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**Emerging role of novel biomarkers in the diagnosis of inflammatory bowel disease**

Soubieres A *et al*. Biomarkers in IBD

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**Abstract**

There is currently no gold standard test for the diagnosis of inflammatory bowel disease. Physicians must rely on a number of diagnostic tools including clinical and endoscopic evaluation as well as histologic, serologic and radiologic assessment. The real difficulty for physicians in both primary and secondary care is differentiating between patients suffering from functional symptoms and those with true underlying inflammatory bowel disease. Alongside this, there is always concern regarding the possibility of a missed, or delayed diagnosis of ulcerative colitis (UC) or Crohn’s disease. Even once the diagnosis of inflammatory bowel disease (IBD) has been made, there is often uncertainty in distinguishing between cases of UC or Crohn’s. As a consequence, in cases of incorrect diagnosis, optimal treatment and management may be adversely affected. Endoscopic evaluation can be uncomfortable and inconvenient for patients. It carries significant risks including perforation and in terms of monetary cost, is expensive. The use of biomarkers to help in the diagnosis and differentiation of IBD has been increasing over time. However, there is not yet one biomarker, which is sensitive of specific enough to be used alone in diagnosing IBD. Current serum testing includes C-reactive protein and erythrocyte sedimentation rate, which are cheap, reliable but non-specific and thus not ideal. Stool based testing such as faecal calprotectin is a much more specific tool and is currently in widespread clinical use. Non-invasive sampling is of the greatest clinical value and with the recent advances in metabolomics, genetics and proteomics, there are now more tools available to develop sensitive and specific biomarkers to diagnose and differentiate between IBD. Many of these new advances are only in early stages of development but show great promise for future clinical use.

**Key words:** Biomarkers; Inflammatory bowel disease; Ulcerative colitis; Crohn’s disease; Indeterminate colitis

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**Core tip:** There is no gold standard test in the diagnosis of inflammatory bowel disease (IBD). Physicians must take into account clinical, endoscopic, and radiologic as well as serologic and histologic evidence in order to correctly diagnose their patients. Endoscopic evaluation is not only expensive, but is uncomfortable for patients and not without significant risk such as perforation. The use of biomarkers to help in the diagnosis and sub classification of IBD is an expanding area. In this review we touch on those non-invasive markers currently in clinical use before focusing on those more novel tests, with the potential to be highly useful in both diagnosis and differentiation of IBD.

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**INTRODUCTION**

The European evidence-based Consensus on the diagnosis and management of inflammatory bowel disease (IBD) states that diagnosis should rely on physicians taking into account a number of factors including clinical and endoscopic evaluation as well as histologic, serologic and radiologic assessment[1,2]. There is no gold standard diagnostic tool.

Abdominal pain with, or without a change in bowel habit is a common presenting symptom in primary care. A majority of these patients will be suffering from functional bowel disorders including functional dyspepsia and irritable bowel syndrome. Indeed, functional bowel disorders make up a significant proportion of referrals to gastroenterology outpatient clinics (up to 60%)[3].

The dilemma for physicians is distinguishing a patient with functional symptoms from one with an underlying diagnosis of inflammatory bowel disease. Up to 50% of patients with a functional diagnosis are referred on for unnecessary endoscopic evaluation[3].

Conversely, there is also often a delay in diagnosis of cases of true Crohn’s disease (CD) and ulcerative colitis (UC), (*i.e.,* time from onset of symptoms to diagnosis). This delay is more marked in the case of ileal CD[4].

Even once a diagnosis of IBD is made, there can still be uncertainty with regard to sub classification into either CD or UC. This is essential, as optimal treatment and management of both conditions is different.

Making this differential diagnosis between CD and UC can be difficult and around 10% of patients are labelled as having an indeterminate colitis (IC)[5].

It is thus clear that even with current available diagnostic tools, as physicians, we still struggle to make accurate diagnoses.

Any investigative test must be acceptable in terms of both cost and comfort to patients. Endoscopic evaluation is not only often uncomfortable as well as expensive, but can be related to significant risk, such as perforation. One recent French study found a rate of between 4.5 and 9.7 cases of perforation per 10000 patients[6].

Radiologic imaging, perhaps most useful in the investigation of small bowel pathology, also has its drawbacks with regard to inter and intra-observer variability, and obviously does not allow for histological sampling[7].

The use of biomarkers to aid the diagnosis of IBD is an ever-expanding investigative area.

A biomarker has been defined as “a characteristic that is objectively\* measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a [n]...intervention.” Example: cholesterol level[8].

As of yet, there is no one biomarker, which is sensitive or specific enough to make a confident diagnosis of IBD on its result alone. Many are indicative of systemic inflammation and so have limitations in their use.

This review will touch on those already well established in their use before focusing on more recent advances in the development of novel biomarkers for both the diagnosis and monitoring of IBD.

**SCOPE OF THIS REVIEW**

This review will focus on more recent advances in the development of novel biomarkers for both the diagnosis and monitoring of IBD.

A large number of biomarkers have been reported in the literature, however,

We have chosen to consider non-invasively obtained biomarkers, as those that are more acceptable to patients, and thus, most promising with regards to clinical utility.

**LITERATURE SEARCH**

This review of the English language literature on novel biomarkers in the diagnosis of IBD is based on papers contained within the PubMed database. Individual searches of the PubMed database were performed with the boolean operator AND, using the terms: “biomarker”, “inflammatory bowel disease”, “Crohn’s disease”, and “ulcerative colitis”.

The abstracts were screened for eligibility and all relevant publications were requested as full-text articles. References used in requested papers were then checked for any further studies of potential interest.

**BIOMARKERS IN WIDESPREAD USE**

***Blood based***

**C-reactive protein:** C-reactive protein (CRP) is produced by hepatocytes in response to inflammation, stimulated by certain cytokines. In the case of active IBD, these cytokines include tumour necrosis factor-alpha (TNF-α), interleukin (IL)-6 and IL-1β[9].

During active IBD, CRP may rise significantly. However, it is not specific and can go up in a variety of conditions including infection, autoimmune conditions, other inflammatory conditions, and malignancy as well as cell necrosis[10].

Elevations in CRP may vary from person to person depending on the individual’s immune response; however, it has been shown that rises in CRP are more common in CD rather than UC. The reason for this is unclear, but may have to do with the deeper, more penetrating inflammation in CD compared with the superficial mucosal inflammation seen in UC. It has also been suggested that disease location, independent of severity may affect the level of rise in CRP[11].

In patients with known IBD, rises in CRP have been shown to correlate with active disease on colonoscopy and severe inflammation on histology, hence can be useful in distinguishing active from quiescent IBD[12].

**Erythrocyte sedimentation rate:** The erythrocyte sedimentation rate (ESR), like CRP is a measure of systemic inflammation and not entirely specific to IBD.

The test measures the distance that erythrocytes have fallen in 1 hour in a vertical column of non-coagulated blood[13]. In comparison to CRP, ESR levels peak later and decrease at a slower rate. In view of this, ESR is better at monitoring disease activity/response to treatment after the first 24 h of onset whilst CRP may be more useful in the first 24 h.

ESR is still very commonly used in monitoring of IBD, despite it usefulness being quite limited. It is influenced by a number of factors including age, gender, anaemia, blood dyscrasias and pregnancy[14].

Yoon *et a*l[15] found that with regard to correlation with endoscopic activity, both CRP and ESR levels correlated only modestly and that the low sensitivities for detecting endoscopic remission suggest that CRP or ESR alone is not sufficient to reflect endoscopic severity accurately.

Another, more recent meta-analysis found that no level of ESR was predictive of IBD. The highest predictive probability of IBD was reported as 1.6% at an ESR level of 200 mm/h[16].

**Antineutrophil cytoplasmic antibodies:** Antineutrophil cytoplasmic antibodies (ANCAs) are antibodies against granules of neutrophil cytoplasm. They are detected using indirect immunofluorescence (IIF) and show three main staining patterns: the cytoplasmic (cANCA), the speckled (sANCA) and the perinuclear (pANCA). Perineuclear ANCA (pANCA) has been shown to increase significantly in UC[17].

Joosens *et al*[18] found in their prospective follow-up study that 64% of UC patients were positive for pANCA (and anti-*Saccharomyces cerevisiae* antibody (ASCA) negative). A further study calculated the rate of pANCA to be 55% in UC and 32% in healthy controls[19].

In UC, the presence of atypical pANCAs has been associated with resistance to treatment of left-sided disease and early surgery. This suggests a role in using the presence of pANCA to identify those UC patients who may require earlier intervention with immunomodulators[20].

**Anti-*Saccharomyces cerevisiae* antibodies:** Anti-*Saccharomyces cerevisiae* antibodies (ASCA) are antibodies for mannan in the cell wall of *Saccharomyces cerevisiae* (*S. cerevisiae*) [21].

In comparison to pANCA, which is found in higher titres in UC, high ASCA levels are more specific for CD. Using the combination test ASCA+/pANCA-, one meta-analysis of 60 studies looking at 7860 IBD patients and 3748 controls demonstrated the ability to differentiate adults with CD from those with UC with 55% sensitivity and 93% specificity[22]. Levels have also been associated with phenotypes corresponding to ileal disease, young age at onset, stricturing, as well as penetrating behavior and multiple bowel surgery[23].

Despite high specificity levels, the low sensitivity of ASCA/pANCA testing has prevented its routine clinical use in distinguishing between CD and UC.

***Stool based***

**Faecal calprotectin:** Alprotectin is a zinc and calcium binding protein belonging to the S100 family that is derived mostly from neutrophils and monocytes, and has also been detected in activated macrophages[24].

Calprotectin is found in serum, saliva, cerebrospinal fluid, urine and faeces[25]. It is an extremely stable protein, and can be found unaltered in stool samples left unprepared for longer than 7 d.

When the inflammatory process is triggered calprotectin is released due to degranulation of neutrophils, making it very specific for gastrointestinal inflammation[26].

Many studies in the literature have focused on faecal calprotectin (FCP) in terms of accuracy in diagnosis and monitoring of IBD. It has now become a widely used test since it was first described in 1980[27]. One meta-analysis calculated sensitivity and specificity of FCP of up to 95% and 91% respectively. In addition they showed that FCP outperformed other serological markers including CRP and ESR[28].

The National Institute for Health and Care Excellence (NICE) recommends the use of FCP as a diagnostic tool to help in the differential diagnosis of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS)[29]. When used in this way in both primary and secondary care, it may help reduce the number of referrals for unnecessary endoscopic evaluation. One meta-analysis of 13 studies concluded that FCP testing would result in a 67% reduction in the number of adults requiring endoscopy, but with a delayed diagnosis in 8% of adults because of false negative results[30]. One area of controversy surrounding FCP testing is the determination of an appropriate cut-off value, above which the result is deemed as positive. In most centres, a relatively low level of 50 μg/g is used.

Tibble *et al*[31] looked at this issue in a cohort of adult patients undergoing faecal calprotectin testing in primary care. At a cut off of 50 μg/g, FCP testing had a negative predictive value (NPV) of 98% and positive predictive value (PPV) of 28%. Increasing the cut off value to 150 μg/g gave a very comparable negative NPV of 97%, but a much higher PPV of 71%.

Given these values, it was calculated that by increasing the cut off value to 150 μg/g, this would reduce colonoscopy and flexible sigmoidoscopy bookings by 10% at the cost of 4 missed cases of inflammatory bowel disease (*n* = 686)[31].

**Faecal lactoferrin:** Lactoferrin is an iron-binding protein; it covers most mucosal surfaces. It is found within neutrophil granulocytes and becomes activated in acute inflammation[32]. Similar to faecal calprotectin, it is stable for up to 5 d in faeces. Levels of faecal lactoferrin increase significantly as neutrophils infiltrate the gastrointestinal tract[33]. Levels of faecal lactoferrin have been found to be significantly higher in active IBD than in inactive IBD, IBS and infectious bowel disease. One study reported the sensitivity and specificity of fecal lactoferrin as 92% and 88%, respectively, for UC, and 92% and 80%, respectively, for CD[34].

Sidhu *et al*[35] looked at the relationship between faecal lactoferrin levels in small bowel Crohn’s in patients undergoing capsule endoscopy. They found positive predictive and negative predictive values of 100% and 83% respectively for faecal lactoferrin in the diagnosis of small bowel Crohn’s disease detected by capsule endoscopy.

Much like faecal calprotectin, faecal lactoferrin is a sensitive and specific marker in measuring IBD activity. It can help in discriminating between inflammatory and non-inflammatory bowel disease as well allowing for the exclusion of IBS in the case of elevated levels.

**Previously studied faecal biomarkers:** Other faecal markers implemented in the diagnosis, assessment of severity and monitoring of response to therapy in IBD include neopterin and PMN-elastase. Nancey *et al*[36] found faecal neopterin to correlate better with endoscopic activity compared with CRP. The authors also found neopterin to be as accurate as faecal calprotectin in the prediction and monitoring of severity of mucosal damage in IBD.

Polymorphonuclear neutrophil (PMN)-elastase has been shown to be able to differentiate active IBD from inactive IBD as well as from IBS, with a diagnostic accuracy of 74.1%, higher than that of CRP (64%)[37].

S100A12 is part of the calcium binding protein family (similar to FCP) and is a stimulator of proinflammatory mediators. It is also stable in room temperature for up to 7 d[38].

S100A12 has been shown to have sensitivity and specificity levels of up to 86% and 96% respectively, higher than FCP. It has also been shown to correlate better with intestinal inflammation in comparison to other biomarkers[39] as well as having the potential to be used in monitoring response to therapy[38].

However, despite its promise, S100A12 is not used routinely in practice, as more studies need to confirm its use in IBD evaluation.

***Emerging novel blood based markers***

**Anti-outer membrane protein C:** Anti-outer membrane protein C (anti-OmpC) is an antibody directed against the outer membrane porin C transport protein of *Escherichia coli*. Anti-OmpC has been reported in 55% of CD patients[40], whilst in UC and healthy controls, rates were insignificant.

It has been suggested that Anti-OmpC may be of value to aid diagnosis of ASCA negative CD patients. In those patients who are ASCA negative, the prevalence of anti-OmpC has been reported as 5%-15%[41].

**Antibodies to flagellin:** Identification of commensal bacterial proteins in colitic mice has found the dominant antigens to be flagellins. A strong immune response was seen in one particular flagellin, anti-CBir1. Percent of 50 patients with CD were found to have IgG reactivity to CBir1 in comparison to 6% of UC patients and 8% of healthy controls[42].

In atypical pANCA positive CD patients, 40%-44% have been found to be positive for anti-CBir1 in comparison to only 4% of atypical pANCA positive UC patients.

Thus, the detection of anti-CBir1 may help in the differentiation between atypical pANCA positive CD and UC patients, independently of ASCA[43].

In addition, anti-CBir1 antibody has been found to be associated with ileal involvement in CD patients independent of other serologic markers and has been suggested to predispose to stenosing and penetrating disease in CD[42].

More recently, Schoepfer demonstrated reactivity towards two new anti-flagellins, anti-A4-Fla2 and anti-Fla-X in 59% and 57% of CD patients as compared to only 6% of UC patients, suggesting a possible role in distinguishing CD from UC[44].

**Anti-I2 antibody:** A fragment of bacterial DNA (I2), has been identified from lamina propria mononuclear cells in active CD and shown to be associated with *Pseudomonas fluorescens*[45].

Anti-I2 positivity has been reported as 30%-50% in CD, 2%-10% in UC, 36%-42% in indeterminate colitis and 4%-8% of healthy controls. Anti-I2 has also been found in patients with other inflammatory enteritis (19%)[40,46].

**Anti-carbohydrate antibodies:** Patients with CD have been found to express antibodies to cell wall carbohydrate epitopes found in different pathogenic bacteria and fungi. These anti-glycan antibodies include anti-laminaribioside carbohydrate antibody (ALCA) (18%-38%), anti-chitobioside carbohydrate antibody (ACCA) (21%-36%), and anti- mannobioside carbohydrate antibody (AMCA) (28%). ALCA, ACCA and AMCA have been found in 18-38%, 21-36% and 28% of CD patients respectively[45-47].

Ferrante *et al*[48] found that patients with CD who were positive for at least one of ALCA, ACCA or gASCA (similar to ASCA) could be differentiated from UC patients with a 77% sensitivity and > 90% specificity. In the differentiation of CD patients from healthy controls however, the specificity fell to 70.3%.

Overall, the sensitivity of these anti-glycan antibodies has been found to be low by a number of studies, which is a limiting factor in their clinical use[48-53].

**Pancreatic antibodies:** Antigen-specific pancreatic antibodies (PABs) against exocrine pancreas have been found to be present in 20%–30% of patients with CD, but in less than 2–9% of patients with UC, and can be found in very few patients with non-IBD related conditions[54,55].

The major zymogen glycoprotein 2 (MZGP2) has recently been identified as the primary autoantigen of PAB[56] and has prompted the development of techniques to allow for its identification in routine practice.

A study from Pavlidis *et al*[57] in 2014 assessed the clinical relevance of PABs by way of a novel ELISA technique in the largest IBD cohort tested in this way to date. They were able to confirm the high specificity of anti-MZGP2 antibodies for CD and their association with disease severity phenotypes. IgA anti-MZGP2 antibodies were more prevalent in CD patients with early disease onset (*P* = 0.011). In addition, anti-MZGP2 positive patients more frequently had extensive disease with ileal involvement. Patients with longer disease duration were more likely to have IgG anti-MZGP2 antibodies[57].

**Alpha-1 antitrypsin and granulocyte colony-stimulating factor:** Soendergaard *et al*[58] looked at serum samples from 65 patients with UC with varying disease activity and from 40 healthy controls. They measured levels of both alpha -1 antitrypsin (AAT) and granulocyte colony-stimulating factor (G-CSF).

AAT levels were able to differentiate between mild, moderate and severe UC, performing better than CRP.

In addition, the authors found that combination measurement of AAT and G-CSF in patients with diagnosed UC held enough statistical power to differentiate between patients with mild, moderate, and severe disease activity.

***Genetics***

In the recent past, a number of genome wide association studies (GWAS) have discovered a number of susceptibility loci in the investigation of UC and CD-specific genomic profiles.

Ellinghaus *et al*[59] found that variants in two genes, PRDM1 and NDP52 determined susceptibility to CD. PRDM1 was found adjacent to a CD interval identified in GWAS and encodes a transcription factor expressed by T and B cells. NDP52 encodes a protein functioning in autophagy of intracellular bacteria and signaling molecules, supporting the role of autophagy in the pathogenesis of CD.

The IBD chip European project looked at a number of CD-single nucleotide polymorphisms to determine their influence on clinical course and phenotype of the disease. The NOD2 gene was found to be the most important genetic factor, being an independent predictive factor for ileal location, stenosing and penetrating CD. It was also associated with a more complicated disease course and the need for surgery[60].

A further recent meta-analysis of CD and UC GWAS reported on significant findings from more than 75000 cases and controls. The authors identified 71 new associations increasing the total number of confirmed IBD susceptibility loci up to 163. They found that most loci contributed to both phenotypes. Interestingly, there was also considerable overlap between susceptibility loci for IBD and mycobacterial infection, suggesting pathways shared between host responses to mycobacteria and those predisposing to IBD[61].

Traditionally, Crohn's disease has been associated with a Th1 cytokine profile, and ulcerative colitis withTh2 cytokines. However this concept has been since challenged by the discovery of Th17 cells and Treg cells. GWAS indicate that IL23R and five additional genes involved in Th17 differentiation (IL12B, JAK2, STAT3, CCR6 and TNFSF15) are associated with susceptibility to Crohn's disease and partly also to ulcerative colitis[62].

In terms of the clinical application of genetics in the diagnosis of IBD, some focus has been made on identifying genetic markers from colonic tissue retrieved from endoscopic biopsy. Von Stein *et al*[63] identified seven genes as differentially expressed in IBD, making it possible to discriminate between patients suffering from UC, CD, or IBS (*P* < 0.0001) using the clinical diagnosis as gold standard.

Much more recently, following on from this work, this same genetic panel was tested on biopsy material from 78 patients with a complicated course (38 probably UC, 18 CD, 22 IBDU). Testing led to a change of the primary diagnosis in a significant number of patients with the initial diagnosis of UC and CD and suggested a clinically probable diagnosis in most of the patients with IBDU and in those with an acute flare of colitis[64].

***Epigenetics***

Epigenetics describes gene-environment interactions affecting gene expression but with no changes in the DNA sequence.

Micro-RNAs (miRs) are single-stranded noncoding RNAs, around 22 nucleotides in length that remain highly conserved throughout evolution[65]. Since they were first described in the 1990s, over 1600 miRs have been described in humans. miRs are transcribed by RNA polymerase into pre-miR, which is then processed in the nucleus and then cytoplasm. 65 miRs regulate gene expression and thus a number of biological processes such as cell proliferation, differentiation and death. Changes in miR expression have been associated with a number of diseases including IBD[66].

Studies have looked at miR profiles in peripheral blood samples from patients with IBD *vs* controls and in CD patients *vs* UC patients. Several miRs have been found to be either up or down regulated. One paediatric study also found differentially expressed levels of certain miRs between serum samples from children with CD compared with healthy controls[67,68].

A recent paper from Schaefer *et al*[69] found CD was associated with altered expression of 6 miRNAs while UC was associated with 9 miRNAs in whole blood. They also found altered expression of different miRNAs in saliva from both UC and CD patients.

They suggest that there are specific miRNA expression patterns associated with UC versus CD, and hence that scrutinizing miRNA expression in saliva and blood samples may be beneficial in monitoring or diagnosing disease in IBD patients.

***Metabolomics***

Metabolomics refers to the study of the many small molecule metabolites present in biological samples, in order to determine the underlying fingerprint of specific cellular processes.

The current main technologies used for metabolomics include 1H NMR spectroscopy, gas chromatography spectrometry (GC-MS) and liquid chromatography- mass spectrometry (LC-MS). These techniques have the advantage of being extremely sensitive and of allowing experiments to be performed in a cost-effective high-throughput manner[70,71].

1H NMR spectroscopy has so far been most widely used in studies on different biofluids from IBD patients. A number of studies have reported differences in metabolic profiles between IBD patients and healthy controls as well as between CD and UC[72,73].

These studies described have mainly focused on the detection of amino acids, TCA cycle intermediates, and on metabolites involved in fatty acid and purine metabolism.

Metabolites of gut bacteria have been detected in urine[74]. Any change in the gut microbiome, which has been shown to be important in the pathogenesis of IBD, may alter the urinary metabolic profile. Thus, urinary metabolites are an attractive option as potential biomarkers for IBD[75].

A study by Williams *et al*[76] looked at the urinary metabolic profiles of CD and UC patients using 1H NMR spectroscopy. They found significant decreases in the levels of hippurate (a metabolite derived from microbiota) in IBD patients.

Other studies have also demonstrated low levels of hippurate in IBD patients using 1H NMR spectroscopy and in addition, have been able to separate between IBD patients and healthy controls[72,77].

Studies have shown that metabolic profiling of serum and plasma by way of 1H NMR spectroscopy is able to discriminate between UC and CD although less reliably than discrimination between UC/CD and healthy controls[72,73].

Further studies have found that profiling of amino acid and TCA cycle-related metabolites can distinguish reliably between UC and CD[78] and also that correlation of metabolic profiles of amino acids with disease activity, suggesting a role in monitoring of IBD[79].

The metabolic profiling of faecal extracts in IBD has shown significantly decreased levels of short chain fatty acids in comparison to healthy controls[80].

Profiling of the gut microbiota as well as the metabolites from faecal extracts may also give further indications to disturbances of gut bacteria in IBD and hence pathogenesis of the disease[81].

Another advance in the field of metabolomics and inflammatory bowel disease is the use of breath testing as a potential biomarker.

A recent review by Kurada *et al*[82] found only 12 (small) studies in the literature, which evaluated the breath metabolome for diagnosis of inflammatory bowel disease. In the case of diagnosis and differentiation of IBD, the volatile organic compounds (VOCs) measured in these studies included mainly pentane, ethane, propane, butane or nitric oxide (NO).

Dryahina *et al*[83] demonstrated elevated levels of pentane in IBD (CD>UC) compared to healthy controls, as did Pelli *et al*[84].

In addition, Pelli *et al*[84] also showed an association between both ethane and propane levels and IBD (*P*= <0.001 for both).

Exhaled NO has been shown to be higher in UC patients compared with CD[85].

With regard to disease activity, one study found a direct correlation between breath pentane levels and WBC scan uptake[86]. Ethane levels have also been shown to correlate with endoscopic activity of disease[87].

Although there have been some promising results from studies, breath analysis is not yet ready for clinical use. Further work is needed to determine the exact breath metabolome patterns in IBD.

***Proteomics***

Proteomics is a more recently advancing area in the identification of new biomarkers. It is based on the analysis of protein expression in healthy and diseased tissues and to carry out protein profiling.

Meuwis *et al*[88] looked at the sera of 120 patients (30 CD, 30 UC, 30 inflammatory controls and 30 healthy controls). They identified 4 proteins of acute phase inflammation (PF4, MRP8, FIBA and Hp­α2).

A much more recent study looked at circulating protein biomarkers in the interleukin-10 knockout [IL-10(-/-)] mouse, a model that develops a time-dependent IBD-like disorder that predominates in the colon[89]. They identified a total of 15 different proteins to be differentially accumulated in serum samples from mid- to late-stage IL-10(-/-) mice compared to early non-inflamed IL-10(-/-) mice, suggesting a role for protein profiling in assessing severity and response to treatment.

**CONCLUSION**

There is a need for more accurate and cost effective biomarkers in the diagnosis and differentiation of IBD. Development of non-invasive biomarkers is paramount in order to be acceptable to patients and to avoid more invasive assessment, such as endoscopy, which is not without risk.

Current serum testing includes CRP and ESR, which are cheap, reliable but non-specific and thus not ideal. Stool based testing such as faecal calprotectin is a much more specific tool and has now a lot of positive evidence behind it to support its use clinically.

It should be highlighted that as of yet, and despite recent advances, there is no biomarker reliable enough to make a confident diagnosis of IBD without going on, in the case of a positive test, to perform confirmatory colonoscopy. Rather, these non-invasive tests are used currently as an adjuvant to endoscopic evaluation; and to avoid unnecessary procedures where a negative test would indicate no underlying inflammation and no pathology of any cause.

Non-invasive sampling is of the greatest clinical value and with the recent advances in metabolomics, genetics and proteomics, there are now more tools available to develop sensitive and specific biomarkers to diagnose and differentiate between IBD.

This review has touched on the great advances, which have been made in the ever-expanding area of biomarkers in inflammatory bowel disease. However, more work is now required to help bring these new techniques into everyday clinical practice.

**REFERENCES**

1 **Van Assche G**, Dignass A, Panes J, Beaugerie L, Karagiannis J, Allez M, Ochsenkühn T, Orchard T, Rogler G, Louis E, Kupcinskas L, Mantzaris G, Travis S, Stange E. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. *J Crohns Colitis* 2010; **4**: 7-27 [PMID: 21122488 DOI: 10.1016/j.crohns.2009.12.003]

2 **Dignass A**, Eliakim R, Magro F, Maaser C, Chowers Y, Geboes K, Mantzaris G, Reinisch W, Colombel JF, Vermeire S, Travis S, Lindsay JO, Van Assche G. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 1: definitions and diagnosis. *J Crohns Colitis* 2012; **6**: 965-990 [PMID: 23040452 DOI: 10.1016/j.crohns.2012.09.003]

3 **British Society of Gastroenterology**. Chronic management: IBS/Functional. Available from: URL: http://www.bsg.org.uk/clinical/commissioning-report/ibs/functional-symptoms.html

4 **Vavricka SR**, Spigaglia SM, Rogler G, Pittet V, Michetti P, Felley C, Mottet C, Braegger CP, Rogler D, Straumann A, Bauerfeind P, Fried M, Schoepfer AM. Systematic evaluation of risk factors for diagnostic delay in inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 496-505 [PMID: 21509908 DOI: 10.1002/ibd.21719]

5 **Tremaine WJ**. Is indeterminate colitis determinable? *Curr Gastroenterol Rep* 2012; **14**: 162-165 [PMID: 22314810 DOI: 10.1007/s11894-012-0244-x]

6 **Blotière PO**, Weill A, Ricordeau P, Alla F, Allemand H. Perforations and haemorrhages after colonoscopy in 2010: a study based on comprehensive French health insurance data (SNIIRAM). *Clin Res Hepatol Gastroenterol* 2014; **38**: 112-117 [PMID: 24268997 DOI: 10.1016/j.clinre.2013.10.005]

7 **Jensen MD**, Ormstrup T, Vagn-Hansen C, Østergaard L, Rafaelsen SR. Interobserver and intermodality agreement for detection of small bowel Crohn's disease with MR enterography and CT enterography. *Inflamm Bowel Dis* 2011; **17**: 1081-1088 [PMID: 21484959 DOI: 10.1002/ibd.21534]

8 **Biomarkers Definitions Working Group.** Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001; **69**: 89-95 [PMID: 11240971 DOI: 10.1067/mcp.2001.113989]

9 **Darlington GJ**, Wilson DR, Lachman LB. Monocyte-conditioned medium, interleukin-1, and tumor necrosis factor stimulate the acute phase response in human hepatoma cells in vitro. *J Cell Biol* 1986; **103**: 787-793 [PMID: 3017995 DOI: 10.1083/jcb.103.3.787]

10 **Pepys MB**, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest* 2003; **111**: 1805-1812 [PMID: 12813013 DOI: 10.1172/JCI200318921]

11 **Florin TH**, Paterson EW, Fowler EV, Radford-Smith GL. Clinically active Crohn's disease in the presence of a low C-reactive protein. *Scand J Gastroenterol* 2006; **41**: 306-311 [PMID: 16497618 DOI: 10.1080/00365520500217118]

12 **Solem CA**, Loftus EV, Tremaine WJ, Harmsen WS, Zinsmeister AR, Sandborn WJ. Correlation of C-reactive protein with clinical, endoscopic, histologic, and radiographic activity in inflammatory bowel disease. *Inflamm Bowel Dis* 2005; **11**: 707-712 [PMID: 16043984 DOI: 10.1097/01.MIB.0000173271.18319.53]

13 **Gabay C**, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999; **340**: 448-454 [PMID: 9971870 DOI: 10.1056/NEJM199902113400607]

14 **Mendoza JL**, Abreu MT. Biological markers in inflammatory bowel disease: practical consideration for clinicians. *Gastroenterol Clin Biol* 2009; **33** Suppl 3: S158-S173 [PMID: 20117339 DOI: 10.1016/S0399-8320(09)73151-3]

15 **Yoon JY**, Park SJ, Hong SP, Kim TI, Kim WH, Cheon JH. Correlations of C-reactive protein levels and erythrocyte sedimentation rates with endoscopic activity indices in patients with ulcerative colitis. *Dig Dis Sci* 2014; **59**: 829-837 [PMID: 24352705 DOI: 10.1007/s10620-013-2907-3]

16 **Menees SB**, Powell C, Kurlander J, Goel A, Chey WD. A meta-analysis of the utility of C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin, and fecal lactoferrin to exclude inflammatory bowel disease in adults with IBS. *Am J Gastroenterol* 2015; **110**: 444-454 [PMID: 25732419 DOI: 10.1038/ajg.2015.6]

17 **Prideaux L**, De Cruz P, Ng SC, Kamm MA. Serological antibodies in inflammatory bowel disease: a systematic review. *Inflamm Bowel Dis* 2012; **18**: 1340-1355 [PMID: 22069240 DOI: 10.1002/ibd.21903]

18 **Joossens S**, Reinisch W, Vermeire S, Sendid B, Poulain D, Peeters M, Geboes K, Bossuyt X, Vandewalle P, Oberhuber G, Vogelsang H, Rutgeerts P, Colombel JF. The value of serologic markers in indeterminate colitis: a prospective follow-up study. *Gastroenterology* 2002; **122**: 1242-1247 [PMID: 11984510 DOI: 10.1053/gast.2002.32980]

19 **Bernstein CN**, El-Gabalawy H, Sargent M, Landers C, Rawsthorne P, Elias B, Targan SR. Assessing inflammatory bowel disease-associated antibodies in Caucasian and First Nations cohorts. *Can J Gastroenterol* 2011; **25**: 269-273 [PMID: 21647462]

20 **Sandborn WJ,** Landers CJ, Tremaine WJ, Targan SR. Association of antineutrophil cytoplasmic antibodies with resistance to treatment of left-sided ulcerative colitis: results of a pilot study. *Mayo Clin Proc* 1996; **71**: 431-436 [PMID: 8628021 DOI: 10.4065/71.5.431]

21 **Sendid B,** Colombel JF, Jacquinot PM, Faille C, Fruit J, Cortot A, Lucidarme D, Camus D, Poulain D. Specific antibody response to oligomannosidic epitopes in Crohn's disease. *Clin Diagn Lab Immunol* 1996; **3:** 219-226 [PMID: 8991640]

22 **Reese GE**, Constantinides VA, Simillis C, Darzi AW, Orchard TR, Fazio VW, Tekkis PP. Diagnostic precision of anti-Saccharomyces cerevisiae antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *Am J Gastroenterol* 2006; **101**: 2410-2422 [PMID: 16952282 DOI: 10.1111/j.1572-0241.2006.00840.x]

23 **Vandewalle-El Khoury P**, Colombel JF, Joossens S, Standaert-Vitse A, Collot M, Halfvarson J, Ayadi A, Landers CJ, Vermeire S, Rutgeerts P, Targan SR, Chamaillard M, Mallet JM, Sendid B, Poulain D. Detection of antisynthetic mannoside antibodies (ASigmaMA) reveals heterogeneity in the ASCA response of Crohn's disease patients and contributes to differential diagnosis, stratification, and prediction. *Am J Gastroenterol* 2008; **103**: 949-957 [PMID: 18047546 DOI: 10.1111/j.1572-0241.2007.01648.x]

24 **Poullis A**, Foster R, Mendall MA, Fagerhol MK. Emerging role of calprotectin in gastroenterology. *J Gastroenterol Hepatol* 2003; **18**: 756-762 [PMID: 12795745 DOI: 10.1046/j.1440-1746.2003.03014.x]

25 **Tibble JA**, Bjarnason I. Fecal calprotectin as an index of intestinal inflammation. *Drugs Today* (Barc) 2001; **37**: 85-96 [PMID: 12783101 DOI: 10.1358/dot.2001.37.2.614846]

26 **Boussac M**, Garin J. Calcium-dependent secretion in human neutrophils: a proteomic approach. *Electrophoresis* 2000; **21**: 665-672 [PMID: 10726775 DOI: 10.1002/(SICI)1522-2683(20000201)21: 3]

27 **Fagerhol MK**, Dale I, Andersson T. A radioimmunoassay for a granulocyte protein as a marker in studies on the turnover of such cells. *Bull Eur Physiopathol Respir* 1980; **16** Suppl: 273-282 [PMID: 7225633]

28 **von Roon AC**, Karamountzos L, Purkayastha S, Reese GE, Darzi AW, Teare JP, Paraskeva P, Tekkis PP. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am J Gastroenterol* 2007; **102**: 803-813 [PMID: 17324124 DOI: 10.1111/j.1572-0241.2007.01126.x]

29 **NICE**. Faecal calprotectin diagnostic tests for inflammatory diseases of the bowel. NICE Diagnostics Guidance 2013; **11**: 1– 58

30 **van Rheenen PF,** Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ* 2010; **341**: c3369. [PMID: 20634346 DOI: 10.1136/bmj.c3369]

31 **Pavlidis P**, Chedgy FJ, Tibble JA. Diagnostic accuracy and clinical application of faecal calprotectin in adult patients presenting with gastrointestinal symptoms in primary care. *Scand J Gastroenterol* 2013; **48**: 1048-1054 [PMID: 23883068 DOI: 10.3109/00365521.2013.816771]

32 **Kane SV**, Sandborn WJ, Rufo PA, Zholudev A, Boone J, Lyerly D, Camilleri M, Hanauer SB. Fecal lactoferrin is a sensitive and specific marker in identifying intestinal inflammation. *Am J Gastroenterol* 2003; **98**: 1309-1314 [PMID: 12818275 DOI: 10.1111/j.1572-0241.2003.07458.x]

33 **Desai D,** Faubion WA, Sandborn WJ. Review article: biological activity markers in inflammatory bowel disease. *Aliment Pharmacol Ther* 2007; **25**: 247-55 [PMID: 17217454 DOI: 10.1111/j.1365-2036.2006.03184.x]

34 **Dai J**, Liu WZ, Zhao YP, Hu YB, Ge ZZ. Relationship between fecal lactoferrin and inflammatory bowel disease. *Scand J Gastroenterol* 2007; **42**: 1440-1444 [PMID: 17852860 DOI: 10.1080/00365520701427094]

35 **Sidhu R**, Sanders DS, Wilson P, Foye L, Morley S, McAlindon ME. Faecal lactoferrin, capsule endoscopy and Crohn's disease. Is there a three way relationship? A pilot study. *J Gastrointestin Liver Dis* 2010; **19**: 257-260 [PMID: 20922188]

36 **Nancey S**, Boschetti G, Moussata D, Cotte E, Peyras J, Cuerq C, Haybrard J, Charlois AL, Mialon A, Chauvenet M, Stroeymeyt K, Kaiserlian D, Drai J, Flourié B. Neopterin is a novel reliable fecal marker as accurate as calprotectin for predicting endoscopic disease activity in patients with inflammatory bowel diseases. *Inflamm Bowel Dis* 2013; **19**: 1043-1052 [PMID: 23511035 DOI: 10.1097/MIB.0b013e3182807577]

37 **Langhorst J**, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. *Am J Gastroenterol* 2008; **103**: 162-169 [PMID: 17916108 DOI: 10.1111/j.1572-0241.2007.01556.x]

38 **van de Logt F**, Day AS. S100A12: a noninvasive marker of inflammation in inflammatory bowel disease. *J Dig Dis* 2013; **14**: 62-67 [PMID: 23146044 DOI: 10.1111/1751-2980.12012]

39 **Kaiser T**, Langhorst J, Wittkowski H, Becker K, Friedrich AW, Rueffer A, Dobos GJ, Roth J, Foell D. Faecal S100A12 as a non-invasive marker distinguishing inflammatory bowel disease from irritable bowel syndrome. *Gut* 2007; **56**: 1706-1713 [PMID: 17675327 DOI: 10.1136/gut.2006.113431]

40 **Landers CJ**, Cohavy O, Misra R, Yang H, Lin YC, Braun J, Targan SR. Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto- and microbial antigens. *Gastroenterology* 2002; **123**: 689-699 [PMID: 12198693 DOI: 10.1053/gast.2002.35379]

41 **Papp M**, Norman GL, Altorjay I, Lakatos PL. Utility of serological markers in inflammatory bowel diseases: gadget or magic? *World J Gastroenterol* 2007; **13**: 2028-2036 [PMID: 17465443 DOI: 10.3748/wjg.v13.i14.2028]

42 **Lodes MJ**, Cong Y, Elson CO, Mohamath R, Landers CJ, Targan SR, Fort M, Hershberg RM. Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest* 2004; **113**: 1296-1306 [PMID: 15124021 DOI: 10.1172/JCI200420295]

43 **Targan SR**, Landers CJ, Yang H, Lodes MJ, Cong Y, Papadakis KA, Vasiliauskas E, Elson CO, Hershberg RM. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterology* 2005; **128**: 2020-2028 [PMID: 15940634 DOI: 10.1053/j.gastro.2005.03.046]

44 **Schoepfer AM**, Schaffer T, Mueller S, Flogerzi B, Vassella E, Seibold-Schmid B, Seibold F. Phenotypic associations of Crohn's disease with antibodies to flagellins A4-Fla2 and Fla-X, ASCA, p-ANCA, PAB, and NOD2 mutations in a Swiss Cohort. *Inflamm Bowel Dis* 2009; **15**: 1358-1367 [PMID: 19253375 DOI: 10.1002/ibd.20892]

45 **Wei B**, Huang T, Dalwadi H, Sutton CL, Bruckner D, Braun J. Pseudomonas fluorescens encodes the Crohn's disease-associated I2 sequence and T-cell superantigen. *Infect Immun* 2002; **70**: 6567-6575 [PMID: 12438326 DOI: 10.1128/IAI.70.12.6567-6575.2002]

46 **Bossuyt X**. Serologic markers in inflammatory bowel disease. *Clin Chem* 2006; **52**: 171-181 [PMID: 16339302 DOI: 10.1373/clinchem.2005.058560]

47 **Dotan I**, Fishman S, Dgani Y, Schwartz M, Karban A, Lerner A, Weishauss O, Spector L, Shtevi A, Altstock RT, Dotan N, Halpern Z. Antibodies against laminaribioside and chitobioside are novel serologic markers in Crohn's disease. *Gastroenterology* 2006; **131**: 366-378 [PMID: 16890590 DOI: 10.1053/j.gastro.2006.04.030]

48 **Ferrante M**, Henckaerts L, Joossens M, Pierik M, Joossens S, Dotan N, Norman GL, Altstock RT, Van Steen K, Rutgeerts P, Van Assche G, Vermeire S. New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. *Gut* 2007; **56**: 1394-1403 [PMID: 17456509 DOI: 10.1136/gut.2006.108043]

49 **Simondi D**, Mengozzi G, Betteto S, Bonardi R, Ghignone RP, Fagoonee S, Pellicano R, Sguazzini C, Pagni R, Rizzetto M, Astegiano M. Antiglycan antibodies as serological markers in the differential diagnosis of inflammatory bowel disease. *Inflamm Bowel Dis* 2008; **14**: 645-651 [PMID: 18240283 DOI: 10.1002/ibd.20368]

50 **Papp M**, Altorjay I, Dotan N, Palatka K, Foldi I, Tumpek J, Sipka S, Udvardy M, Dinya T, Lakatos L, Kovacs A, Molnar T, Tulassay Z, Miheller P, Norman GL, Szamosi T, Papp J, Lakatos PL. New serological markers for inflammatory bowel disease are associated with earlier age at onset, complicated disease behavior, risk for surgery, and NOD2/CARD15 genotype in a Hungarian IBD cohort. *Am J Gastroenterol* 2008; **103**: 665-681 [PMID: 18047543 DOI: 10.1111/j.1572-0241.2007.01652.x]

51 **Lakatos PL**, Papp M, Rieder F. Serologic antiglycan antibodies in inflammatory bowel disease. *Am J Gastroenterol* 2011; **106**: 406-412 [PMID: 21245832]

52 **Seow CH**, Stempak JM, Xu W, Lan H, Griffiths AM, Greenberg GR, Steinhart AH, Dotan N, Silverberg MS. Novel anti-glycan antibodies related to inflammatory bowel disease diagnosis and phenotype. *Am J Gastroenterol* 2009; **104**: 1426-1434 [PMID: 19491856 DOI: 10.1038/ajg.2009.79]

53 **Rieder F**, Schleder S, Wolf A, Dirmeier A, Strauch U, Obermeier F, Lopez R, Spector L, Fire E, Yarden J, Rogler G, Dotan N, Klebl F. Association of the novel serologic anti-glycan antibodies anti-laminarin and anti-chitin with complicated Crohn's disease behavior. *Inflamm Bowel Dis* 2010; **16**: 263-274 [PMID: 19653286 DOI: 10.1002/ibd.21046]

54 **Klebl FH**, Bataille F, Huy C, Hofstädter F, Schölmerich J, Rogler G. Association of antibodies to exocrine pancreas with subtypes of Crohn's disease. *Eur J Gastroenterol Hepatol* 2005; **17**: 73-77 [PMID: 15647645]

55 **Joossens S**, Vermeire S, Van Steen K, Godefridis G, Claessens G, Pierik M, Vlietinck R, Aerts R, Rutgeerts P, Bossuyt X. Pancreatic autoantibodies in inflammatory bowel disease. *Inflamm Bowel Dis* 2004; **10**: 771-777 [PMID: 15626896]

56 **Roggenbuck D**, Hausdorf G, Martinez-Gamboa L, Reinhold D, Büttner T, Jungblut PR, Porstmann T, Laass MW, Henker J, Büning C, Feist E, Conrad K. Identification of GP2, the major zymogen granule membrane glycoprotein, as the autoantigen of pancreatic antibodies in Crohn's disease. *Gut* 2009; **58**: 1620-1628 [PMID: 19549613 DOI: 10.1136/gut.2008.162495]

57 **Pavlidis P**, Shums Z, Koutsoumpas AL, Milo J, Papp M, Umemura T, Lakatos PL, Smyk DS, Bogdanos DP, Forbes A, Norman GL. Diagnostic and clinical significance of Crohn's disease-specific anti-MZGP2 pancreatic antibodies by a novel ELISA. *Clin Chim Acta* 2015; **441**: 176-181 [PMID: 25512163 DOI: 10.1016/j.cca.2014.12.010]

58 **Soendergaard C**, Nielsen OH, Seidelin JB, Kvist PH, Bjerrum JT. Alpha-1 antitrypsin and granulocyte colony-stimulating factor as serum biomarkers of disease severity in ulcerative colitis. *Inflamm Bowel Dis* 2015; **21**: 1077-1088 [PMID: 25803506 DOI: 10.1097/MIB.00000000000000348]

59 **Ellinghaus D**, Zhang H, Zeissig S, Lipinski S, Till A, Jiang T, Stade B, Bromberg Y, Ellinghaus E, Keller A, Rivas MA, Skieceviciene J, Doncheva NT, Liu X, Liu Q, Jiang F, Forster M, Mayr G, Albrecht M, Häsler R, Boehm BO, Goodall J, Berzuini CR, Lee J, Andersen V, Vogel U, Kupcinskas L, Kayser M, Krawczak M, Nikolaus S, Weersma RK, Ponsioen CY, Sans M, Wijmenga C, Strachan DP, McArdle WL, Vermeire S, Rutgeerts P, Sanderson JD, Mathew CG, Vatn MH, Wang J, Nöthen MM, Duerr RH, Büning C, Brand S, Glas J, Winkelmann J, Illig T, Latiano A, Annese V, Halfvarson J, D'Amato M, Daly MJ, Nothnagel M, Karlsen TH, Subramani S, Rosenstiel P, Schreiber S, Parkes M, Franke A. Association between variants of PRDM1 and NDP52 and Crohn's disease, based on exome sequencing and functional studies. *Gastroenterology* 2013; **145**: 339-347 [PMID: 23624108 DOI: 10.1053/j.gastro.2013.04.040]

60 **Cleynen I**, González JR, Figueroa C, Franke A, McGovern D, Bortlík M, Crusius BJ, Vecchi M, Artieda M, Szczypiorska M, Bethge J, Arteta D, Ayala E, Danese S, van Hogezand RA, Panés J, Peña SA, Lukas M, Jewell DP, Schreiber S, Vermeire S, Sans M. Genetic factors conferring an increased susceptibility to develop Crohn's disease also influence disease phenotype: results from the IBDchip European Project. *Gut* 2013; **62**: 1556-1565 [PMID: 23263249 DOI: 10.1136/gutjnl-2011-300777]

61 **Jostins L,** Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, Lee JC, Schumm LP, Sharma Y, Anderson CA, Essers J, Mitrovic M, Ning K, Cleynen I, Theatre E, Spain SL, Raychaudhuri S, Goyette P, Wei Z, Abraham C, Achkar JP, Ahmad T, Amininejad L, Ananthakrishnan AN, Andersen V, Andrews JM, Baidoo L, Balschun T, Bampton PA, Bitton A, Boucher G, Brand S, Büning C, Cohain A, Cichon S, D’ Amato M, De Jong D, Devaney KL, Dubinsky M, Edwards C, Ellinghaus D, Ferguson LR, Franchimont D, Fransen K, Gearry R, Georges M, Gieger C, Glas J, Haritunians T, Hart A, Hawkey C, Hedl M, Hu X, Karlsen TH, Kupcinskas L, Kugathasan S, Latiano A, Laukens D, Lawrance IC, Lees CW, Louis E, Mahy G, Mansfield J, Morgan AR, Mowat C, Newman W, Palmieri O, Ponsioen CY, Potocnik U, Prescott NJ, Regueiro M, Rotter JI, Russell RK, Sanderson JD, Sans M, Satsangi J, Schreiber S, Simms LA, Sventoraityte J, Targan SR, Taylor KD, Tremelling M, Verspaget HW, De Vos M, Wijmenga C, Wilson DC, Winkelmann J, Xavier RJ, Zeissig S, Zhang B, Zhang CK, Zhao H; International IBD Genetics Consortium (IIBDGC), Silverberg MS, Annese V, Hakonarson H, Brant SR, Radford-Smith G, Mathew CG, Rioux JD, Schadt EE, Daly MJ, Franke A, Parkes M, Vermeire S, Barrett JC, Cho JH. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012; **491**: 119-124 [PMID: 23128233 DOI: 10.1038/nature11582]

62 **Brand S**. Crohn's disease: Th1, Th17 or both? The change of a paradigm: new immunological and genetic insights implicate Th17 cells in the pathogenesis of Crohn's disease. *Gut* 2009; **58**: 1152-1167 [PMID: 19592695 DOI: 10.1136/gut.2008.163667]

63 **von Stein P,** Lofberg R, Kuznetsov NV, Gielen AW, Persson JO, Sundberg R, Hellstrom K, Eriksson A, Befrits R, Ost A, von Stein OD. Multigene analysis can discriminate between ulcerative colitis, Crohn’s disease, and irritable bowel syndrome. *Gastroenterology* 2008; **134**: 1869-1881; quiz 2153-2154 [PMID: 18466904 DOI: 10.1053/j.gastro.2008.02.083]

64 **Janczewska I**, Kapraali M, Saboonchi F, Nekzada Q, Wessulv Å, Khoshkar J, Marouf F, Gorsetman J, Risberg D, Lissing M, Wirström G, Sandstedt B. Clinical application of the multigene analysis test in discriminating between ulcerative colitis and Crohn's disease: a retrospective study. *Scand J Gastroenterol* 2012; **47**: 162-169 [PMID: 22229803 DOI: 10.3109/00365521.2011.647065]

65 **O'Connell RM**, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol* 2010; **10**: 111-122 [PMID: 20098459 DOI: 10.1038/nri2708]

66 **Dalal SR**, Kwon JH. The Role of MicroRNA in Inflammatory Bowel Disease. *Gastroenterol Hepatol (N Y)* 2010; **6**: 714-722 [PMID: 21437020]

67 **Wu F**, Guo NJ, Tian H, Marohn M, Gearhart S, Bayless TM, Brant SR, Kwon JH. Peripheral blood microRNAs distinguish active ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis* 2011; **17**: 241-250 [PMID: 20812331 DOI: 10.1002/ibd.21450]

68 **Zahm AM**, Thayu M, Hand NJ, Horner A, Leonard MB, Friedman JR. Circulating microRNA is a biomarker of pediatric Crohn disease. *J Pediatr Gastroenterol Nutr* 2011; **53**: 26-33 [PMID: 21546856 DOI: 10.1097/MPG.0b013e31822200c]

69 **Schaefer JS**, Attumi T, Opekun AR, Abraham B, Hou J, Shelby H, Graham DY, Streckfus C, Klein JR. MicroRNA signatures differentiate Crohn's disease from ulcerative colitis. *BMC Immunol* 2015; **16**: 5 [PMID: 25886994 DOI: 10.1186/s12865-015-0069-0]

70 **Jansson J**, Willing B, Lucio M, Fekete A, Dicksved J, Halfvarson J, Tysk C, Schmitt-Kopplin P. Metabolomics reveals metabolic biomarkers of Crohn's disease. *PLoS One* 2009; **4**: e6386 [PMID: 19636438 DOI: 10.1371/journal.pone.0006386]

71 **Zhang X**, Choi FF, Zhou Y, Leung FP, Tan S, Lin S, Xu H, Jia W, Sung JJ, Cai Z, Bian Z. Metabolite profiling of plasma and urine from rats with TNBS-induced acute colitis using UPLC-ESI-QTOF-MS-based metabonomics--a pilot study. *FEBS J* 2012; **279**: 2322-2338 [PMID: 22520047 DOI: 10.1111/j.1742-4658.2012.08612.x]

72 **Schicho R**, Shaykhutdinov R, Ngo J, Nazyrova A, Schneider C, Panaccione R, Kaplan GG, Vogel HJ, Storr M. Quantitative metabolomic profiling of serum, plasma, and urine by (1)H NMR spectroscopy discriminates between patients with inflammatory bowel disease and healthy individuals. *J Proteome Res* 2012; **11**: 3344-3357 [PMID: 22574726 DOI: 10.1021/pr300139q]

73 **Williams HR**, Willsmore JD, Cox IJ, Walker DG, Cobbold JF, Taylor-Robinson SD, Orchard TR. Serum metabolic profiling in inflammatory bowel disease. *Dig Dis Sci* 2012; **57**: 2157-2165 [PMID: 22488632 DOI: 10.1007/s10620-012-2127-2]

74 **Ackerman AB**, Milde P. Naming acquired melanocytic nevi. Common and dysplastic, normal and atypical, or Unna, Miescher, Spitz, and Clark? *Am J Dermatopathol* 1992; **14**: 447-453 [PMID: 1415964]

75 **Takaishi H**, Matsuki T, Nakazawa A, Takada T, Kado S, Asahara T, Kamada N, Sakuraba A, Yajima T, Higuchi H, Inoue N, Ogata H, Iwao Y, Nomoto K, Tanaka R, Hibi T. Imbalance in intestinal microflora constitution could be involved in the pathogenesis of inflammatory bowel disease. *Int J Med Microbiol* 2008; **298**: 463-472 [PMID: 17897884]

76 **Williams HR,** Cox IJ, Walker DG, North BV, Patel VM, Marshall SE, Jewell DP, Gjosh S, Thomas HJ, Teare JP. Jakobovits S, Zeki S, Welsh KI, Taylor-Robinon SD, Orchard TR. Characterization of inflammatory bowel disease with urinary metabolic profiling. *Am J Gastroenterol* 2009; **104**: 1435–1444 [PMID: 19491857 DOI: 10.1038/ajg.2009.175]

77 **Stephens NS**, Siffledeen J, Su X, Murdoch TB, Fedorak RN, Slupsky CM. Urinary NMR metabolomic profiles discriminate inflammatory bowel disease from healthy. *J Crohns Colitis* 2013; **7**: e42-e48 [PMID: 22626506 DOI: 10.1016/j.crohns.]

78 **Ooi M**, Nishiumi S, Yoshie T, Shiomi Y, Kohashi M, Fukunaga K, Nakamura S, Matsumoto T, Hatano N, Shinohara M, Irino Y, Takenawa T, Azuma T, Yoshida M. GC/MS-based profiling of amino acids and TCA cycle-related molecules in ulcerative colitis. *Inflamm Res* 2011; **60**: 831-840 [PMID: 21523508 DOI: 10.1007/s00011-011-0340-7]

79 **Hisamatsu T**, Okamoto S, Hashimoto M, Muramatsu T, Andou A, Uo M, Kitazume MT, Matsuoka K, Yajima T, Inoue N, Kanai T, Ogata H, Iwao Y, Yamakado M, Sakai R, Ono N, Ando T, Suzuki M, Hibi T. Novel, objective, multivariate biomarkers composed of plasma amino acid profiles for the diagnosis and assessment of inflammatory bowel disease. *PLoS One* 2012; **7**: e31131 [PMID: 22303484 DOI: 10.1371/journal.pone.0031131]

80 **Marchesi JR**, Holmes E, Khan F, Kochhar S, Scanlan P, Shanahan F, Wilson ID, Wang Y. Rapid and noninvasive metabonomic characterization of inflammatory bowel disease. *J Proteome Res* 2007; **6**: 546-551 [PMID: 17269711]

81 **Le Gall G**, Noor SO, Ridgway K, Scovell L, Jamieson C, Johnson IT, Colquhoun IJ, Kemsley EK, Narbad A. Metabolomics of fecal extracts detects altered metabolic activity of gut microbiota in ulcerative colitis and irritable bowel syndrome. *J Proteome Res* 2011; **10**: 4208-4218 [PMID: 21761941 DOI: 10.1021/pr2003598]

82 **Kurada S**, Alkhouri N, Fiocchi C, Dweik R, Rieder F. Review article: breath analysis in inflammatory bowel diseases. *Aliment Pharmacol Ther* 2015; **41**: 329-341 [PMID: 25523187 DOI: 10.1111/apt.13050]

83 **Dryahina K**, Španěl P, Pospíšilová V, Sovová K, Hrdlička L, Machková N, Lukáš M, Smith D. Quantification of pentane in exhaled breath, a potential biomarker of bowel disease, using selected ion flow tube mass spectrometry. *Rapid Commun Mass Spectrom* 2013; **27**: 1983-1992 [PMID: 23939966 DOI: 10.1002/rcm.6660]

84 **Pelli MA**, Trovarelli G, Capodicasa E, De Medio GE, Bassotti G. Breath alkanes determination in ulcerative colitis and Crohn's disease. *Dis Colon Rectum* 1999; **42**: 71-76 [PMID: 10211523]

85 **Koek GH**, Verleden GM, Evenepoel P, Rutgeerts P. Activity related increase of exhaled nitric oxide in Crohn's disease and ulcerative colitis: a manifestation of systemic involvement? *Respir Med* 2002; **96**: 530-535 [PMID: 12194639]

86 **Kokoszka J**, Nelson RL, Swedler WI, Skosey J, Abcarian H. Determination of inflammatory bowel disease activity by breath pentane analysis. *Dis Colon Rectum* 1993; **36**: 597-601 [PMID: 8500379]

87 **Sedghi S**, Keshavarzian A, Klamut M, Eiznhamer D, Zarling EJ. Elevated breath ethane levels in active ulcerative colitis: evidence for excessive lipid peroxidation. *Am J Gastroenterol* 1994; **89**: 2217-2221 [PMID: 7977245]

88 **Meuwis MA**, Fillet M, Geurts P, de Seny D, Lutteri L, Chapelle JP, Bours V, Wehenkel L, Belaiche J, Malaise M, Louis E, Merville MP. Biomarker discovery for inflammatory bowel disease, using proteomic serum profiling. *Biochem Pharmacol* 2007; **73**: 1422-1433 [PMID: 17258689]

89 **Viennois E,** Baker MT, Xiao B, Wang L, Laroui H, Merlin D. Longitudinal study of circulating protein biomarkers in inflammatory bowel disease. *J Proteomic* 2015; **112**: 166-179 [PMID: 25230104 DOI: 10.1016/j.jprot.2014.09.002]

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