Review Article

Novel Biomarkers and the Future Potential of Biomarkers in Inflammatory Bowel Disease

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There is increasing importance placed upon noninvasive assessment of gut inflammation. These tools are likely to be the key in differentiating intestinal inflammatory disease from functional disorders and in monitoring the response to intervention in individuals with known inflammatory conditions. Although various noninvasive markers are currently available, they have limitations and do not provide ideal utility. This review focuses on emerging markers of gut inflammation, highlighting the potential of specific markers.

1. Introduction

Historically, noninvasive assessments of gut inflammation have utilized tests such as the detection of fecal white blood cells [1] and whole stool lavage. However, these provide inadequate assessment of gut inflammation. The need to improve assessment of gut inflammation has driven investigation into additional biomarkers of inflammation. Calprotectin, S100A12, and lactoferrin have generally been well described as fecal biomarkers in inflammatory bowel disease (IBD) [2]. However, other less well-established fecal biomarkers include M2-pyruvate kinase, osteoprotegerin, myeloperoxidase, HMGB1, chitinase 3-like 1, defensins, matrix metalloproteinases, and human nucleic acid: most of these have been assessed in single cohorts and require further extensive evaluations and validation. This review will focus on these novel fecal biomarkers, with mention of the future potential of biomarkers in diagnosis, prognosis, and monitoring of disease activity in IBD.

2. M2-PK

Pyruvate kinase, a key enzyme in the glycolytic pathway [3], can be present in skeletal muscle, heart or brain as a tetramer (M1), or in undifferentiated and proliferating tissues as a dimer (termed M2-PK) [4, 5]. M2-PK can be measured in serum or stool and is stable in stool for up to two days [6]. Fecal M2-PK concentrations are increased in colorectal carcinoma [7], but also in gut inflammation [3] reflecting increased cell turnover. Although, it is postulated that intestinal epithelial cells may be protected against apoptosis by the upregulation of M2-PK through the Bcl-xl pathway in Crohn’s disease (CD) [8].

High levels of M2-PK were documented in 81 adults diagnosed with IBD [3] (Table 1). This cohort was compared to a group of 43 subjects with irritable bowel syndrome (IBS) and 7 with colorectal carcinoma. M2-PK concentrations were higher in patients with ulcerative colitis (UC) or CD than in the controls. Furthermore, higher levels were evident in indi-
viduals with active IBD than in those with quiescent disease. In a further study, M2-PK was assessed in 105 adults presenting with undifferentiated gastrointestinal symptoms and 94 healthy controls [9]. The 14 adults subsequently diagnosed with organic diseases (only 10 with IBD) had higher fecal concentrations of M2-PK than those with functional symptoms or the controls. M2-PK measurement provided sensitivity of 67% and specificity of 88% in distinguishing between organic and functional diagnoses.

Fecal M2-PK was assessed in a group of Polish children with IBD [4]. Seventy-five children with UC, 32 with CD, and 35 healthy control children provided stool samples. M2-PK levels were higher in children with IBD, and levels correlated with pediatric Crohn disease activity index (PCDAI [10, 11]) scores in the 32 children with CD. Although mean M2-PK levels were higher in those with active disease, 47% of the children with IBD judged to be in remission also had elevated M2-PK (Table 2(a)).

In a recent Australian study, mean fecal M2-PK levels were also higher in 17 children with active CD than in 21 healthy controls (p = 0.0007) [12]. However, M2-PK levels did not correlate with PCDAI scores or serum inflammatory markers. There was no relationship between fecal M2-PK and fecal S100A12 levels in the children with active CD. The children with ileocolonic disease tended to have higher M2-PK concentrations than those with isolated colonic or ileal disease (Table 2(b)).

High fecal M2-PK levels also were demonstrated in children with active UC [13]. M2-PK and three other fecal markers (calprotectin, S100A12, and lactoferrin) were evaluated as indicators of the response to first line medical therapy in 101 children with acute severe UC. M2-PK was found to be superior to the other markers in identifying those who subsequently failed intravenous corticosteroids.

In 2014, Czub et al. [14] directly compared M2-PK and calprotectin in assessing the severity and activity of pediatric

| Ref | Age | Population | N | Comparison groups | Cut-off | Sn (%) | Sp (%) | PPV (%) | NPV (%) | p value |
|-----|-----|------------|---|------------------|---------|-------|-------|--------|--------|---------|
| [3] | P   | HC/CD/UC  | 142 | HC versus IBD    | 4 U/g   | 96.2  | 94.3  | 96.2   | 94.3   | <0.0001 |
|     |     |            |     | HC versus CD     | 4 U/g   | 100   | 94.3  | 89.5   | 100    |         |
|     |     |            |     | HC versus UC     | 4 U/g   | 94.3  | 94.3  | 94.3   |        |         |
| [10]| P   | HC/CD     | 38  | HC versus CD     | 5 U/g   | 94.4  | 97.1  | 97.1   | 94.4   |         |

### Table 1: Use of novel fecal markers as diagnostic test for IBD.

**M2 pyruvate kinase (M2-PK)**

| Ref | Age | Population | N | Comparison groups | Cut-off | Sn (%) | Sp (%) | PPV (%) | NPV (%) | p value |
|-----|-----|------------|---|------------------|---------|-------|-------|--------|--------|---------|
| [3] | P   | HC/CD/UC  | 142 | HC versus IBD    | 4 U/g   | 96.2  | 94.3  | 96.2   | 94.3   | <0.0001 |
| [10]| P   | HC/CD     | 38  | HC versus CD     | 5 U/g   | 94.4  | 97.1  | 97.1   | 94.4   |         |

**Osteoprotegerin (OPG)**

| Ref | Age | Population | N | Comparison groups | Cut-off | Sn (%) | Sp (%) | PPV (%) | NPV (%) | p value |
|-----|-----|------------|---|------------------|---------|-------|-------|--------|--------|---------|
| [29]| P   | HC/CD     | 127| HC versus CD     | 0.065 U/ml | 89    | 51    | 89     | 51     | <0.0001 |

**Myeloperoxidase (MPO)**

| Ref | Age | Population | N | Comparison groups | Cut-off | Sn (%) | Sp (%) | PPV (%) | NPV (%) | p value |
|-----|-----|------------|---|------------------|---------|-------|-------|--------|--------|---------|
| [34]| A   | HC/UC     | 74 | HC versus UC     | 0.065 U/ml | NA    |       |       |       |         |
| [36]| A   | HC/UC     | 109| HC versus UC     | 0.065 U/ml | <0.001|       |       |       |         |
| [38]| A   | HC/CD/UC  | 51 | HC versus CD     | 0.065 U/ml | <0.0001|       |       |       |         |
|     |     |            |     | HC versus UC     | 0.065 U/ml | <0.0001|       |       |       |         |

**High-mobility group box (HMGB1)**

| Ref | Age | Population | N | Comparison groups | Cut-off | Sn (%) | Sp (%) | PPV (%) | NPV (%) | p value |
|-----|-----|------------|---|------------------|---------|-------|-------|--------|--------|---------|
| [39]| P   | HC/CD/UC  | 54 | HC versus CD     | 0.065 U/ml | 84.7  | 88.9  | 96.8   | 59.3   |         |
|     |     |            |     | HC versus UC     | 0.065 U/ml | 81.6  | 80    | 93.9   | 53.3   |         |
|     |     |            |     | HC versus UC     | 0.065 U/ml | 88.2  | 100   | 100    | 66.7   |         |

**Chitinase 3-like 1 (CHI3L1)**

| Ref | Age | Population | N | Comparison groups | Cut-off | Sn (%) | Sp (%) | PPV (%) | NPV (%) | p value |
|-----|-----|------------|---|------------------|---------|-------|-------|--------|--------|---------|
| [46]| P   | HC/CD/UC  | 237| HC versus IBD    | 13.7 ng/g | 84.7  | 88.9  | 96.8   | 59.3   |         |
|     |     |            |     | HC versus CD     | 81.6    | 80    | 93.9   | 53.3   |         |
|     |     |            |     | HC versus UC     | 88.2    | 100   | 100    | 66.7   |         |

**Human β defensin (HBD) 2**

| Ref | Age | Population | N | Comparison groups | Cut-off | Sn (%) | Sp (%) | PPV (%) | NPV (%) | p value |
|-----|-----|------------|---|------------------|---------|-------|-------|--------|--------|---------|
| [49]| P   | HC/CD/IC/UC| 46 | HC versus IBD    | 0.065 U/ml | NA    |       |       |       | <0.001 |
|     |     |            |     | HC versus CD     | 0.065 U/ml | <0.001|       |       |       | <0.001 |
|     |     |            |     | HC versus IC     | 0.065 U/ml | <0.001|       |       |       | <0.001 |
|     |     |            |     | HC versus UC     | 0.065 U/ml | <0.001|       |       |       | <0.001 |
|     |     |            |     | CD versus UC     | 0.065 U/ml | <0.001|       |       |       | <0.001 |

**Human β defensin (HBD) 2**

| Ref | Age | Population | N | Comparison groups | Cut-off | Sn (%) | Sp (%) | PPV (%) | NPV (%) | p value |
|-----|-----|------------|---|------------------|---------|-------|-------|--------|--------|---------|
| [50]| A   | HC/IBS/UC | 100| HC versus IBD    | 0.065 U/ml | NA    |       |       |       | <0.001 |
|     |     |            |     | HC versus UC     | 0.065 U/ml | <0.001|       |       |       | <0.001 |
|     |     |            |     | IBS versus UC    | 0.065 U/ml | 0.165 |       |       |       |         |

Age: A: adult; P: paediatric; N: number of patients; Sn: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; HC: healthy controls; IBD = CD + UC(+IC); CD: Crohn’s disease; UC: ulcerative colitis; IC: inflammatory colitis; NA: not available.
IBD. Truelove-Witts score was used to describe disease severity in UC patients, and PCDAI was used to assess CD patients. The performance of M2-PK was described as inferior to calprotectin to identify IBD, UC, and CD from healthy controls. In addition, M2-PK was inferior to calprotectin in identifying UC and CD in remission amongst healthy controls. It was postulated that calprotectin reflects paediatric IBD severity and activity better than M2-PK. However, this is in contradiction of the observation of Roszak et al. [15] who state that M2-PK is a more sensitive marker than calprotectin.
Osteoprotegerin, also known as osteoprotegrin, (OPG) is a basic glycoprotein that is found either as a 60 kDa monomer or as a 120 kDa dimer. OPG is a cytokine receptor and belongs to the TNF superfamily [16, 17]. OPG can be produced by a wide range of cell types, including osteoblasts, B lymphocytes, dendritic cells, bone marrow stromal cells, epithelial cells, and monocytes/macrophages [17, 18]. OPG production may be regulated by proinflammatory mediators [16, 19, 20]. The cellular sources of OPG are distinct to those of the established inflammatory markers calprotectin, lactoferrin, and S100A12.

OPG has a well-established role in bone turnover. The equilibrium of osteoclast and osteoblast activity is coordinately regulated by the receptor activator of nuclear factor-κB (RANK) and its ligand (RANKL). OPG acts as a decoy receptor for RANK [16, 18, 20–22]. In this role as a decoy receptor, OPG inhibits the differentiation, survival, and function of osteoclasts by competitively blocking the interaction between RANK and RANKL [18] promoting bone formation as a counter regulatory response to factors such as inflammatory cytokines (IL-1, TNFα) [23]. This is of importance in IBD where there is an established increased fracture risk associated with the disease [24]. However, the impact of intestinal-derived OPG upon bone loss in the context of IBD is yet to be directly established [25].

OPG also may have a role in IBD pathogenesis, quite separate to the role in bone metabolism. The OPG/RANKL/RANK triad may contribute to mucosal and systemic inflammation [17, 20, 22]. RANK, RANKL, and OPG decrease the functional capacity of dendritic cells (DC) and activated T cells but enhance B cell maturation [26–28].

Recently, a small number of studies have demonstrated that OPG can be a useful marker of inflammation in the context of IBD. Nahidi et al. [27] evaluated OPG in children with CD and control children without evidence of underlying gut disease (Table 2(b)). OPG was detected in serum, mucosal biopsies, and in the stool. Levels of OPG were greatly increased in stool samples collected from the children with CD and in the endoscopically obtained mucosal biopsies. In addition, serum levels of OPG were markedly elevated in those children with severe CD compared to control values. Furthermore, serum and fecal levels fell substantially with remission induction therapy in a subset of children (exclusive enteral nutrition in this instance). In this group of children with CD, those children with isolated colonic involvement had greater levels than a group with ileocolonic disease and serum and fecal OPG did not correlate. Although OPG did not correlate with the PCDAI scores, levels did correlate with modified PCDAI scores (includes the serum inflammatory marker components of the PCDAI, with the addition of a score for CRP [29]). In addition, fecal OPG also correlated with fecal S100A12 and serum CRP at diagnosis of IBD, yet did not correlate following treatment.

Galliera et al. [30] measured serum OPG and RANKL levels following the administration of the anti-TNF-α inhibitor infliximab in adult patients with IBD. In this study, OPG levels also decreased with treatment, in correlation with falling CRP levels. It is important to note that OPG levels did not fall acutely and were only significantly lower 22 weeks after the commencement of therapy. The authors suggest that in this setting, OPG is representative of the inflammatory response and not bone turnover.

In addition, Sylvester et al. [31] recently investigated whether fecal OPG was able to act as a predictive marker for the treatment of UC in a large group of children. They reported that fecal OPG was elevated in patients who had failed first-line corticosteroid therapy and required infliximab or colectomy. Further, OPG was superior in predicting response to therapy compared to lactoferrin or S100A12. These reports indicate that OPG may be useful in monitoring the inflammatory response in IBD. However, the authors note that OPG is rapidly degraded in stool at room temperature and, therefore, optimal stool collection and storage conditions must be used to accurately assess fecal OPG.

There are, however, conflicting reports on fecal OPG expression. In a preliminary investigation, Skinner et al. [32] report that fecal OPG is elevated at diagnosis of UC but not of CD, and therefore, may have potential as a UC specific marker. This is in contrast to Nahidi et al. [27] who report that OPG is elevated at diagnosis of CD, albeit levels were more elevated with severe disease. Nevertheless, the expression pattern of OPG appears unique amongst the stool inflammatory markers investigated to date. Consequently, OPG has the potential to enhance knowledge of the intestinal inflammatory picture. However, it is clear that further investigation into the settings of OPG expression in the intestinal mucosa is required to advance our understanding of how OPG fits into the inflammatory cascade and its role in the pathogenesis of the inflammatory response in IBD.

4. Myeloperoxidase

Myeloperoxidase (MPO) is one of a number of proteins stored in and released from neutrophil secretary granules: it is stable for at least 3 days in stool [33]. One potential limitation of myeloperoxidase as an inflammatory marker is its cationic charge, which may lead it to bind to fecal particles, limiting reliability as a disease marker [34].

MPO levels were greatly increased in a cohort of 55 Indian patients with UC compared to 74 healthy controls (0.42 units versus 0.06 units: p < 0.001) [35] (Table 1). In differentiating between UC and no inflammation, a sensitivity of 89% but specificity of just 51.4% was observed. Levels correlated with endoscopic severity scores and fell following therapeutic intervention. However, levels did not correlate with endoscopic extent or histological severity scores [36] (Table 2(b)).

Sangfelt et al. [37] also showed significant correlation between MPO concentrations and endoscopic and clinical activity in a study of 11 Swedish patients with UC. A Japanese study involving 33 patients with UC and 32 patients with CD again illustrated a strong relationship between MPO and
endoscopic extent or histological grade [38] (Table 2(a)). In this instance, MPO levels fell in response to therapy and as suggested earlier fecal MPO may be used as a noninvasive biomarker for the response to treatment [36].

5. HMGB1

The nuclear protein, high-mobility group box (HMGB) 1, is released from immune cells in the setting of inflammation. It has been described as an alarmin, with key in
digesting this to be the source of the increased production.

High cytoplasmic levels of HMGB1 in mucosal biopsies, sug-

increased in 40 children with IBD, whilst being undetectable

with IBD [39]. Fecal levels of HMGB1 protein were greatly

required before HMGB1 could be considered further.

Alimentary invasion and adhesion to epithelial cells. Increased serum

urine [60] levels have also been reported in IBD patients.

levels (Table 2(a), Table 2(b)). Finally, in a subset of 11 children

with paired samples before and after therapy to induce remis-

sion, fecal levels of CHI3L1 were noted to fall (p = 0.01). In

addition to its presumed role in IBD, CHI3L1 was suggested by Chen et al. [47] to play a role in inflammation-associated neoplastic modification in colonic epithelial cells.

Although there is some rationale to consider that this protein may be a useful marker, further assessment and val-

idation is required.

7. Human Beta Defensin 2

Defensins are innate antimicrobial peptides that are pro-
duced at epithelial borders and contribute to host defence

[48]. Several members of the β defensin group are produced in the colon by epithelial cells and plasma cells. An initial study completed in French children demonstrated that human β defensin (HBD) 2 could be measured in stool samples [49]. Although able to be detected in control children, levels were substantially higher in those with IBD (p = 0.0002), especially those with UC (Table 1).

One further study has evaluated fecal levels of HBD2 in a
group of adults [50]. HBD2 was elevated in 30 adult patients

with UC compared to 24 healthy controls (106.9 versus 29.9:

p = 0.001) (Table 1). However, levels were also increased in a group of 46 patients with IBS compared to the control group, but not different to those with UC. These results suggest a

proinflammatory activation of the mucosal immune system in patients with IBS as well as IBD and suggest that this marker may not be specific to IBD.

8. Matrix Metalloproteinase

The matrix metalloproteinases (MMP) are a family of key

biological mediators involved in tissue degradation and resti-
tution. A number of studies have examined the roles of MMP

proteins in IBD [51, 52]: these include regulation, inflam-

mation, and tissue destruction. Furthermore, the balance

between MMPs and their inhibitors (tissue inhibitors of

metalloproteinases: TIMPs) may influence fibrosis and stric-

ture development.

MMPs are expressed in areas of inflammation and ulcer-

ation in the gut, and several MMPs are overexpressed in IBD

[51, 53–57]. MMP-2 has been reported to be elevated in the

inflamed tissue from IBD patients [58]. Gao et al. [58]

reported increased tissue mRNA levels that correlated with

severity of inflammation. Immunohistochemistry studies

revealed that MMP-2 was present in the extracellular matrix of the submucosa. Furthermore, elevated serum [59] and

urine [60] levels have also been reported in IBD patents.

MMP was also monitored in patients with CD managed

with infliximab. Interestingly, MMP-2 serum levels increased,

both in responders and nonresponders to treatment, and this was hypothesised to be due to increased intestinal cell turn-

over [59]. Garg et al. [61] reported the findings of an experi-

cental colitis model using dextran sodium sulphate-

induced colitis in MMP-2-ablated mice. In this model, the

MMP-2-ablated mice developed a more severe colitis than the

control animals indicating that MMP-2 has a protective
role against developing colitis and may explain elevated levels in response to treatment.

Most interest in MMPs as disease markers of IBD has focused on MMP-9. Kofla-Dlubacz et al. [62] showed that serum MMP-9 concentrations correlated with CRP levels and with disease activity (using PCDAI scores) in 82 children with CD [63] and in 31 children with UC. Annahazi et al. [64] have subsequently delineated fecal levels in 47 patients with IBD, 23 with IBS, and 24 control patients. In the subjects with UC, MMP-9 concentrations correlated with endoscopic and clinical severity scores ($p < 0.001$ for both relationships) and were also associated with CRP levels ($p = 0.002$). Furthermore, MMP-9 levels correlated with fecal calprotectin ($p = 0.014$).

Despite MMP-9 being elevated in both CD and UC, the performance of MMP-9 as a disease marker appears to be better in UC and pouchitis [65]. Kolho et al. [66] compared the performance of MMP-9 to that of calprotectin in distinguishing IBD from non-IBD subjects. Although MMP-9 performance was comparable to calprotectin in those with UC, MMP-9 was inferior to calprotectin in CD [66]. There are differing reports regarding the response of MMP-9 to therapy. Gao et al. [59] reported that serum MMP-9 levels fell in response to a single dose of infliximab; however, Makitalo et al. [67] reported that serum MMP-9 levels were not altered with therapy. Overall, it appears that MMP-9 may be a useful biomarker in the assessment of undifferentiated gut symptoms and in the evaluation of mucosal inflammatory activity although further work is needed to more fully describe these expression patterns.

Several other MMP’s are also elevated in IBD. MMP-7 and MMP-13 mRNA levels were elevated in biopsy specimens from CD and UC patients [68]. Serum MMP-3 [62] and MMP-8 [67] levels are also elevated in IBD. However, the role of MMP as markers in IBD may not be restricted to reporters of inflammation and tissue repair. MMP expression is also associated with colorectal tumors. MMP-7 mRNA had been detected in cancerous intestinal tissue [69] and has been proposed to be involved in the growth of tumors [70]. Therefore, MMP-7 may also have a potential role in colorectal cancer screening, including in the context of colitis-associated cancer. However, fecal evaluation of this marker is not yet described.

9. Human Nucleic Acid (DNA and miRNA)

One study has assessed human deoxyribonucleic acid levels in stool of 36 individuals with UC [71]. Excretion of DNA correlated with clinical disease activity and endoscopic severity (Table 2(b)). In differentiating between active disease and remission, this test provided sensitivity of 67% and specificity of 100%. The same group subsequently assessed this biomarker in a cohort of 54 adults with inactive UC [72] (Table 2(a)). Over a 12-month period of observation, 23 of the subjects relapsed. Fecal DNA levels remained stable in those who remained well, whilst levels increased in the patients with relapse, providing a predictive marker.

In addition to DNA, RNA or more specifically microRNA (miRNA) has the potential to be used as a disease marker in IBD. miRNAs are small noncoding RNA molecules that are capable of regulating gene expression at the posttranscriptional level. Expression patterns have been described in intestinal biopsies collected from IBD patients with a number of specific miRNA reported to be upregulated in both CD and UC [73–75]. In CD, it has been documented by Wu et al. [75] that the pattern of expression of miRNA in ileal and colonic biopsies differs significantly, meaning that no miRNA expressions were overlapping. In CD and UC, when compared to healthy controls, specific upregulated miRNA (CD [76], UC [77]) and downregulated miRNA (CD [78], UC [76]) were described. Importantly, differentially expressed miRNA were also detected in the serum of IBD patients indicating the potential of these molecules to be used as disease markers.

However, there are several difficulties that need to be overcome to compare these studies. For example, concurrent medications, the inflammatory status, and the location of the biopsy need to be considered when interpreting the role of miRNAs [79, 80]. Further investigation of the detection of miRNA in stool is required. In addition, the full role of these molecules in IBD is still required; however, differential expression of specific miRNA have been implicated in disease pathogenesis [74].

10. Future Potential of Biomarkers in IBD

A biomarker can be described as a product that provides a measurable indication of the presence and/or severity of disease or physiological state of an organism. The holy grail of a biomarker is that it is disease specific, correlates highly with disease severity, and can provide both diagnostic and prognostic indications. To date, several promising fecal biomarkers for IBD have been identified. However, none of the currently described markers are disease specific so the search for better biomarkers of IBD continues. A number of promising novel biomarkers have been identified, and this article has described a portion of the most promising novel markers. However, there are many more potential biomarkers of IBD and further work is required to determine if any of the newly identified markers will be equal to, or surpass, the utility of currently available markers. The aims of the tables are to describe and compare the current knowledge in the use of novel markers: as markers of diagnostic tests for IBD (Table 1), as markers to assess disease activity (inactive versus active) in IBD (Table 2(a)), and finally, as markers of disease severity in IBD (Table 2(b)).

The initial identification of calprotectin as a biomarker of IBD was greeted with great enthusiasm and promise that it would impact clinical care. However, after nearly 30 years of calprotectin research, the incorporation of this biomarker into a routine clinical care has been slow. Calprotectin has not replaced current nonspecific inflammatory markers, but when used, it is generally used as an adjunct disease marker. Therefore, it is reasonable to suspect a long wait for new IBD biomarkers to appear in the clinic. Nevertheless, biomarkers of IBD have a bright future, in research studies at least, as prognostic indicators and in the search for personalised and improved IBD clinical care.
11. Conclusion

There also are several promising markers described here that likely reflect different cellular sources and different aspects of the IBD response. Currently, no single marker appears to be sought after highly sensitive, diagnostic, and prognostic IBD specific indicator. However, a number of novel biomarkers have been evaluated in just one or two cohorts: these all require more extensive assessments in various settings to establish their roles in identifying, monitoring, and predicting disease behaviour.

More clinical studies are required to ascertain the full potential of fecal biomarkers in IBD. Although such evaluations should focus on specific biomarkers, comparative assessments are also required, yet there remains plenty of potential for novel biomarkers to impact clinical care.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] L. Huicho, M. Campos, J. Rivera, and R. L. Guerrant, “Fecal screening tests in the approach to acute infectious diarrhea: a scientific overview,” The Pediatric Infectious Disease Journal, vol. 15, no. 6, pp. 486–494, 1996.

[2] R. N. Lopez, S. T. Leach, D. A. Lemberg, G. Duvoisin, R. B. Gearry, and A. S. Day, “Fecal biomarkers in inflammatory bowel disease,” Journal of Gastroenterology and Hepatology, vol. 32, no. 3, pp. 577–582, 2016.

[3] G. Chung-Faye, B. Hayee, S. Maestranzi, N. Donaldson, I. Forgacs, and R. Sherwood, “Fecal M2-pyruvate kinase (M2-PK): a novel marker of intestinal inflammation,” Inflammatory Bowel Diseases, vol. 13, no. 11, pp. 1374–1378, 2007.

[4] E. Czub, K.-H. Herzig, A. Szafalarska-Popawska et al., “Fecal pyruvate kinase: a potential new marker for intestinal inflammation in children with inflammatory bowel disease,” Scandinavian Journal of Gastroenterology, vol. 42, no. 10, pp. 1147–1150, 2007.

[5] J. Walkowiak, T. Banasiewicz, P. Krołkowicz, R. Hansdorfer-Korzon, M. Drews, and K.-H. Herzig, “Fecal pyruvate kinase (M2-PK): a new predictor for inflammation and severity of pouchitis,” Scandinavian Journal of Gastroenterology, vol. 40, no. 12, pp. 1493–1496, 2005.

[6] U. Haug, M. N. Wente, C. M. Seiler, D. Rothenbacher, M. W. Buchler, and H. Brenner, “Tumor M2 pyruvate kinase as a stool marker for colorectal cancer: stability at room temperature and implications for application in the screening setting,” Clinical Chemistry, vol. 52, no. 4, pp. 782–784, 2006.

[7] P. D. Hardt, S. Mazurek, M. Toepfer et al., “Fecal tumour M2 pyruvate kinase: a new, sensitive screening tool for colorectal cancer,” British Journal of Cancer, vol. 91, no. 5, pp. 980–984, 2005.

[8] Q. Tang, Q. Ji, W. Xia et al., “Pyruvate kinase M2 regulates apoptosis of intestinal epithelial cells in Crohn’s disease,” Digestive Diseases and Sciences, vol. 60, no. 2, pp. 393–404, 2015.

[9] J. Jeffery, S. J. Lewis, and R. M. Ayling, “Fecal dimeric M2-pyruvate kinase (tumor M2-PK) in the differential diagnosis of functional and organic bowel disorders,” Inflammatory Bowel Diseases, vol. 15, no. 11, pp. 1630–1634, 2009.

[10] J. S. Hyams, G. D. Ferry, F. S. Mandel et al., “Development and validation of a pediatric Crohn’s disease activity index,” Journal of Pediatric Gastroenterology and Nutrition, vol. 12, no. 4, pp. 439–447, 1991.

[11] J. Hyams, J. Markowitz, A. Otley et al., “Evaluation of the pediatric crohn disease activity index: a prospective multicenter experience,” Journal of Pediatric Gastroenterology and Nutrition, vol. 41, no. 4, pp. 416–421, 2005.

[12] A. S. Day, T. Judd, D. A. Lemberg, and S. T. Leach, “Fecal M2-PK in children with Crohn’s disease: a preliminary report,” Digestive Diseases and Sciences, vol. 57, no. 8, pp. 2166–2170, 2012.

[13] T. A. Judd, A. S. Day, D. A. Lemberg, D. Turner, and S. T. Leach, “Update of fecal markers of inflammation in inflammatory bowel disease,” Journal of Gastroenterology and Hepatology, vol. 26, no. 10, pp. 1493–1499, 2011.

[14] E. Czub, J. K. Nowak, A. Szafalarska-Popawska et al., “Comparison of fecal pyruvate kinase isoform M2 and calprotectin in assessment of pediatric inflammatory bowel disease severity and activity,” Acta Biochimica Polonica, vol. 61, no. 1, pp. 99–102, 2014.

[15] D. Roszak, M. Galeyka, W. Cichy, and P. Szachta, “Determination of faecal inflammatory marker concentration as a noninvasive method of evaluation of pathological activity in children with inflammatory bowel diseases,” Advances in Medical Sciences, vol. 60, no. 2, pp. 246–252, 2015.

[16] A. R. Moschen, A. Kaser, B. Enrich et al., “The RANKL/OPG system is activated in inflammatory bowel disease and correlates to the state of bone loss,” Gut, vol. 54, no. 4, pp. 479–487, 2005.

[17] K. Vidal, P. Serrat, B. Schlosser, P. van den Broek, F. Lorget, and A. Donnet-Hughes, “Osteoprotegerin production by human intestinal epithelial cells: a potential regulator of mucosal immune responses,” American Journal of Physiology, Gastrointestinal and Liver Physiology, vol. 287, no. 4, pp. G836–G844, 2004.

[18] N. Franchimont, C. Reenaers, C. Lambert et al., “Increased expression of receptor activator of NF-κappaB ligand (RANKL),” its receptor RANK and its decoy receptor osteoprotegerin in the colon of Crohn’s disease patients,” Clinical and Experimental Immunology, vol. 138, no. 3, pp. 491–498, 2004.

[19] C. N. Bernstein and W. D. Leslie, “The pathophysiology of bone disease in gastrointestinal disease,” European Journal of Gastroenterology & Hepatology, vol. 15, no. 8, pp. 857–864, 2003.

[20] H. Tilg, A. R. Moschen, A. Kaser, A. Pines, and I. Dotan, “Gut, inflammation and osteoporosis: basic and clinical concepts,” Gut, vol. 57, no. 5, pp. 684–694, 2008.

[21] W. C. Dougall and M. Chaisson, “The RANKL/RANKL/OPG triad in cancer-induced bone diseases,” Cancer Metastasis Reviews, vol. 25, no. 4, pp. 541–549, 2006.

[22] L. Rodriguez-Bores, J. Barahona-Garrido, and J. K. Yamamoto-Furusho, “Basic and clinical aspects of osteoporosis in inflammatory bowel disease,” World Journal of Gastroenterology, vol. 13, no. 46, pp. 6156–6165, 2007.

[23] C. N. Bernstein, M. Sargent, and W. D. Leslie, “Serum osteoprotegerin is increased in Crohn’s disease: a population-based case control study,” Inflammatory Bowel Diseases, vol. 11, no. 4, pp. 325–330, 2005.
factor kappaB-ligand/osteoprotegerin associated with bone deterioration in patients with Crohn’s disease,” European Journal of Gastroenterology & Hepatology, vol. 21, no. 2, pp. 159–166, 2009.

[25] C. M. Schulte, “Review article: bone disease in inflammatory bowel disease,” Alimentary Pharmacology & Therapeutics, vol. 20, Supplement 4, pp. 43–49, 2004.

[26] Y. Y. Kong, H. Yoshida, I. Sarosi et al., “OPGL is a key regulator of osteclastogenesis, lymphocyte development and lymph-node organogenesis,” Nature, vol. 397, no. 6717, pp. 315–323, 1999.

[27] L. Nahidi, S. T. Leach, M. A. Sidler, A. Levin, D. A. Lemberg, and A. S. Day, “Osteoprotegerin in pediatric Crohn’s disease and the effects of exclusive enteral nutrition,” Inflammatory Bowel Diseases, vol. 17, no. 2, pp. 516–523, 2011.

[28] T. Ueland, A. Yndestad, E. Oie et al., “Dysregulated osteoprotegerin/RANK ligand/RANK axis in clinical and experimental heart failure,” Circulation, vol. 111, no. 19, pp. 2461–2468, 2005.

[29] S. T. Leach, L. Nahidi, S. Tilakaratne, A. S. Day, and D. A. Lemberg, “Development and assessment of a modified paediatric Crohn disease activity index,” Journal of Pediatric Gastroenterology & Nutrition, vol. 51, no. 2, pp. 232–236, 2010.

[30] E. Galliera, L. de Girolamo, G. Dogliotti, C. De Salvo, G. Tosetti, and L. Pastorelli, “Circulating OPG levels are reduced following infliximab treatment and correlate with CRP levels: is serum OPG a potential marker of IBD disease activity?” Inflammatory Bowel Diseases, vol. 17, no. 6, pp. E59–E60, 2011.

[31] F. A. Sylvester, D. Turner, A. Draghi 2nd et al., “Fecal osteoprotegerin may guide the introduction of second-line therapy in hospitalized children with ulcerative colitis,” Inflammatory Bowel Diseases, vol. 17, no. 8, pp. 1726–1730, 2011.

[32] A. Skinner, T. Lerer, N. Wyzga, A. Viswanathan, and F. Sylvester, “51225 fecal osteoprotegerin: a marker for pediatric ulcerative colitis at diagnosis - a pilot study,” Gastroenterology, vol. 134, 4, Supplement 1, p. A-205, 2008.

[33] C. G. B. Peterson, E. Eklund, Y. Taha, Y. Raab, and M. Carlson, “A new method for the quantification of neutrophil and eosinophil cationic proteins in feces: establishment of normal levels and clinical application in patients with inflammatory bowel disease,” The American Journal of Gastroenterology, vol. 97, no. 7, pp. 1755–1762, 2002.

[34] W. M. Nauseef and H. L. Malech, “Analysis of the peptide subunits of human neutrophil myeloperoxidase,” Blood, vol. 67, no. 5, pp. 1504–1507, 1986.

[35] I. Masoodi, R. Kochhar, U. Dutta et al., “Fecal lactoferrin, myeloperoxidase and serum C-reactive are effective biomarkers in the assessment of disease activity and severity in patients with idiopathic ulcerative colitis,” Journal of Gastroenterology and Hepatology, vol. 24, no. 11, pp. 1768–1774, 2009.

[36] I. Masoodi, R. Kochhar, U. Dutta et al., “Evaluation of fecal myeloperoxidase as a biomarker of disease activity and severity in ulcerative colitis,” Digestive Diseases and Sciences, vol. 57, no. 5, pp. 1336–1340, 2012.

[37] P. Sangfelt, M. Carlson, M. Thorn, L. Loof, and Y. Raab, “Neutrophil and eosinophil granule proteins as markers of response to local prednisolone treatment in distal ulcerative colitis and proctitis,” The American Journal of Gastroenterology, vol. 96, no. 4, pp. 1085–1090, 2001.

[38] T. Saiki, “Myeloperoxidase concentrations in the stool as a new parameter of inflammatory bowel disease,” The Kurume Medical Journal, vol. 45, no. 1, pp. 69–73, 1998.

[39] R. Vitali, L. Stronati, A. Negroni et al., “Fecal HMGB1 is a novel marker of intestinal mucosal inflammation in pediatric inflammatory bowel disease,” The American Journal of Gastroenterology, vol. 106, no. 11, pp. 2029–2040, 2011.

[40] F. Palone, R. Vitali, S. Cucchiara et al., “Role of HMGB1 as a suitable biomarker of subclinical intestinal inflammation and mucosal healing in patients with inflammatory bowel disease,” Inflammatory Bowel Diseases, vol. 20, no. 8, pp. 1448–1457, 2014.

[41] A. Spichalova, I. Spichal, P. Chmelarova, and I. Trebichavsky, “Alarmin HMGB1 is released in the small intestine of gnotobiotic piglets infected with enteric pathogens and its level in plasma reflects severity of sepsis,” Journal of Clinical Immunology, vol. 31, no. 3, pp. 488–497, 2011.

[42] R. Vitali, F. Palone, S. Cucchiara et al., “Dipotassium glycyrrhizate inhibits HMGB1-dependent inflammation and ameliorates colitis in mice,” PloS One, vol. 8, no. 6, Article ID e66527, 2013.

[43] S. H. Dave, J. S. Tilstra, K. Matsuoka et al., “Ethyl pyruvate decreases HMGB1 release and ameliorates murine colitis,” Journal of Leukocyte Biology, vol. 86, no. 3, pp. 633–643, 2009.

[44] E. Mizoguchi, “Chitinase 3-like-1 exacerbates intestinal inflammation by enhancing bacterial adhesion and invasion in colonic epithelial cells,” Gastroenterology, vol. 130, no. 2, pp. 398–411, 2006.

[45] I. Vind, J. S. Johansen, P. A. Price, and P. Munkholm, “Serum YKL-40, a potential new marker of disease activity in patients with inflammatory bowel disease,” Scandinavian Journal of Gastroenterology, vol. 38, no. 6, pp. 599–605, 2003.

[46] T. Aomatsu, H. Imaeda, K. Matsumoto et al., “Facial chitinase 3-like-1: a novel biomarker of disease activity in paediatric inflammatory bowel disease,” Alimentary Pharmacology & Therapeutics, vol. 34, no. 8, pp. 941–948, 2011.

[47] C. C. Chen, J. Pekow, V. Llado et al., “Chitinase 3-like-1 expression in colonic epithelial cells as a potentially novel marker for colitis-associated neoplasia,” The American Journal of Pathology, vol. 179, no. 3, pp. 1494–1503, 2011.

[48] M. Ramasundara, S. T. Leach, D. A. Lemberg, and A. S. Day, “Defensins and inflammation: the role of defensins in inflammatory bowel disease,” Journal of Gastroenterology and Hepatology, vol. 24, no. 2, pp. 202–208, 2009.

[49] N. Kapel, N. Benahmed, A. Morali et al., “Fecal beta-defensin-2 in children with inflammatory bowel diseases,” Journal of Pediatric Gastroenterology & Nutrition, vol. 48, no. 1, pp. 117–120, 2009.

[50] J. Langhorst, A. Junge, A. Rueffer et al., “Elevated human beta-defensin-2 levels indicate an activation of the innate immune system in patients with irritable bowel syndrome,” The American Journal of Gastroenterology, vol. 104, no. 2, pp. 404–410, 2009.

[51] M. D. Baugh, G. S. Evans, A. P. Hollander et al., “Expression of matrix metalloproteases in inflammatory bowel disease,” Annals of the new York Academy of Sciences, vol. 859, no. 1, pp. 249–253, 1998.

[52] C. Medina and M. W. Radomski, “Role of matrix metalloproteinases in intestinal inflammation,” The Journal of Pharmacology and Experimental Therapeutics, vol. 318, no. 3, pp. 933–938, 2006.

[53] M. D. Baugh, M. J. Perry, A. P. Hollander et al., “Matrix metalloproteinase levels are elevated in inflammatory bowel disease,” Gastroenterology, vol. 117, no. 4, pp. 814–822, 1999.
[54] R. B. Heuschkel, T. T. MacDonald, G. Monteleone, M. Bajaj-Elliott, J. A. Smith, and S. L. Pender, "Imbalance of stromelysin-1 and TIMP-1 in the mucosal lesions of children with inflammatory bowel disease," *Gut*, vol. 47, no. 1, pp. 57–62, 2000.

[55] E. Louis, C. Ribbens, A. Godon et al., "Increased production of matrix metalloproteinase-3 and tissue inhibitor of metalloproteinase-1 by inflamed mucosa in inflammatory bowel disease," *Clinical and Experimental Immunology*, vol. 120, no. 2, pp. 241–246, 2000.

[56] M. T. Salmela, T. T. MacDonald, D. Black et al., "A..."  

[57] B. von Lampe, B. Barthel, S. E. Coupland, E. O. Riecken, and S. Rosewicz, "Differential expression of matrix metalloproteinases and their tissue inhibitors in colon mucosa of patients with inflammatory bowel disease," *Gut*, vol. 47, no. 1, pp. 63–73, 2000.

[58] Q. Gao, M. J. Meijer, F. J. Kubben et al., "Expression of matrix metalloproteinases-2 and -9 in intestinal tissue of patients with inflammatory bowel diseases," *Digestive and Liver Disease*, vol. 37, no. 8, pp. 584–592, 2005.

[59] Q. Gao, M. J. Meijer, U. G. Schluter et al., "Infliximab treatment influences the serological expression of matrix metalloproteinase (MMP)-2 and -9 in Crohn’s disease," *Inflammatory Bowel Diseases*, vol. 13, no. 6, pp. 693–702, 2007.

[60] M. A. Manfredi, D. Zurakowski, P. A. Rufo, T. R. Walker, V. L. Fox, and M. A. Moses, "Increased incidence of urinary matrix metalloproteinases as predictors of disease in pediatric patients with inflammatory bowel disease," *Inflammatory Bowel Diseases*, vol. 14, no. 8, pp. 1091–1096, 2008.

[61] P. Garg, M. Rojas, A. Ravi et al., "Selective ablation of matrix metalloproteinase-2 exacerbates experimental colitis: contrasting role of gelatinases in the pathogenesis of colitis," *Journal of Immunology*, vol. 177, no. 6, pp. 4103–4112, 2006.

[62] A. Kofla-Dłubacz, M. Matusiewicz, E. Krzesieck, L. Noga, and B. Iwanczak, "Metalloproteinase-3 and -9 as novel markers in the evaluation of ulcerative colitis activity in children," *Advances in Clinical and Experimental Medicine*, vol. 23, no. 1, pp. 103–110, 2014.

[63] A. Kofla-Dłubacz, M. Matusiewicz, M. Krzystek-Korpacka, and B. Iwanczak, "Correlation of MMP-3 and MMP-9 with Crohn’s disease activity in children," *Digestive Diseases and Sciences*, vol. 57, no. 3, pp. 706–712, 2012.

[64] A. Annahazi, T. Molnar, K. Farkas et al., "Fecal MMP-9: a new noninvasive differential diagnostic and activity marker in ulcerative colitis," *Inflammatory Bowel Diseases*, vol. 19, no. 2, pp. 316–320, 2013.

[65] K. Farkas, Z. Sarodi, A. Balint et al., "The diagnostic value of a new fecal marker, matrix metalloproteinase-9, in different types of inflammatory bowel diseases," *Journal of Crohn’s & Colitis*, vol. 9, no. 3, pp. 231–237, 2015.

[66] K. L. Kolho, T. Sipponen, E. Valtonen, and E. Savilahti, "Fecal calprotectin, MMP-9, and human beta-defensin-2 levels in pediatric inflammatory bowel disease," *International Journal of Colorectal Disease*, vol. 29, no. 1, pp. 43–50, 2014.

[67] L. Makitalo, H. Rintamaki, T. Tervahartiala, T. Sorsa, and K. L. Kolho, "Serum MMPs 7-9 and their inhibitors during glucocorticoid and anti-TNF-alpha therapy in pediatric inflammatory bowel disease," *Scandinavian Journal of Gastroenterology*, vol. 47, no. 7, pp. 785–794, 2012.

[68] T. Rath, M. Roderfeld, J. Graf et al., "Enhanced expression of MMP-7 and MMP-13 in inflammatory bowel disease: a precancerous potential?" *Inflammatory Bowel Diseases*, vol. 12, no. 11, pp. 1025–1035, 2006.

[69] M. Yoshimoto, F. Itoh, H. Yamamoto, Y. Hinoda, K. Imai, and A. Yachi, "Expression of MMP-7(PUMP-1) mRNA in human colorectal cancers," *International Journal of Cancer*, vol. 54, no. 4, pp. 614–618, 1993.

[70] K. J. Newell, L. M. Matrisian, and D. K. Driman, "Matrilysin (matrix metalloproteinase-7) expression in ulcerative colitis-related tumorigenesis," *Molecular Carcinogenesis*, vol. 34, no. 2, pp. 59–63, 2002.

[71] F. Casellas, M. Antolin, E. Varela et al., "Fecal excretion of human deoxyribonucleic acid as an index of inflammatory activity in ulcerative colitis," *Clinical Gastroenterology and Hepatology*, vol. 2, no. 8, pp. 683–689, 2004.

[72] F. Casellas, N. Borruel, M. Antolin et al., "Fecal excretion of deoxyribonucleic acid in long-term follow-up of patients with inactive ulcerative colitis," *Inflammatory Bowel Diseases*, vol. 13, no. 4, pp. 386–390, 2007.

[73] M. Coskun, J. T. Bjerrum, J. T. Troelsen, J. Olsen, and O. H. Nielsen, "miR-20b, miR-98, miR-125b-1, and let-7e as new potential diagnostic biomarkers in ulcerative colitis," *World Journal of Gastroenterology*, vol. 19, no. 27, pp. 4289–4299, 2013.

[74] G. Koukos, C. Polytarchou, J. L. Kaplan et al., "MicroRNA-124 regulates STAT3 expression and is down-regulated in colon tissues of pediatric patients with ulcerative colitis," *Gastroenterology*, vol. 145, no. 4, pp. 842–852, 2013, e2.

[75] F. Wu, S. Zhang, T. Dassopoulou et al., "Identification of microRNAs associated with ileal and colonic Crohn’s disease," *Inflammatory Bowel Diseases*, vol. 16, no. 10, pp. 1729–1738, 2010.

[76] M. Fasseu, X. Treton, C. Guichard et al., "Identification of restricted subsets of mature microRNA abnormally expressed in inactive colonic mucosa of patients with inflammatory bowel disease," *PloS One*, vol. 5, no. 10, Article ID e13160, 2010.

[77] T. Takagi, Y. Naito, K. Mizushima et al., "Increased expression of microRNA in the inflamed colonic mucosa of patients with active ulcerative colitis," *Journal of Gastroenterology and Hepatology*, vol. 25, Supplement 1, pp. S129–S133, 2010.

[78] Z. Huang, T. Shi, Q. Zhou et al., "miR-141 regulates colonic leukocytic trafficking by targeting CXCL12beta during murine colitis and human Crohn’s disease," *Gut*, vol. 63, no. 8, pp. 1247–1257, 2014.

[79] M. Iborra, F. Bernuzzi, C. Correale et al., "Identification of serum and tissue micro-RNA expression profiles in different stages of inflammatory bowel disease," *Clinical and Experimental Immunology*, vol. 173, no. 2, pp. 250–258, 2013.

[80] S. R. Whiteoak, R. Felwick, T. Sanchez-Elsner, and J. R. Fraser Cummings, "MicroRNAs in inflammatory bowel diseases: paradoxes and possibilities," *Inflammatory Bowel Diseases*, vol. 21, no. 5, pp. 1160–1165, 2015.