflammatory cytokines.

Finally, both disorders are functionally characterized by impaired nutrient absorption and increased intestinal permeability coupled with abnormal epithelial junctional protein expression at molecular level.[36,37] In contrast, microbial translocation seems to be an environmental enteropathy-specific feature.[38,39]

1.2 | Mucosal proteome and transcriptome as possible host-mucosal derived markers

The small bowel mucosal damage and inflammation described above obviously have a profound impact on tissue proteome and transcriptome (Figure 1). Therefore, studies addressing these are of interest in terms of faecal mucosal-derived biomarkers in coeliac disease and environmental enteropathy. Regarding coeliac disease, few studies focusing on small bowel mucosal proteome have so far been presented,[40–43] and these report for instance reduced expression of proteins involved in nutrient metabolism and enterocyte function and increased expression of proteins involved in plasma cell-related processes and IFN-γ and type I interferon pathways in untreated coeliac disease. Of particular interest in untreated coeliac disease mucosa is the higher expression of the two subunits of calprotectin, S1008A and S1009A, produced by neutrophils and monocytes/macrophages.[41] To the best of our knowledge, no studies have so far been presented describing the proteome of small bowel mucosal samples in environmental enteropathy.

The small bowel mucosal transcriptomic studies in coeliac disease are heterogeneous in terms of patient groups, methods used and gene coverage but despite this variation they have all reported upregulation of genes encoding for chemokines and adhesion molecules as well as proteins involved in IFN-γ response.[23] A study comparing mucosal transcriptome between coeliac disease and environmental enteropathy reported that while expression changes in αβ – and γδ T lymphocyte, B cell, cell cycle and mitosis, brush border functions and lipid and retinol metabolism pathways are common to both diseases, upregulation of the genes involved in pathways related to response to microbiota and innate immune function seems specific to environmental enteropathy.[33] These findings raise interesting scenarios about the faecal gut exfoliome as a proxy for small bowel damage.

2 | METHODS

2.1 | Search strategy and selection criteria

Studies on PubMed (MEDLINE) until 1 March 2024 were searched. The following terms in title or abstract were used in the systematic literature search: 'celiac' OR 'coeliac' OR 'gluten enteropath*' OR 'gluten sensitive enteropath*' OR 'gluten intolerance' OR 'non-tropical sprue' OR 'environmental enteropath*' OR 'enteric dysfunction' OR 'tropical sprue' OR 'tropical enteropath*' OR 'subclinical malabsorption' AND 'faecal' OR 'faecal' OR 'stool' OR 'faeces' OR 'faeces' AND 'marker*' OR 'biomarker*' OR 'assay*' OR 'test*' OR 'immunoglobulin*' OR 'antibod*'. In addition, publications found in the references of included articles or otherwise known to be relevant were included. Detailed search strategies are given as Supplementary Material.

Inclusion criteria for the papers were original studies, English language and full text available. Exclusion criteria were in vitro and animal studies, and human studies lacking data on the condition of the small intestine or verification of the diagnoses based on histology, lactulose–mannitol ratio or corresponding method, studies evaluating biomarkers only in blood/serum/urine samples, and publications presenting only study protocols. In addition, as the focus of this review was on small intestinal mucosal damage, studies addressing microbiota (including short-chain fatty acids, metabolome and volatile organic compounds reflecting metabolic processes and microbial activities), occult blood, gluten immunogenic peptides describing dietary gluten use and faecal elastase reflecting exocrine pancreatic function were excluded. Also, studies on α-1-antitrypsin were excluded as this protein is produced mainly by hepatocytes and only in small amounts by intestinal mucosal cells.[44] Although faecal α-1-antitrypsin clearance is commonly used in the diagnostics of protein-losing enteropathy with various aetiology including CeD and environmental enteropathy, it primarily reflects the loss of proteins from the bloodstream to the gut rather than disease activity itself.[45]

Two authors (LK and KS) screened separately the abstracts and full texts, and LK performed the data extraction. Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia, available at www.covidence.org) was used for screening the search results and data extraction from the selected studies. The findings of the review were reported according to the PRISMA guidelines. The study protocol was pre-registered in OSF (available at osf.io/ubgh4).

3 | RESULTS

The literature search resulted in 361 studies on coeliac disease and 130 studies on environmental enteropathy, two additional relevant studies were found among the references and one other relevant study was included (Supplementary Figure). After screening, 36 coeliac disease studies and 29 environmental enteropathy studies were assessed for eligibility, and finally 19 original studies on coeliac disease and 16 on environmental enteropathy were included in the review (Supplementary Figure, Tables 1 and 2). The studies assessed, in addition to host mucosal transcripts, 14 different markers reflecting especially cell-mediated, innate and gluten-induced immune responses, and unspecific and neutrophil associated inflammation (Table 3).

TABLE 1 Summary of the studies evaluating host-mucosal derived faecal biomarkers in biopsy-proven coeliac disease (CeD).

Study, country	Age group	Number of participants: Untreated CeD + treated CeD + controls	Main results
Antibodies			
Positive findings			
Haas 1993, France[46]	Adults	19 + 0 + 12	Higher AGA in untreated CeD than in controls (median 24 vs. 5 ng/mL, $p<0.01$). Correlation between serum and faecal IgA AGA.
Haas 1995, France[47]	Adults	19 + 0 + 17	Higher IgA1 (median 22 vs. 1 ng/mL) and IgA2 (56 vs. 1 ng/mL) IgA2 AGA in untreated CeD than in controls ($p<0.005$).
Picarelli 2002, Italy[48]	Adults	21 + 10 + 14	IgA class antibody positivity is more common in untreated than in treated CeD patients or in controls (EmA 100% vs. 0% vs. 0%; TGA 81% vs. 20% vs. ND; AGA 67% vs. 0% vs. ND).
Di Tola 2016, Italy[49]	Adults	25 + 0 + 12	IgA class TGA and DGP levels higher[a] and antibody positivity more common in untreated CeD than in controls (EmA 44% vs. 0%, $p=0.007$; TGA 76% vs. 17%, $p=0.001$; DGP 68% vs. 0%). Correlation between faecal and serum IgA TGA and DGP levels. No significant difference in IgG class levels.
Non-significant results			
Halblaub 2004, Germany[50]	Children & adults	4 + 13 + 160	No significant difference in antibody positivity between untreated and treated CeD (EmA 25% vs. 15%; TGA 25% vs. 23%; AGA 0% vs. 31%), ND from controls. Partially overlapping levels between the groups.
Kappler 2006, Germany[51]	Children	20 + 6 (same patients, follow-up) + 62	No significant difference in IgA class antibody positivity between untreated and treated CeD and controls (TGA 10% vs. 0% vs. 3%; AGA 6% vs. 6% vs. 2%).
Calprotectin			
Positive findings			
Ertekin 2010, Turkey[52]	Children	29 + 29 (same patients, follow-up) + 10	Higher calprotectin levels in untreated than in treated CeD or controls (mean 13.4 vs. 4.6 vs. 4.3 mg/L, $p=0.004$) and in patients with total vs. partial villous atrophy (13.8 vs. 3.7 mg/L, $p=0.005$).
Balamtekın 2012, Turkey[53]	Children	31 + 33 + 34	Higher calprotectin levels in untreated than in treated CeD or controls (median 117 vs. 4 vs. 10 µg/g, $p<0.001$), in patients with GI-symptoms than in those without (142.8 vs. 79.7 µg/g, $p=0.04$). No correlation with Marsh grades or degree of neutrophilic infiltration. Decreased from 113.7 to 4.2 µg/g in nine patients during follow-up ($p<0.01$).
Biskou 2016, United Kingdom[54]	Children	17 + 46 + 92	Higher calprotectin levels in untreated than in treated CeD or controls (median 36 vs. 25 vs. 25 mg/kg, $p=0.045$), decreased significantly in 13 untreated patients in 6-mo follow-up on a GFD from median 37 to 25 mg/kg, $p=0.012$. Not associated with TGA, PedsQL or GI symptoms.
Rajani 2016, Canada[55]	Children	88 + 73 (same patients, follow-up) + 0	Calprotectin was elevated in untreated CeD (median 67.5 [range: 4.9–3068] µg/g) but not in treated CeD (33 [1.11–736.5] µg/g) compared to the laboratory cut-off (<50 µg/g).

TABLE 1 (Continued)

Study, country	Age group	Number of participants: Untreated CeD + treated CeD + controls	Main results
Non-significant results			
Montalto 2007, Italy[56]	Adults	28 + 0 + 30	Non-significant difference between untreated CeD and controls (45.02 vs. 36.51 μg/g, $p = 0.163$). No significant association with symptoms, histology or neutrophil infiltration.
Capone 2014, Italy[57]	Adults	50 + 0 + 50	Non-significant difference between untreated CeD and controls (mean 58 vs. 45 μg/g, p = NS). No association with symptoms, histology or serum TGA.
Szaflarska-Popławska 2020, Poland[58]	Children	55 + 17 + 0	Non-significant difference between untreated and treated CeD (mean 101 vs. 62 μg/g, $p = 0.183$). No difference between clinical forms of untreated CeD or severity of small intestinal damage.
Calprotectin and other			
Positive findings			
Kamilova 2022, Uzbekistan[59]	Children	76 + 0 + 32	Higher mean calprotectin (35.4 vs. 19.95 μg/g, $p < 0.05$) and β-defensin-2 (99.6 vs. 64.3 ng/mL, $p < 0.02$) levels in untreated CeD than in controls.
Sutton 2024, the United States[60]	Children	73 + 0 + 18	Lipocalin-2 was higher in CeD than in controls (estimated from the figure 1500 vs. 750 mg/mL, $p = 0.007$) but the levels were comparable between seropositive patients with Marsh 0 and 3 lesion. Calprotectin was comparable between CeD patients and controls.
Gallego 2024, Spain[61]	Children	23 + 23 (same patients, follow-up) + 39	Higher median levels of calprotectin (29.8 vs. 17.4 vs. 13.9 μg/g) and zonulin-related proteins (348 vs. 157 vs. 178 ng/mL) in untreated CeD than in treated patients or controls, respectively. No association between the markers and serum TGA or symptoms.
Other			
Positive findings			
Letizia 1996, Italy[62]	Children & adults	20 + 18 + 15	ACE activity was significantly higher in untreated CeD than in treated patients or controls (113 vs. 37 vs. 21 nmol/min/100 g, respectively, $p < 0.05$).
Palone 2018, Italy[63]	Children	39 + 24 + 20	HMGB1 was increased in untreated CeD, decreased markedly in treated patients and was undetectable in controls. No absolute numbers given. HMGB1 correlated with S-TGA (Spearman's $\rho = 0.79$).
Francavilla 2023, Italy[64]	Adults	3 + 63 + 66 (controls-1) + 40 (controls-2)	109 miRNAs expressed differentially between CeD patients and controls-1, and 77 of these were confirmed in a comparison to controls-2. Five miRNAs expressed differently in both TGA-positive and TGA-negative-treated patients compared to controls, and 5 miRNAs associated with the length of gluten-free diet. 16 other sncRNAs were upregulated in TGA-positive-treated patients compared to controls-1 and TGA-negative-treated patients, and of these five were upregulated also when compared to controls-2.

Abbreviations: ACE, angiotensin-converting enzyme; AGA, anti-gliadin antibodies; EmA, endomysial antibodies; miRNA, micro-RNA; NA, not available; ND, no data; piRNA, piwi-interacting RNA; sncRNA, small non-coding RNA; TGA, tissue transglutaminase antibodies; tRNA, transfer RNA.

[a]Results given as absorbance 450 nm.

(Continues)

All the studies on faecal markers in coeliac disease were case–control studies, seven of them included adults, 10 paediatric patients and two both age groups. Studies focused mainly on calprotectin ($n=10$) or faecal antibodies ($n=6$) and single studies evaluated human β-defensin-2 (HBD2), high-mobility group box 1 (HMGB1) lipocalin-2, transcripts, zonulin-related proteins and angiotensin-converting enzyme (ACE) (Table 1).

Studies on faecal markers in environmental enteropathy were either cohort or case–controls studies including young children evaluated during the first years of life partly from the same patient cohorts. Studies included comparisons of intestinal permeability against myeloperoxidase (MPO) ($n=10$), neopterin ($n=10$), transcripts ($n=6$) or calprotectin ($n=4$), and one study also lactoferrin and regenerating islet-derived protein 1 (Reg1) (Table 2). Of note, many environmental enteropathy studies were excluded due to lacking comparison of faecal markers against lactulose–mannitol ratio, and no studies comparing faecal markers against small intestinal histology were identified.

3.1 | Antibodies

TGA, EmA and DGP measured from serum are routinely used in coeliac disease diagnostics.[7–10] Historically, serum anti-gliadin antibodies (AGA) targeted towards native gliadin have also been utilized, but they are unspecific and no longer recommended in clinical practice. Secretion of the antibodies in gut lumen has been demonstrated in the studies focusing on intestinal lavage fluid.[81,82]

Six studies on the usefulness of faecal antibodies in coeliac disease focused on TGA, EmA, DGP and/or AGA and compared either the antibody positivity or their levels between 4 and 25 untreated and 0–13 treated coeliac disease patients and 12–160 controls (Table 1). Picarelli et al. (2002) and Di Tola et al. (2016) reported faecal EmA and TGA to be higher and more often positive in untreated adult coeliac disease patients than in treated patients or controls, whereas Halblaub et al. (2004) reported non-significant differences in TGA and EmA positivity, and Kappler comparable TGA positivity between the three groups including both children and adults.[48–51] Di Tola et al. (2016) reported faecal DGP to be higher and more often positive in adult untreated coeliac disease patients than in controls.[49] Haas et al. (1993 and 1995) and Picarelli et al. (2002) reported faecal AGA to be associated with untreated coeliac disease in adults, whereas Halblaub et al. (2004) and Kappler et al. (2006) reported no differences in AGA positivity between untreated/treated coeliac disease and controls in studies including children and adults[46–48,50,51] (Table 1).

In environmental enteropathy, no studies on antibodies targeting distinct antigens were found.

3.2 | Calprotectin

Calprotectin is a dimer of S100A8 and A9 proteins found primarily in the cytosol of neutrophils, monocytes and macrophages which have functions in antimicrobial and antifungal processes.[83] Largely due to its stability at room temperature for several days, faecal calprotectin is widely used in clinical practice to evaluate intestinal inflammation and neutrophil exudation, for example, in inflammatory bowel diseases and to differentiate this from non-inflammatory conditions such as irritable bowel syndrome.[83,84]

Ten studies, each including <100 patients, were found to evaluate faecal calprotectin in coeliac disease with inconsistent results (Table 1). This is somewhat surprising given the abundance of calprotectin in the above-mentioned duodenal proteomic study.[41] Six studies conducted on children reported calprotectin levels to be higher in untreated coeliac disease patients than in treated patients or in controls.[52–55,59,61] In contrast, two studies on adults and two on children reported non-significant difference in calprotectin levels between untreated and coeliac disease patients and controls.[56–58,60] Ertekin et al. reported higher calprotectin levels in patients with more severe duodenal damage, whereas four other studies found no significant association between calprotectin and histological findings[52,53,56–58] (Table 1). In 2021, the European Society for Paediatric Gastroenterology Hepatology and Nutrition Gastroenterology Committee recommended not using faecal calprotectin as a marker in coeliac disease.[80]

Four publications including 35–80 participants from partly overlapping study cohorts reported non-significant difference in faecal calprotectin levels in children with and without environmental enteropathy.[71–74] (Table 2). In faecal transcript studies, the results for calprotectin were inconsistent.[66–69] According to a duodenal transcriptome study, the S100A8 subunit was differentially expressed between environmental enteropathy and controls,[33] whereas no proteomic studies were conducted.

3.3 | Host transcripts

Studies on faecal host transcripts in coeliac disease and environmental enteropathy have addressed messenger RNAs (mRNAs) and small non-coding RNAs (sncRNAs) including microRNAs (miRNAs), piwi-interacting RNAs (piRNA) and transfer RNAs (tRNAs).

Francavilla et al. (2023) evaluated stool sncRNA profiles together with the microbiome and identified 77 differentially expressed miRNAs between 66 coeliac disease patients and two different sets of controls ($n=106$)[64] (Table 1). Target enrichment analysis highlighted associations between the differentially expressed miRNAs characteristics for the pathways of inflammation, absorption and transmembrane transport, cell apoptosis and glucose transport, immune response and endoplasmic reticulum stress.

Six studies evaluated faecal mRNA transcripts as potential biomarkers in environmental enteropathy including 70–658 children (Table 2). In whole transcriptome profiling, Yu et al. identified 51 transcripts as associated with environmental enteropathy and 12 with its severity measured with lactulose permeability.[67] Most identified transcripts code for proteins or cytokines participating in immune responses to microbes, and some also epithelial junction proteins. Twenty-four of all identified faecal transcripts were also

TABLE 2 Summary of the studies evaluating host-mucosal-derived faecal biomarkers in environmental enteropathy (EE) defined by abnormal sugar absorption tests. All the studies included <4-year-old children.

Study, country	Study groups	Children in the groups	Main results
mRNA transcripts			
Positive findings			
Agapova 2013, Malawi[65]	EE: LMR > 0.24 Controls: LMR < 0.13	$n = 36$ $n = 34$	Of five pre-selected transcripts, one (REG4) differed between children with EE and controls.
Ordiz 2016, Malawi[66]	Severe EE: LMR ≥ 0.45 Moderate EE: LMR 0.16–0.44 Normal: LMR <= 0.15	$n = 134$ $n = 524$ $n = 150$	Of 18 pre-selected transcripts, four differentiated by EE severity (CD53, TNF, HLA-DRA, MUC12) and seven predicted EE in the model (CD53, S100A8, TNF, CDX1, HLA-DRA, MUC12, REG1A).
Yu 2016, Malawi[67]	Severe EE: %L > 0.7 Intermediate EE: %L 0.2–0.7 Normal: %L < 0.2	$n = 42$ $n = 157$ $n = 60$	Of the whole transcriptome profile, 51 were associated with EE and 12 were associated with EE severity.
Ordiz 2018, Malawi[68]	Severe EE: %L ≥ 0.45 Normal: %L < 0.45	$n = 59$ $n = 281$	Of seven pre-selected transcripts, two were associated with %L (CDX1, REG1A) but these did not predict severe EE in random forest models.
Singh 2021a, Sierra Leone[69]	Severe EE: %L ≥ 0.45 Medium EE: %L 0.2–0.44 Normal: %L < 0.2	EE: $n = 323$ Normal: $n = 97$	Of 15 pre-selected transcripts, eight identified EE and differentiated its severity (SELL, BIRC3, IFI30, AQP9, PIK3AP1, DECR1, DEFA6, REG3A)
mRNA transcripts, MPO, neopterin			
Non-significant results			
Singh 2021b, Sierra Leone[70]	EE: %L ≥ 0.2 Normal: %L < 0.2	$n = 406$ $n = 114$	15 pre-selected transcripts were clustered into three groups reflecting gut inflammation, structure and defence. LMR was not associated with transcript scores in these groups or faecal myeloperoxidase or neopterin. %L was associated with gut defence score.
MPO, neopterin, calprotectin			
Non-significant results			
Long 2019, Bangladesh[71]	EE: LMR ≥ 0.09 Normal: LMR < 0.09	$n = 40$ $n = 40$	No significant difference in median levels between the groups in calprotectin (128 vs. 153 mg/dL, $p = \text{NS}$), MPO (1317 vs. 1750 µg/mL, $p = \text{NS}$) or neopterin (582 vs. 1000 ng/mL, $p = \text{NS}$).
Mondal 2019, Bangladesh[72]	EE: LMR ≥ 0.09 Normal: LMR < 0.09	$n = 18–20$ $n = 17–20$[a]	No significant difference in median levels between the groups in calprotectin (115 vs. 64 µg/g, $p = 0.21$), MPO (1161 vs. 668 ng/g, $p = 0.70$) or neopterin (1026 vs. 954 nmol/L, $p = 0.67$).
Shivakumar 2022, India[73]	EE: LRR ≥ 0.068 Normal: LRR < 0.068	$n = 27$ $n = 19$	No significant difference in median levels between the groups in calprotectin (244 vs. 135 µg/g, $p = 0.287$), MPO (71,668 vs. 59,221 ng/mL, $p = 0.204$) or neopterin (1107 vs. 1151 nmol/L, $p = 0.599$).
Shivakumar 2023, India[74]	EE: LRR ≥ 0.068 Normal: LRR < 0.068	n = 17 $n = 24$[b]	No significant difference in median levels between the groups in calprotectin (317 vs. 181 mg/g, $p = 0.363$), MPO (66,655 vs. 38,460 ng/mL, $p = 0.101$) or neopterin (1150 vs. 1392 nmol/L, $p = 0.921$).
MPO, neopterin or other			
Positive findings			
Guerrant 2016, Brazil[75]	Malnutrition study	$n = 375$[c]	LMR correlated with MPO (Pearson's correlation coefficient 0.183, $p = 0.003$) and lactoferrin ($n = 77$, 0.297, $p = 0.026$) but not with neopterin (0.037, $p = 0.593$) or REG1 (−0.065, $p = 0.322$).
Campbell 2017, Bangladesh[5]	EE: LMR > 0.07 Normal: LMR ≤ 0.07	$n = 210$ $n = 329$	LMR correlated weakly with MPO (Pearson's correlation coefficient 0.15, $p \leq 0.01$) and neopterin (0.11, $p \leq 0.05$).

(Continues)

TABLE 2 (Continued)

Study, country	Study groups	Children in the groups	Main results
Kosek 2017, Multi-centre[76]	MAL-ED birth cohort study[d,e]	$n = 1253$	At 1 year of age: MPO negatively associated with L:M z-score, neopterin not associated. At 2 years of age: MPO or neopterin not associated with L:M z-score. No more detailed data given.
Richard 2019, Multi-centre[77]	MAL-ED birth cohort study[f,g]	$n = 1017$	MPO was associated with LMR z-score at 6 months (coefficient −0.24, $p < 0.05$) and 9 months (−0.25, $p < 0.05$) of age, but not at 3 months (−0.22, $p =$ NS) or 15 months (−0.20, $p =$ NS).
Non-significant results			
Campbell 2004, Gambia[78]	Children with enteropathy	$n = 72$	No significant correlation between neopterin and intestinal permeability ($p = 0.11$).
McCormick 2019, Multi-centre[79]	MAL-ED birth cohort study[d,h]	$n = 1283$	MPO and neopterin did not correlate with LMR z-score ($\rho \sim 0.1$–0.2).

Note: Study cohort overlapping with [a]Long et al. (2019) and [b]Shivakumar et al. (2022); [c]274 with available data about the markers; [d]Bangladesh, Brazil, India, Nepal, Peru, Pakistan, Tanzania; [e]focus at 1 and 2 years of age; [f]Bangladesh, Brazil, India, Nepal, Peru, South Africa and Tanzania; [g]follow-up until 2 years of age; [h]focus on age of 9–15 months.

Abbreviations: %L, lactulose permeability; IQR, intra-quartile range; LMR, lactulose–mannitol ratio; LRR, lactulose recovery ratio; MAL-ED, Aetiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health; MPO, myeloperoxidase; mRNA, messenger RNA; ND, no data; NS, non-significant.

listed in the duodenal transcriptome study in environmental enteropathy by Haberman et al.[33] Other studies focused on partially overlapping pre-selected faecal transcripts, of which calprotectin subunit S100A8 was included in all studies. Other frequently included transcripts also found by duodenal or faecal transcriptome were CD53, IFI30, SELL and BIRC3. However, their association with environmental enteropathy was inconsistent between the studies (Table 2).

3.4 | Myeloperoxidase

MPO belongs to a family of haeme peroxidase enzymes. It is a major component of the primary granules in neutrophils and is seen in lower concentrations in monocytes and macrophages.[85] Similarly to calprotectin, it has been suggested to be used to reflect neutrophil-associated inflammation. MPO is usually not found in extravascular tissues without inflammation, but activated neutrophils release MPO that produces oxidants to kill invading pathogens. In chronic inflammatory conditions, increased extracellular amounts of MPO may result in host tissue damage.[85]

Ten studies on seven partly overlapping patient cohorts ($n = 35$–1283) were identified to study the association between faecal MPO and environmental enteropathy as defined by lactulose–mannitol ratio (Table 2). Four studies reported a weak correlation with low correlation coefficients between MPO and lactulose–mannitol ratio or found an association only at a certain age, whereas six studies reported no differences (Table 2). Of note, the Malnutrition and Enteric Disease (MAL-ED) longitudinal cohort study including 22,846 stool samples collected from 2076 children at 0–24 months of age reported poor environmental conditions, age during the first 2 years, recent breastmilk intake and enteric infections to affect faecal MPO levels.[76,86]

No studies evaluating faecal MPO in coeliac disease were found. Density of neutrophils is known to be increased in the intestinal mucosa of coeliac disease patients,[27] although their exact role in pathogenesis is poorly understood. In a proteomic study, MPO was expressed in the small intestinal mucosa of coeliac disease patients with differences during a 3-day gluten challenge and between the samples from treated and untreated patients.[41] Furthermore, Hällgren et al. reported MPO levels in jejunal perfusion fluid to be higher in seven coeliac disease patients than in 16 controls, and Maluf reported higher MPO levels in the peripheral blood of 47 coeliac disease patients than in the blood of 31 controls.[87,88]

3.5 | Neopterin

Neopterin is a breakdown product of guanosine triphosphate and its synthesis and release from macrophages and monocytes is activated by local interferons, especially IFN-γ secreted by helper T1 cells.[89,90] Neopterin can be measured from serum or, as it is excreted by the kidneys, from urine. Moreover, neopterin levels in other body fluids have been suggested to reflect local immune activation.[90]

Ten studies from seven partly overlapping patient cohorts ($n = 35$–1283) evaluated the association between environmental enteropathy and faecal neopterin (Table 2). Campbell et al. reported a very weak correlation between faecal neopterin and lactulose–mannitol ratio,[5] whereas the other nine publications detected no differences between neopterin levels in children with high and low lactulose–mannitol ratio or lactulose permeability (Table 2). However, similarly to MPO, age (increasing during the first 2 years), recent breastmilk

TABLE 3 Summary of the host-mucosal derived faecal biomarkers evaluated in coeliac disease (CeD) and environmental enteropathy (EE) defined by small intestinal histology or abnormal sugar absorption test.

Marker	Method(s)[a]	Confounding factors[b]	Evidence as faecal marker in CeD/EE
Cellular immune response			
Neopterin	ELISA	Age, breastfeeding, infections.	EE: weak association with LMR in one study, not associated in nine studies.[5,70–75,78,79] CeD: no studies identified
Gluten-dependent antibody response (CeD)			
AGA	ELISA, IIF	ND	CeD: associated with untreated CeD in three studies, not associated in two studies.[46–48,50,51]
DGP			CeD: levels higher and more often positive in untreated CeD than in controls according to one study.[49]
EmA			CeD: associated with untreated CeD in two studies, not associated in one study.[48–50]
TGA			CeD: associated with untreated CeD in two studies, not associated in two studies.[48–51]
Inflammation, neutrophil activity			
Calprotectin	ELISA	Age, drugs, infections, GI bleeding.	CeD: inconsistent results from 10 studies.[52–61] ESPGHAN does not recommend the use in CeD.[80] EE: no difference between children with high and low LMR according to four studies.[71–74]
Lactoferrin	ELISA	Age, breastfeeding, infections.	EE: correlated with LMR in one study.[75]
MPO	ELISA	Age, breastfeeding, infections.	EE: Weak correlation with LMR in two studies, age-related association in two studies and no association in six studies.[5,70–77,79]
Inflammation, unspecific			
Lipocalin-2	ELISA	Infections, drugs, tumours.	CeD: higher levels in CeD than in controls, but no difference in seropositive patients with Marsh 0 and 3 according to one study.[60]
Innate immunity, alarmins			
HBD2	ELISA	Infections, metabolic, physical, chemical and thermal stimuli.	CeD: higher levels in untreated CeD than in controls according to one study.[59]
HMGB1	Western blotting		CeD: levels increased in untreated CeD versus controls, decreased during GFD, correlated with S-TGA according to one study.[63]
Other			
ACE	Colorimetric method	ND	CeD: higher activity in untreated than in treated CeD or in controls according to one study.[62]
Host transcripts	qPCR, transcriptome array	Microbiota, diet.	CeD: differentially expressed miRNA between treated CeD patients with positive or negative TGA and controls, and association with duration of GFD according to one study.[64] EE: various potential mRNA transcripts recognized and used in models to predict EE and its severity; inconsistent results according to six studies.[65–70]
Reg1	ELISA	ND	EE: no correlation with LMR according to one study.[75]
ZRP	ELISA	Infections.	CeD: levels higher in untreated CeD than controls, decreased on a GFD according to one study.[61]

Abbreviations: ACE, angiotensin-converting enzyme; AGA, anti-gliadin antibodies; DGP, deamidated gliadin peptide antibodies; ELISA, Enzyme-Linked Immunosorbent Assay; EmA, endomysial antibodies; GFD, gluten-free diet; GI, gastrointestinal; HBD2, human β-defensin-2; HMGB1, high mobility group box 1; IIF, indirect immunofluorescence; LMR, lactulose–mannitol ratio; MPO, myeloperoxidase; qPCR, qualitative polymerase chain reaction; Reg1, regenerating islet-derived protein 1; S-TGA, serum transglutaminase-2 antibodies; ZRP, zonulin-related proteins.

[a]Used in the studies included to the review.

[b]Potentially affecting the biomarker levels in general.

intake and enteric infections were reported to affect faecal neopterin levels in the previously mentioned MAL-ED study cohort.[76,86] As far as we know, faecal neopterin has not been studied in coeliac disease, but Fuchs et al. (1983) reported urine neopterin levels to be higher in untreated, symptomatic coeliac disease patients than in asymptomatic patients and those on a gluten-free diet.[91]

3.6 | Other markers

3.6.1 | Alarmins

Alarmins are endogenous peptides or proteins released due to cell damage. They stimulate both the cells of innate immunity and antigen-presenting cells resulting in subsequent inflammation.[92] Because of this role, they can also be considered to be damage-associated molecular patterns (DAMPs). Interest in the possible significance of alarmins in immune-mediated conditions including coeliac disease has increased.[92,93]

HBD2 is a granule-derived alarmin and antimicrobial peptide expressed particularly by the epithelial cells of the skin, respiratory and gastrointestinal tract in response to bacterial products and cytokines, including IFN-γ.[94] In the intestine, it is believed to have an anti-inflammatory and microbiota regulating role.[93] Kamilova et al. (2022) reported faecal HBD2 to be higher in 76 recently (<1 year) diagnosed paediatric coeliac disease patients than in 32 healthy controls[59] (Table 1). However, studies on duodenal transcripts and proteomics have reported inconsistent results about the possible expression of HBD2 in coeliac disease.[33,41,95–98]

HMGB1, also called amphoterin, is a non-histone, DNA binding protein which has various functions depending on its cellular position and posttranslational modifications. Damaged cells including monocytes, macrophages and epithelial cells, may release HMGB1 in the extracellular space and act as an alarmin activating innate immunity and promoting inflammation.[92] Palone et al. (2018) reported faecal HMGB1 to be elevated in 39 children with untreated coeliac disease, to decrease during 12 months on a gluten-free diet and to be undetectable in 20 age- and sex-matched controls.[63] Levels correlated with serum TGA (Table 1). Also, serum HMGB1 levels have been reported to be higher in paediatric coeliac disease patients than in controls and possibly associated with clinical features and severity of villous atrophy.[99,100] No studies evaluating HBD2 or HMGB1 in environmental enteropathy were found.

3.6.2 | Angiotensin-converting enzyme

ACE is an enzyme with an important role in the renin–angiotensin system regulating blood pressure and body fluid balance by converting angiotensin I to II and degrading bradykinin. ACE can be found in various tissues, especially the lungs and kidneys, but also in the intestine where it may have a role in absorption.[101] Letizia et al. (1996) reported significantly higher faecal ACE activity in 20 untreated coeliac disease patients compared to 18 treated patients and 15 controls[62] (Table 1), but no more recent studies on ACE were identified either in coeliac disease or environmental enteropathy.

3.6.3 | Lactoferrin

Lactoferrin is a heat stable glycoprotein found not only in various body fluids including breast milk, bile and saliva but also in epithelial cells and neutrophil granules where it is released during inflammation or infection.[102] In addition to immune defence, lactoferrin has antimicrobial functions and participates in iron metabolism. The study by Guerrant et al. (2016) was the only one to evaluate faecal lactoferrin in environmental enteropathy and reported it to correlate with lactulose–mannitol ratio in 375 participants.[75] (Table 2). Fine et al. (1998) reported faecal lactoferrin to be undetectable in 92% of 13 coeliac disease patients, but the study did not report the criteria used for diagnosing coeliac disease and thus did not meet the inclusion criteria of the current review.[103]

3.6.4 | Lipocalin-2

Lipocalin-2, also called neutrophil gelatinase-associated lipocalin (NGAL), is a small glycoprotein released by various cell types in response to cell injury, for example, in intestinal and metabolic inflammation, infection and ischaemia.[104] Although the precise pathophysiological role of lipocalin-2 remains unclear, many of its functions are related to iron metabolism, for example, in protection combating infections.[104] Sutton et al. reported faecal lipocalin-2 to be higher in 73 adult coeliac disease patients than in 18 controls, whereas seropositive patients with Marsh 0 lesion presented with lipocalin-2 levels comparable to those with Marsh 3[60] (Table 1). Of note, plasma lipocalin-2 levels have been comparable between coeliac disease patients and controls.[60,105] According to a proteomic study, lipocalin-2 expression was detected in the mucosa of coeliac disease patients and changed during a 3-day gluten challenge but was not significantly different in samples from treated and untreated patients.[41] No studies evaluating faecal lipocalin-2 in environmental enteropathy were found, but in a study on mucosal genomics, Haberman et al. reported lipocalin-2 to be up-regulated in environmental enteropathy even more specifically than in coeliac disease.[33]

3.6.5 | Regenerating islet-derived protein 1

Reg1 is a β-cell growth factor belonging to the regenerating gene protein family and having a role not only in pancreatic islet regeneration and cancer but also in other pathologies including intestinal inflammation.[106] During inflammation, notably secretory cells such as Paneth and enterochromaffin-like cells seem to express

Reg1 according to evidence from inflammatory bowel diseases.[106] Guerrant et al. (2016) reported faecal Reg1 not to correlate with lactulose–mannitol ratio in a study on 375 children with environmental enteropathy and malnutrition[75] (Table 2). No studies evaluating Reg1 in coeliac disease were identified.

3.6.6 | Zonulin-related proteins

Zonulin is a precursor to haptoglobin 2, which reportedly regulates the integrity of intestinal epithelial tight junction proteins.[107] Increased zonulin release has been suggested to reflect increased intestinal permeability and to be associated for example with infections and gluten. However, there are significant limitations in commercially available zonulin ELISA tests as many of these may indicate the presence of proteins other than zonulin.[108,109] Gallego et al. (2024) reported the levels of faecal zonulin-related proteins to be higher in 23 children with untreated coeliac disease than in 39 controls, and to decrease on a gluten-free diet[61] (Table 1). Elevated zonulin levels have also been reported in the intestinal biopsies and serum of untreated coeliac disease patients and in serum samples preceding preclinical coeliac disease than in the samples from controls.[107,110,111] However, all these studies used ELISA kits with unknown reliability for measuring zonulin.

4 | DISCUSSION

To summarize, the evidence on faecal markers reflecting the degree of small intestinal damage is still scarce (Table 3). In coeliac disease, there were single studies with positive findings about antibodies and protein markers but also contradictory results. In environmental enteropathy, even the markers used widely in research, such as neopterin and MPO, seemed to lack the evidence about the association with intestinal damage. In only a few of the environmental enteropathy studies, the main aim was to study the association of the markers with intestinal damage, and most of them focused primarily on nutritional aspects and linear growth in young children. Furthermore, all the environmental enteropathy studies included here used lactulose–mannitol ratio or lactulose permeability as a proxy for small intestinal damage although these have significant practical limitations and reflect mainly permeability and absorption; it is unclear whether they correlate with inflammation and morphological changes.[19,20,112,113]

Proteomic and transcriptomic studies of small intestinal mucosa provide important background on the molecules possibly indicating intestinal damage.[23] However, these methods also have limitations and do not directly reflect the possible findings in faeces. In proteomic studies, high- and medium-abundant proteins are usually well-recognized whereas small and low-abundant proteins may not be detected.[23] While RNA sequencing is currently the most used method, sequencing depth and coverage may cause significant variation in the results. Also, some of the molecules detected in mucosal samples, such as cytokines, have a short half-life and are thus not candidates for faecal measurement.[114] Furthermore, the degradation of molecules starting already in the small intestine, continuing through the large intestine and persisting after defecating affects what remains measurable in faeces. Whether the extent or localization of mucosal damage is associated with the amount of molecules released in faeces is not known.[115,116] We could speculate that if, for example, calprotectin was released in higher volumes in the lower than in the upper intestine, this might explain why it was found in duodenal proteomic studies, while the results from faecal marker studies were conflicting.

Molecules remaining in faeces after degradation should be relatively stable, enabling practical sample collection, transportation and storage. However, these aspects are not well validated for many of the markers studied and the potential influence of stability on the results reported remains unclear. Furthermore, the possible significance of stool frequency and consistency are not known. Patients with diarrhoea were excluded from some of the studies, and, for example, faecal dry weight to reflect watery content more specifically was rarely measured. This method may be time consuming, and while stool consistency classification could also be used to reflect watery content, there is limited evidence on its utility.[117,118] Faecal sample processing for the analyses is also an important step,[119] but whether, for example, normalizing the amount of proteins compared to faecal weight results in better outcomes is not known.[120] Finally, simple enough methodology to measure the markers or their fragments from faecal samples is needed, and as was seen here, ELISA was used in many of the studies.

Most of the markers of interest, except for antibodies in coeliac disease, are unspecific and their utility has also been studied in other gastrointestinal conditions including inflammatory bowel diseases, necrotizing enterocolitis and colorectal conditions, such as cancer and polyps.[121–126] These markers mainly reflect inflammation and immune responses in the mucosa and some of them have been reported to be affected, for example, by infections,[127,128] age,[76,86] dietary factors and gut microbiota.[129,130] To overcome the lack of specificity, a combination of different markers assessing various aspects of intestinal damage may be needed. In environmental enteropathy, the combination of, for example, MPO, neopterin and α-1-antitrypsin has been suggested to reflect the complex damage pathways and pathology better than single marker.[131] Finally, when talking about faecal transcripts, it is crucial to note that most of them originate from microbes. That said, only a fraction of stool seems to be relevant from the biomarker aspect.

In clinical practice, the need for novel non-invasive markers to reflect the condition of small intestinal mucosal is clear. Feasible tools for monitoring disease activity would help to focus the invasive downstream evaluations only on those in need. Also, non-invasive, and reliable endpoints are much needed in coeliac disease and environmental enteropathy intervention studies as repeated biopsies are not justified, especially for paediatric patients. In addition to these aspects, although environmental enteropathy is defined as a small intestinal enteropathy, it is rarely diagnosed on the basis of mucosal biopsies. Improved biomarkers are thus needed to define and characterize environmental enteropathy.

5 | CONCLUSIONS AND FUTURE RESEARCH

Stool remains an interesting and relatively little utilized source of information on conditions of the small intestine. More evidence about the molecules released from intestinal mucosa and their potential to be measurable in faeces is needed. After this, important steps towards utilizing stool samples more effectively would be the validation of sample collection and processing, and ensuring the availability of clinically feasible measurement methods for relevant markers. Finally, the utility of the markers should be compared directly against the findings from intestinal biopsies to study their reliability. It is possible that a combination of several markers rather than a single marker would be needed to describe the various characteristics of small intestinal mucosal health.

AUTHOR CONTRIBUTIONS

Laura Kivelä: Conceptualization; writing – original draft; funding acquisition; methodology; visualization; formal analysis; data curation. **Katri Lindfors:** Conceptualization; writing – original draft; writing – review and editing; visualization; methodology. **Knut E. A. Lundin:** Conceptualization; writing – review and editing; methodology. **Ketil Størdal:** Conceptualization; methodology; writing – review and editing; supervision; resources.

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CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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REFERENCES

1. Keusch GT, Denno DM, Black RE, Duggan C, Guerrant RL, Lavery JV, et al. Environmental enteric dysfunction: pathogenesis, diagnosis, and clinical consequences. Clin Infect Dis. 2014;59:S207–S212.
2. Lindfors K, Ciacci C, Kurppa K, Lundin KEA, Makharia GK, Mearin ML, et al. Coeliac disease. Nat Rev Dis Primers. 2019;5:3.
3. Singh P, Arora A, Strand TA, Leffler DA, Catassi C, Green PH, et al. Global prevalence of celiac disease: systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2018;16:823–836.e2.
4. Hossain MI, Haque R, Mondal D, Mahfuz M, Ahmed AMS, Islam MM, et al. Undernutrition, vitamin a and iron deficiency are associated with impaired intestinal mucosal permeability in young Bangladeshi children assessed by lactulose/mannitol test. PLoS One. 2016;11:e0164447.
5. Campbell RK, Schulze KJ, Shaikh S, Mehra S, Ali H, Wu L, et al. Biomarkers of environmental enteric dysfunction among children in rural Bangladesh. J Pediatr Gastroenterol Nutr. 2017;65:40–6.
6. Kelly P, Menzies I, Crane R, Zulu I, Nickols C, Feakins R, et al. Responses of small intestinal architecture and function over time to environmental factors in a tropical population. Am J Trop Med Hyg. 2004;70:412–9.
7. Ludvigsson JF, Bai JC, Biagi F, Card TR, Ciacci C, Ciclitira PJ, et al. Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology. Gut. 2014;63:1210–28.
8. Rubio-Tapia A, Hill ID, Semrad C, Kelly CP, Greer KB, Limketkai BN, et al. American College of Gastroenterology guidelines update: diagnosis and management of celiac disease. Am J Gastroenterol. 2023;118:59–76.
9. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al. European society Paediatric gastroenterology, hepatology and nutrition guidelines for diagnosing coeliac disease 2020. J Pediatr Gastroenterol Nutr. 2020;70:141–56.
10. Al-Toma A, Volta U, Auricchio R, Castillejo G, Sanders DS, Cellier C, et al. European Society for the Study of coeliac disease (ESsCD) guideline for coeliac disease and other gluten-related disorders. United European Gastroenterol J. 2019;7:583–613.
11. Donaldson MR, Firth SD, Wimpee H, Leiferman KM, Zone JJ, Horsley W, et al. Correlation of duodenal histology with tissue transglutaminase and endomysial antibody levels in pediatric celiac disease. Clin Gastroenterol Hepatol. 2007;5:567–73.
12. Taavela J, Kurppa K, Collin P, Lähdeaho M–L, Salmi T, Saavalainen P, et al. Degree of damage to the small bowel and serum antibody titers correlate with clinical presentation of patients with celiac disease. Clin Gastroenterol Hepatol. 2013;11:166–171.e1.
13. Ziv-Baran T, Dubov Y, Weinberger R, Guz-Mark A, Shamir R, Assa A. Anti-tissue transglutaminase titers are associated with endoscopic findings and severity of mucosal damage in children with celiac disease. Eur J Pediatr. 2021;180:263–9.
14. Patel N, Leffler DA, Al-Toma A, Mulder CJ, Elli L, Gan G, et al. Clinical data do not reliably predict duodenal histology at follow-up in celiac disease: a 13 center correlative study. Am J Surg Pathol. 2024;48:212–20.
15. Silvester JA, Kurada S, Szwajcer A, Kelly CP, Leffler DA, Duerksen DR. Tests for serum transglutaminase and endomysial antibodies do not detect most patients with celiac disease and persistent villous atrophy on gluten-free diets: a meta-analysis. Gastroenterology. 2017;153:689–701.e1.
16. Ludvigsson JF, Ciacci C, Green PH, Kaukinen K, Korponay-Szabo IR, Kurppa K, et al. Outcome measures in coeliac disease trials: the Tampere recommendations. Gut. 2018;67:1410–24.
17. Raju SA, Shiha MG, Penny HA. Monitoring coeliac disease in 2024, time to change practice? Curr Opin Gastroenterol. 2024;40:190–5.
18. Korpe PS, Petri WA. Environmental enteropathy: critical implications of a poorly understood condition. Trends Mol Med. 2012;18:328–36.
19. Campbell DI, Murch SH, Elia M, Sullivan PB, Sanyang MS, Jobarteh B, et al. Chronic T cell-mediated enteropathy in rural west african children: relationship with nutritional status and small bowel function. Pediatr Res. 2003;54:306–11.
20. Denno DM, VanBuskirk K, Nelson ZC, Musser CA, Hay Burgess DC, Tarr PI. Use of the lactulose to mannitol ratio to evaluate

childhood environmental enteric dysfunction: a systematic review. Clin Infect Dis. 2014;59:S213–S219.

21. Kosek M, Guerrant RL, Kang G, Bhutta Z, Yori PP, Gratz J, et al. Assessment of environmental enteropathy in the MAL-ED cohort study: theoretical and analytic framework. Clin Infect Dis. 2014;59(Suppl 4):S239–S247.
22. Thompson AJ, Bourke CD, Robertson RC, Shivakumar N, Edwards CA, Preston T, et al. Understanding the role of the gut in undernutrition: what can technology tell us? Gut. 2021;70:1580–94.
23. Stamnaes J. Insights from tissue "omics" analysis on intestinal remodeling in celiac disease. Proteomics. 2021;21:e2100057.
24. Khan M, Jamil Z, Ehsan L, Zulqarnain F, Srivastava S, Siddiqui S, et al. Quantitative morphometry and machine learning model to explore duodenal and rectal mucosal tissue of children with environmental enteric dysfunction. Am J Trop Med Hyg. 2023;108:672–83.
25. Syed S, Yeruva S, Herrmann J, Sailer A, Sadiq K, Iqbal N, et al. Environmental enteropathy in undernourished Pakistani children: clinical and histomorphometric analyses. Am J Trop Med Hyg. 2018;98:1577–84.
26. Marie C, Ali A, Chandwe K, Petri WA, Kelly P. Pathophysiology of environmental enteric dysfunction and its impact on oral vaccine efficacy. Mucosal Immunol. 2018;11:1290–8.
27. Iversen R, Sollid LM. The immunobiology and pathogenesis of celiac disease. Annu Rev Pathol. 2023;18:47–70.
28. Popp A, Taavela J, Graziano P, Parente P, Covelli C, Lamacchia C, et al. New intraepithelial γδ T-lymphocyte marker for celiac disease classification in formalin-fixed paraffin-embedded (FFPE) duodenal biopsies. Dig Dis Sci. 2021;66:3352–8.
29. Veitch AM, Kelly P, Zulu IS, Segal I, Farthing MJG. Tropical enteropathy: a T-cell-mediated crypt hyperplastic enteropathy. Eur J Gastroenterol Hepatol. 2001;13:1175–81.
30. Hüe S, Mention J-J, Monteiro RC, Zhang SL, Cellier C, Schmitz J, et al. A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. Immunity. 2004;21:367–77.
31. Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, et al. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity. 2004;21:357–66.
32. Mayassi T, Ladell K, Gudjonson H, McLaren JE, Shaw DG, Tran MT, et al. Chronic inflammation permanently reshapes tissue-resident immunity in celiac disease. Cell. 2019;176:967–981.e19.
33. Haberman Y, Iqbal NT, Ghandikota S, Mallawaarachchi I, Braun T, Dexheimer PJ, et al. Mucosal genomics implicate lymphocyte activation and lipid metabolism in refractory environmental enteric dysfunction. Gastroenterology. 2021;160:2055–2071.e0.
34. Di Niro R, Mesin L, Zheng N-Y, Stamnaes J, Morrissey M, Lee J-H, et al. High abundance of plasma cells secreting transglutaminase 2–specific IgA autoantibodies with limited somatic hypermutation in celiac disease intestinal lesions. Nat Med. 2012;18:441–5.
35. Steinsbø Ø, Dunand CJH, Huang M, Mesin L, Salgado-Ferrer M, Lundin KEA, et al. Restricted VH/VL usage and limited mutations in gluten-specific IgA of coeliac disease lesion plasma cells. Nat Commun. 2014;5:4041.
36. Cardoso-Silva D, Delbue D, Itzlinger A, Moerkens R, Withoff S, Branchi F, et al. Intestinal barrier function in gluten-related disorders. Nutrients. 2019;11:2325.
37. Kreutz JM, Adriaanse MPM, van der Ploeg EMC, Vreugdenhil ACE. Narrative review: nutrient deficiencies in adults and children with treated and untreated celiac disease. Nutrients. 2020;12:500.
38. Kaonga P, Kaimoyo E, Besa E, Zyambo K, Sinkala E, Kelly P. Direct biomarkers of microbial translocation correlate with immune activation in adult Zambians with environmental enteropathy and hepatosplenic schistosomiasis. Am J Trop Med Hyg. 2017;97:1603–10.
39. Lee GO, McCormick BJJ, Seidman JC, Kosek MN, Haque R, Olortegui MP, et al. Infant nutritional status, feeding practices, enteropathogen exposure, socioeconomic status, and illness are associated with gut barrier function as assessed by the lactulose mannitol test in the MAL-ED birth cohort. Am J Trop Med Hyg. 2017;97:281–90.
40. Simula MP, Cannizzaro R, Canzonieri V, Pavan A, Maiero S, Toffoli G, et al. PPAR signaling pathway and cancer-related proteins are involved in celiac disease-associated tissue damage. Mol Med. 2010;16:199–209.
41. Tutturen AEV, Dørum S, Clancy T, Reims HM, Christophersen A, Lundin KEA, et al. Characterization of the small intestinal lesion in celiac disease by label-free quantitative mass spectrometry. Am J Pathol. 2018;188:1563–79.
42. Stamnaes J, Stray D, Stensland M, Sarna VK, Nyman TA, Lundin KEA, et al. In well-treated celiac patients low-level mucosal inflammation predicts response to 14-day gluten challenge. Adv Sci. 2021;8:2003526.
43. Johansen A, Sandve GKF, Ibsen JH, Lundin KEA, Sollid LM, Stamnaes J. Biopsy proteome scoring to determine mucosal remodeling in celiac disease. Gastroenterology. 2024;167:493–504.e10.
44. Strnad P, McElvaney NG, Lomas DA. Alpha-1-antitrypsin deficiency. N Engl J Med. 2020;382:1443–55.
45. Levitt DG, Levitt MD. Protein losing enteropathy: comprehensive review of the mechanistic association with clinical and subclinical disease states. Clin Exp Gastroenterol. 2017;10:147–68.
46. Haas L, Meillet D, Kapel N, Rostoker G, Gobert JG. Increased concentrations of fecal anti-gliadin IgA antibodies in untreated celiac disease. Clin Chem. 1993;39:696–7.
47. Haas L, Petit-Phar M, Terzidis H, Kapel N, Meillet D, Gobert JG, et al. IgA subclass distribution of IgA anti-gliadin antibodies in feces of patients with coeliac disease. Adv Exp Med Biol. 1995;371B:1349–53.
48. Picarelli A, Sabbatella L, Di TM, Di CT, Vetrano S, Anania MC. Antiendomysial antibody detection in fecal supernatants: in vivo proof that small bowel mucosa is the site of antiendomysial antibody production. Am J Gastroenterol. 2002;97:95–8.
49. Di Tola M, Marino M, Casale R, Di Battista V, Borghini R, Picarelli A. Extension of the celiac intestinal antibody (CIA) pattern through eight antibody assessments in fecal supernatants from patients with celiac disease. Immunobiology. 2016;221:63–9.
50. Halblaub JM, Renno J, Kempf A, Bartel J, Schmidt-Gayk H. Comparison of different salivary and fecal antibodies for the diagnosis of celiac disease. Clin Lab. 2004;50:551–7.
51. Kappler M, Krauss-Etschmann S, Diehl V, Zeilhofer H, Koletzko S. Detection of secretory IgA antibodies against gliadin and human tissue transglutaminase in stool to screen for coeliac disease in children: validation study. BMJ. 2006;332:213–4.
52. Ertekin V, Selimoğlu MA, Turgut A, Bakan N. Fecal calprotectin concentration in celiac disease. J Clin Gastroenterol. 2010;44:544–6.
53. Balamtekın N, Baysoy G, Uslu N, Orhan D, Akçören Z, Özen H, et al. Fecal calprotectin concentration is increased in children with celiac disease: relation with histopathological findings. Turk J Gastroenterol. 2012;23:503–8.
54. Biskou O, Gardner-Medwin J, Mackinder M, Bertz M, Clark C, Svolos V, et al. Faecal calprotectin in treated and untreated children with coeliac disease and juvenile idiopathic arthritis. J Pediatr Gastroenterol Nutr. 2016;63:e112–e115.
55. Rajani S, Huynh HQ, Shirton L, Kluthe C, Spady D, Prosser C, et al. A Canadian study toward changing local practice in the diagnosis of pediatric celiac disease. Can J Gastroenterol Hepatol. 2016;2016:6234160.

56. Montalto M, Santoro L, Curigliano V, D'Onofrio F, Cammarota G, Panunzi S, et al. Faecal calprotectin concentrations in untreated coeliac patients. Scand J Gastroenterol. 2007;42:957–61.
57. Capone P, Rispo A, Imperatore N, Caporaso N, Tortora R. Fecal calprotectin in coeliac disease. World J Gastroenterol. 2014;20:611–2.
58. Szaflarska-Popławska A, Romańczuk B, Parzęcka M. Faecal calprotectin concentration in children with coeliac disease. Prz Gastroenterol. 2020;15:44–7.
59. Kamilova AT, Azizova GK, Umarnazarova ZE, Abdullaeva DA, Geller SI. The activity of antimicrobial peptides in pediatric celiac disease. Front Pediatr. 2022;10:873793.
60. Sutton KA, He M, Ma C, Liu TC, Faubion WA, Hoffmann J, et al. Non-invasive markers of inflammation and protein loss augment diagnosis of pediatric celiac disease. Clin Transl Gastroenterol. 2024;15:e00695.
61. Martínez Gallego MÁ, Crespo Sánchez MG, Serrano Olmedo MG, Buño Soto A, Álvarez Casasempere S, Nozal P, et al. Trends in faecal zonulin concentrations in paediatric patients with celiac disease at baseline and on a gluten-free diet: exploring correlations with other faecal biomarkers. Nutrients. 2024;16:684.
62. Letizia C, Picarelli A, De Ciocchis A, Di Giovambattista F, Greco M, Cerci S, et al. Angiotensin-converting enzyme activity in stools of healthy subjects and patients with celiac disease. Dig Dis Sci. 1996;41:2268–71.
63. Palone F, Vitali R, Trovato CM, Montuori M, Negroni A, Mallardo S, et al. Faecal high mobility group box 1 in children with celiac disease: a pilot study. Dig Liver Dis. 2018;50:916–9.
64. Francavilla A, Ferrero G, Pardini B, Tarallo S, Zanatto L, Caviglia GP, et al. Gluten-free diet affects fecal small non-coding RNA profiles and microbiome composition in celiac disease supporting a host-gut microbiota crosstalk. Gut Microbes. 2023;15:2172955.
65. Agapova S, Stephenson K, Manary M, Weisz A, Tarr PI, Mkakosya R, et al. Detection of low-concentration host mRNA transcripts in Malawian children at risk for environmental enteropathy. J Pediatr Gastroenterol Nutr. 2013;56:66–71.
66. Ordiz MI, Shaikh N, Trehan I, Maleta K, Stauber J, Shulman R, et al. Environmental enteric dysfunction is associated with poor linear growth and can be identified by host fecal mRNAs. J Pediatr Gastroenterol Nutr. 2016;63:453–9.
67. Yu J, Ordiz MI, Stauber J, Shaikh N, Trehan I, Barnell E, et al. Environmental enteric dysfunction includes a broad spectrum of inflammatory responses and epithelial repair processes. Cell Mol Gastroenterol Hepatol. 2016;2:158–174.e1.
68. Ordiz MI, Wold K, Kaimila Y, Divala O, Gilstrap M, Lu HZ, et al. Detection and interpretation of fecal host mRNA in rural Malawian infants aged 6-12 months at risk for environmental enteric dysfunction. Exp Biol Med. 2018;243:985–9.
69. Singh A, Potani I, Griswold SP, Suri D, Langlois B, Shen Y, et al. Host fecal mRNAs predicted environmental enteric dysfunction among children with moderate acute malnutrition in Sierra Leone. Am J Trop Med Hyg. 2021;105:1376–82.
70. Singh A, Ghosh S, Ward H, Manary MJ, Rogers BL, Rosenberg IH. Biomarkers of environmental enteric dysfunction are differently associated with recovery and growth among children with moderate acute malnutrition in Sierra Leone. Am J Clin Nutr. 2021;113:1556–64.
71. Long JM, Mondal P, Westcott JE, Miller LV, Islam MM, Ahmed M, et al. Zinc absorption from micronutrient powders is low in Bangladeshi toddlers at risk of environmental enteric dysfunction and may increase dietary zinc requirements. J Nutr. 2019;149:98–105.
72. Mondal P, Long JM, Westcott JE, Islam MM, Ahmed M, Mahfuz M, et al. Zinc absorption and endogenous fecal zinc losses in Bangladeshi toddlers at risk for environmental enteric dysfunction. J Pediatr Gastroenterol Nutr. 2019;68:874–9.
73. Shivakumar N, Hsu JW, Kashyap S, Thomas T, Kurpad AV, Jahoor F. Tryptophan oxidation in young children with environmental enteric dysfunction classified by the lactulose rhamnose ratio. Am J Clin Nutr. 2022;116:970–9.
74. Shivakumar N, Kashyap S, Jahoor F, Devi S, Preston T, Thomas T, et al. The systemic availability of indispensable amino acids from orally ingested algal and legume protein in young children at risk of environmental enteric dysfunction. Am J Clin Nutr. 2023;118:96–102.
75. Guerrant RL, Leite AM, Pinkerton R, Medeiros PHQS, Cavalcante PA, DeBoer M, et al. Biomarkers of environmental enteropathy, inflammation, stunting, and impaired growth in children in Northeast Brazil. PLoS One. 2016;11:e0158772.
76. Kosek MN, Ahmed T, Bhutta Z, Caulfield L, Guerrant R, Houpt E, et al. Causal pathways from enteropathogens to environmental enteropathy: findings from the MAL-ED birth cohort study. EBioMedicine. 2017;18:109–17.
77. Richard SA, McCormick BJJ, Murray-Kolb LE, Lee GO, Seidman JC, Mahfuz M, et al. Enteric dysfunction and other factors associated with attained size at 5 years: MAL-ED birth cohort study findings. Am J Clin Nutr. 2019;110:131–8.
78. Campbell DI, McPhail G, Lunn PG, Elia M, Jeffries DJ. Intestinal inflammation measured by fecal neopterin in Gambian children with enteropathy: association with growth failure, Giardia lamblia, and intestinal permeability. J Pediatr Gastroenterol Nutr. 2004;39:153–7.
79. McCormick BJJ, Murray-Kolb LE, Lee GO, Schulze KJ, Ross AC, Bauck A, et al. Intestinal permeability and inflammation mediate the association between nutrient density of complementary foods and biochemical measures of micronutrient status in young children: results from the MAL-ED study. Am J Clin Nutr. 2019;110:1015–25.
80. Koninckx CR, Donat E, Benninga MA, Broekaert IJ, Gottrand F, Kolho KL, et al. The use of fecal calprotectin testing in paediatric disorders: a position paper of the European Society for Paediatric Gastroenterology and Nutrition Gastroenterology Committee. J Pediatr Gastroenterol Nutr. 2021;72:617–40.
81. Dahele A, Aldhous MC, Kingstone K, Humphreys K, Bode J, McIntyre M, et al. Gut mucosal immunity to tissue transglutaminase in untreated celiac disease and other gastrointestinal disorders. Dig Dis Sci. 2002;47:2325–35.
82. O'Mahony S, Barton JR, Crichton S, Ferguson A. Appraisal of gut lavage in the study of intestinal humoral immunity. Gut. 1990;31:1341–4.
83. Jukic A, Bakiri L, Wagner EF, Tilg H, Adolph TE. Calprotectin: from biomarker to biological function. Gut. 2021;70:1978–88.
84. Røseth AG, Fagerhol MK, Aadland E, Schjønsby H. Assessment of the neutrophil dominating protein calprotectin in feces: a methodologic study. Scand J Gastroenterol. 1992;27:793–8.
85. Davies MJ, Hawkins CL. The role of myeloperoxidase in biomolecule modification, chronic inflammation, and disease. Antioxid Redox Signal. 2020;32:957–81.
86. McCormick BJJ, Lee GO, Seidman JC, Haque R, Mondal D, Quetz J, et al. Dynamics and trends in fecal biomarkers of gut function in children from 1–24 months in the MAL-ED study. Am J Trop Med Hyg. 2017;96:465–72.
87. Hällgren R, Colombel JF, Dahl R, Fredens K, Kruse A, Jacobsen NO, et al. Neutrophil and eosinophil involvement of the small bowel in patients with celiac disease and Crohn's disease: studies on the secretion rate and immunohistochemical localization of granulocyte granule constituents. Am J Med. 1989;86:56–64.
88. Maluf SW, Wilhelm Filho D, Parisotto EB, Medeiros GS, Pereira CHJ, Maraslis FT, et al. DNA damage, oxidative stress, and inflammation in children with celiac disease. Genet Mol Biol 2020; 43: e20180390.

89. Huber C, Batchelor JR, Fuchs D, Hausen A, Lang A, Niederwieser D, et al. Immune response-associated production of neopterin. Release from macrophages primarily under control of interferon-gamma. J Exp Med. 1984;160:310–6.
90. Müller MM, Curtius H-C, Herold M, Huber CH. Neopterin in clinical practice. Clin Chim Acta. 1991;201:1–16.
91. Fuchs D, Granditsch G, Hausen A, Reibnegger G, Wachter H. Urinary neopterin excretion in coeliac disease. Lancet. 1983;322:463–4.
92. Danieli MG, Antonelli E, Piga MA, Claudi I, Palmeri D, Tonacci A, et al. Alarmins in autoimmune diseases. Autoimmun Rev. 2022;21:103142.
93. Rizzi A, Di Gioacchino M, Gammeri L, Inchingolo R, Chini R, Santilli F, et al. The emerging role of innate lymphoid cells (ILCs) and alarmins in celiac disease: an update on pathophysiological insights, potential use as disease biomarkers, and therapeutic implications. Cells. 2023;12:1910.
94. Pazgier M, Hoover DM, Yang D, Lu W, Lubkowski J. Human β-defensins. Cell Mol Life Sci. 2006;63:1294–313.
95. Boniotto M, Pirulli D, Verga Falzacappa MV, Trevisiol C, Gerarduzzi T, Crovella S. Localization and expression of two human b-defensins (HBD-1 and HBD-2) in intestinal biopsies of celiac patients. Eur J Histochem. 2003;47:389–92.
96. Forsberg G, Fahlgren A, Horstedt P, Hammarstrom S, Hernell O, Hammarstrom M-L. Presence of bacteria and innate immunity of intestinal epithelium in childhood celiac disease. Am J Gastroenterol. 2004;99:894–904.
97. Taha AS, Faccenda E, Angerson WJ, Balsitis M, Kelly RW. Natural antibiotic expression in celiac disease? Correlation with villous atrophy and response to a gluten-free diet. Dig Dis Sci. 2005;50:791–5.
98. Vordenbäumen S, Pilic D, Otte J, Schmitz F, Schmidt-Choudhury A. Defensin-mRNA expression in the upper gastrointestinal tract is modulated in children with celiac disease and helicobacter pylori –positive gastritis. J Pediatr Gastroenterol Nutr. 2010;50:596–600.
99. Manti S, Cuppari C, Tardino L, Parisi G, Spina M, Salpietro C, et al. HMGB1 as a new biomarker of celiac disease in children: a multicenter study. Nutrition. 2017;37:18–21.
100. Yagci M, Aydemir Y, Baris Z. HMGB1 is related to disease activity in children with celiac disease. Clin Res Hepatol Gastroenterol. 2023;47:102175.
101. Ward PE, Sheridan MA, Hammon KJ, Erdös EG. Angiotensin I converting enzyme (kininase II) of the brush border of human and swine intestine. Biochem Pharmacol. 1980;29:1525–9.
102. Ward PP, Paz E, Conneely OM. Lactoferrin: multifunctional roles of lactoferrin: a critical overview. Cell Mol Life Sci. 2005;62:2540–8.
103. Fine KD, Ogunji F, George J, Niehaus MD, Guerrant RL. Utility of a rapid fecal latex agglutination test detecting the neutrophil protein, lactoferrin, for diagnosing inflammatory causes of chronic diarrhea. Am J Gastroenterol. 1998;93:1300–5.
104. Moschen AR, Adolph TE, Gerner RR, Wieser V, Tilg H. Lipocalin-2: a master mediator of intestinal and metabolic inflammation. Trends Endocrinol Metab. 2017;28:388–97.
105. Janas RM, Rybak A, Wierzbicka-Rucińska A, Socha P, Śnitko R, Szaflarska-Popławska A, et al. Serum concentrations of insulin, ghrelin, adiponectin, leptin, leptin receptor and lipocalin-2 in children with celiac disease who do and do not adhere to a gluten-free diet. Gut Liver. 2016;10:587–94.
106. Edwards J-A, Tan N, Toussaint N, Ou P, Mueller C, Stanek A, et al. Role of regenerating islet-derived proteins in inflammatory bowel disease. World J Gastroenterol. 2020;26:2702–14.
107. Fasano A. Zonulin, regulation of tight junctions, and autoimmune diseases. Ann N Y Acad Sci. 2012;1258:25–33.
108. Massier L, Chakaroun R, Kovacs P, Heiker JT. Blurring the picture in leaky gut research: how shortcomings of zonulin as a biomarker mislead the field of intestinal permeability. Gut. 2021;70:1801–2.
109. Sollid LM, Koning F. Lack of relationship of AT1001 to zonulin and prehaptoglobin-2: clinical implications. Gut. 2021;70:2211–2.
110. Fasano A, Not T, Wang W, Uzzau S, Berti I, Tommasini A, et al. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. Lancet. 2000;355:1518–9.
111. DaFonte TM, Valitutti F, Kenyon V, Locascio JJ, Montuori M, Francavilla R, et al. Zonulin as a biomarker for the development of celiac disease. Pediatrics. 2024;153:e2023063050.
112. Sullivan PB, Lunn PG, Northrop-Clewes C, Crowe PT, Marsh MN, Neale G. Persistent diarrhea and malnutrition—the impact of treatment on small bowel structure and permeability. J Pediatr Gastroenterol Nutr. 1992;14:208–15.
113. Iqbal TH, Lewis KO, Gearty JC, Cooper BT. Small intestinal permeability to mannitol and lactulose in the three ethnic groups resident in west Birmingham. Gut. 1996;39:199–203.
114. Liu C, Chu D, Kalantar-Zadeh K, George J, Young HA, Liu G. Cytokines: from clinical significance to quantification. Adv Sci. 2021;8:2004433.
115. Shaoul R, Sladek M, Turner D, Paeregaard A, Veres G, Wauters GV, et al. Limitations of fecal calprotectin at diagnosis in untreated pediatric Crohn's disease. Inflamm Bowel Dis. 2012;18:1493–7.
116. Sipponen T, Kolho K-L. Faecal calprotectin in children with clinically quiescent inflammatory bowel disease. Scand J Gastroenterol. 2010;45:872–7.
117. Blake MR, Raker JM, Whelan K. Validity and reliability of the Bristol stool form scale in healthy adults and patients with diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther. 2016;44:693–703.
118. Nordin E, Hellström PM, Brunius C, Landberg R. Modest conformity between self-reporting of Bristol stool form and fecal consistency measured by stool water content in irritable bowel syndrome and a FODMAP and gluten trial. Am J Gastroenterol. 2022;117:1668–74.
119. Gangadoo S, Rajapaksha Pathirannahalage P, Cheeseman S, Dang YTH, Elbourne A, Cozzolino D, et al. The multiomics analyses of fecal matrix and its significance to coeliac disease gut profiling. Int J Mol Sci. 2021;22:1965.
120. de Moura Gondim Prata M, Havt A, Bolick DT, Pinkerton R, Lima A, Guerrant RL. Comparisons between myeloperoxidase, lactoferrin, calprotectin and lipocalin-2, as fecal biomarkers of intestinal inflammation in malnourished children. J Transl Sci. 2016;2:134–9.
121. Di Ruscio M, Vernia F, Ciccone A, Frieri G, Latella G. Surrogate fecal biomarkers in inflammatory bowel disease: rivals or complementary tools of fecal calprotectin? Inflamm Bowel Dis. 2018;24:78–92.
122. Vernia F, Viscido A, Di Ruscio M, Stefanelli G, Valvano M, Latella G. Fecal lactoferrin and other putative fecal biomarkers in Crohn's disease: do they still have a potential clinical role? Digestion. 2021;102:833–44.
123. Langhorst J, Junge A, Rueffer A, Wehkamp J, Foell D, Michalsen A, et al. Elevated human β-defensin-2 levels indicate an activation of the innate immune system in patients with irritable bowel syndrome. Am J Gastroenterol. 2009;104:404–10.
124. Liu X-C, Li L-Q, Ling K-R, Guo L, Hu X-Y, Li C. Fecal HBD-2 and Claudin-3 may be potential biomarkers to predict the deterioration of necrotizing enterocolitis: a prospective study. Front Pediatr. 2022;10:1062798.

125. Chalkias A, Nikotian G, Koutsovasilis A, Bramis J, Manouras A, Mystrioti D, et al. Patients with colorectal cancer are characterized by increased concentration of fecal Hb-hp complex, myeloperoxidase, and secretory IgA. Am J Clin Oncol. 2011;34:561–6.
126. Kilciler AG, Kutluana U, Mizrak S. Fecal neopterin level determination: can be a useful screening test for colorectal polyps? Eur J Gastroenterol Hepatol. 2021;33:655–61.
127. Gazi MA, Alam MA, Fahim SM, Wahid BZ, Khan SS, Islam MO, et al. Infection with Escherichia Coli pathotypes is associated with biomarkers of gut enteropathy and nutritional status among malnourished children in Bangladesh. Front Cell Infect Microbiol. 2022;12:901324.
128. George CM, Burrowes V, Perin J, Oldja L, Biswas S, Sack D, et al. Enteric infections in young children are associated with environmental enteropathy and impaired growth. Trop Med Int Health. 2018;23:26–33.
129. Bouzid Y, Alkan Z, Stephensen C, Lemay D. Low intake of dietary fiber is associated with gastrointestinal inflammation in healthy U.S. adults. Curr Dev Nutr. 2022;6:974.
130. Kortekangas E, Fan Y-M, Chaima D, Lehto KM, Malamba-Banda C, Matchado A, et al. Associations between gut microbiota and intestinal inflammation, permeability and damage in young Malawian children. J Trop Pediatr. 2022;68:fmac012.
131. Kosek M, Haque R, Lima A, Babji S, Shrestha S, Qureshi S, et al. Fecal markers of intestinal inflammation and permeability associated with the subsequent acquisition of linear growth deficits in infants. Am J Trop Med Hyg. 2013;88:390–6.

SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

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