Literature Review

A Review of Breath Metabolic Profiling for Non-invasive Testing in Inflammatory Bowel Disease Patients.

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160 Character summary: This review summarises the current literature on volatile organic molecule breath testing as a potential avenue for safer and more reliable diagnosis and monitoring of inflammatory bowel disease.

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Abstract

This review aims to summarise the current literature on employing exhaled breath volatile organic molecules (VOMs) as novel biomarkers for non-invasive testing in inflammatory bowel disease (IBD) patients.

Inflammatory bowel disease is a multifactorial disease that significantly diminishes the quality of life of affected individuals. Currently, the tools employed in IBD diagnosis and monitoring are numerous, imprecise and invasive for patients. This has necessitated the need to develop new biomarkers that are accurate. The use of VOM breath testing is one such potential modality. This review discusses the efficacy of current IBD testing modalities and the principles of metabolic profiling. It evaluates the use of breath VOM profiling in IBD testing and postulates its implications for future practice. The VOM profiles of IBD patients are different to those of healthy individuals. VOM profiles also differ between IBD subcategories and correlate to disease severity. VOM profiling via the breath headspace is accurate, non-invasive and has the potential for point-of-care testing. VOM profiling offers an exciting avenue as a frontline diagnostic and monitoring tool for IBD patients and thus merits further research.

Introduction

Inflammatory bowel disease (IBD) is a chronic, relapsing inflammatory condition of the gastrointestinal tract, comprising of two subcategories: Crohn's disease (CD) and ulcerative colitis (UC) [1,2]. While both forms have commonalities in their clinical presentation, mainly abdominal pain, diarrhoea and weight loss, CD and UC can be differentiated by their histological features and extent of colonic involvement. CD can affect the entirety of the gastrointestinal tract and is characterised by discontinuous transmural lesions. In UC, inflammation is continuous, affecting only the superficial mucosal layer of the large intestine [3,4].

The pathophysiology of IBD is largely elusive. It is thought to arise from an inappropriately heightened mucosal immunological response to environmental stimuli in individuals who are genetically predisposed to the condition [5,6].

Currently, there are no gold standards for IBD diagnostic and monitoring tools [7]. Traditional approaches to diagnosis involve eliciting a detailed patient history along with physical examination, serology, faecal biomarker investigations, imaging studies, endoscopic investigations and histology [8]. This regime is substandard for patients due to its invasive nature. Furthermore, there are often high rates of misdiagnosis, delayed diagnosis and incorrect sub-categorization [8,9].

The highly debilitating nature of IBD, increasing incidence and subsequent healthcare expenditure inflation necessitates the development of new diagnostic and monitoring modalities [2,3]. One such modality is the use of metabolic profiling. This involves the analysis of volatile organic molecules (VOMs) using urine, stool and exhaled breath samples [6]. There is surmounting evidence that metabolic profiling can be used to successfully diagnose conditions, in which there is increased oxidative stress. The emerging technology has currently been employed in the diagnosis of breast cancer, lung cancer, diabetes, and tuberculosis, so it offers great potential as a diagnostic modality for gastrointestinal diseases [9].

This review will first focus on current clinical practice before evaluating the use of exhaled VOMs as novel biomarkers for IBD diagnosis and management.

Current efficacy of IBD diagnostics

Physicians often face difficulty in diagnosing IBD based on clinical presentation alone, given that symptoms of IBD can overlap with those for irritable bowel syndrome (IBS) [1]. Consequently, few patients being referred for endoscopic evaluation have IBD [10], with up to 50% of patients with IBS being referred for unnecessary endoscopic evaluation [11]. To complicate things further, IBD diagnosis is often missed or delayed. Since timely treatment is critical to halt disease progression, a mean diagnostic delay of nine months places IBD patients at a greater risk of surgical intervention [12]. Once a diagnosis of IBD has been made, sub-categorisation into CD or UC can pose another challenge for physicians, with subcategory reclassification rates of 10% [7]. In 10-15% of patients, sub-categorisation is indiscernible, which results in an undifferentiated diagnosis of indeterminate colitis [7,13]. Accurate sub-categorisation is essential in establishing a prognosis, evaluating a patient's risk of complications and implementing optimal management strategies [6,7]. Incorrect diagnosis can lead to diminished quality of life and places a heavy burden on the healthcare system [14]. This underpins the need for a non-invasive and accessible tool to prioritise patients for colonoscopy and reduce unnecessary investigations in those with IBS.

Current diagnostic and monitoring tools in clinical practice

Endoscopic investigations and subsequent histological findings are most often used in clinical practice to diagnose and monitor disease activity in patients with IBD. Colonoscopies are favourable for diagnosis as physicians are able to simultaneously diagnose IBD via biopsy, investigate its complications and remove colonic polyps. For the patient, these investigations are invasive due to bowel purgation and the need for anaesthetic. As such, frequent endoscopic evaluations are not suitable for monitoring disease activity [15]. Furthermore, endoscopic procedures do not come without risk [7]. Gastrointestinal perforation occurs in 4.5-9.7 cases per 10,000 patients during such investigations [16].

Radiological assessment is often used in IBD diagnosis and monitoring [7]. While CT scanning is accessible and minimally invasive, it exposes a typically young cohort of patients to ionizing radiation. As such, repeated scanning to monitor disease activity should be avoided. The quality of imaging can be limited by the intraluminal localisation of contrast. Likewise, ultrasound is dependent upon sonographer experience as it is difficult to follow the entirety of the intestines [5]. MR enterography is often used to monitor disease activity and response to treatment for IBD patients. It is favourable in that it gives physicians a full transmural view of the bowel wall, as well as extra-intestinal complications, and does not expose patients to radiation; however, it is problematic for patients who are claustrophobic and risky in those with renal insufficiency due to the need for large volumes of contrast [17].

Active periods of IBD are accompanied by an immune response that is detectable by blood-based biomarkers, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). These serological tests can be used to confirm diagnosis and for monitoring response to treatment. They are cheap, rapid and easily accessible [2]. CRP is produced by hepatocytes in response to pro-inflammatory cytokines and its short half-life makes it a responsive indicator of acute inflammation [18]. Being a systemic marker of inflammation, CRP is non-specific and can rise in response to a range of inflammatory conditions [1,9]. Conversely, up to 50% of patients with endoscopically active IBD do not have an elevated CRP level [19]. Since CRP only rises during active inflammation, diagnosis can be missed in those with quiescent IBD [11]. ESR, another systemic inflammatory indicator, rises due to an increase in

plasma viscosity as a result of acute phase protein generation. ESR peaks later than CRP and is more indicative of chronic inflammation. It is, unfortunately, equally non-specific and can be affected by haematocrit, reducing its accuracy [18].

Stool samples can be analysed for white cell proteins [9]. They are cost-effective investigations and highly specific for inflammatory conditions localised to the bowel [19]. Classified as a danger-associated molecular pattern, faecal calprotectin is a protein derived from neutrophils, monocytes and macrophages during inflammation [18]. It is raised in IBD and sensitive for differentiating IBD from healthy controls, but it can be raised in gastrointestinal infections and as a result of non-steroidal anti-inflammatory drug use [1]. Notably, 8% of patients receive false negative results and not all IBD patients have raised faecal calprotectin [11]. Another stool-based biomarker, faecal lactoferrin, an iron-binding protein released upon neutrophil degranulation, is also increased in IBD. Reluctance of patients to provide samples, storage of faeces outside of optimal temperature, high intra-individual variability and false positive results reduce its effectiveness as a diagnostic and monitoring tool [1].

The principles of metabolic profiling

Volatile organic molecules are disease-specific gas phase biomarkers that characterise the interactions of colonocytes with intestinal microbiota and pathogens [8]. While VOM profiling is in its early stages of development, it is clear that the VOM profiles of IBD patients are different from those of healthy individuals. This is thought to result from the associated alterations in metabolic processes, inflammatory changes and microbial dysbiosis of IBD [14,16]. Profiles also differ between IBD subcategories, with many VOMs being upregulated in CD, allowing for subcategory classification. This most likely reflects the transmural nature and potentially greater extent of inflammation associated with CD [14]. There is also evidence that these biomarkers differ during periods of active disease and remission in patients with previously diagnosed IBD.

Intestinal VOMs can be excreted in faeces and urine or transported via the bloodstream into the lungs and exhaled in breath [3]. VOMs are identified by gas chromatography or via a selective ion flow tube and measured by mass spectrometry or flame ionisation [7]. While VOMs can be measured from all three samples, patients are often reluctant to provide urine or stool samples and collection may not be feasible in patients experiencing diarrhoeal symptoms [6]. Testing is also highly dependent upon sample storage, since temperature and contamination can compromise sample quality [1,2]. Metabolic profiling via breath samples is advantageous due to many factors (Table 1). Breath samples can be obtained spontaneously via a non-invasive process that is comfortable and convenient for patients and where storage issues are non-existent. Other advantages of using breath samples is that patients are not exposed to ionising radiation and bowel preparation or contrast is not required [6]. As such, breath testing offers a more patient-centred approach to IBD diagnosis and monitoring. It is more likely to be accepted by patients, improving compliance and satisfaction, as well as generating more accurate results than other VOM profiling headspaces. For these reasons, breath samples offer the most promising avenue of metabolic profiling for IBD diagnosis and will be the focus of this review.

Distinguishing IBD patients from healthy controls

The most widely studied exhaled VOMs are alkanes. These include pentane, butane, ethane, and propane. Like many VOMs, alkanes are produced as a result of excessive reactive oxygen species-induced lipid peroxidation during an inflammatory response [1,14]. A study led by Pelli et al. [20] revealed significantly increased ethane, propane and pentane concentrations in the exhaled breath of IBD patients compared to healthy controls. Another breath analysis of 487 VOMs showed that increased exhaled alkanes were unique to IBD patients. In this study CD active individuals and healthy controls were differentiated with a sensitivity of 96% and a specificity of 97%. Heptadecane was upregulated in CD groups compared to healthy controls, 2,2,4-trimethylpentane was higher in CD active groups compared to CD remission and healthy controls and 2,2,4,4-tetramethyloctane and 2,4,4-trimethylhexane were higher in active CD than remission. This study also implicated aldehydes as a potential VOM biomarker for IBD diagnosis. It found that breath samples of IBD patients contained higher aldehyde concentrations than healthy controls [1]. This is consistent with results from Hicks where exhaled butanal and nonanal were increased in both CD and UC, with CD patients having the highest concentrations across all cohorts [6]. Patel et al.'s [2] study did find, however, that hydrogen sulphide concentrations were reduced in IBD patient breath samples compared to healthy controls, with significance in a paediatric population. These results were consistent with studies on adult IBD populations by Hicks et al. and Reider et al. [6,12]. However, since hydrogen sulphide is produced by the intestinal microbiota, it may not be suitable to differentiate between IBD and IBS, limiting its use [6].

Distinguishing IBD patients from IBS patients

Condensed cytokines, namely interleukins, such as IL-1B, IL-6, IL-8, and tumour necrosis factor alpha (TNF-a) were in greater abundance in the exhaled breath of IBD patients compared to healthy controls [21]. Since immune cells release these pro-inflammatory cytokines during intestinal inflammation, cytokine profiling is a potential avenue for IBD diagnosis, particularly when distinguishing IBD from non-inflammatory conditions such as IBS. A 2017 study showed that an IL-1B and a TNF-like cytokine were increased in IBD patients compared to IBS patients, distinguishing them with a sensitivity of 50% and specificity of 80%. [22]

Distinguishing CD patients from UC patients

Dryahina et al. [3] showed significant differences between the exhaled pentane concentrations of CD and UC patients, with CD patients having much higher readings, although sensitivity and specificity were low. Patel et al. [2] showed that the more widespread the intestinal inflammation of IBD patients, the higher exhaled pentane concentration. They found that exhaled pentane levels were higher in CD patients with known ileocolonic disease, and thus more extensive inflammation, compared to UC. As such, it can be said that exhaled pentane has the potential to offer greater clinical value as a biomarker to diagnose IBD, differentiate between CD and UC, and determine disease location in patients with known CD. However, the only known study on paediatric breath testing shows that these correlations are not significant in children [2]. Further studies are needed to establish the efficacy of pentane as a biomarker in paediatric IBD diagnosis.

Use in IBD management

The use of metabolic profiling is not limited to IBD diagnosis. Pentane has been found to correlate with the severity of inflammation seen in IBD. IBD patients with severe intestinal inflammation was confirmed by imaging studies showed much greater exhaled pentane

concentrations (4.3 nmol/L) than those with moderate inflammation (3.1 nmol/L) and than those with absent inflammation (2.1 nmol/L) [23]. It has also been demonstrated that VOM levels normalise following treatment and where remission is achieved. Walton's [24] multivariate analysis on faecal VOMs of bacterial origin found that, following therapy, IBD VOM profiles normalised to levels found in healthy controls. As such, metabolic profiling is a promising individualised and non-invasive approach of mapping disease activity and monitoring responsiveness to treatment. Future studies should be pursued in this context, as metabolic profiling of breath samples would provide great clinical value in facilitating the management of IBD patients, leading to better health outcomes for these individuals.

Implications for future practice

The use of VOMs as an investigative modality for IBD has the potential to relieve the burdens associated with current diagnostic and monitoring regimes. While metabolic profiling should not replace current diagnostic modalities, it may have a role in distinguishing IBS from IBD, thus prioritising patients for endoscopic evaluation, speeding up the diagnostic process and reducing the number of unnecessary colonoscopies currently seen in clinical practice. It may also offer an alternative to current monitoring tools in patients with established IBD. However, despite surmounting evidence that metabolic profiling can be used to distinguish healthy adults and IBS patients from those with IBD, its use is limited by minimal studies (Table 1). It is unclear whether metabolic profiling can be applied to a paediatric cohort. Furthermore, it has not been fully established whether it can provide sufficient discrimination between CD and UC. It has not been fully established if VOM profiles can accurately follow disease progress. Limited studies report the effects of antibiotics and/or other drugs or having other inflammatory conditions on VOM profiles. Currently, traditional VOM testing modalities cannot be employed at the bedside. Further research of VOMs is needed and may lead to the development of timely and non-invasive point-of-care testing for all patients. A 2015 preliminary study utilised a field asymmetric ion mobility spectroscopy (FAIMS) portable device to detect VOMs in the exhaled breath of 76 patients. Patients with IBD were distinguished from healthy controls with a sensitivity and specificity of 74% and 75%, respectively, while differentiating between CD and UC with a specificity and sensitivity of 67%. Since FAIMS technology is 10-20% of the cost of traditional VOM testing devices, the study manifests the potential of this technology to provide much needed non-invasive point-of-care bedside testing for IBD patients [8].

Conclusion

Inflammatory bowel disease is a multifactorial, debilitating disease of the gastrointestinal tract. Current tools employed in IBD diagnostics and monitoring are substandard with notable limitations, placing a heavy physiological and psychological burden on patients. Analysing the VOM composition in the exhaled breath of IBD patients is a highly promising approach that has the potential to be used in clinical practice. While complementing existing diagnostic tools, VOMs may be employed in the future as a means of delivering non-invasive point of care testing for IBD diagnosis, monitoring disease activity and responsiveness to treatment. VOMs such as alkanes, aldehydes, hydrogen sulphide, and cytokines have been implicated as potential biomarkers, with concentrations correlating to disease activity in adult IBD patients. While it is known that VOM concentrations differ with IBD severity, it is unclear if a single VOM biomarker that is subcategory-specific exists in the breath headspace of patients. In the future, further understanding of VOMs may lead to the identification of such CD-specific or UC-specific biomarkers, which would be revolutionary to clinical practice. This may help prioritise patients and reduce the current diagnostic delay experienced by some patients, leading to a restoration of their quality of life, which is of paramount importance. Moreover, the VOM profiles of paediatric patients differ to those of adults and more studies are needed to ascertain the efficacy of profiling in these patients. The preliminary evidence implicates breath metabolic profiling as an encouraging diagnostic and monitoring modality for IBD. The challenge remains to develop disease activity indices for VOM breath testing that are subcategory-specific in both adult and paediatric populations, with the ability to provide point of care testing. If achieved, VOM profiling has the potential to offer patients with IBD much needed optimism regarding the management of their condition and maximise their quality of life.

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Table 1. Advantages and disadvantages of VOM profiling via breath samples [6].

ADVANTAGES	**DISADVANTAGES**
Non-invasive (no bowel preparation, anaesthetic, or contrast is required)	The effects of antibiotics and co-morbidities on VOM profiles are not known
Does not expose patient to radiation	Not widely studied in paediatric cohorts
Minimal risks	
Potential for point-of-care bedside testing	