Expert Review of Clinical Immunology

ISSN: 1744-666X (Print) 1744-8409 (Online) Journal homepage: informahealthcare.com/journals/ierm20

# Fecal volatile organic compounds: a novel, cheaper method of diagnosing inflammatory bowel disease?

Chris SJ Probert, Sophie Reade & Iftikhar Ahmed

**To cite this article:** Chris SJ Probert, Sophie Reade & Iftikhar Ahmed (2014) Fecal volatile organic compounds: a novel, cheaper method of diagnosing inflammatory bowel disease?, Expert Review of Clinical Immunology, 10:9, 1129-1131, DOI: 10.1586/1744666X.2014.943664

**To link to this article:** https://doi.org/10.1586/1744666X.2014.943664

Published online: 28 Jul 2014.

# Fecal volatile organic compounds: a novel, cheaper method of diagnosing inflammatory bowel disease?

*Expert Rev. Clin. Immunol.* 10(9), 1129–1131 (2014)

**Chris SJ Probert**

*Author for correspondence:*
*Department of Gastroenterology, Institute of Translational Medicine, University of Liverpool, Liverpool, L69 3BX, UK*
*Tel.: +44 151 795 4010*
*Fax: +44 151 794 6825*
*mdcsjp@liverpool.ac.uk*

**Sophie Reade**

*Department of Gastroenterology, Institute of Translational Medicine, University of Liverpool, Liverpool, L69 3BX, UK*

**Iftikhar Ahmed**

*Department of Gastroenterology, University Hospital Southampton, Tremona Road, Hampshire, SO16 6YD, UK*

The investigation of a novel, cheaper method of diagnosing inflammatory bowel disease (IBD) is an area of active research. Recently, investigations into the metabolomic profile of IBD patients and animal models of colitis compared to healthy controls has begun to receive considerable attention and correlations between the fecal volatile organic compound (VOC) metabolome and IBD is merging. Patients and clinicians have often reported a change in odor of feces during relapse of IBD. Therefore, this article will focus specifically on the fecal VOC metabolome and its potential role in identifying a novel diagnostic method for IBD.

Inflammatory bowel disease (IBD) has two major phenotypes, Crohn's disease (CD) and ulcerative colitis (UC), and is characterized by chronic inflammation of the GI tract. It has been widely hypothesized that the pathogenesis of IBD results from an interaction of environmental factors (e.g., smoking, oral contraceptives, high-fat diet) affecting the intestinal microbiota and an inappropriate immune response in a genetically predisposed person. Clinically, patients diagnosed with UC or CD present with symptoms such as severe abdominal pain, diarrhea, rectal bleeding and weight loss. The disease predominantly affects the younger population, and due to its remitting and relapsing nature, has significant impact on their educational, social, professional and family life [1]. Therefore, there is an ever-increasing need to focus on methods to control the underlying pathology and diagnosis of IBD in order to improve the quality of life and treatment for patients.

The diagnosis and care of IBD remains difficult for clinicians and is commonly based on the presenting symptoms and a range of investigations including laboratory tests, endoscopy, histology and radiology [2]. These current diagnostic methods are often invasive, expensive and are found unpleasant by the patients. The serum markers such as C-reactive protein are non-specific, lack specificity and are raised in other inflammatory diseases [3]. Consequently, non-invasive methods of assessing the disease process have become the 'holy grail' for research into diagnostics. Calprotectin and lactoferrin are neutrophil proteins that pass into the lumen and enter the feces when the intestine is inflamed and can be measured directly in the feces of patients with active IBD [4]. However, although these fecal markers are informative when diagnosing IBD, they have a slow turnaround and are also raised in the presence of fecal blood (e.g., hemorrhoids, polyps or gut infections such as *Clostridium difficile*); so may be unable to distinguish between infectious diseases and IBD [5].

Patients often report an abnormal odor emitted from their feces during disease relapse, yet there has been little research performed to investigate the composition of fecal gases. The human gut microbiota is comprised of numerous species of bacteria which, during health, are thought to contribute to the mucosal integrity, the maintenance of

EXPERT REVIEWS

**Keywords:** diagnostic • feces • inflammatory bowel disease • novel • volatile organic compound

 10.1586/1744666X.2014.943664  ISSN 1744-666X 

host health and thus provide protection against invading organisms [6]. This resident microbiota are responsible for the fermentation of undigested foods in the colon, producing putrefactive compounds such as ammonia, aliphatic amines, branched chain fatty acids, indole, phenol and volatile sulfur-containing compounds, thus affecting both the colonic and metabolic health [6,7]. Therefore, interruption of the host gut microbiota in IBD patients may result in an alteration of the odor of their feces, as observed by patients and health care staff.

Recently, the assessment of volatile organic compounds (VOCs) present in the gas emitted from biological samples using techniques such as gas chromatography–mass spectrometry or gas electronic sensors [8], from both healthy and diseased patients, has led to the identification of disease-specific patterns and potential biomarkers [9,10]. Therefore, the use of VOC metabolomic analysis is beginning to receive considerable attention as a novel, non-invasive and cheaper method of IBD diagnosis.

VOCs are, by definition, a large and highly diverse group of carbon-based chemicals, related by their volatility at room temperature and include hydrocarbons, alcohols, aldehydes, ketones, esters and organic acids. Most vapors emitted from biological samples (e.g., breath, sweat, blood, urine and feces) contain odorous and non-odorous VOCs and those that vary may be used as an indication of cellular metabolism leading to, or indicative of, a diseased state [11]. Using urine samples recruited by the University of Warwick, VOCs were detected with an electronic sensor and a field asymmetric ion mobility spectrometer. Both instruments showed separation between IBD and controls with a >75% accuracy ($p < 0.001$); and between active disease and those in remission [12]. Another group, from Cleveland Clinic, USA, collected breath samples from children (5–21 yrs; n = 62) with IBD plus healthy controls (n = 55). Using a selective ion flow tube mass spectrometer, they were able to develop a model successfully tested on all 62 IBD samples with only four misclassifications, concluding that exhaled breath VOC analysis is a promising non-invasive method of diagnosing IBD [13].

Based on current research, it has become apparent that the accurate reproducible detection of VOCs from biological samples has great potential to develop into a non-invasive diagnostic test for IBD. However, to date, there seems to be a limited number of published works investigating the VOCs present in the feces, in comparison to breath or urine, of IBD patients, compared to healthy donors [12,14–17]. Human stool samples represent the end-product of diet, digestive and excretory processes, and colonic bacterial metabolism [6]. The analysis of VOCs in the feces of patients is therefore likely to offer a more favurable approach to diagnosing IBD non-invasively.

We have previously performed a cohort and a longitudinal study using fecal samples from healthy volunteers, giving us an overview of the VOCs present in health, and how they varied from day to day. Later, the patterns of VOCs identified in the healthy cohort were compared to the VOCs detected from samples of patients with UC, *Campylobacter jejuni* and *C. difficile*. The profile of VOCs from the feces of patients with UC, *C. difficile* and *C. jejuni* was all significantly different to that of the healthy controls. The number of VOCs detected in fecal samples from the diseased groups was considerably less diverse than those in healthy donors with the levels of acids, alcohols and ester compounds varying between the two groups. An extensive literature search has revealed that this was the first study to investigate the VOCs present in the feces of IBD patients using the sampling technique, solid-phase microextraction coupled to gas chromatography–mass spectrometry. We have reported an extensive catalogue of fecal VOCs that will provide a foundation for all future studies of gastrointestinal disease-specific changes occurring within the human gut [16].

Walton *et al.* (2013) recently took this one step further by reporting the quantitative changes of VOCs in the feces of patients with IBD. They recruited 87 subjects comprised of 19 healthy volunteers, 22 CD, 20 UC and 26 irritable bowel syndrome (IBS) patients. Fecal samples were collected from each group before and after treatment. Findings showed that the abundance of specific VOCs significantly differed between CD and other gastrointestinal diseases, with a significant increase in the concentrations of ester and alcohol derivatives of short-chain fatty acids and indole in CD patients compared to healthy, UC and IBS. Interestingly, the concentrations of several VOCs identified in the diseased groups significantly decreased after treatment, becoming more similar to the levels observed in the healthy group. These results may provide further evidence that the different patterns of VOCs produced in the feces of IBD patients are a result of variations in the gut microbiota, indicative of the specific disease processes [18]. However, this study did not consider the entire fecal VOC patient metabolome but selected a smaller number of candidate compounds to render statistical analysis tractable [15].

We have since reported the first comprehensive study to evaluate the entire fecal VOC metabolome from samples of 30 patients with diarrhea predominant IBS, 217 with IBD (CD = 117, UC = 100) and 109 healthy individuals. Fecal VOCs were extracted and identified using our standard method [19,20]. In total, 350 VOCs were identified and a discriminative model was developed to separate active IBD cases from those in remission, IBS and healthy controls. This model was able to successfully differentiate between active CD and UC from healthy controls and also active IBD from inactive IBD and IBS, with overall sensitivity of 90% and specificity of 80%. These results show that VOC analysis has the potential to provide not only a non-invasive method of diagnosing IBD, but also a good method to monitor the disease activity and to differentiate it from diarrhea-predominant IBS [17].

To summarize, it is apparent that the study of fecal VOC analysis offers a promising tool to diagnose IBD and it is hoped that this will lay down the foundations for the development of a novel, simple and cheaper method of diagnosing IBD. Specifically, these recent studies have commonly reported an increase in ester and alcohol derivatives of short-chain fatty

acids in the headspace of feces from IBD patients. A high-quality review discusses in detail the literature surrounding specific VOCs in urine and feces with reference to a large number of diseases [11]. The authors have reported distinct changes in the fecal VOC patterns in IBS, IBD and healthy controls using the most commonly used technique of VOC detection, gas chromatography–mass spectrometry. The authors' most recent work has led to the development of a novel device called the 'OdoReader', which utilizes a gas chromatography column coupled to a metal oxide sensor system with pattern recognition software at the University of West England, Bristol. Currently, the system is able to distinguish between fecal samples from patients with IBS and IBD with a sensitivity and specificity of 76 and 88%, respectively, with an overall accuracy of 76% [21].

In conclusion, there is sufficient evidence to warrant further study to develop this disease-sniffing technology as an improvement on the current IBD diagnostic methods with the hope to offer a faster method of screening patients who require further investigation.

**Financial & competing interests disclosure**

*CSJ Probert declares that he owns IP/patent to protect the OdoReader device to facilitate commercialization. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.*

*No writing assistance was utilized in the production of this manuscript.*

## References

1. Knights D, Lassen KG, Xavier RJ. Advances in inflammatory bowel disease pathogenesis: linking host genetics and the microbiome. Gut 2013;62(10):1505-10
2. Canavese G, Bassotti G, Astegiano M, et al. Inflammatory bowel disease: a proposal to facilitate the achievement of an unequivocal diagnosis. World J Gastroenterol 2013; 19(3):426-8
3. Nappo A, Iacoviello L, Fraterman A, et al. High-sensitivity C-reactive protein is a predictive factor of adiposity in children: results of the identification and prevention of dietary- and lifestyle-induced health effects in children and infants (IDEFICS) study. J Am Heart Assoc 2013; 2(3)):e000101
4. Waugh N, Cummins E, Royle P, et al. Faecal calprotectin testing for differentiating amongst inflammatory and non-inflammatory bowel diseases: systematic review and economic evaluation. Health Technol Asses 2013;17(55)):xv-xix.1-211
5. Probert CSJ. Role of faecal gas analysis for the diagnosis of IBD. Biochem Soc Trans 2011;39:1079-80
6. Probert CSJ, Ahmed I, Khalid T, et al. Volatile organic compounds as diagnostic biomarkers in gastrointestinal and liver diseases. J Gastrointest Liver Dis 2009; 18(3):337-43
7. Arasaradnam RP, Covington JA, Harmston C, Nwokolo CU. Review article: next generation diagnostic modalities in gastroenterology – gas phase volatile compound biomarker detection. Aliment Pharmacol Ther 2014;39(8):780-9
8. Ratcliffe NM, de Lacy Costello B, Shepherd S. The use of MS for the investigation of irritable bowel syndrome and inflammatory bowel disease. Clin Lab Int Magazine 2013;37:18-20
9. Altomare DF, Di Lena M, Porcelli F, et al. Exhaled volatile organic compounds identify patients with colorectal cancer. Br J Surg 2013;100(1):144-50
10. Khalid T, White P, De Lacy Costello B, et al. A pilot study combining a GC-sensor device with a statistical model for the identification of bladder cancer from urine headspace. PloS One 2013;8(7)):e69602
11. de Lacy Costello B, Ratcliffe N M. Volatile organic compounds (VOCs) found in urine and stool. In: Volatile biomarkers: non-invasive diagnosis in physiology and medicine 1st edn. Amann A, Smith D, Editors Amsterdam: Elsevier; 2013. pp 405-62
12. Arasaradnam RP, Ouaret N, Thomas MG, et al. A novel tool for noninvasive diagnosis and tracking of patients with inflammatory bowel disease. Inflamm Bowel Dis 2013; 19(5):999-1003
13. Patel N, Cikach F, Eng K, et al. Metabolomic analysis of exhaled breath to identify volatile organic compounds (VOCs) in children with inflammatory bowel disease. Gastroenterology 2013;144(5): S710-0
14. Jayasena H, Khalid T, Probert CS. PTU-076 Diagnostic potential of volatile organic compounds as faecal biomarkers in inflammatory bowel disease. Gut 2013; 62(Suppl 1)):A75-6
15. Walton C, Fowler DP, Turner C, et al. Analysis of volatile organic compounds of bacterial origin in chronic gastrointestinal diseases. Inflamm Bowel Dis 2013;19(10): 2069-78
16. Garner CE, Smith S, Costello BD, et al. Volatile organic compounds from feces and their potential for diagnosis of gastrointestinal disease. FASEB J 2007; 21(8):1675-88
17. Ahmed I, Greenwood R, Costello BD, et al. An investigation of fecal volatile organic metabolites in irritable bowel syndrome. PloS One 2013;8(3)):e58204
18. Kinross JM, Darzi AW, Nicholson JK. Gut microbiome-host interactions in health and disease. Genome Med 2011;3(3):14
19. Ahmed I, Smith S, Probert CS. Volatile organic compounds as diagnostic faecal biomarkers in inflammatory bowel disease method development. Gut 2009;58:A63-3
20. Reade S, Mayor A, Aggio R, et al. Optimisation of sample preparation for direct SPME–GC–MS analysis of murine and human faecal volatile organic compounds for metabolomic studies. J Anal Bioanal Tech 2014;5(2)
21. Shepherd SF, McGuire ND, de Lacy Costello BP, et al. The use of a gas chromatograph coupled to a metal oxide sensor for rapid assessment of stool samples from irritable bowel syndrome and inflammatory bowel disease patients. J Breath Res 2014;8(2):026001