Luminally expressed gastrointestinal biomarkers

Luminally-expressed Gastrointestinal Biomarkers

G. Cummins[1], D.E. Yung[2], B.F. Cox[3], A. Koulaouzidis[2], M.P.Y. Desmulliez[1], S. Cochran[4]

1. *Institute of Sensors, Signals and Systems, School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh, EH14 4AS, United Kingdom*
2. *The Royal Infirmary of Edinburgh, Endoscopy Unit, Edinburgh, EH16 4SA, United Kingdom*
3. *School of Medicine, University of Dundee, Dundee, DD1 9SY, United Kingdom*
4. *Medical and Industrial Ultrasonics, School of Engineering, University of Glasgow, G12 8QQ, United Kingdom*

Corresponding author details:

Email: G.Cummins@hw.ac.uk

Journal:

Expert review of gastroenterology and hepatology

1. Abstract

Introduction

A biomarker is a measurable indicator of normal biologic processes, pathogenic processes or pharmacological responses. The identification of a useful biomarker is challenging, with several hurdles to overcome before clinical adoption. This review gives a general overview of a range of biomarkers associated with inflammatory bowel disease or colorectal cancer along the gastrointestinal tract.

Areas covered

These markers include those that are already clinically accepted, such as inflammatory markers such as faecal calprotectin, S100A12 (Calgranulin C), Fatty Acid Binding Proteins (FABP), malignancy markers such as Faecal Occult Blood, Mucins, Stool DNA, Faecal microRNA (miRNA), other markers such as Faecal Elastase, Faecal alpha-1-antitrypsin, Alpha2-macroglobulin and possible future markers such as microbiota, volatile organic compounds and pH.

Expert commentary

There are currently a few biomarkers that have been sufficiently validated for routine clinical use at present such as FC. However, many of these biomarkers continue to be limited in sensitivity and specificity for various GI diseases. Emerging biomarkers have the potential to improve diagnosis and monitoring but further study is required to determine efficacy and validate clinical utility.

2. Keywords

biomarker
gastrointestinal disease
faecal calprotectin
lactoferrin
phagocyte derived protein,
faecal occult blood testing,
mucins,
fatty acid binding proteins,
microbiome,
volatilome

3. Introduction

A biomarker is defined as an objectively measurable characteristic that is objectively measured and evaluated as an indicator of normal biologic or pathogenic processes, or pharmacological responses to a specified therapeutic intervention [1]ï [3]. Ideally it should be measured from easily obtained bodily fluids and waste such as plasma, serum, urine and faecal matter for improved patient acceptance; if not feasible then invasive techniques such as tissue biopsy are acceptable. An ideal biomarker would be sensitive to a specific condition. A useful biomarker has the potential to improve diagnosis, discriminate ill patients according to disease, reduce healthcare costs, expedite drug development and monitor therapeutic efficacy.

In this review, a general overview is given of established and potentially new biomarkers found within the intestinal lumen, such as proteins, enzymes, microbes and their metabolic products (summarised in Table I) are assessed in terms of their usefulness for the diagnosis of conditions such as colorectal cancer (CRC) and inflammatory bowel disease (IBD). Combined with advances in point of care diagnostics and minimally invasive therapies, these biomarkers may be expected to improved patient outcomes in the future.

4. Luminally expressed gastrointestinal biomarkers

4.1. Markers of inflammation

4.1.1. Phagocyte-derived proteins as markers of inflammation

Markers derived from phagocytes are useful as indicators of inflammation, with those expressed only by phagocytes being more specific. Markers which are also inducible in epithelial cells are more sensitive, but their levels can be raised by other non-inflammatory but still physiologically stressful conditions, such as lactose intolerance. Neutrophil activation markers have been found to be involved in the pathogenesis of inflammatory bowel disease (IBD), therefore their levels often show good correlation with disease activity. However, cytokines such as the interleukins and tumour necrosis factor-alpha (TNFŬ) tend to be short-lived and are unstable molecules, and methods for their detection are unreliable [4], [5].

Lactoferrin, polymorphonuclear (PMN)-elastase, myeloperoxidase (MPO) and human neutrophil lipocalin are markers of neutrophil degranulation, which can be detected in stool, and have therefore been proposed as markers of gastrointestinal (GI) tract inflammation. Lactoferrin, an iron binding glycoprotein, is considered the most accurate of these, but is also found in epithelial cells and consequently has limited specificity. In a large meta-analysis [6], lactoferrin was found to have a sensitivity of 82% and specificity of 79% for IBD, compared to a sensitivity of 49% and specificity of 92% seen in C-reactive protein (CRP). How lactoferrin is involved in inflammatory pathways is not well-established [7]ï [9]. PMN-elastase has been used to determine the severity of acute pancreatitis, with the advantage of being elevated earlier than conventional markers such as CRP. Markers of eosinophil degranulation, mainly eosinophilic cationic protein and eosinophilic protein X, have been found to have some use in intestinal hypersensitivity and eosinophilic inflammation [4].

A major drawback of using phagocyte-derived proteins as markers of inflammation is their lack of specificity to the GI tract, as they are expressed during any inflammatory process. For example, neutrophil MPO has found use in assessing other inflammatory conditions including lung inflammation in smokers [10], [11].

4.1.2. Faecal Calprotectin

The S100 proteins are a family of phagocyte-specific damage-associated molecular pattern molecules (DAMPs). They encompass a family of over 20 known calcium-binding proteins with tissue-specific expression patterns. S100A8, S100A9 and S100A12 are specifically linked to innate immune functions by their expression in phagocytes [4].

The S100A8/S100A9 complex is better known as faecal calprotectin (FC). FC is a calcium and zinc binding heterodimer protein with a regulatory role in the inflammatory process, and is released during cell activation and cell death. It constitutes about 60% of soluble proteins in human neutrophilic cytosol and is also found in monocytes, macrophages and ileal tissue eosinophils. It is excreted into the gut lumen as a response to inflammatory stimuli and is therefore six times more concentrated in faeces than in plasma and other bodily fluids [12].

FC is currently in wide clinical use as a marker of IBD activity, both in screening patients with suspected IBD and monitoring disease activity in those with an established diagnosis. In daily clinical practice, FC has proven useful due to the ease of collection of stool samples; the complex is stable at room temperature, resistant to degradation and homogeneously distributed in stool [8], [9], [12]ï [14]. The current literature supports the use of FC as a screening and monitoring biomarker for both main forms of IBD ï ulcerative colitis (UC), characterised by mucosal colonic inflammation, and Crohnôs disease (CD), which can affect all parts of the gastrointestinal tract with transmural inflammation. The cut-off point for ñnormalò FC is usually taken to be 50 µg/g of stool, as specified by the test manufacturers; however, cut-off values vary widely depending on clinical situation and even these values are still a matter of debate and study. Conventionally, cut-off values of 50-100 µg/g have been reported in the literature, depending on individually calculated receiver operative curves in different studies. The management of patients with ñborderlineò FC levels (50-150 µg/g) remains unclear as many such patients are not eventually diagnosed with IBD [15]. A summary of the

various reported FC levels corresponding to different clinical scenarios in IBD is given in Table II.

The use of FC as a screening test in suspected IBD has been shown to reduce the number of endoscopies with negative results ï in a meta-analysis by van Rheenen *et al* [6], the pooled sensitivity of FC for IBD in adults was 93%, with pooled specificity 96%, and the authors estimated that screening with FC would reduce the number of endoscopies required in these adults by 67%. Another meta-analysis [16] found that using a cut-off of FC of 50 µg/g gave lower sensitivity and specificity (89% and 81%, respectively) compared to FC of 100 µg/g (sensitivity 98%, specificity 91%). Gisbert and McNicholl [17] reported that FC had a higher diagnostic accuracy for CD (sensitivity 83%, specificity 85%) than UC (sensitivity 72%, specificity 74%). Interestingly, these studies and other subsequent ones [18] have found a lower specificity of FC for IBD in children of about 70% although sensitivities were comparable.

FC is also useful in monitoring disease activity in patients with established IBD. Studies have shown that FC correlates well with endoscopic disease activity measured using a number of different indices including the Rachmilewitz index [19], the modified Baron index in UC [20], and Crohnôs Disease Index of Severity (CDIS) [21], [22]. The normalisation of FC has good correlation with mucosal healing which is an important prognostic indicator in IBD [23]ï [27]. By now, several studies have demonstrated that patients with both UC and CD who were in remission following medical treatment also had normalisation of FC levels to below 50 µg/g, whereas non-responders continued to have elevated FC [28]. Conversely, rising FC concentrations in patients with clinically quiescent disease have been found to predict clinical relapse with up to 90% sensitivity and 82% specificity. A large meta-analysis by Mosli *et al* in 2015 [6] showed overall sensitivity of 88% and specificity 73% for endoscopically active IBD. Sensitivities were similar between UC (88%) and CD (87%), but the specificity of FC was lower in CD (67%) than UC (79%); this is in line with previous studies [12], [29]. Furthermore, mildly elevated levels of FC in unaffected relations of individuals with IBD show its sensitivity to even subclinical GI tract inflammation [30], [31].

In general, the optimal levels or cut-offs of FC to use vary widely across different clinical scenarios and studies. Furthermore, there has been some emerging evidence that FC levels may display intra-individual variability from day to day, or even within the same day, observed in individuals with active IBD [32], [33], quiescent IBD [34] and also normal individuals [35]. Therefore it is suggested that more attention needs to be paid to timing of sample collection and the practicalities of sample storage [32], and also that multiple or serial FC measurements [33] are obtained. Optimal cut-offs may also differ by disease (UC, CD), brand of assay used, distribution of inflammation, and age of patient [36]. Clinical guidance from various organisations including the British Society of Gastroenterology [37] and the European Crohnôs and Colitis Organisation [38] does not contain a clear-cut definition of ñnormalò FC and recommendations seem to be guided by the trend of an individual patientôs FC levels, i.e. increasing or normalising.

Despite its ease of use and wide adoption, FC is not specific to IBD. Other causes of GI tract inflammation can result in elevated FC, including use of non-steroidal anti-inflammatory drugs (NSAIDs) [39]ï [41] and malignancy [42]ï [44]. Another limitation of FC is its inability to localise pathology [43], [44]. It is currently thought that FC is more reflective of disease activity in UC than in CD, as discussed above, and in colonic CD compared to small bowel CD [31]. However, a recent meta-analysis has shown that elevated FC in patients with normal ileocolonoscopy was associated with active small bowel inflammation [45].

4.1.3. S100A12 (Calgranulin C)

S100A12 (calgranulin C) has recently emerged and may be more specific for IBD than FC. Studies have shown that, unlike FC and lactoferrin, it is elevated in IBD but not in conditions such as gastroenteritis. This is thought to be because S100A12 is expressed only by granulocytes and acts independently of FC in calcium-dependent signalling, hence also being thought to be involved in the pathogenesis of IBD [14], [46], [47]. Elevated S100A12 >10 mg/kg

has been reported to have sensitivity of 96% and specificity of 92% for IBD in children, and sensitivity of 86% and specificity of 96% in adults [28].

4.1.4. Other markers

Potential biomarkers are constantly being discovered [48]ï [50]. Recent studies on neopterin demonstrated its ability to identify patients with IBD with active mucosal lesions and could potentially be used to assess the severity their mucosal damage [51]. It was shown that FC and neopterin concentrations correlated closer with endoscopic scores for UC ($r = 0.75$ and $r = 0.72$, respectively; $p < 0.0001$ for both) than in CD ($r = 0.53$ and $r = 0.47$, respectively; $p < 0.0001$ for both). Neopterin has a similar overall accuracy to FC in predicting endoscopic activity in IBD patients when cut-offs of 250 ε g/g for FC and 200 pmol/g for Neopterin were used.

4.2. Fatty acid binding proteins (FABP)

Fatty acid binding proteins (FABPs) are low molecular weight cytosolic proteins found in tissues involved in the uptake and consumption of fatty acids. Three forms have been found to be expressed by enterocytes: intestinal FABP (I-FABP), liver FABP (L-FABP) and ileal-bile acid binding protein (I-BABP). As they are found in the tips of villi, the presence of FABPs in the circulation or urine has been considered as a marker of early enterocyte damage [52].

I-FABP levels have been shown to increase with acute intestinal ischaemia and inflammation, implying correlation with ischaemia-reperfusion injury [52]. For example, a rise in urinary I-FABP following cardiopulmonary bypass was found to be associated with increased risk of postoperative complications [53]. A significant inverse association has also been demonstrated between I-FABP and I-BABP and mean arterial pressure, thought to be due to intestinal mucosal cell injury secondary to systemic hypotension and consequent hypo-perfusion [52]. Elevated plasma I-FABP is also associated with poorer outcomes in patients with abdominal sepsis [54].

The usefulness of these FABPs as GI biomarkers is due to their specificity for gut pathology and early elevation following enterocyte damage, allowing a rapid response in time-critical conditions such as sepsis. Their excretion in urine is also helpful, especially in the paediatric setting.

4.3. Markers of malignancy

4.3.1. Faecal Occult Blood Testing

Faecal occult blood testing (FOBT) detects traces of blood in stool. The earliest form of FOBT, guaiac (gFOBT), is nonspecific for human blood as it is based on the detection of pseudoperoxidase activity [55], [56]. Consequently it has relatively low sensitivity and specificity; for example, false positives could be obtained due to recent consumption of red meat [57]. Therefore, faecal immunochemical tests (FITs), which specifically and quantitatively detect human blood through antibody reaction with human globin, are now in wider use. FITs are also thought to be more specific for distal GI blood loss such as in CRC. Another form of FIT is haemoglobin-haptoglobin (Hb/Hpt) complex testing. Haptoglobin forms a stable soluble complex with haemoglobin which is detected via an immunoradiometric assay [58]. A large systematic review on the various available forms of FOBT found a wide range of reported sensitivities, from 6.2-83.3% for gFOBT and 5.4-62.6% for FITs [59] . Specificity performed better, from 65.0-99.0% for gFOBT and 89.4-98.5% for FITs.

FOBT is widely used worldwide to screen for adenomas and CRC. The use of FOBT has been shown to increase early detection of CRC and improve survival [57], [60], [61]. A Cochrane review in 2008 [61] concluded that FOBT reduced the relative risk of mortality from CRC by 16%, with risk reduction increased to 25% when results were adjusted for screening attendance. A more recent Cochrane review [62] compared FOBT to flexible sigmoidoscopy for the detection of CRC; both modes of screening reduced the relative risk of death from CRC (0.72 in flexible sigmoidoscopy vs 0.86 with FOBT). Although flexible sigmoidoscopy

understandably performed better, FOBT offers advantages as it is cheap and easily implemented and therefore suitable for screening large numbers of people efficiently.

There is little published data on the value of FOBT in predicting small bowel pathology, although it is thought to be less useful [63]. 40-60% of subjects with positive FOBT have no lesions found in the colon or rectum with colonoscopy, raising the possibility of small bowel bleeding [63], [64].

4.3.2. Mucins: MUC1, MUC2, MUC5AC, small-intestinal mucin antigen, CDX2, villin

Mucins are high molecular weight glycoproteins expressed in various types of epithelium. Specific mucins such as MUC1, MUC2 and MUC5AC have been used as markers for small bowel and CRC, as the development and progression of neoplasia are associated with aberrant mucin expression [65]ï [68]. Similarly, small-intestinal mucin antigen (SIMA) is an oncofetal glycoprotein present only in normal small bowel in adults; its abnormal expression is associated with adenocarcinomas of the GI tract [65], [69]. Therefore, such markers can be useful in distinguishing the types of detected neoplasia in patients and help to guide further management.

Villin is an actin-binding protein involved in brush border maintenance that is specific for epithelial neoplasms in the GI tract. It has proven useful in distinguishing between GI tract cancer and ovarian, bladder and prostate cancers. Another sensitive marker for GI adenomas and especially CRC is CDX2, a homeobox nuclear transcription factor [65]. However there remains a dearth of information about the accuracy parameters and therefore utility of these markers and they are currently not in routine use.

4.4. Faecal or Stool DNA (sDNA)

During the evolution of normal colonocytes into CRC, an accumulation of genetic mutations and epigenetic alterations occurs within aberrant colonocytes [70]. These cells are continuously exfoliated into the lumen and passed in faeces [71].

Genetic variations are represented by various mutations [72] such as KRAS and APC. Additional changes are represented by epigenetic aberrant hyper- or hypo-methylation [73]. Aside from their role in the genesis and propagation of CRC, these modified molecules have a demonstrated role as biomarkers. This is due in part to their presence in plasma/serum [74], urine [75] and stool [76]. Furthermore it has been noted that the mutated genetic material remains stable in the stool [77] and can be further stabilised *ex vivo* using buffers [78]. Amplification and means of detection employ variations of polymerase chain reaction (PCR) [79], [80].

As a screening modality, sDNA has demonstrated variable sensitivity and specificity with performance linked to the markers analysed and in what combination they are analysed. A meta-analysis by Zhai et al [81], including 53 studies, calculated a pooled sensitivity of 48% and specificity of 97% for single gene testing. Multigene testing experienced an improved pooled sensitivity of 77.8% with a slightly lower specificity of 92.7%. The authors speculate that the improved multigene sensitivity is due to evaluating sDNA more broadly while simultaneously maintaining a precise mutation search. Compared to FIT, multigene sDNA demonstrated superior sensitivity but lower specificity when performed in an asymptomatic, average risk individual [82]. In a large cross-sectional study (n = 9,989), Imperiale and colleagues compared FIT against multitarget sDNA (MT-sDNA) for aberrant KRAS, NDRG4, BMP3 and ɓ-actin for DNA quantity reference. Post stool analysis, participants underwent colonoscopy as the reference standard. Sensitivity for CRC detection was 92.3% for MT-sDNA and 73.8% for FIT ($p = 0.002$). Detection of advanced precancerous lesions measuring $\geq$ 1 cm, including advanced adenomas and sessile serrated polyps, resulted in sensitivities of 42.4% and 23.8% for MT-sDNA and FIT, respectively ($p < 0.001$). Specificity of sDNA was 86.6% and 94.9% for FIT ($p < 0.001$). Approval by the FDA of MT-sDNA testing was granted in August, 2014 [83].

In addition to its utility in screening for CRC, sDNA may have a potential role in prognosis and as a predictive biomarker [84]. This opinion is echoed by Okugawa et al [17] with particular

attention directed towards CpG island methylator phenotype (CIMP) as a promising prognostic indicator. This is due to CIMP positive tumours correlating with a negative prognosis as indicated by Phipps et al [85]. Furthermore CIMP positive status was employed as a predictor of 5-FU response [86] though this has since been refuted [87]. However a meta-analysis by Juo et al [88] was inconclusive due to lack of standardisation and recommended further follow-up with a RCT.

Comparative-effectiveness studies are now needed to clarify the role of stool DNA testing with respect to programmatic screening with other test options. Only through a better understanding of other key factors, such as the screening interval, adherence, cost, and diagnostic evaluation of positive results, can we determine the appropriate place for stool DNA testing on the screening menu [89].

4.5. Faecal microRNA (miRNA)

MicroRNA (miRNA) is a short single stranded string of non-coding RNA [90]. This family is 18-25 nucleotides in length and plays a role in the regulation of oncogenes and tumour suppressors [91]. Altered miRNA expression, in the form of up or down regulation, has been linked to a number of cancers including CRC [92], as well as to IBD [93]. The small hairpin structure of miRNA renders it resistant to RNAse. It thus demonstrates a high degree of stability in a number of media including blood and faeces [94], [95] and can be recovered from fixed and frozen tissue [96]. Detection and quantitation is by PCR and microarray analysis with other means being investigated [97].

Several groups have run pilot studies aimed at investigating miRNA profiles and biomarker potentials in human stool samples [95], [98], [99]. Wu at al compared miRNA-21 and -92a levels in pre- and post-surgery CRC patients, individuals with polyps and healthy controls [100]. It was first noted that stool based miRNA-92a detection was robust and reproducible over a 72 hour time span. Significantly higher miRNA levels were noted in CRC patients than in healthy controls ($p < 1.01$ and $p < 0.0001$ for miRNA-21 and -92a respectively). Furthermore, miRNA-92a was significantly elevated in the patients with polyps group versus

the healthy controls ($p < 0.0001$). Interestingly, tumour removal resulted in a reduction of both miRNA-21 and -92a ($p < 0.01$) and a decrease in miRNA-92a for advanced adenoma excision ($p < 0.05$). Further investigation compared miRNA-135b amongst four different groups, which included patients with CRC, adenomas, IBD and healthy controls [100]. The use of an IBD group was to restrict markers to individuals with neoplastic disease. From an array analysis, miRNA-31 and 135b were identified as the most unregulated. MiRNA-135b was found to be significantly higher in patients with CRC ($p < 0.0001$) and adenomas ($p < 0.0001$) than in the IBD and control groups. Furthermore, an increasing trend was noted along the adenoma to carcinoma spectrum ($p < 0.0001$). Additional results showed that miRNA was indifferent to proximal or distal location of the lesion and target stool levels dropped post excision. The sensitivity and specificity of miRNA-135b for CRC were 78% and 68%, respectively. Sensitivity for adenomas was 65% and further improved to 73% for advanced adenomas.

Additional evaluation of miRNA and comparison to FIT was done by Koga et al [98]. Tests were carried out on confirmed CRC patients and healthy controls. Sensitivity and specificity for miRNA-106a alone were 34.2% and 97.2% respectively versus significant ($p = 0.001$) FIT results of 60.7% and 98.1%, respectively. However when combined, sensitivity was elevated to 70.9% with a slight drop in specificity to 96.3%. Further investigation by Ahmed et al discovered the over and under-expression of a number of miRNA targets [99]. These results illustrate the feasibility of miRNA as a non-invasive biomarker for colon-related neoplasia screening, due to the feasibility of collecting stool as well as the change in miRNA expression levels along the adenoma-carcinoma spectrum, correlating with TNM staging

4.6. Markers of other pathology

4.6.1. Faecal Elastase

Faecal elastase-1 is an enzyme specific to the human pancreas. It is currently used as a test for exocrine pancreatic insufficiency as it is not degraded during intestinal transport and is therefore testable in a stool sample [101], [102]. Its faecal concentration has been found to correlate with levels of pancreatic enzyme secretion but sensitivity and specificity have been

shown to vary. Faecal elastase performs better in moderate to severe pancreatic insufficiency but has been shown to be superior to other tests of pancreatic function [102]ï [104].

4.6.2. Faecal alpha-1-antitrypsin

Faecal alpha-1-antitrypsin is a marker of GI plasma loss or protein losing enteropathy, with the advantage that it is less invasive than the use of radiolabelled macromolecules. Furthermore, it shows good correlation with protein loss and is a relatively inexpensive test [5]. It was previously used as a marker of CD but its use has declined with the introduction of FC and other more specific markers [105], [106].

4.6.3. Alpha2-macroglobulin

Alpha2-macroglobulin is a protease which has been used as a marker for protein losing enteropathy and pancreatitis. It has been suggested that increased protease activity and consumption in certain inflammatory conditions such as acute pancreatitis, sepsis and IBD could cause its levels to fall [5]. However, it is highly non-specific and there is little conclusive evidence to support its routine use.

4.7. Microbiota

There are approximately 100 x 10^{12} microorganisms within the GI tract, an order of magnitude greater than the number of somatic cells within the body. These microorganisms primarily reside within the large intestine and include bacteria, yeasts, single eukaryotes and more. This section will primarily discuss the role of bacteria on GI health. Several factors aid bacterial growth within the large intestine, such as the slow transit time of the colon, neutral pH and low bile salt concentration [107]. Bacteria are located within two sections of the large intestine [107]ï [109]. As the colon is devoid of oxygen, most of this bacterial population is anaerobic. The bacteria within the large intestine provide several useful functions such as protection of epithelial cells, aid the immune system, stimulation of intestinal angiogenesis, and fermentation of nondigestible dietary fibre, cellulose, resistant starches, gums and pectins, leading to the production of short-chain fatty acids (SCFA) which are an energy source for colonocytes [89],[90].

Whilst reflective of the community in the colonic lumen, the microbiome in the luminal section may not reflect the composition of the epithelial and cryptal community. The microbiome in the mucosa is a dense community of bacteria that adheres to the surface. That can withstand the hydrodynamic shear forces present [111]. Adherent resident bacteria may play a role in the development of IBD and CRC.

The Human Microbiome Project and other research show that there is a high degree of variation in the bacterial populations between individuals [2] [112] and studies have shown that factors such as age, diet, ethnicity and environment may contribute to this variation. Despite the dissimilarities, many of these bacteria that carry out common metabolic activities are similar between different individuals [113]. Commonly found genera of bacteria within the adult large intestine include *Bifidobacterium, Eubacterium, Lactobacillus, Bacteroides, Clostridium, Escherichia, Streptococcus and Ruminococcus. Bacteroides i*s one of the most abundant within the GI tract, with *Eubacterium, Bifidobacterium, Peptostreptococcus, and Ruminococcus* also widespread [107],[114].

An increasing number of studies have demonstrated that there is a relationship between changes in the gut microbial population and the development of diseases such as IBD [96]ï [101] and CRC [107], [110], [111], [122]ï [137]. A summary of some of these studies is shown in Table III. Many of the trials shown in this table use a relatively small number of samples, which limits the wider applicability of the results. Additionally, several studies have identified changes in different bacterial populations to be linked to the same GI disease. One contributing factor may be that many of the studies shown in Table III were conducted using stool samples as a proxy for mucosal samples due to ease of collection. However it is unknown to what extent the faecal and mucosal microbiomes differ, which may have influenced the results of these studies [138]. Besides the use of faecal biota as opposed to mucosal biota, other factors that may limit the widespread applicability of these studies may include lack of information regarding microbiota patchiness, and heterogeneity between different anatomical

niches along the colon, difference due to ages and genders, and other factors, limit the usefulness and [2].

It is unclear, whether the changes in gut microbiota contribute to disease pathogenesis or whether they occur due to local inflammation [139]. The bacterial driver-passenger model proposed by Tjalsma could explain the influence of microbiota on the development of GI disease [140]. This model suggests that certain populations of driver bacteria with pro-carcinogenic features damage intestinal epithelium DNA, leading to tumorigenesis, which in turn alters the intestinal environment leading to a decline in homeostasis due to overgrowth of opportunistic passenger bacteria.

Study of the gut microbiome is still in its infancy and the lack of a holistic understanding of this complex environment and its role in gut pathology presents a major barrier to the identification of bacteria or combinations of bacteria as potentially reliable, specific and sensitive biomarkers for CRC and IBD. Currently, identification of the microbial constituents can be performed through use of molecular fingerprinting methods and sequence analysis of cloned microbial ribosomal DNA due to difficulties in cultivation. These methods are expensive and take time, which currently impedes the recognition of widely accepted biomarkers for clinical usage. The development of inexpensive, minimally invasive, rapid and reliable technologies for direct sampling and analysis of the microbiome will aid the design and operation of clinical trials necessary in identifying these biomarkers and utilising them to aid diagnosis in future.

4.8. Volatile Organic Compounds

Direct intra-luminal measurement of microbiota population and demographics may not be possible with current technology. However, the competition between different populations of bacteria for resources such as substrates or hydrogen can lead to deviations in the concentrations of metabolic by-products such acetic, propionic and butyric acids, CO_2, H_2, CH_4, NH_3, H_2S and volatile fatty acids [141]. These changes in metabolic activity may be reflected in changes in the constituent gases of the patients of breath. Diagnosis by breath analysis is an area of much interest as measurements are non-invasive and potentially near

real time, making it ideal for screening or point of care diagnosis. However, this is not trivial as human breath is a complex mixture of gases containing at least 3000 different compounds [142], which includes more than 800 volatile organic compounds (VOCs). Changes in the relative concentration of the VOCs may correlate with pathological or physiological changes [143].

Several studies have proposed various VOC biomarkers for CRC or IBD [144]ï [157] with the results of some of these studies shown in Table IV. However, the sample size for these studies has been small, which may be a factor in different biomarkers being identified for the same pathologies. Further study is needed amongst a larger population to identify useful, widely applicable biomarkers for the detection of GI disease.

The rapid development and expansion of capsule endoscopy functionality through increased use of microengineering has enabled integration of various sensing modalities, such as gas sensors into these diagnostic tools [158], [159]. While, the prospect of direct or indirect sampling and subsequent analysis of the VOCs produced within the GI tract for clinical diagnosis is attractive, there is a need for further large, randomised and blinded studies before reliable, sensitive and specific VOC markers can be definitively identified for the diagnosis of GI pathology in clinical applications.

4.9. pH

Changes in luminal pH along the GI tract is an established subject of interest. The pH values of a typical, healthy GI tract is generally agreed to be as shown in Table V, showing an increase from the duodenum to the terminal ileum, a decrease in the caecum and a slow rise along the colon to the rectum. The values can change based on time since time since ingestion, diet, age and other factors [160],[161]. Changing pH along the GI tract occurs for several reasons, such as the absorption of acetate, propionate, butyrate and other short chain fatty acids, fermentation, and production of alkaline metabolites [114].

It has been proposed that UC and to some extent other IBDs such as CD can cause a decrease in pH due to mucosal inflammation within the colon [158]-[160], but this is subject to debate [162]. Drawing conclusions is difficult due to the small size of the studies, variations in dietary intake, extent and severity of the colitis, and possible signal loss or drift due to the use of endoscopic capsules. Several studies have shown that CRC is not linked with any significant change in pH [163]ï [165] despite an earlier hypothesis [166] that acidification of the bowel may play a role in the aetiology of CRC.

5. Expert Commentary

Endoluminal biomarkers such as FC and lactoferrin have changed the management of GI disease, allowing detection at an earlier stage or improved diagnostic certainty. The increased understanding of GI disease pathogenesis and progression due to the emergence of *omics* in recent years has led to the identification of thousands of potential biomarkers. However, there remain significant limitations to their use. These limitations include the low sensitivity and/or specificity of tests like FOBT and current lack of comprehensive understanding of the complexities of the gut microbiome. Other barriers to clinical adoption include clinician education in the conduct and interpretation of tests, and the relevant costs. Therefore, only a few biomarkers have been sufficiently validated for routine clinical use at present. Nevertheless, emerging biomarkers have the potential to improve clinical practice, for example accurate VOC biomarkers could improve the rate of screening of CRC in the wider population due to the non-invasive and rapid nature of breath analysis, various stool biomarkers could offer non-invasive testing with quicker results than serum markers. The study of these biomarkers and development of testing methods could also further increase understanding of GI disease mechanisms and therefore improve management, patient care and outcomes.

6. Five Year View

The transfer of biomarkers from benchtop to clinical practice is not trivial. To become clinically approved, an effective biomarker must be validated using hundreds of samples, be specific -

able to correctly identify a high proportion of true negative rates; sensitive - able to correctly identify a high proportion of true positives and also be reproducible - able to achieve the same level of detection under similar conditions multiple times. Validation of biomarkers in independent cohorts from several different institutions will help this process. To date, no marker has been found with 100% sensitivity and specificity for any GI disease. However, several solutions have been proposed to help biomarkers progress from the initial preclinical exploratory stage to final control studies. These include improving the assay used for detection, effective use of biomarker combinations and identification of the subpopulations in which the biomarker is most effective for use as a stratified diagnostic tool. This will be helpful for verifying potential VOC and microbiota biomarkers that have so far been identified in small studies, prone to bias due to various factors such as diet, ethnicity and age. Over the next five years, the increasing adoption of inexpensive, point of care diagnostic tools in labs will enable quicker screening and characterisation of effective biomarkers or combinations of biomarkers for various GI diseases, thereby speeding up the route to clinical adoption.

7. Key Issues

- There are currently well-established biomarkers such as FC. However, biomarkers continue to be limited in sensitivity and specificity for various GI diseases.
- Several biomarkers show potential but further study is needed to determine their efficacy, validate their use and overcome barriers to clinical adoption.
- New and emerging GI biomarkers could improve disease diagnosis and monitoring through better sensitivity, specificity and/or ease and speed of measurement.
- A greater understanding of the role of the microbiome in GI disease is important for future biomarker identification and more effective use of existing but not yet well-established biomarkers.
- The ideal biomarker is easy to obtain from patients (e.g. stool, urine or breath samples as opposed to serum) and has an inexpensive test, which is easy to conduct and interpret, with excellent sensitivity and specificity.

Table I: Summary of biomarkers

Type	Biomarker	Possible Cause	Advantages	Disadvantages	References
Inflammation markers	Phagocyte derived protein	Inflammatory bowel disease	Good sensitivity to IBD.	Lack of specificity to inflammation of the GI tract	[5], [8]ï [10], [167], [168][6]
	Faecal Calprotectin	Inflammation	Well established, relatively inexpensive	Moderate specificity for IBD	[8], [11]ï [15],[17]ï [29][6] [15] [16] [17] [18] [19], [20], [21], [22]. [23]ï [27].[28]
	S100A12 (Calgranulin C)	Inflammatory bowel disease	Initial studies show greater specificity than faecal calprotectin for IBDA	Larger studies required	[14], [46], [47]
	Fatty acid binding proteins	Inflammation associated with early stage enterocyte damage	Good specificity for gut pathology Useful for time critical conditions such as sepsis due to early elevation following enterocyte damage Excretion of urine useful for sample collection	Larger studies required	[52]ï [54]
	Faecal Occult Blood Testing	Distal GI blood loss	Popular and inexpensive method of screening	Low sensitivity and specificity	[33]ï [37], [38], [39], [58] [59] [61] [62]

Malignancy markers				Little published work on its value as a predictive marker	
	Mucins	Small bowel and colorectal adenocarcinomas		Lack of comprehensive information regarding accuracy and utility of mucins as a biomarker	[65]ï [69]
Faecal DNA	KRAS	Colorectal cancer	Initial studies show moderate sensitivity and good specificity. Trend in reduction in cost of analytical equipment.	Larger studies required.	[45]ï [55], [56], [57], [59]ï [63]
	APC				
	CIMP				
Faecal microRNA	miRNA-21	Colorectal cancer	Initial studies show good sensitivity and specificity Trend towards reduction in cost of analytical equipment	Larger studies required	[90]ï [99] [100].
	miRNA-92a				
	miRNA-106a				
	miRNA-135b				
Enzymes	Faecal Elastase-1	Pancreatic function	Good correlation with pancreatic enzyme secretion Demonstrated superior performance to other tests of pancreatic function.	Varied sensitivity and specificity. Limited to moderate and severe pancreatic insufficiency	[101]ï [104]
	Faecal alpha-1-antitrypsin	GI plasma loss or protein losing enteropathy	Good correlation with protein loss. Relatively inexpensive to perform	Less specific than faecal calprotectin and other markers that have	[105], [106][5]

				superceded it in terms of usage	
	Alpha2-macroglobulin	Protein losing enteropathy and pancreatic, sepsis and inflammatory bowel disase	Good correlation with acute pancreatitis, sepsis and IBD	Non specific Little conclusive evidence to support routine use	[5][171]
Other	Microbiota	Colorectal cancer, inflammatory bowel disease	See Table III for further information	Knowledge of the role of microbiota on GI pathology still in its infancy Further studies required to identify reliable microbiota biomarkers See Table III for further information	[107], [110], [111], [122]ï [127], [129]ï [137], [139], [171]ï [177]
	Volatile Organic Compounds		Potential for minimally invasive detection of GI disease without need to handle stool or urine samples. See Table IV for further information	Knowledge of the volatilome still basic, requiring further studies to link changes in gas concentration to pathology. More accurate and inexpensive methods of gas detection also required. See Table IV for further information	[144]ï [157]
	pH	Inflammatory bowel disease		Further study required due to confounding factors such as age, diet and other factors.	[162]ï [166], [178]ï [180]

Table II: Summary of use of faecal calprotectin in IBD

Clinical scenario	FC levels
Diagnosis	>50 usually used to distinguish between IBD and functional disorders; however 50-150 is generally considered a grey area >100 has better specificity but sensitivity varies between studies; is considered ñstrongly positiveò
Active vs inactive IBD	>100: endoscopically active CD >250: large ulcers seen on endoscopy <250: mucosal healing on endoscopy/ endoscopic remission In general, normalisation of FC correlates with mucosal healing.
Treatment response	Rise in FC >100 predicts clinical relapse Normalisation of FC or at least <75% reduction from baseline can be used as surrogate marker for mucosal healing Normalisation of FC to below 100 may predict clinical remission
Postoperative recurrence in CD	>100-200 (depending on study) In general, a rise in FC is considered significant FC expected to normalise within 2 months after uncomplicated ileocaecal resection; persistently raised FC could reflect ongoing inflammation
Relapse	>250 measured 3 months after diagnosis: high relapse risk in UC >500: thought to distinguish high relapse risk from low relapse risk (paediatric study)

Table III: Summary of experiments demonstrating relationship between changes in microbiome and presence of disease

Disease	Bacteria	Sample	Subjects	Clinical Outcome	Reference
Crohn's Disease	*Faecalibacterium prausnitzzi, Escherichia coli*	Biopsy samples	DNA analysis of biopsy collected from 5 locations between ileum and rectum from 6 discordant monozygotic twin pairs against 4 concordant pairs	Patients with ileal Crohn's disease had a reduced population of *Faecalibacterium prausnitzzi (p<0.001)* and an abundance of *Escherichia coli (p<0.03)* compared to healthy concordant twins and those with colon localised Crohn's	[172]
Crohn's Disease	*Escherichia coli*	Biopsy samples	28 patients were studied: 13 with Crohn's disease involving the ileum, 8 with Crohn's disease restricted to the colon but with a a normal ileum versus 7 healthy patients	Ileal mucosa found to have a population of *Escherichia coli* (P<0.001), but relatively depleted in a subset of *Clostridiales* (P<0.05) and negative for *Mycobacterium avium subspecies paratuberculosis*, *Shigella* and *Listeria*. The population of Escherichia coli was found to positively correlate with severity of Crohn's disease (P<0.001).	[181]
Inflammatory Bowel Disease	*Butyricicoccus pullicaecorum*	Stool samples	51 patients with Crohn's disease, 25 with Ulcerative Colitis versus 88 healthy controls	The average number of *Butyricicoccus* bacteria was significantly (p<0.0001) lower in the stools of IBD group versus healthy controls. A significantly lower level of *Butyricicoccus* species was observed in the faecal microbiota of patients with active CD compared with CD in remission (p<0.0188)	[173]
Inflammatory Bowel Disease	*Bdellovibrio bacteriovorus*	Biopsy samples	9 patients with Crohn's, 6 patients with Ulcerative Colitis versus 8 healthy controls	Statistically higher population of B. bacteriovorus in ileum, colon, and rectum of control biopsies with respect to CD (p<0.0001). No difference was found among overall UC and control samples p=0.6760).	[171]
Ulcerative Colitis	*Faecalibacterium prausnitzii Roseburia hominis*	Stool samples	127 patients with Ulcerative Colitis (39 active, 88 in remission) versus 87 healthy controls	Real-time PCR analysis revealed a lower abundance of *Roseburia hominis* (p<0.0001) and *Faecalibacterium prausnitzii* (p<0.0001) in UC patients compared to controls	[174]
Colorectal cancer	*Roseburia Microbacterium Anoxybacilus*	Biopsy samples	8 patients (4 with colon cancer and 4 with rectal cancer). Study was performed with 16 tissue samples taken during colonoscopy, 8 taken from healthy tissue and 8 taken from cancerous tissue.	50% of Chinese CRC patients, we found a significant increase of Roseburia (p = 0.017), and a concurrent decrease of both Microbacterium (p = 0.009) and Anoxybacillus (p = 0.009) in tumor tissue	[175]
Colorectal cancer	*Fusobacteria Eubacteriaceae Clostridiales* Family XI. *Incertae sedis Staphylococcaceae Bacteroides Campylobacteraceae Porphyromonadaceae Enterococcaceae*	Stool samples	19 patients with CRC versus healthy controls	Increased population of *Fusobacteria* (p<0.01), *Eubacteriaceae* (p=0.037), *Clostridiales* Family XI. *Incertae sedis* (p = 0.004), *Staphylococcaceae* (p=0.011), *Bacteroides* (p=0.046) compared to healthy control group. Reduced population of *Campylobacteraceae* (p=0.014) and *Porphyromonadaceae* (p=0.001) families compared to healthy control group. No	[127]

				significant change in *Enterococcaceae* (p=0.062).	
Colorectal cancer	*Bacteroides/Prevotella*	Stool samples	Stool samples taken from 60 patients with confirmed colorectal cancer after colonoscopy versus healthy controls	Elevated population of *Bacteroides/Prevotella* in CRC patients compared to healthy controls (p = 0.009)	[124]
Colorectal cancer	*Bacteroides* *Roseburia* *Alistipes* *Eubacterium* *Parasutterella* *Porphyromonas* *Escherichia/Shigella* *Enterococcus* *Streptococcus* *Peptostreptococcus*	Stool samples	46 patients with CRC versus healthy controls	Elevated populations of *Bacteroides* (p=0.005), *Roseburia* (p=0.003), *Alistipes* (p=0.039), *Eubacterium* (p=0.028), *Parasutterella* (p=0.032) and reduced populations of *Porphyromonas* (p=0.02), *Escherichia/Shigella* (p<0.01), *Enterococcus* (p<0.01), *Streptococcus* (p=0.018), *Peptostreptococcus* (p<0.01) compared to healthy controls	[176]
Colorectal cancer	*Atopobium* *Clostridia* *Fusobacterium* *Porphyromonas*	Stool samples	47 patients with colorectal cancer versus healthy controls	Elevated populations of Bacteroidetes observed and a reduced population fo Firmicutes (p=0.05) and overall microbial diversity was reduced compared to healthy controls (p=0.002). Genera such as Clostridia were reduced in numbers (p=0.005), while increased numbers of Fusobacterium (p=0.004), Atipobium (p<0.001) and Porphyromonas (p=0.05) observed, when compared to healthy controls.	[136]
Colorectal cancer	*Acidaminobacter* *Phascolaractobacterium* *Citrobacter farmer* *Akkermanasia mucinphillia* *Bacteroides finegoldii* *Bacteroides intestinalis* *Bacteroides capillosis* *Prevotella copri* *Prevotella oris* *Ruminococcus abeum*	Stool samples	CRC group had decreased overall microbial community diversity (p = .02). Taxonomical analysis showed reduced relative population of *Clostridia* (p=0.005), increased *Fusobacterium* (p=0.004), *Atopobium* (p<0.001) and *Porphyromonas* (p=0.001) compared to control group.	Elevated populations of *Acidaminobacter* (p=0.0045), *Phascolarctobacterium* (p=0.00), *Citrobacter farmer* (p=0.0050), *Akkermanasia mucinphilia* (p=0.0032), compared to healthy controls. Elevated populations of *Bacteroides finegoldii* (p=0.0032), *Bacteroides intestinalis* (p=0.0063), *Prevotella copri* (p=0.00), *Bacteroides capillosis* (p=0.0057), *Prevotella oris* (p=0.001), *Ruminococcus abeum* (p=0009), amongst many others in healthy controls compared in to CRC group.	[133]

Table IV: Summary of experiments demonstrating relationship between presence of disease and specific volatile organic compounds on breath

Disease	Volatile Organic Compounds	Method of Analysis	Subjects	Clinical Outcome	Reference
Colorectal cancer	Methane	-	45 patients with colorectal cancer versus healthy controls	No significant difference between colorectal cancer and control groups	[144]
Colorectal cancer	Methane	-	59 patients with unresected colorectal cancer versus healthy controls	No significant difference between colorectal cancer and control groups	[145]
Colorectal cancer	Methane	GC	55 patients with unresected colorectal cancer versus healthy controls	No significant difference observed between patients with colorectal cancer and healthy controls	[146]
Colorectal cancer	Methane	GC	47 patients with unresected colorectal cancer, 36 patients with resected colorectal cancer, 7 with nonresectable cancer, 29 with non-malignant diseases of colon	The majority of patients (91.4%) with unresected colorectal cancer produced more methane than healthy controls ($p<0.001$) and patients with benign diseases of the colon ($p<0.001$)	[147]
Colorectal cancer	Ethanol, Acetone, Ethyl acetate, 4-methyl octane	GC-MS	65 patients with colorectal cancer, 22 with adenoma versus health controls	Increased Acetone and Ethyl actete in CRC patients ($p = 0.010$, $p = 0.005$ respectively) versus healthy controls. Reduced Ethanol and 4-methyl octane in CRC patients ($p<0.001$, $p=0.004$ repectively) versus healthy controls.	[148]
Colorectal cancer	Non-anal 4-Methyl-2-pentanone Decanal 2-Methylbutane 1,2-Pentadiene 2-Methylpentane 3-Methylpentane Methylcyclopentane Cyclohexane Methylcyclohexane 1,3-Dimethylbenzene 4-Methyloctane 1,4-Dimethylbenzene 4-methylundecane trimethyldecane	GC-MS	37 patients with colorectal cancer versus controls	Elevated concentrations detected in CRC patients compared to control	[149]

Colorectal cancer	1,10-(1-butenylidene)bis benzene 1,3-dimethyl benzene 1-iodo nonane [(1,1-dimethylethyl)thio] acetic acid 4-(4-propylcyclohexyl)-40-cyano[1,10-biphenyl]-4-yl ester benzoic acid 2-amino-5-isopropyl-8-methyl-1-azulenecarbonitrile	GC-MS	26 patients with colorectal cancer versus controls	Elevated concentrations detected in CRC patients compared to controls	[150]
Inflammatory Bowel Disease	Pentane	GC	33 inflammatory bowel disease patients	Correlation with disease activity	[151]
Inflammatory bowel disease[iii]	1-Octene, 1-Nonene, 1-Decene, z3- Methylhexane, (E)-2-Nonene, Hydrogen sulphide	SIFT-MS	62 patients with inflammatory bowel disease versus controls	Elevated readings of 1-octene, 1-decene, 3-methylhexane and reduced readings of 1-Nonene, (E)-2-Nonene, hydrogen sulphide in IBD patients compared to control. P<0.001	[152]
Inflammatory Bowel Disease	2-propanol, acrylonitrile, carbon disulfide, dimethylsulfide, ethanol, isoprene, triethylamine	SIFT-MS	24 patients with Crohnôs disease and 11 patients with Ulcerative Colitis versus healthy controls	Changes in concentrations of gases observed compared to healthy controls (p<0.001 for all gases). There was no significant difference in any VOC levels between CD and UC	[153]
Irritable Bowel Syndrome	Hydrogen Sulphide	MS	27 patients with diarrhoea predominant IBS versus healthy controls	Patients confirmed to have small bowel intestinal overgrowth (SIBO) in irritable bowel syndrome showed higher concentrations of H2S compared to those that were negative SIBO (p<0.001)	[154]
Ulcerative colitis	Ethane, Pentane	GC	17 active ulcerative colitis patients versus controls	Significantly higher compared to controls (p<0.013). Positive correlation of ethane with endoscopic score, symptom score, disease activity and chemiluminescence in rectal tissue. Pentane levels did not correlate with any of the clinical measurements	[155]
Ulcerative colitis and Crohn's disease	Ethane, Propane, Butane, Isoprene	GC	10 patients with UC and 10 patients with CD versus HC	Significant difference between elevated patients with IBD and controls were found for ethane, propane, and pentane (p ranges between <0.05 to <0.001), but no	[156]

				significance found for butane and isoprene.	
Ulcerative Colitis, Colorectal cancer, Crohn's disease	Methane	GC	20 patients with unresected colorectal cancer, 40 patients with ulcerative colitis and 40 patients with Crohn's disease versus healthy controls	Decreased methane concentration on breath of UC and CD patients versus healthy controls ($p<0.001$, $p<0.001$). Increased methane concentration on breath of CC patients versus healthy controls ($p<0.005$)	[157]

Table V: Typical pH along GI tract

Organ	pH
Stomach	2
Small Intestine	7
Caecum	6
Colon	6.5

References:

[1] T. O. Keku, S. Dulal, A. Deveaux, B. Jovov, and X. Han, ñThe gastrointestinal microbiota and colorectal cancer,ò*Am. J. Physiol. - Gastrointest. Liver Physiol.*, vol. 308, no. 5, pp. G351ï G363, Mar. 2015.

[2] P. B. Eckburg *et al.*, ñDiversity of the human intestinal microbial flora.,ò*Science*, vol. 308, no. 5728, pp. 1635ï 8, 2005.

[3] J. Atkinson A.J. *et al.*, ñBiomarkers and surrogate endpoints: Preferred definitions and conceptual framework,ò*Clinical Pharmacology and Therapeutics*, vol. 69, no. 3. pp. 89ï 95, 2001.

[4] D. Foell, H. Wittkowski, and J. Roth, ñMonitoring disease activity by stool analyses: from occult blood to molecular markers of intestinal inflammation and damage,ò*Gut*, vol. 58, no. 6, pp. 859ï 868, Jun. 2009.

[5] A. Poullis, R. Foster, T. C. Northfield, and M. A. Mendall, ñFaecal markers in the assessment of activity in inflammatory bowel disease,ò*Aliment. Pharmacol. Ther.*, vol. 16, no. 4, pp. 675ï 681, Apr. 2002.

[6] M. H. Mosli *et al.*, ñC-Reactive Protein, Fecal Calprotectin, and Stool Lactoferrin for Detection of Endoscopic Activity in Symptomatic Inflammatory Bowel Disease Patients: A Systematic Review and Meta-Analysis,ò*Am. J. Gastroenterol.*, vol. 110, no. 6, pp. 802ï 819, Jun. 2015.

[7] T. Sipponen *et al.*, ñCorrelation of faecal calprotectin and lactoferrin with an endoscopic score for Crohnôs disease and histological findings,ò*Aliment. Pharmacol. Ther.*, vol. 28, no. 10, pp. 1221ï 1229, 2008.

[8] R. Dôincà *et al.*, ñCalprotectin and lactoferrin in the assessment of intestinal inflammation and organic disease,ò*Int. J. Colorectal Dis.*, vol. 22, no. 4, pp. 429ï 437, 2007.

[9] J. Langhorst, S. Elsenbruch, J. Koelzer, A. Rueffer, A. Michalsen, and G. J. Dobos, ñNoninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: Performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices,ò*Am. J. Gastroenterol.*, vol. 103, no. 1, pp. 162ï 169, 2008.

[10] R. B. Bridges, M. C. Fu, and S. R. Rehm, ñIncreased neutrophil myeloperoxidase activity associated with cigarette smoking.,ò*Eur. J. Respir. Dis.*, vol. 67, no. 2, pp. 84ï 93, Aug. 1985.

[11] A. Ekberg-Jannson *et al.*, ñNeutrophil-associated activation markers in healthy smokers relates to a fall in DLCO and to emphysematous changes on high resolution CT,ò*Respir. Med.*, vol. 95, no. 5, pp. 363ï 373, May 2001.

[12] S. Vermeire, G. Van Assche, and P. Rutgeerts, ñLaboratory markers in IBD: useful, magic, or unnecessary toys?,ò*Gut*, vol. 55, no. 3, pp. 426ï 31, 2006.

[13] F. Costa *et al.*, ñRole of faecal calprotectin as non-invasive marker of intestinal inflammation,ò*Dig. Liver Dis.*, vol. 35, no. 9, pp. 642ï 647, 2003.

[14] R. A. Sherwood, ñFaecal markers of gastrointestinal inflammation.,ò*J. Clin. Pathol.*, vol. 65, no. 11, pp. 981ï 5, Nov. 2012.

[15] N. Waugh *et al.*, ñFaecal calprotectin testing for differentiating amongst inflammatory and non-inflammatory bowel diseases: systematic review and economic evaluation,ò*Health Technol. Assess. (Rockv).*, vol. 17, no. 55, Nov. 2013.

[16] A. C. von Roon *et al.*, ñDiagnostic Precision of Fecal Calprotectin for Inflammatory Bowel Disease and Colorectal Malignancy,ò*Am. J. Gastroenterol.*, vol. 102, no. 4, pp. 803ï 813, Apr. 2007.

[17] J. P. Gisbert and A. G. McNicholl, ñQuestions and answers on the role of faecal calprotectin as a biological marker in inflammatory bowel disease,ò*Dig. Liver Dis.*, vol. 41, no. 1, pp. 56ï 66, Jan. 2009.

[18] P. Henderson, N. H. Anderson, and D. C. Wilson, ñThe Diagnostic Accuracy of Fecal Calprotectin During the Investigation of Suspected Pediatric Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis,ò *Am. J. Gastroenterol.*, vol. 109, no. 5, pp. 637ï 645, May 2014.

[19] A. M. Schoepfer *et al.*, ñFecal Calprotectin Correlates More Closely With the Simple Endoscopic Score for Crohnôs Disease (SES-CD) than CRP, Blood Leukocytes, and the CDAI,ò *Am. J. Gastroenterol.*, vol. 105, no. 1, pp. 162ï 169, Jan. 2010.

[20] A. M. Schoepfer *et al.*, ñFecal Calprotectin More Accurately Reflects Endoscopic Activity of Ulcerative Colitis than the Lichtiger Index, C-reactive Protein, Platelets, Hemoglobin, and Blood Leukocytes,ò *Inflamm. Bowel Dis.*, vol. 19, no. 2, pp. 332ï 341, Feb. 2013.

[21] T. Lobatón, A. López-García, F. Rodríguez-Moranta, A. Ruiz, L. Rodríguez, and J. Guardiola, ñA new rapid test for fecal calprotectin predicts endoscopic remission and postoperative recurrence in Crohnôs disease,ò *J. Crohn's Colitis*, vol. 7, no. 12, pp. e641ï e651, Dec. 2013.

[22] T. Sipponen, E. Savilahti, K.-L. Kolho, H. Nuutinen, U. Turunen, and M. Färkkilä, ñCrohnõs disease activity assessed by fecal calprotectin and lactoferrin: Correlation with Crohnõs disease activity index and endoscopic findings,ò *Inflamm. Bowel Dis.*, vol. 14, no. 1, pp. 40ï 46, Jan. 2008.

[23] A. G. Røseth, E. Aadland, J. Jahnsen, and N. Raknerud, ñAssessment of Disease Activity in Ulcerative Colitis by Faecal Calprotectin, a Novel Granulocyte Marker Protein,ò *Digestion*, vol. 58, no. 2, pp. 176ï 180, Feb. 1997.

[24] A. G. Røseth, E. Aadland, and K. Grzyb, ñNormalization of faecal calprotectin: a predictor of mucosal healing in patients with inflammatory bowel disease,ò *Scand. J. Gastroenterol.*, vol. 39, no. 10, pp. 1017ï 1020, Jan. 2004.

[25] T. Sipponen, C.-G. A. Björkesten, M. Färkkilä, H. Nuutinen, E. Savilahti, and K.-L. Kolho, ñFaecal calprotectin and lactoferrin are reliable surrogate markers of endoscopic response during Crohnôs disease treatment,ò*Scand. J. Gastroenterol.*, vol. 45, no. 3, pp. 325ï 331, Mar. 2010.

[26] P. F. van Rheenen, E. Van de Vijver, and V. Fidler, ñFaecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis,ò*BMJ*, vol. 341, p. c3369, 2010.

[27] A. K. Jonscher, ñDielectric relaxation in solids,ò*J. Phys. D. Appl. Phys.*, vol. 32, no. 14, pp. R57ï R70, 1999.

[28] J. D. Lewis, ñThe utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease,ò*Gastroenterology*, vol. 140, no. 6, pp. 1817ï 1826, 2011.

[29] R. Mao *et al.*, ñFecal calprotectin in predicting relapse of inflammatory bowel diseases: A meta-analysis of prospective studies,ò*Inflamm. Bowel Dis.*, vol. 18, no. 10, pp. 1894ï 1899, Oct. 2012.

[30] B. Thjodleifsson *et al.*, ñSubclinical intestinal inflammation: an inherited abnormality in Crohnôs disease relatives?,ò*Gastroenterology*, vol. 124, no. 7, pp. 1728ï 1737, 2003.

[31] J. P. Gisbert and a. G. McNicholl, ñQuestions and answers on the role of faecal calprotectin as a biological marker in inflammatory bowel disease,ò*Dig. Liver Dis.*, vol. 41, no. 1, pp. 56ï 66, 2009.

[32] A. Lasson *et al.*, ñThe intra-individual variability of faecal calprotectin: A prospective study in patients with active ulcerative colitis,ò*J. Crohn's Colitis*, vol. 9, no. 1, pp. 26ï 32, 2014.

[33] M. Calafat, E. Cabré, M. Mañosa, T. Lobatón, L. Marín, and E. Domènech, ñHigh within-day variability of fecal calprotectin levels in patients with active ulcerative colitis: what is the best timing for stool sampling?,ò*Inflamm. Bowel Dis.*, vol. 21, no. 5, pp.

1072ï 6, 2015.

[34] G. D. Naismith *et al.*, ñA prospective single-centre evaluation of the intra-individual variability of faecal calprotectin in quiescent Crohnôs disease,ò*Alimentary Pharmacology and Therapeutics*, vol. 37, no. 6. pp. 613ï 621, 2013.

[35] E. Husebye, H. Tøn, and B. Johne, ñBiological variability of fecal calprotectin in patients referred for colonoscopy without colonic inflammation or neoplasm,ò*Am. J. Gastroenterol.*, vol. 96, no. 9 SUPPL., pp. 2683ï 2687, 2001.

[36] D. J. Robertson and T. F. Imperiale, ñStool Testing for Colorectal Cancer Screening,ò *Gastroenterology*, vol. 149, no. 5, pp. 1286ï 1293, Oct. 2015.

[37] C. Mowat *et al.*, ñGuidelines for the management of inflammatory bowel disease in adults.,ò*Gut*, vol. 60, no. 5, pp. 571ï 607, 2011.

[38] European Crohnôs and Colitis Organisation, ñPublished ECCO Guidelines,ò2017. .

[39] J. A. Tibble *et al.*, ñHigh prevalence of NSAID enteropathy as shown by a simple faecal test.,ò*Gut*, vol. 45, no. 3, pp. 362ï 6, Sep. 1999.

[40] T. R. Meling, L. Aabakken, A. Røseth, and M. Osnes, ñFaecal Calprotectin Shedding after Short-Term Treatment with Non-Steroidal Anti-Inflammatory Drugs,ò*Scand. J. Gastroenterol.*, vol. 31, no. 4, pp. 339ï 344, Jan. 1996.

[41] Z. Rendek *et al.*, ñEffect of oral diclofenac intake on faecal calprotectin,ò*Scand. J. Gastroenterol.*, vol. 51, no. 1, pp. 28ï 32, Jan. 2016.

[42] J. B. Johne, O. Kronborg, H. I. Tøn, ñA New Fecal Calprotectin Test for Colorectal Neoplasia: Clinical Results and Comparison with Previous Method,ò*Scand. J. Gastroenterol.*, vol. 36, no. 3, pp. 291ï 296, Jan. 2001.

[43] A. Damms and S. C. Bischoff, ñValidation and clinical significance of a new calprotectin rapid test for the diagnosis of gastrointestinal diseases,ò*Int. J. Colorectal*

Dis., vol. 23, no. 10, pp. 985ï 992, Oct. 2008.

[44] C. B. Summerton, M. G. Longlands, K. Wiener, and D. R. Shreeve, ñFaecal calprotectin: a marker of inflammation throughout the intestinal tract.,ò*Eur. J. Gastroenterol. Hepatol.*, vol. 14, no. 8, pp. 841ï 5, 2002.

[45] U. Kopylov *et al.*, ñFecal calprotectin for the prediction of small-bowel Crohnôs disease by capsule endoscopy,ò*Eur. J. Gastroenterol. Hepatol.*, p. 1, Jul. 2016.

[46] D. Foell *et al.*, ñNeutrophil derived human S100A12 (EN-RAGE) is strongly expressed during chronic active inflammatory bowel disease.,ò*Gut*, vol. 52, no. 6, pp. 847ï 53, 2003.

[47] T. Kaiser *et al.*, ñFaecal S100A12 as a non-invasive marker distinguishing inflammatory bowel disease from irritable bowel syndrome,ò*Gut*, vol. 56, no. 1458ï 3288 (Electronic), pp. 1706ï 1713, 2007.

[48] K. Farkas *et al.*, ñThe Diagnostic Value of a New Fecal Marker, Matrix Metalloprotease-9, in Different Types of Inflammatory Bowel Diseases,ò*J. Crohn's Colitis*, vol. 9, no. 3, pp. 231ï 237, Mar. 2015.

[49] W. Jiang and X. Li, ñMolecular Analysis of Inflammatory Bowel Disease: Clinically Useful Tools for Diagnosis, Response Prediction, and Monitoring of Targeted Therapy,ò*Mol. Diagn. Ther.*, vol. 19, no. 3, pp. 141ï 158, Jun. 2015.

[50] A. Buisson *et al.*, ñFaecal chitinase 3-like 1 is a reliable marker as accurate as faecal calprotectin in detecting endoscopic activity in adult patients with inflammatory bowel diseases,ò*Aliment. Pharmacol. Ther.*, vol. 43, no. 10, pp. 1069ï 1079, May 2016.

[51] S. Nancey *et al.*, ñNeopterin Is a Novel Reliable Fecal Marker as Accurate as Calprotectin for Predicting Endoscopic Disease Activity in Patients with Inflammatory Bowel Diseases,ò*Inflamm. Bowel Dis.*, vol. 19, no. 5, pp. 1043ï 1052, Apr. 2013.

[52] J. P. Derikx, M. D. Luyer, E. Heineman, and W. A. Buurman, ñNon-invasive markers

of gut wall integrity in health and disease,ò*World J. Gastroenterol.*, vol. 16, no. 42, pp. 5272ï 5279, 2010.

[53] J. H. Holmes *et al.*, ñElevated Intestinal Fatty Acid Binding Protein and Gastrointestinal Complications Following Cardiopulmonary Bypass: A Preliminary Analysis,ò*J. Surg. Res.*, vol. 100, no. 2, pp. 192ï 196, 2001.

[54] J. J. de Haan *et al.*, ñRapid development of intestinal cell damage following severe trauma: a prospective observational cohort study,ò*Crit. Care*, vol. 13, no. 3, p. R86, 2009.

[55] H. Brenner and S. Tao, ñSuperior diagnostic performance of faecal immunochemical tests for haemoglobin in a head-to-head comparison with guaiac based faecal occult blood test among 2235 participants of screening colonoscopy,ò*Eur. J. Cancer*, vol. 49, pp. 3049ï 3054, 2013.

[56] L. Hol *et al.*, ñScreening for colorectal cancer: randomised trial comparing guaiac-based and immunochemical faecal occult blood testing and flexible sigmoidoscopy.,ò *Gut*, vol. 59, no. 1, pp. 62ï 8, 2010.

[57] G. Vart, R. Banzi, and S. Minozzi, ñComparing participation rates between immunochemical and guaiac faecal occult blood tests: A systematic review and meta-analysis,ò*Prev. Med. (Baltim).*, vol. 55, pp. 87ï 92, 2012.

[58] A. Shiotani *et al.*, ñApplication of fecal hemoglobin ï haptoglobin complex testing for small bowel lesions,ò*Scand. J. Gastroenterol.*, vol. 49, pp. 539ï 544, 2014.

[59] J. A. Burch *et al.*, ñDiagnostic accuracy of faecal occult blood tests used in screening for colorectal cancer: a systematic review,ò*J. Med. Screen.*, vol. 14, no. 3, pp. 132ï 137, Sep. 2007.

[60] M. Zorzi *et al.*, ñImpact on colorectal cancer mortality of screening programmes based on the faecal immunochemical test.,ò*Gut*, vol. 64, no. 5, pp. 784ï 90, 2015.

[61] P. Hewitson, P. Glasziou, E. Watson, B. Towler, and L. Irwig, ñCochrane Systematic Review of Colorectal Cancer Screening Using the Fecal Occult Blood Test (Hemoccult): An Update,ò*Am. J. Gastroenterol.*, vol. 103, no. 6, pp. 1541ï 1549, Jun. 2008.

[62] Ø. Holme, M. Bretthauer, A. Fretheim, J. Odgaard-Jensen, and G. Hoff, ñFlexible sigmoidoscopy versus faecal occult blood testing for colorectal cancer screening in asymptomatic individuals,òin *Cochrane Database of Systematic Reviews*, Ø. Holme, Ed. Chichester, UK: John Wiley & Sons, Ltd, 2013.

[63] H. Chiba, M. Sekiguchi, T. Ito, Y. Tsuji, and K. Ohata, ñIs It Worthwhile to Perform Capsule Endoscopy for Asymptomatic Patients with Positive Immunochemical Faecal Occult Blood Test?,ò*Dig. Dis. Sci.*, vol. 56, pp. 3459ï 3462, 2011.

[64] J. Jun, P. Jae, and H. Cheon, ñSmall Bowel Evaluation in Asymptomatic Fecal Immunochemical Test-Positive Patients with a Negative Colonoscopy: Is It Necessary?,ò*Dig. Dis. Sci.*, vol. 56, pp. 2773ï 2775, 2011.

[65] M. Q. Zhang, F. Lin, P. Hui, Z. M. E. Chen, J. H. Ritter, and H. L. Wang, ñExpression of mucins, SIMA, villin, and CDX2 in small-intestinal adenocarcinoma,ò*Am. J. Clin. Pathol.*, vol. 128, no. 5, pp. 808ï 816, 2007.

[66] D. L. Ouyang, J. J. Chen, R. H. Getzenberg, and R. E. Schoen, ñNoninvasive Testing for Colorectal Cancer: A Review,ò*Am. J. Gastroenterol.*, vol. 100, no. 6, pp. 1393ï 1403, Jun. 2005.

[67] D. W. Kufe, ñMucins in cancer: function, prognosis and therapy,ò*Nat. Rev. Cancer*, vol. 9, no. 12, pp. 874ï 885, Dec. 2009.

[68] Y. H. Sheng, S. Z. Hasnain, T. H. J. Florin, and M. A. McGuckin, ñMucins in inflammatory bowel diseases and colorectal cancer,ò*J. Gastroenterol. Hepatol.*, vol. 27, no. 1, pp. 28ï 38, Jan. 2012.

[69] S. Hundt, U. Haug, and H. Brenner, ñBlood Markers for Early Detection of Colorectal Cancer: A Systematic Review,ò*Cancer Epidemiol. Prev. Biomarkers*, vol. 16, no. 10, 2007.

[70] A. Amaro, S. Chiara, and U. Pfeffer, ñMolecular evolution of colorectal cancer: from multistep carcinogenesis to the big bang,ò*Cancer Metastasis Rev.*, vol. 35, no. 1, pp. 63ï 74, 2016.

[71] D. A. Ahlquist, J. J. Harrington, L. J. Burgart, and P. C. Roche, ñMorphometric analysis of the ómucocellular layerôoverlying colorectal cancer and normal mucosa: relevance to exfoliation and stool screening.,ò*Hum. Pathol.*, vol. 31, no. 1, pp. 51ï 57, 2000.

[72] B. T. Dickinson, J. Kisiel, D. a Ahlquist, and W. M. Grady, ñMolecular markers for colorectal cancer screening.,ò*Gut*, vol. 64, no. 9, pp. 1485ï 94, 2015.

[73] Y. Okugawa, W. M. Grady, and A. Goel, ñEpigenetic Alterations in Colorectal Cancer: Emerging Biomarkers,ò*Gastroenterology*, vol. 149, no. 5, p. 1204ï 1225e12, 2015.

[74] F. Diehl *et al.*, ñCirculating mutant DNA to assess tumor dynamics.,ò*Nat. Med.*, vol. 14, no. 9, pp. 985ï 990, 2008.

[75] Y.-H. Su *et al.*, ñHuman Urine Contains Small, 150 to 250 Nucleotide-Sized, Soluble DNA Derived from the Circulation and May Be Useful in the Detection of Colorectal Cancer,ò*J. Mol. Diagnostics*, vol. 6, no. 2, pp. 101ï 107, May 2004.

[76] L. J. W. Bosch *et al.*, ñAnalytical sensitivity and stability of DNA methylation testing in stool samples for colorectal cancer detection,ò*Cell. Oncol.*, vol. 35, no. 4, pp. 309ï 315, 2012.

[77] H. Zou, J. J. Harrington, K. K. Klatt, and D. A. Ahlquist, ñA sensitive method to quantify human long DNA in stool: Relevance to colorectal cancer screening,ò*Cancer Epidemiol. Biomarkers Prev.*, vol. 15, no. 6, pp. 1115ï 1119, 2006.

[78] J. Olson, D. H. Whitney, K. Durkee, and A. P. Shuber, ñDNA stabilization is critical for maximizing performance of fecal DNA-based colorectal cancer tests,ò *Diagn.Mol.Pathol.*, vol. 14, no. 3, pp. 183ï 191, 2005.

[79] H. Zou *et al.*, ñQuantification of methylated markers with a multiplex methylation-specific technology,ò*Clin. Chem.*, vol. 58, no. 2, pp. 375ï 383, 2012.

[80] F. Diehl *et al.*, ñAnalysis of Mutations in DNA Isolated From Plasma and Stool of Colorectal Cancer Patients,ò*Gastroenterology*, vol. 135, no. 2, p. 489ï 498.e7, Aug. 2008.

[81] R.-L. Zhai *et al.*, ñThe Diagnostic Performance of Stool DNA Testing for Colorectal Cancer,ò*Medicine (Baltimore)*., vol. 95, no. 5, p. e2129, Feb. 2016.

[82] T. F. Imperiale *et al.*, ñMultitarget stool DNA testing for colorectal-cancer screening.,ò *N. Engl. J. Med.*, vol. 370, no. 14, pp. 1287ï 97, 2014.

[83] J. S. Lin *et al.*, ñScreening for Colorectal Cancer: A Systematic Review for the U.S. Preventive Services Task Force: (Evidence Syntheses, No. 135.),ò2016.

[84] B. George and S. Kopetz, ñPredictive and prognostic markers in colorectal cancer,ò *Curr. Oncol. Rep.*, vol. 13, no. 3, pp. 206ï 215, 2011.

[85] A. I. Phipps *et al.*, ñAssociation between molecular subtypes of colorectal cancer and patient survival,ò*Gastroenterology*, vol. 148, no. 1, p. 77ï 87.e2, 2015.

[86] B.-H. Min *et al.*, ñThe CpG island methylator phenotype may confer a survival benefit in patients with stage II or III colorectal carcinomas receiving fluoropyrimidine-based adjuvant chemotherapy,ò*BMC Cancer*, vol. 11, no. 1, p. 344, Dec. 2011.

[87] R. Jover *et al.*, ñ5-Fluorouracil adjuvant chemotherapy does not increase survival in patients with CpG island methylator phenotype colorectal cancer,ò*Gastroenterology*, vol. 140, no. 4, pp. 1174ï 1181, 2011.

[88] Y. Y. Juo *et al.*, ñPrognostic value of CpG island methylator phenotype among colorectal cancer patients: a systematic review and meta-analysis.,ò*Ann. Oncol.*, vol. 25, no. 12, pp. 2314ï 27, 2014.

[89] D. J. Robertson and J. A. Dominitz, ñStool DNA and Colorectal-Cancer Screening,ò*N. Engl. J. Med.*, vol. 370, no. 14, pp. 1350ï 1351, Apr. 2014.

[90] D. P. Bartel, ñMicroRNAs: Genomics, Biogenesis, Mechanism, and Function,ò*Cell*, vol. 116, no. 2. pp. 281ï 297, 2004.

[91] O. Slaby, M. Svoboda, J. Michalek, and R. Vyzula, ñMicroRNAs in colorectal cancer: translation of molecular biology into clinical application.,ò*Mol. Cancer*, vol. 8, p. 102, 2009.

[92] S. Volinia *et al.*, ñA microRNA expression signature of human solid tumors defines cancer gene targets.,ò*PNAS*, vol. 103, no. 7, pp. 2257ï 2261, 2006.

[93] R. Kalla *et al.*, ñMicroRNAs: new players in IBD.,ò*Gut*, vol. 64, no. 3, pp. 504ï 17, 2015.

[94] P. S. Mitchell *et al.*, ñCirculating microRNAs as stable blood-based markers for cancer detection,ò*Proc. Natl. Acad. Sci.*, vol. 105, no. 30, pp. 10513ï 10518, Jul. 2008.

[95] C. W. Wu *et al.*, ñIdentification of microrna-135b in stool as a potential noninvasive biomarker for colorectal cancer and adenoma,ò*Clin. Cancer Res.*, vol. 20, no. 11, pp. 2994ï 3002, 2014.

[96] W. Meng *et al.*, ñComparison of MicroRNA Deep Sequencing of Matched Formalin-Fixed Paraffin-Embedded and Fresh Frozen Cancer Tissues,ò*PLoS One*, vol. 8, no. 5, p. e64393, May 2013.

[97] T. Tian, J. Wang, and X. Zhou, ñA review: microRNA detection methods,ò*Org. Biomol. Chem.*, vol. 13, no. 8, pp. 2226ï 2238, 2015.

[98] Y. Koga *et al.*, ñFecal miR-106a Is a Useful Marker for Colorectal Cancer Patients with False-Negative Results in Immunochemical Fecal Occult Blood Test,ò *Cancer Epidemiol. Biomarkers Prev.*, vol. 22, no. 10, pp. 1844ï 1852, Oct. 2013.

[99] F. E. Ahmed *et al.*, ñDiagnostic microRNA markers to screen for sporadic human colon cancer in stool: I. Proof of principle.,ò *Cancer Genomics Proteomics*, vol. 10, no. 3, pp. 93ï 113, 2013.

[100] C. W. Wu *et al.*, ñDetection of miR-92a and miR-21 in stool samples as potential screening biomarkers for colorectal cancer and polyps,ò *Gut*, vol. 61, no. 5, pp. 739ï 745, May 2012.

[101] C. Loser, A. Mollgaard, and U. R. Folsch, ñFaecal elastase 1: a novel, highly sensitive, and specific tubeless pancreatic function test.,ò *Gut*, vol. 39, no. 4, pp. 580ï 586, Oct. 1996.

[102] S. Lüth, S. Teyssen, K. Forssmann, C. Kölbel, F. Krummenauer, and M. V Singer, ñFecal elastase-1 determination: ógold standardôof indirect pancreatic function tests?,ò *Scand. J. Gastroenterol.*, vol. 36, no. 10, pp. 1092ï 1099, 2001.

[103] D. Rothenbacher *et al.*, ñPrevalence and determinants of exocrine pancreatic insufficiency among older adults: Results of a population-based study.,ò *Scand. J. Gastroenterol.*, vol. 40, pp. 697ï 704, 2005.

[104] P. G. Lankisch *et al.*, ñFaecal elastase 1: not helpful in diagnosing chronic pancreatitis associated with mild to moderate exocrine pancreatic insufficiency,ò *Gut*, vol. 42, no. 4, pp. 551ï 554, Apr. 1998.

[105] U. Karbach, K. Ewe, and H. Bodenstein, ñAlpha 1-antitrypsin, a reliable endogenous marker for intestinal protein loss and its application in patients with Crohnôs disease.,ò *Gut*, vol. 24, no. 8, pp. 718ï 23, Aug. 1983.

[106] O. Saitoh *et al.*, ñIntestinal Protein Loss and Bleeding Assessed by Fecal Hemoglobin,

Transferrin, Albumin, and Alpha-1-Antitrypsin Levels in Patients with Colorectal Diseases,ò*Digestion*, vol. 56, no. 1, pp. 67ï 75, Feb. 1995.

[107] E. Nistal, N. Fernández-Fernández, S. Vivas, and J. L. Olcoz, ñFactors Determining Colorectal Cancer: The Role of the Intestinal Microbiota.,ò*Front. Oncol.*, vol. 5, no. October, p. 220, 2015.

[108] R. E. Ley, D. A. Peterson, and J. I. Gordon, ñEcological and Evolutionary Forces Shaping Microbial Diversity in the Human Intestine,ò*Cell*, vol. 124, no. 4, pp. 837ï 848, 2006.

[109] F. Guarner *et al.*, ñGut flora in health and disease,ò*The Lancet*, vol. 361, no. 9356, pp. 512ï 519, Feb. 2003.

[110] P. Louis, G. L. Hold, and H. J. Flint, ñThe gut microbiota, bacterial metabolites and colorectal cancer,ò*Nat. Rev. Microbiol.*, vol. 12, no. 10, pp. 661ï 672, 2014.

[111] T. O. Keku, S. Dulal, A. Deveaux, B. Jovov, and X. Han, ñThe gastrointestinal microbiota and colorectal cancer.,ò*Am. J. Physiol. Gastrointest. Liver Physiol.*, vol. 308, no. 5, pp. G351-63, Mar. 2015.

[112] A. B. Shreiner, J. Y. Kao, and V. B. Young, ñThe gut microbiome in health and in disease,ò*Curr. Opin. Gastroenterol.*, vol. 31, no. 1, pp. 69ï 75, Jan. 2015.

[113] C. Huttenhower *et al.*, ñStructure, function and diversity of the healthy human microbiome,ò*Nature*, vol. 486, no. 7402, pp. 207ï 214, Jun. 2012.

[114] M. Conlon and A. Bird, ñThe Impact of Diet and Lifestyle on Gut Microbiota and Human Health,ò*Nutrients*, vol. 7, no. 1, pp. 17ï 44, Dec. 2014.

[115] C. E. West *et al.*, ñThe gut microbiota and inflammatory noncommunicable diseases: Associations and potentials for gut microbiota therapies,ò*J. Allergy Clin. Immunol.*, vol. 135, no. 1, pp. 3ï 13, 2015.

[116] B. P. Willing *et al.*, ñA Pyrosequencing Study in Twins Shows That Gastrointestinal Microbial Profiles Vary With Inflammatory Bowel Disease Phenotypes,ò *Gastroenterology*, vol. 139, no. 6, p. 1844ï 1854.e1, 2010.

[117] E. S. Wills, D. M. A. E. Jonkers, P. H. Savelkoul, A. A. Masclee, M. J. Pierik, and J. Penders, ñFecal microbial composition of ulcerative colitis and Crohnôs disease patients in remission and subsequent exacerbation,ò *PLoS One*, vol. 9, no. 3, pp. 1ï 10, 2014.

[118] P. Marteau, ñBacterial flora in inflammatory bowel disease.,ò *Dig. Dis.*, vol. 27 Suppl 1, no. suppl 1, pp. 99ï 103, 2009.

[119] E. Papa *et al.*, ñNon-invasive mapping of the gastrointestinal microbiota identifies children with inflammatory bowel disease,ò *PLoS One*, vol. 7, no. 6, 2012.

[120] G. Cammarota, G. Ianiro, R. Cianci, S. Bibbò, A. Gasbarrini, and D. Currò, ñThe involvement of gut microbiota in inflammatory bowel disease pathogenesis: Potential for therapy,ò *Pharmacol. Ther.*, vol. 149, pp. 191ï 212, 2015.

[121] R. B. Sartor, ñMicrobial Influences in Inflammatory Bowel Diseases,ò *Gastroenterology*, vol. 134, no. 2, pp. 577ï 594, 2008.

[122] Q. Feng *et al.*, ñGut microbiome development along the colorectal adenoma-carcinoma sequence.,ò *Nat. Commun.*, vol. 6, p. 6528, 2015.

[123] C. L. Sears and W. S. Garrett, ñMicrobes, microbiota, and colon cancer,ò *Cell Host Microbe*, vol. 15, no. 3, pp. 317ï 328, 2014.

[124] I. Sobhani *et al.*, ñMicrobial dysbiosis in colorectal cancer (CRC) patients,ò *PLoS One*, vol. 6, no. 1, 2011.

[125] M. Bonnet *et al.*, ñColonization of the human gut by E. coli and colorectal cancer risk,ò *Clin. Cancer Res.*, vol. 20, no. 4, pp. 859ï 867, 2014.

[126] S. Dulal and T. O. Keku, ñGut microbiome and colorectal adenomas.,ò*Cancer J.*, vol. 20, no. 3, pp. 225ï 31, 2014.

[127] N. Wu *et al.*, ñDysbiosis Signature of Fecal Microbiota in Colorectal Cancer Patients,ò*Microb. Ecol.*, vol. 66, no. 2, pp. 462ï 470, 2013.

[128] T. Irraz??bal, A. Belcheva, S. E. Girardin, A. Martin, and D. J. Philpott, ñThe multifaceted role of the intestinal microbiota in colon cancer,ò*Mol. Cell*, vol. 54, no. 2, pp. 309ï 320, 2014.

[129] J. Sun and I. Kato, ñGut microbiota, inflammation and colorectal cancer,ò*Genes Dis.*, vol. 3, no. 2, pp. 130ï 143, Jun. 2016.

[130] R. Sinha *et al.*, ñFecal Microbiota, Fecal Metabolome, and Colorectal Cancer Interrelations,ò*PLoS One*, vol. 11, no. 3, p. e0152126, 2016.

[131] J. P. Zackular, N. T. Baxter, G. Y. Chen, and P. D. Schloss, ñManipulation of the Gut Microbiota Reveals Role in Colon Tumorigenesis,ò*mSphere*, vol. 1, no. 1, pp. e00001-15, 2015.

[132] A. D. Kostic *et al.*, ñFusobacterium nucleatum Potentiates Intestinal Tumorigenesis and Modulates the Tumor-Immune Microenvironment,ò*Cell Host Microbe*, vol. 14, no. 2, pp. 207ï 215, Aug. 2013.

[133] T. L. Weir, D. K. Manter, A. M. Sheflin, B. A. Barnett, A. L. Heuberger, and E. P. Ryan, ñStool Microbiome and Metabolome Differences between Colorectal Cancer Patients and Healthy Adults,ò*PLoS One*, vol. 8, no. 8, 2013.

[134] C. M. Hester, V. R. Jala, M. G. Langille, S. Umar, K. A. Greiner, and B. Haribabu, ñFecal microbes, short chain fatty acids, and colorectal cancer across racial/ethnic groups.,ò*World J. Gastroenterol.*, vol. 21, no. 9, pp. 2759ï 69, 2015.

[135] L. Flanagan *et al.*, ñFusobacterium nucleatum associates with stages of colorectal neoplasia development, colorectal cancer and disease outcome,ò*Eur. J. Clin.*

Microbiol. Infect. Dis., vol. 33, no. 8, pp. 1381ï 1390, 2014.

[136] J. Ahn *et al.*, ñHuman gut microbiome and risk for colorectal cancer,ò*J. Natl. Cancer Inst.*, vol. 105, no. 24, pp. 1907ï 1911, 2013.

[137] J. P. Zackular, M. a. M. Rogers, M. T. Ruffin, and P. D. Schloss, ñThe human gut microbiome as a screening tool for colorectal cancer,ò*Cancer Prev. Res.*, vol. 7, no. November, pp. 1112ï 1121, 2014.

[138] Y. Momozawa, V. Deffontaine, E. Louis, and J. F. Medrano, ñCharacterization of bacteria in biopsies of colon and stools by high throughput sequencing of the V2 region of bacterial 16s rRNA gene in human,ò*PLoS One*, vol. 6, no. 2, 2011.

[139] A. D. Kostic, R. J. Xavier, and D. Gevers, ñThe Microbiome in Iflammatory Bowel Diseases: Current Status and the Future Ahead,ò*Gastroenterology*, vol. 146, no. 6, pp. 1489ï 1499, 2015.

[140] H. Tjalsma, A. Boleij, J. R. Marchesi, and B. E. Dutilh, ñA bacterial driverï passenger model for colorectal cancer: beyond the usual suspects,ò*Nat. Rev. Microbiol.*, vol. 10, no. 8, pp. 575ï 582, Jun. 2012.

[141] M. Wolin, ñFermentation in the rumen and human large intestine,ò*Science (80-.).*, vol. 213, no. 4515, pp. 1463ï 1468, Sep. 1981.

[142] S. Kurada, N. Alkhouri, C. Fiocchi, R. Dweik, and F. Rieder, ñReview article: breath analysis in inflammatory bowel diseases.,ò*Aliment. Pharmacol. Ther.*, vol. 41, no. 4, pp. 329ï 41, Feb. 2015.

[143] B. de Lacy Costello *et al.*, ñA review of the volatiles from the healthy human body.,ò*J. Breath Res.*, vol. 8, no. 1, p. 14001, 2014.

[144] H. Kashtan, M. Rabau, Y. Peled, A. Milstein, and T. Wiznitzer, ñMethane production in patients with colorectal carcinoma.,ò*Isr. J. Med. Sci.*, vol. 25, no. 11, pp. 614ï 6, Nov. 1989.

[145] S. M. Sivertsen, A. Bjørneklett, H. P. Gullestad, and K. Nygaard, ñBreath Methane and Colorectal Cancer,ò*Scand. J. Gastroenterol.*, vol. 27, no. 1, pp. 25ï 28, Jul. 2009.

[146] D. a Karlin, R. D. Jones, J. R. Stroehlein, a J. Mastromarino, and G. D. Potter, ñBreath methane excretion in patients with unresected colorectal cancer.,ò*J. Natl. Cancer Inst.*, vol. 69, no. 3, pp. 573ï 6, 1982.

[147] J. M. Pique, M. Pallares, E. Cuso, J. Vilar-Bonet, and M. A. Gassull, ñMethane production and colon cancer,ò*Gastroenterology*, vol. 87, no. 3, pp. 601ï 605, 1984.

[148] H. Amal *et al.*, ñBreath testing as potential colorectal cancer screening tool,ò*Int. J. Cancer*, vol. 138, no. 1, pp. 229ï 236, Jan. 2016.

[149] D. F. Altomare *et al.*, ñExhaled volatile organic compounds identify patients with colorectal cancer,ò*Br. J. Surg.*, vol. 100, no. 1, pp. 144ï 150, Jan. 2013.

[150] G. Peng *et al.*, ñDetection of lung, breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors.,ò*Br. J. Cancer*, vol. 103, no. 4, pp. 542ï 51, 2010.

[151] J. Kokoszka, R. L. Nelson, W. I. Swedler, J. Skosey, and H. Abcarian, ñDetermination of inflammatory bowel disease activity by breath pentane analysis,ò*Dis. Colon Rectum*, vol. 36, no. 6, pp. 597ï 601, Jun. 1993.

[152] N. Patel *et al.*, ñMetabolomic analysis of breath volatile organic compounds reveals unique breathprints in children with inflammatory bowel disease: A pilot study,ò *Aliment. Pharmacol. Ther.*, vol. 40, no. 5, pp. 498ï 507, 2014.

[153] F. Rieder *et al.*, ñA Distinct Colon-Derived Breath Metabolome is Associated with Inflammatory Bowel Disease, but not its Complications.,ò*Clin. Transl. Gastroenterol.*, vol. 7, no. 11, p. e201, 2016.

[154] G. D. Banik *et al.*, ñHydrogen sulphide in exhaled breath: a potential biomarker for small intestinal bacterial overgrowth in IBS,ò*J. Breath Res.*, vol. 10, no. 2, p. 26010,

2016.

[155] S. Sedghi, A. Keshavarzian, M. Klamut, D. Eiznhamer, and E. J. Zarling, ñElevated Breath Ethane Levels in Active Ulcerative Colitis: Evidence for Excessive Lipid Peroxidation,ò*Am. J. Gastroenterol.*, vol. 89, no. 12, pp. 2217ï 2222, 1994.

[156] M. A. Pelli, G. Trovarelli, E. Capodicasa, G. E. De Medio, J.-G. Bassotti, and D. S. Ph, ñBreath Mkanes Determination in Ulcerative Colitis and Crohn âú™ s Disease,òvol. 42, no. 1, pp. 71ï 76, 1999.

[157] L. F. McKay, M. A. Eastwood, and W. G. Brydon, ñMethane excretion in man--a study of breath, flatus, and faeces.,ò*Gut*, vol. 26, no. 1, pp. 69ï 74, 1985.

[158] J. Z. Ou, C. K. Yao, A. Rotbart, J. G. Muir, P. R. Gibson, and K. Kalantar-zadeh, ñHuman intestinal gas measurement systems: in vitro fermentation and gas capsules.,ò*Trends Biotechnol.*, vol. 33, no. 4, pp. 208ï 13, 2015.

[159] K. Kalantar-zadeh *et al.*, ñIntestinal Gas Capsules: A Proof-of-Concept Demonstration,ò*Gastroenterology*, vol. 150, no. 1, pp. 37ï 39, Jan. 2016.

[160] S. Clarysse, J. Tack, F. Lammert, G. Duchateau, C. Reppas, and P. Augustijns, ñPostprandial Evolution in Composition and Characteristics of Human Duodenal Fluids in Different Nutritional States,ò*J. Pharm. Sci.*, vol. 98, no. 3, pp. 1177ï 1192, Mar. 2009.

[161] J. P. F. Bai, G. J. Burckart, and A. E. Mulberg, ñLiterature Review of Gastrointestinal Physiology in the Elderly, in Pediatric Patients, and in Patients with Gastrointestinal Diseases,ò*J. Pharm. Sci.*, pp. 1ï 11, 2015.

[162] K. Ewe, S. Schwartz, S. Petersen, and A. G. Press, ñInflammation does not decrease intraluminal pH in chronic inflammatory bowel disease,ò*Dig. Dis. Sci.*, vol. 44, no. 7, pp. 1434ï 1439, 1999.

[163] G. Pye, D. F. Evans, S. Ledingham, and J. D. Hardcastle, ñGastrointestinal

intraluminal pH in normal subjects and those with colorectal adenoma or carcinoma.,ò *Gut*, vol. 31, no. 12, pp. 1355ï 1357, 1990.

[164] H. Hove, M. Rye Clausen, and P. Brøbech Mortensen, ñLactate and pH in faeces from patients with colonic adenomas or cancer.,ò *Gut*, vol. 34, no. 5, pp. 625ï 629, 1993.

[165] W. Van Dokkum and B. De Boer, ñDiet, faecal pH and colorectal cancer,ò *J. Cancer*, pp. 109ï 110, 1983.

[166] J. R. THORNTON, ñHIGH COLONIC pH PROMOTES COLORECTAL CANCER,ò *Lancet*, vol. 317, no. 8229, pp. 1081ï 1083, May 1981.