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Review

Application of Fecal Volatile Organic Compound Analysis in Clinical Practice: Current State and Future Perspectives

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Abstract: Increasing interest is noticed in the potential of volatile organic compound (VOC) analysis as non-invasive diagnostic biomarker in clinical medical practice. The spectrum of VOCs, originating from (patho)physiological metabolic processes in the human body and detectable in bodily excrements, such as exhaled breath, urine and feces, harbors a magnificent source of information. Thus far, the majority of studies have focused on VOC analysis in exhaled breath, aiming at identification of disease-specific VOC profiles. Recently, an increasing number of studies have evaluated the usability of VOC present in the headspace of feces in the diagnostic work-up of a wide range of gastrointestinal diseases. Promising results have been demonstrated particularly in those diseases in which microbiota alterations are considered to play a significant etiological role, such as colorectal carcinoma, inflammatory bowel disease, irritable bowel syndrome, celiac disease and infectious bowel diseases. In addition, fecal VOC analysis seems to have potential as a diagnostic biomarker for extra-intestinal diseases, including bronchopulmonary dysplasia and sepsis. Different methods for VOC analysis have been used in medical studies, such as gas-chromatography mass spectrometry, selected-ion flow tube-mass spectrometry, ion-mobility spectrometry, and electronic nose devices. In this review, the available literature on the potential of fecal VOCs as diagnostic biomarker, including an overview of relevant VOC detection techniques, is discussed. In addition, future hurdles, which need to be taken prior to implementation of VOC analysis in daily clinical practice, are outlined.

Keywords: volatile organic compounds; disease biomarkers; electronic nose; VOC; feces

1. Introduction

In a significant number of diseases, alternative disease-detection technologies are warranted, for example in gastrointestinal diseases where colonoscopies, tissue biopsies and microbial culture tests remain a mandatory diagnostic tool in the majority of cases. These techniques are commonly invasive, expansive, time-consuming, and carry a high burden on patients. Furthermore, currently used biomarkers of disease activity, such as in inflammatory bowel disease (i.e., Crohn's disease and ulcerative colitis) in which C-reactive protein (CRP) and fecal calprotectin (FCP) are often used, lack specificity, which underlines the urgent need for development of alternative disease-specific biomarkers. Numerous potential fecal biomarkers have been studied in the search for new

disease-specific biomarkers, such as Fecal immunochemical test for hemoglobin (FIT) [1,2], DNA [3] and microRNA [4] for early detection of colorectal cancer (CRC). Furthermore, inflammation related proteins, such as beta-glucuronidase, have been studied as a potential biomarker in inflammatory bowel disease [5]. These potential biomarkers show great potential, especially fecal DNA and microRNA hold potential in facilitating more individualized treatment approaches. However, a major concern of these biomarkers is the lack of specificity, therefore preventing their application in clinical diagnostic work-up [3,6].

In the past decades, an increasing number of studies have assessed the usability of volatile organic compound (VOC) analysis as a non-invasive, high-throughput and low-cost diagnostic biomarker. VOCs are carbon-based chemicals which under normal indoor atmospheric conditions of temperature and pressure evaporate to the ambient air. They are emitted from the body as a product of both physiological and pathophysiological metabolic processes, and can be detected in sweat, urine, exhaled breath, feces, blood and vaginal secretions [7]. In addition, the intestinal microbiota excrete volatile metabolites which contribute to the (fecal) VOC composition, and microbial changes are therefore considered to be reflected by VOC alterations [8]. Consequently, VOC analysis hypothetically harbors an extensive source of information to be used in the diagnostic work-up of a wide range of diseases. In numerous proof-of-principle studies, VOC profiles have been shown to differentiate healthy state from a variety of diseases, including metabolic, infectious, inflammatory, endocrine diseases and malignancies [9].

Sample acquisition for VOC analysis is relatively easy and, in most cases, non-invasive. In many studies, different considerations are made for the choice of analytic excreta (e.g., urine, feces, and exhaled air), which is mainly based on the disease of interest and readiness of patients to collect certain bodily excrements. Initially, many studies preferred usage of exhaled breath, since it is relatively easy to collect and little patient resistance is experienced. However, when studying gastrointestinal diseases, in particular those diseases where microbiota alterations are considered to play an etiological role, analysis of VOCs captured form the headspace of fecal samples may provide a more integral view on disease activity. Every bodily excrement possesses its own advantages and disadvantages. Whereas exhaled breath is easy to use for real-time analysis, difficulties are found in storage of exhaled breath for offline analysis. There are no difficulties in the collection and storage of fecal and urine derived samples, however, in the case of fecal collection, in some cultures, there is still a taboo on feces, which can result in decreased sampling compliance.

In this review, we provide an overview of studies on fecal VOC analyses in gastrointestinal and extra-intestinal diseases. Over time, numerous VOC analyzing techniques have been introduced, including gas-chromatography mass spectrometry (GC-MS), ion flow tube-mass spectrometry (SIFT-MS), field asymmetric ion mobility spectrometry (FAIMS) and electronic nose devices (eNose). To increase reader comprehensibility, the background of the analytical techniques applied in the reviewed fecal VOC studies is discussed in more detail. Furthermore, hurdles that need to be overcome prior to clinical implementation of VOC analysis as a non-invasive diagnostic mean are outlined.

2. Fecal Volatile Organic Compound Analytical Methods

Roughly, available VOC detection techniques can be divided into two different categories. Chemical analytical techniques, residing at one end of the spectrum, allow for the quantitative and qualitative detection of individual VOCs, whereas, at the other end of the spectrum, electronic devices containing an array of different VOC sensors allow for discrimination between gaseous mixtures based on pattern recognition algorithms.

Gas-chromatography mass spectrometry (GC-MS) is currently considered the gold standard in VOC detection and can be placed under the chemical analytical techniques. In GC-MS, compounds are volatized and transported by a carrier gas over a coated capillary column, thereby promoting maximum separation of VOCs based on the ability of individual compounds to interact with the column surface. Subsequently, after separation of individual analyte molecules by the GC, the specimen molecules

are ionized using electron impact ionization and pushed through an electrical field, manipulating the velocity of the charged molecules by varying the voltage value. Eventually, separated by the electrical field, these charged molecules reach a detector plate, resulting in a spectrum of peaks. In this "mass spectrum" each peak represents a particular molecule mass, with increasing peak height demonstrating a higher concentration present [10]. GC-MS provides very good specificity and reliable quantitation, however is time-consuming, expensive, labor intensive, requires highly trained operating personnel and has high maintenance requirements.

Another chemical analytical technique increasingly used in clinical research setting is selected ion flow tube mass spectrometry (SIFT-MS), allowing for real-time analysis of complex gaseous mixtures. SIFT-MS first chemically ionizes VOCs with precursor ions, resulting in less fragmentation compared to GC-MS. Subsequently, these product ions are separated and quantified by a downstream (analytical) quadrupole mass spectrometer [11]. Although fast results are ensured, SIFT-MS devices provide less detailed information compared to GC-MS.

A third chemical analytical technique is field asymmetric ion mobility spectrometry (FAIMS). With this technique, VOCs are ionized before being pushed through two metal plates. Over such metal plates, an asymmetric high voltage waveform is applied, allowing for separation of individual VOCs based on their ion mobility. Depending on the applied electrical field, only ions with a specific mobility value reach the end of the drifting tube onto the detector plate. Using a gradient of electrical fields, a spectrum of individual peaks is generated [12].

Electronic nose devices, residing the other end of the spectrum, come in many different shapes and sizes. They enable real-time and high-throughput analysis, entail inexpensive purchase and measurement costs and produce relatively easy to interpret outcomes. A complex gaseous VOC mixture is presented to an array of different sensors, influencing the measurable attribute (for example, electrical resistance or oscillation frequency) of each individual sensor. Based on pattern recognition algorithms, obtained VOC-profiles could be compared and potentially discriminated from each other. Therefore, different studies assessed usability of eNose devices in medical diagnostics of various diseases [13–17]. However, the main limitation of such eNose devices is their inability to identify individual VOCs and their concentration, consequently hampering inter-device outcome comparison. Furthermore, reproducibility of obtained results is hampered by sensor drift, preventing from reliable comparison of study results and application as a bed-side tool in clinical practice. Table 1 provides an overview of the advantages and disadvantages of previous described analytical techniques.

Table 1. Overview of advantages and disadvantages of different analytical techniques.

Analytical Technique	Advantages	Disadvantages
eNose device	Real-time measurements possible Easy to use High-throughput analysis possible Low purchase and measurement costs Portable and suitable for bedside use Sensitivity in ppb range	Identification of specific individual VOCS not possible Inter-device outcome comparison not possible Over time sensor drift
GC-MS	Identification of specific individual VOCs Sensitivity possible in ppb range Reproducible	Time consuming and labor intensive Expensive Requires highly trained operating personnel High maintenance requirements System immobile Real-time measurements not possible Not suitable for clinical use

Table 1. *Cont.*

Analytical Technique	Advantages	Disadvantages
FAIMS	Sensitivity in ppm range Mobile system and suitable for clinical use Easy to use Low cost	Identification of specific individual VOCs not possible Real-time measurements not possible
SIFT-MS	Real-time measurements possible Fast Sensitivity in ppb range Mobile system	Identification of specific individual VOCs is not possible

Abbreviations: eNose, electronic nose; GC-MS, gas chromatography mass spectrometry; FAIMS, field asymmetric ion mobility spectrometry; SIFT-MS, selected ion flow tube mass spectrometry; VOC, volatile organic compound; ppb, parts per billion; ppm, parts per million.

3. Fecal VOC in Gastrointestinal DISEASES

3.1. Infectious Diseases

The gold standard for identification of pathogens in infectious diseases comprises of microbiological culture, in specific cases combined with detection of either bacterial DNA, bacterial antigens or microbial toxins. However, results of culture are commonly obtained after at least 48 h, while rapid diagnosis is necessary to apply appropriate therapeutic interventions, such as prescription of targeted antibiotics and isolation of patients [18]. Most studies on application of fecal VOC analysis in infectious disease have focused on *Clostridium difficile* [19–23]. Other studies include detection of *Campylobacter jejuni*, cholera, giardiasis and rotavirus [21,23–26].

Clostridium difficile infection is particularly prevalent in elderly and in patients who have received antimicrobial agents. This infectious disease is highly contagious and development of more severe forms have emerged. Outbreak in hospitals have resulted in ward closures and extensive infection control measures. Rapid diagnosis of *C. difficile* infection can lead to quick application of therapeutic and hygienic/preventive strategies [27].

Bomers and colleagues assessed differences in fecal VOC profiles of 43 *C. difficile* negative stool samples and 35 *C. difficile* positive stool samples of adults, with a subdivision in presence of toxins, by means of FAIMS. In addition, the diagnostic accuracy of FAIMS for direct detection of *C. difficile* in stool samples was determined. Analysis by FAIMS allowed for differentiation of *C. difficile* positive and negative stool samples (Table 2) [28]. To date, four studies assessed fecal VOC composition in *C. difficile* positive stools compared to controls by means of GC-MS. One of these studies demonstrated abundance of three VOCs, which were found to be discriminant between *C. difficile* positive and negative stool samples (Table 2). Unique in this study was usage of enzyme specific substrate, 3-fluoro-hydroxyphenylacetatic acid, which is converted by an enzyme produced by *C. difficile* to the VOC 2-fluoro-4-methylphenol. Addition of this substrate resulted in a high sensitivity and specificity (Table 2). In absence of 2-fluoro-4-methylphenol, discrimination was not possible between *C. difficile* positive and negative samples [20]. In addition, three other studies demonstrated that, by means of GC-MS, *C. difficile* positive fecal samples could be distinguished from controls based on their VOC profiles. Specific VOCs, sensitivity and specificity are listed in Table 2 [19,21,23].

These studies underline the potential of fecal VOC analysis for detection of *C. difficile*, although a uniform VOC profile for *C. difficile* is yet to be identified. Advantage of VOC targeted diagnostics over culture include opportunities for rapid, bedside detection of *C. difficile*, allowing for timely initiation of therapy and hygienic/preventive strategies. However, additional studies on standardization of sampling methods and assessment of environmental factors are needed, especially the influence of dietary intake on fecal VOC composition, since these effects are still largely unknown, hampering

clinical application. In addition, identification of discriminant VOCs in *C. difficile* positive patients is needed to develop tailor-made sensors, before clinical application of fecal VOC analysis is possible.

Other Infectious Diseases

A pilot study on detection of adult cholera patients by fecal VOC analysis using GC-MS, demonstrated the presence of two different VOCs in cholera positive fecal samples, whereas these were absent in healthy controls (Table 2). Overall, chromatograms of cholera samples showed a lower number of individual VOCS: 23 in cholera cases (n = 6) vs. 36 in healthy controls (n = 3). However, sample size in this study was too small to draw conclusions [29].

Al-Kateb and colleagues studied fecal VOC profiles of samples of 53 children from Malawi, comprising 27 rotavirus positive and 26 stool samples of children with unspecified gastrointestinal problems, by means of GC-MS. In particular, two VOCs were found with a higher frequency and an higher abundance in rotavirus infected samples (Table 2) [25]. Another study found one specific VOC to be ubiquitous in rotavirus positive fecal samples, namely ethyl dodecanoate, however only five stool samples were analyzed [23].

Bond et al. compared fecal VOCs from 16 adult patients infected with *Giardia lamblia* with 17 controls with diarrhea without *Giardia lamblia* by means of GC-MS. The two groups could be separated based on five different VOCs listed in Table 2, with AUCs ranging from 0.80 to 0.93. Additional larger studies by means of GC-MS are needed to assess the potential of fecal VOCs as a bedside diagnostic tool for rotavirus and *Giardia lamblia* detection [26].

In a study on VOC profiles in *Campylobacter jejuni* positive fecal samples, it was found that the presence of one specific VOC was positively associated with *C. jejuni* [8]. Another study demonstrated the absence of two types of VOCS were strongly associated with *C. jejuni*, as listed in Table 2 [10].

3.2. Inflammatory Bowel Disease and Irritable Bowel Syndrome

Inflammatory bowel disease (IBD) is a chronic relapsing condition of the gastrointestinal tract which usually develops in the teens or young adulthood, and comprises the phenotypes ulcerative colitis (UC) and Crohn's disease (CD). Chronic active mucosal inflammation is associated with a variety of severe complications, emphasizing the importance of early detection and adequate monitoring of disease activity. In the diagnostic work up and follow-up, endoscopic investigation is necessary, which is an invasive and costly procedure for children and adults [30,31]. The burden is especially high in children, who need hospitalization for colonic lavage and administration of laxatives by nasogastric tube prior to ileocolonoscopy, which is performed under general anesthesia. In clinical practice, it can be challenging to differentiate between IBD and irritable bowel syndrome (IBS) based on clinical symptoms. To exclude IBD, FCP is commonly used as noninvasive biomarker. FCP is characterized by a high sensitivity for mucosal inflammation (0.98, 95% CI 0.95–0.99) but also by a low specificity (0.68, 95% CI 0.50–0.86), consequently leading to the performance of unnecessary endoscopies [32]. For these reasons, the search for novel noninvasive biomarkers in both the diagnostic workup and follow-up of IBD patients remains warranted. Fecal VOCs seem to hold potential for this purpose in both pediatric and adult IBD patients.

In 2013, Ahmed et al. compared fecal VOCs of 30 diarrhea-predominant adult IBS patients to 110 adult IBD patients by means of GC-MS [33]. They found that three types of VOCs were more abundant in the IBS group, while the abundance of five types of VOCs were higher in the IBD group (Table 3). Adult patients with IBS could be discriminated from active CD, UC and healthy controls (HC) with a high sensitivity and specificity, which are listed in Table 3.

Table 2. Overview of studies on fecal VOC analysis in infectious diseases.

Disease	Ref.	Case/Control No.	Analytical Technique	VOC Biomarker [a,b]	Sensitivity	Specificity	AUC (95% CI)
Clostridium difficile	Bomers et al. (2015)	26/50	FAIMS	VOC profile	92.3%	86.0%	0.93 (0.85–1.00)
	Tait et al. (2014)	77/23	GC-MS	↑ 2-fluoro-4-methylphenol (10 μg/mL) ↑ p-cresol (0.59 μg/mL) ↑ isocaproic acid (87 μg/mL)	83.1%	100%	- -
	Garner et al. (2007)	22/30	GC-MS	* 2-ethanol; * toluene; * 6-methyl-3,5-heptadiene-2-one; * hexanoic acid	- -	- -	- -
	Probert et al. (2004)	6/38	GC-MS	↑ 5-methyl-2-furancarboxyaldehyde	83%	97%	- -
	McGuire et al. (2014)	50/50	GC-MS	VOC profile	85%	80%	- -
Campylobacter jejuni	Garner et al. (2007)	31/30	GC-MS	↑ 1-butoxy-2-propanol; * 3-methyl furan; * dimethylsulfide	- -	- -	- -
	Probert et al. (2004)		GC-MS	* Terpenes; * hydrocarbons	100%	92%	- -
Cholera	Garner et al. (2009)	6/3	GC-MS	↑ dimethyl disulfide; ↑ p-menth-1-en-8-ol	- -	- -	- -
Giardiasis	Bond et al. (2015)	16/17	GC-MS	↓ acetone (16.423 vs. 18.268) [c] ↓ 2-butanone (17.454 vs. 18.889) [c] ↑ 2-methylphenol ↑ 4-methylphenol (20.522 vs. 19.355) [c]	- -	- -	Tetramethylocatane: 0.93 1-propanol: 0.85 Acetic acid: 0.82 Pentamethylheptane: 0.81 Acetone: 0.80
Rotavirus	Al-Kateb et al. (2012)	27/26	GC-MS	↑ aldehydes; ↑ 2-3-butanedione	- -	- -	- -
	Probert et al. (2004)	5/38	GC-MS	↑ ethyl dodecanoate	100%	97%	- -

Symbol meanings: [a] biomarkers are noted for study cases. [b] when concentrations of individual VOCs were available, mean concentration is noted in parentheses. [c] Mean abundance, no concentration units were mentioned in original paper. ↑, increased VOCs. ↓, decreased VOCs. *, absent VOCs. - -, not mentioned in the original study. Abbreviations: GC-MS, gas chromatography mass spectrometry; FAIMS, field asymmetric ion mobility spectrometry; AUC, area under the curve; CI, confidence interval; VOC, volatile organic compound; No., number.

Table 3. Overview of studies on fecal VOC analysis in gastrointestinal diseases.

Disease [1]	Ref.	Study No.	Analytical Technique	VOC Biomarker [a]	Sensitivity	Specificity	AUC (95% CI)
Adult IBD and IBS	Ahmed et al. (2013)	110 IBD (62 CD and 48 UC) 30 IBS 109 HC	GC-MS	**CD:** ↑ aldehydes; ↑ ketones **UC:** ↑ 1-propanol, 2-methyl; ↑ undecane; ↑ methoxy-phenyl-oxime **IBS:** ↑ Esters of SCFAs; ↑ cyclohexanecarbocylic acid and its derivatives	IBS vs. IBD: 96% IBS vs. CD: 94% IBS vs. UC: 96% IBS vs. HC: 90%	IBS vs. IBD: 80% IBS vs. CD: 82% IBS vs. UC: 80% IBS vs. HC: 80%	IBS vs. IBD: 0.98 IBS vs. CD: 0.97 IBS vs. UC: 0.96 IBS vs. HC: 0.94
	Walton et al. (2013)	42 IBD (22 CD and 20 UC) 26 IBS 19 HC	GC-MS	**CD:** ↑ esters; ↑ indole; ↑ alcohol derivatives of SCFAs	- -	- -	- -
	Shepherd et al. (2014)	34 IBS 101 IBD (42 CD and 59 UC) 46 HC	GC-MOS	VOC profile	IBS vs. IBD: 76%	IBS vs. IBD: 88%	- -
	Ahmed et al. (2016)	217 IBD (117 CD and 100 UC) 109 HC	GC-MS	**a-CD:** ↑ heptanal; ↑ 1-octen-3-ol; ↑ 2-piperidinone; ↑ 6-methyl-2-heptanone; ↑ decane; ↓ methanethiol; ↓ 3-methyl-phenol; ↓ alpha-pinene **a-UC:** ↑ Bicyclohexane; ↑ 4-methylene-1-methylethyl	- -	- -	- -
	Aggio et al. (2017)	33 a-IBD 50 i-IBD 28 IBS 41 HC	GC-MS	VOC profiles	a-IBD vs. IBS: 93% IBS vs. HC: 91% IBD vs. HC: 78%	a-IBD vs. IBS: 90% IBS vs. HC: 54% IBD vs. HC: 79%	a-CD vs. IBS: 87% (84–89%) IBS vs. HC: 78% (76–80%)
Pediatric IBD and IBS	de Meij et al. (2014)	55 IBD (29 CD and 26 UC) 28 HC	eNose	VOC profiles	a-UC vs. HC: 100% a-CD vs. HC: 86% r-UC vs. HC: 94% r-CD vs. HC: 94% a-CD vs. a-UC: 97% r-CD vs. r-UC: 88%	a-UC vs. HC: 100% a-CD vs. HC 67% r-UC vs. HC: 94% r-CD vs. HC: 92% a-CD vs. a-UC: 92% r-CD vs. r-UC: 72%	a-UC vs. HC: 1.00 (±0.00) a-CD vs. HC: 0.85 (±0.05) r-UC vs. HC: 0.94 (±0.05) r-CD vs. HC: 0.94 (±0.06) a-CD vs. a-UC: 0.96 (±0.03) r-CD vs. r-UC: 0.81 (±0.08)
	van Gaal et al. (2017)	36 IBD (23 CD and 13 UC) 24 HC	FAIMS	VOC profiles	IBD vs. HC: 79% CD vs. HC: 83% UC vs. HC: 77% CD vs. UC: 65%	IBD vs. HC: 78% CD vs. HC: 83% UC vs. HC: 75% CD vs. UC: 62%	IBD vs. HC: 0.76 (±0.14) CD vs. HC: 0.90 (±0.10) UC vs. HC: 0.74 (±0.19) CD vs. UC: 0.67 (± 0.19)
	Bosch et al. (2018)	30 IBD (15 CD and 15 UC) 15 IBS 30 HC	FAIMS	VOC profiles	IBS vs. IBD: 100% IBS vs. HC: 60% IBD vs. HC: 93%	IBS vs. IBD: 87% IBS vs. HC: 63% IBD vs. HC: 97%	IBD vs. IBS: 0.94 (0.88–1) IBS vs. HC: 0.59 (0.41–0.77) IBD vs. HC: 0.96 (0.9–1)

Table 3. *Cont.*

Disease [1]	Ref.	Study No.	Analytical Technique	VOC Biomarker [a]	Sensitivity	Specificity	AUC (95% CI)
CRC	de Meij et al. (2014)	40 CRC 60 AA 57 HC	eNose	VOC profiles	CRC vs. HC: 85% AA vs. HC: 62% AA vs. CRC: 75%	CRC vs. HC: 87% AA vs. HC: 86% AA vs. CRC: 73%	CRC vs. HC: 0.92 (±0.03) AA vs. HC: 0.79 (±0.04) AA vs. CRC: 0.82 (±0.04)
	Batty et al. (2015)	31 High risk (AA + CRC) 31 Low risk (HC)	SIFT-MS	↑ hydrogen sulfide; ↑ dimethyl sulfide; ↑ dimethyl disulfide	High vs. low risk: 72%	High vs. low risk: 78%	- -
NEC	Garner et al. (2009)	6 NEC 7 HC	GC-MS	* 2-ethylhexyl acetic ester; * decanoic acid ethyl ester; * dodecanoic acid ethyl ester; * hexadecanoic acid ethyl ester	- -	- -	- -
	de Meij et al. (2015)	13 NEC 31 sepsis 14 HC	eNose	VOC profiles	NEC vs. HC †: 88.9% NEC vs. sepsis †: 88.9%	NEC vs. HC †: 88.9% NEC vs. sepsis †: 56.5%	NEC vs. HC †: 0.99 (±0.04) NEC vs. sepsis †: 0.64 (±0.18)
Celiac disease	Di Cagno et al. (2009)	7 t-CD 7 u-CD 7 HC	GC-MS	**u-CD:** ↑ alcohols; ↑ aldehydes; ↑ sulfur compounds; ↑ hydrocarbons **t-CD:** ↑ SCFA	- -	- -	- -
	Di Cagno et al. (2011)	19 t-CD 15 HC	GC-MS	↑ Alcohols; ↓ Esters; ↓ Sulfur compounds; ↓ Ketones ↓ hydrocarbons ↓ aldehydes; ↓ aromatic organic compounds; ↓ heptane; ↓ SCFA	- -	- -	- -
Adult NAFLD	Bailey et al. (2009)	7 suspected NAFLD 9 HC	GC-MS	VOC profile	- -	- -	- -
	Raman et al. (2013)	30 NAFLD 30HC	GC-MS	↑ Short chain aliphatic alcohols; ↑ carboxylic acids	- -	- -	- -
Pediatric NAFLD	Del Chierico et al. (2017)	27 NAFLD 26 NASH 8 obese no steatosis 54 HC	GC-MS	**NAFLD/NASH/obese:** ↑ alcohols; ↑ acids; ↑ aldehydes; ↑ ketones; ↑ amines; ↑ esters; ↓ aromatic hydrocarbons; ↓ hydrazines	- -	- -	- -

Symbol meanings: [a] biomarkers are noted for study cases. ↑, increased VOCs. ↓, decreased VOCs. *, absent VOCs. "- -", not mentioned in the original study. [1] Disease abbreviations: IBD, Inflammatory Bowel Disease; IBS, Irritable Bowel Disease; CRC, colorectal carcinoma; NEC, Necrotizing Enterocolitis; NAFLD, Non-Alcoholic Fatty Liver Disease; NASH, Non-alcoholic steatohepatitis. Other abbreviations: GC-MOS, gas chromatography single metal oxide sensor; GC-MS, gas chromatography mass spectrometry; FAIMS, field asymmetric ion mobility spectrometry; eNose, electronic nose; AUC, area under the curve; CI, confidence interval; VOC, volatile organic compound; CD, Crohn's Disease; UC, ulcerative colitis; HC, healthy controls; t-CD, treated celiac disease; u-CD, untreated celiac disease; a-CD, active Crohn's disease; a-UC, active ulcerative colitis; a-IBD, active IBD; i-IBD, inactive IBD; r-CD, remission Crohn's disease; r-UC, remission ulcerative colitis; AA, advanced adenoma; No., number; SCFA, short chain fatty acids.

Others compared fecal VOC composition of 42 adult IBD patients, with 26 adult IBS patients and 19 HC, by means of GC-MS, and found a significant increase in the abundance of three types of VOCs in CD patients (Table 3) [34]. In addition, several VOCS were more abundant in UC (i.e., butanoic acid) and IBS (i.e., propanoic acid, indole, dimethyl sulfide and 3 methylbutanoic acid), however, differences were not significant. Furthermore, concentrations of several VOCs in the CD group normalized to "healthy concentrations" in the after targeted treatment. In 2014, differences in VOC patterns rather than specific metabolites between 34 adult IBS, 42 CD, 59 UC and 46 HC were assessed using GC with a single metal oxide sensor, coupled to an artificial neural network software [35]. Based on a cross-validation network set, differentiation between IBS and IBD was feasible (Table 3). Ahmed et al. found outcomes in line with their previous study, in a cohort consisting of 217 adult IBD patients (117 CD, 100 UC) and 109 HC using GC-MS [36]. They found upregulation of three VOCs and downregulation of two VOCs in patients with active CD compared to inactive CD and HC. The model could also discriminate between CD patients with colonic and small bowel localization, but was less accurate in separating active and inactive UC and HC (Table 3). A recent study by Aggio et al. investigated the potential of fecal VOCs as novel noninvasive IBD biomarker for adult IBD [37]. In this study, GC with a metal oxide gas sensor and a computer algorithm was used to identify electrical resistance, to compare resistance patterns among 33 adults with active IBD, 50 adults with inactive IBD, 28 adult IBS patients and 41 HC. They demonstrated significant differences between active CD vs. IBS and IBS vs. HC (Table 3). Both the sensitivity and specificity for the discrimination of IBS from HC and IBS from IBD are in line with a previous conducted study by Ahmed et al. [33].

Over the last few years, pediatric IBD and IBS have also been subject of several fecal VOC studies. In 2014, de Meij et al. studied differences in fecal VOC patterns between pediatric de novo treatment-naïve IBD patients and 29 HC by means of an eNose (Cyranose 320®) at baseline and upon achieving remission (at 6 weeks of follow-up) [38]. Both active UC and CD could be distinguished from healthy controls based on their VOC profiles (Table 3). Upon remission, UC and CD could still be discriminated from HC with high diagnostic accuracy. In addition, CD differed from UC with a high accuracy during active disease and upon clinical remission (Table 3).

In a study by van Gaal et al., VOC patterns of fecal samples from de novo, treatment-naïve pediatric IBD patients were compared to 24 matched HC using FAIMS [39]. IBD could be distinguished from HC with a high overall accuracy. In addition, CD could also be differentiated from HC with a high diagnostic accuracy, whereas the diagnostic accuracy to discriminate between UC and HC was moderate (Table 3). Notably, in that study no significant differences were seen between CD and UC, in contrast to the previous study performed by an eNose [38]. This contrast might be explained by the usage of different analytical techniques. Hypothetically, minor differences in VOC profiles as measured by an eNose device may induce significant differences, whereas these differences are not (significantly) detected by FAIMS, and vice versa. Recently, Bosch et al. published a study comparing fecal VOC patterns of 15 pediatric IBS patients with 30 de novo, treatment naïve IBD patients and 30 HC by means of FAIMS [40]. In line with previous results, IBD could be distinguished from HC with a high diagnostic accuracy. In addition, IBD and IBS could also be discriminated with a high accuracy, whereas no significant differences were found between IBS and HC (Table 3).

In summary, all performed studies on fecal VOC analysis in IBD, using both metabolite-specific and pattern-based methods, have confirmed the potential of fecal VOCs as noninvasive biomarkers for IBD detection and even to monitor disease activity. VOC analysis seems to add value to discriminate IBS from IBD, but not from HC. Future studies should focus on the validation of these differences in a large cohort, comparing the diagnostic value of VOCs to FCP. By identifying specific volatiles and/or VOC patterns, an IBD-specific gas sensor can be developed, which may replace the currently used FCP, possibly lowering the need for endoscopies for IBD exclusion and detection of exacerbations.

3.3. Colorectal Cancer

Colorectal cancer (CRC) is one of the most prevalent malignancies in the industrialized world and is an important cause of cancer-related mortality [41,42]. Between 70% and 90% of CRC originates from adenomatous polyps, and early detection and removal of these precancerous adenomas significantly decrease CRC incidence and mortality [43]. Therefore, current CRC screening aims for early detection of CRC, and those adenomas with a high risk of malignant transformation (advanced adenomas) [44]. In screening tests, individuals with a positive fecal immunological test (FIT) are referred for colonoscopy (gold standard). However, performance of this test is suboptimal, with sensitivity for CRC and advanced adenomas of 66–87% and 27–38%, respectively, depending on cut-off value used [45–48]. In addition, screening with FIT results in a substantial number of false positive tests, and as a consequence, unneeded colonoscopies. Because of these limitations, an unmet need exists for a more sensitive and specific test to select only high risk individuals for colonoscopy. VOCs originating from breath, feces, blood and urine are increasingly considered as potential noninvasive biomarkers for CRC and advanced adenomas. Up to now, two studies have focused on the analysis of feces for this purpose.

In 2014, fecal VOC patterns of 40 CRC patients, 60 patients with advanced adenomas (AA) and 57 healthy controls were analyzed by means of an eNose [49]. They found that both patients with CRC and AA could be discriminated from controls (Table 3). In a second study, fecal VOC profiles of patients with a positive fecal occult blood test (FOBT) were assessed by means of SIFT-MS [50]. By colonoscopy, patients were classified into risk groups (e.g., low risk and high risk) based on their histopathology. Of 62 FOBT positive patients 31 were classified as high risk, meaning they were diagnosed with AA or CRC, while the remaining 31 patients were classified as low risk. This study demonstrated that classification based on VOC profiles was possible with good sensitivity and specificity (Table 3).

The results of these studies emphasize the potential of fecal VOCs as non-invasive biomarkers for the detection of colonic neoplasia, in particular of precancerous adenomas. Whether this could lead to earlier cancer detection and consequently to lower (unnecessary) endoscopy rates and a higher curation chance should be studied in larger validation settings, comparing the diagnostic accuracy of the currently used FIT screening with VOC patterns. Furthermore, many studies focused on the identification of disease specific VOCs, however, limited studies report quantitative data which might be used in the determination of cut-off values for example CRC.

3.4. Necrotizing Enterocolitis

Necrotizing enterocolitis is one of the leading causes of morbidity and mortality in neonatal intensive care units [51]. While the overall survival of extremely preterm infants is improving, incidence of NEC increases, varying from 2% to 7% in preterm infants with a gestational age <32 weeks and 5–22% in very low birth weight infants [51]. Mortality in high-income countries varies from 15.9% to 42% and 20–40% eventually require surgical interventions [51,52]. Nowadays, establishment of the diagnosis is reached by a combination of clinical, radiographic and surgical features, where early diagnosis and initiation of therapy are of pivotal prognostic importance. Up until now, two studies focused on fecal VOCs as a noninvasive biomarker for preclinical detection of NEC.

In 2009, Garner et al. conducted a pilot case-control study analyzing fecal VOCs of six NEC and seven control infants by means of GC-MS [24]. Fecal samples of different time points prior to clinical manifestation of NEC were analyzed. The number of different extracted VOCs became more numerous as the infant matures (0.49 extra VOCs per day), while this trend was absent in NEC cases. This phenomenon likely reflects the increasing intestinal microbial diversity. In addition, up to four days prior to clinical manifestation of NEC, absence of four different ethers were noticed (Table 3). The non-NEC samples, mostly contained at least one of these esters. The authors hypothesized that the loss of some VOCs before clinical manifestation of NEC may reflect the change in intestinal physiology and intestinal microbiota. Another research group conducted a prospective multicenter case-control study including 13 NEC cases, 31 sepsis cases and 14 controls [14]. Fecal samples collected in the

days prior to clinical onset of NEC and sepsis were analyzed using an eNose (Cyranose 320®). It was possible to discriminate fecal VOC profiles of NEC cases from controls up to three days prior to clinical onset of NEC. In addition, the differences in VOC profiles of the two groups increased even further toward onset of NEC (Table 3).

Both studies underline the potential of fecal VOC analysis in early detection of infants who develop NEC, as a non-invasive, low-cost and user friendly diagnostic mean. Furthermore, in clinical practice, it is yet found difficult to diagnose NEC at an early stage, whereas early diagnosis is correlated with a better outcome. Before clinical implementation of VOC analysis as a diagnostic mean, larger multicenter validation studies need to be carried out, by means of both GC-MS and eNose, preferably linked to longitudinal microbiota analysis to assess origin of the VOCs, increasing understanding of NEC pathophysiology.

3.5. Other Gastrointestinal Diseases

3.5.1. Celiac Disease

Celiac disease is a chronic autoimmune disorder mainly affecting the small intestine, resulting in villous atrophy and related malabsorption. The immune system is triggered by ingestion of gluten from wheat, rye and barley in genetically susceptible individuals [53]. Approximately 1.4% of people worldwide are affected by celiac disease [54]. Nowadays, gluten-free diet (GFD) is the only effective treatment currently available, however, compliance to this dietary therapy is complex. In search for a potential role of probiotics in addition to current therapy, two studies have been conducted using fecal VOC analyses [55,56].

Firstly, Di Cago et al. aimed to study the effect of celiac disease pathology on the fecal microbiotas of children, by comparing microbiota and VOC patterns between seven treated and seven untreated children with celiac disease and seven healthy children, by means of GC-MS [56]. There was a significantly higher concentration of one VOC in treated celiac disease and healthy controls vs. untreated celiac disease. It is described that these results are linked to absence of *Lactobacillus* and *Bifidobacterium* in untreated celiac disease, whereas these two bacterial strains play a major role in the metabolism of SCFAs. The same research group conducted a follow-up study aiming at studying differences in fecal VOC profiles between 19 treated children with celiac disease (two year treatment with GFD) and 15 children without celiac disease and other known food intolerances whom underwent upper endoscopy for symptoms related to functional dyspepsia [55]. Higher concentrations of one type of VOC in healthy controls were found compared to treated celiac disease (Table 3). Furthermore, higher concentrations of three types of VOCS were found in treated celiac disease compared to healthy controls (Table 3). These results might indicate that, after a period of two years GFD treatment, microbiota and volatile metabolome composition of children with celiac disease is still not fully restored.

In summary, studies mentioned above showed the potential of fecal VOCs analysis to assess disease activity and therapeutic efficacy in celiac disease. However, since sensitivity and specificity of anti-transglutaminase type 2 and anti-endomysium antibodies are very high, the need for novel biomarkers such as VOC analysis to assess therapeutic efficacy in clinical practice could be questioned.

3.5.2. Non-Alcoholic Fatty Liver Disease

Obesity prevalence is dramatically rising in Western society, and is accompanied by different comorbidities, including nonalcoholic fatty liver disease (NAFLD). Prevalence of NAFLD in affluent societies varies within 20–30% [57]. Alterations in microbiome composition are one of the prevailing assumptions on development and maintenance of obesity and NAFLD. Some bacterial strains, such as *Escherichia coli*, produce a significant amount of ethanol, which may reach the liver via the bloodstream in a continuous fashion and promote steatosis and liver injury [58]. Several studies assessed microbiome composition and fecal VOC composition in obesity related NAFLD [58–60].

Bailey and colleagues conducted a study to characterize and compare fecal microbiota and bacteria derived fecal VOCs in obese NAFLD and healthy subjects by GC-MS [59]. Concentration of two different VOCs varied widely between obese NAFLD patients and healthy controls. Additional information on the study could not be outlined, since only a conference abstract is available. In 2013, Raman et al. analyzed fecal microbiota and bacteria-derived VOC metabolites of 30 obese patients with NAFLD (BMI > 30 kg/m^2) and 30 controls (BMI < 25 kg/m^2), by means of GC-MS [58]. It was found that 12 fecal VOCS were significantly less, and 18 VOCs more abundant in NAFDL subjects (Table 3). Recently, Del Chierico and colleagues aimed to assess the structural and functional role of gut microbiota in onset and progression of pediatric NAFLD by analysis of fecal microbiota composition and bacterial derived fecal VOCs, by means of GC-MS-SPME [60]. In total, 61 children were included, comprising of 27 (NAFLD), 26 nonalcoholic steatohepatitis (NASH), 8 obese subjects without steatosis and 54 controls. They found that 26 VOCs were up-regulated in NASH, NAFLD and obese subjects. A combination of high levels 2-butanone and low abundance of *Oscillospira* seems to be specific for NAFLD subjects compared to healthy controls.

The results of previous discussed studies indicate that the found VOC profiles might be associated with a compositional shift in the microbiome of obese NAFLD patients, which is hypothesized to be part of the etiology of NAFLD in obese patients. In addition, these studies underline the potential of fecal VOC analysis in further unraveling of the role of microbiota in development of NAFLD in obese patients and application of VOC analysis as novel non-invasive biomarkers for diagnosing and evaluating NAFLD.

4. Extra-Intestinal Diseases

4.1. Late Onset Sepsis

In the past decades, the incidence of late-onset sepsis (LOS, onset >72 h after birth) in premature infants has increased towards 20%, despite advanced technology in specialized neonatal intensive care units [61,62]. LOS is characterized by a high morbidity and mortality. The origin of LOS has traditionally been linked to colonization of intravascular catheters, however, recent studies demonstrated genetic incongruity between organisms isolated from blood culture and bacteria cultured from intravascular catheter tip, suggesting another origin [63–65]. Studies shown genetic similarity between cultured LOS pathogens and isolates from the gastrointestinal tract, indicating LOS pathogens might originate from the gut [66–72]. Therefore, Berkhout et al. studied fecal VOC profiles in 36 preterm infants with confirmed LOS and compared these to 40 non–LOS controls. Fecal samples were analyzed up to five days prior to sepsis onset by means of an eNose (Cyranose 320®) [15]. Fecal VOC profiles of sepsis subjects could significantly be discriminated up to three days prior to clinical onset from healthy controls (Table 4). The same research group conducted a similar study consisting of 127 LOS cases and 127 matched preterm born infants, in which fecal samples up to three days prior to clinical onset where analyzed by means of FAIMS. Discrimination between LOS cases and healthy controls was possible one day prior to clinical onset irrespective of the cultured pathogen. In the sub analysis, it was demonstrated that fecal VOC profiles of LOS caused by *Staphylococcus aureus, Staphylococcus epidermidis* and *Escherichia coli* differed significantly from matched healthy controls up to three days prior to clinical onset (Table 4) [73].

Both studies underline the potential of fecal VOC analysis as a non-invasive diagnostic tool which allows for preclinical detection of LOS in preterm infants. Notably, each sepsis-pathogen seems to be reflected by different fecal VOC patterns. Further studies are needed to address these pathogen-VOCs by means of GC-MS in order to develop tailor-made eNose sensors to be used in clinical use. Since identification of pathogen specific VOCs can lead to early detection of specific bacterial pathogens, appropriate antimicrobial therapy can be administrated in an early stage. In addition, proof of absence of specific bacterial pathogens by VOC analysis can be achieved, therefore preventing administration of unnecessary antimicrobial therapy.

Table 4. Overview of studies on fecal VOC analysis in extra-intestinal diseases.

Disease [1]	Ref.	Case/Control No.	Analytical Technique	VOC Biomarker [a]	Sensitivity	Specificity	AUC (95% CI)
LOS	Berkhout et al. (2017)	36/40	eNose	VOC profile	T_{-1}: 64.3% T_{-2}: 75.0% T_{-3}: 57.1%	T_{-1}: 64.3% T_{-2}: 70.8% T_{-3}: 61.5%	T_{-1}: 70.4 (49.6–91.3) T_{-2}: 77.7 (62.7–92.7) T_{-3}: 70.2 (52.2–88.3)
	Berkhout et al. (2018)	127/127	FAIMS	VOC profile	- -	- -	*S. aureus* T_{-3}: 0.85 *S. aureus* T_{-2}: 0.70 *S. aureus* T_{-1}: 0.80 *E. coli* T_{-3}: 0.88 *E. coli* T_{-2}: 0.99 *E. coli* T_{-1}: 0.86 *S. epidermidis* T_{-3}: 0.90 *S. epidermidis* T_{-2}: 0.78 *S. epidermidis* T_{-1}: 0.63
BPD	Berkhout et al. (2018)	15/15	eNose	VOC profile	T_7: 43.5% T_{14}: 60.0% T_{21}: 66.7% T_{28}: 69.2%	T_7: 54.5% T_{14}: 73.3% T_{21}: 73.3% T_{28}: 69.2%	T_7: 0.58 (0.33–0.83) T_{14}: 0.72 (0.54 0.90) T_{21}: 0.71 (0.52–0.90) T_{28}: 0.78 (0.59–0.96)

Symbol meanings: [a] biomarkers are noted for study cases. - -, not mentioned in the original study. [1] Disease abbreviations: LOS, late onset sepsis; BPD, bronchopulmonary dysplasia. Other abbreviations: FAIMS, field asymmetric ion mobility spectrometry; eNose, electronic nose; CoNS, coagulase negative staphylococcus; *S. aureus*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; AUC, area under the curve; CI, confidence interval; VOC, volatile organic compound; No., number; T_{-n}, days of life prior clinical onset (e.g., T_{-1}); T_n, day of life (e.g., T_7).

4.2. Bronchopulmonary Dysplasia

One of the most common adverse outcomes in preterm very low birthweight infants (<1500 g) is BPD, with an overall incidence in this specific population of approximately 30% [74]. Infants who survive BPD commonly suffer from impaired cognitive functions and lung function abnormalities. Early identification of preterm infants at risk of developing BPD, allows for timely initiation of interventions [13]. Since BPD is a lung disease, exhaled VOCs may be preferred to perform VOC analysis, however, infants suffering from BPD are commonly mechanically ventilated which makes collection of exhaled breath complicated. Several studies have demonstrated the existence of vital cross-talks between different mucosal sites of the body, including bidirectional interaction between gut and lungs, the so-called gut-lung axis [75,76]. Therefore, Berkhout et al. aimed to evaluate the potential of fecal VOC by means of eNose (Cyranose 320®) as early non-invasive biomarker for BPD [13]. Fecal VOC profiles were identified at four different time points (7, 14, 21, and 28 days postnatally) of 15 preterm infants with severe BPD and 15 matched healthy controls. Discrimination of preterm infants with severe BPD from healthy controls was feasible at 14, 21, and 28 days (Table 4). Notably, no differences were found in intestinal microbiota composition between the two groups as estimated at similar time points, indicating that observed differences in VOC profiles were not caused by alterations in microbiota composition but presumably reflect local (lungs) and systemic metabolic and inflammatory pathways associated with BPD. Next step would be to identify BPD-specific volatiles to increase understanding of BPD pathophysiology, possibly providing windows of opportunities to develop strategies aimed at prevention of BPD.

5. Sampling Methods

Since VOC analysis is a premature research field, protocols for best suiting sampling methods and evaluation of environmental effects on VOC outcome are yet to be identified. To date, only two studies have focused on human fecal sample handling methods for different types of analytical techniques, which will shortly be discussed below.

5.1. GC-MS

Reade and colleagues aimed to assess sampling methods by analyzing 33 neonatal fecal samples by means of SPME-GC-MS [77]. They observed a positive correlation between sample weight and

the number of identified VOCs, especially when sample mass was increased from 100 mg to both 450 mg and 700 mg. Addition of salt to the fecal samples prior to analysis did not improve results. Furthermore, no differences were seen between keeping samples at 1 °C for 14 h instead of immediate analysis after storage at −20 °C, using vials of 2 mL or 10 mL, and in VOC outcome between different SPME fiber coatings (CAR/PDMS and DVB/CAR/PDMS). Based on these study results, it is advised for future studies to take effects of sample mass on VOC detection into account, whereas larger sample mass results in more VOC detection. In addition, small sample mass can be used (50–100 mg) for analysis, however reanalysis of the same headspace VOCs is not possible, since little VOCs remain in the headspace after analysis for detection.

5.2. Electronic Nose Device

To date, one study assessed the effects of sampling conditions on fecal VOC outcome by means of an eNose device (Cyranose 320®) [78]. Subsamples were obtained from one fecal sample derived from one healthy control. Variables were compared to baseline characteristics, defined as undiluted fecal samples weighing 0.50 g, stored at −20 °C upon collection and gradually heated in an incubator to 37 °C one hour prior to VOC analysis. This study demonstrated that alteration of assessed variables (e.g., sample mass (0.2 g, 0.5 g and 2 g) and samples heated to 4 °C, 21 °C) resulted in a significantly different VOC profile, with the exception of fecal samples which were diluted in a ratio of 1:5 compared to dilutions of 1:1 of 1:2 (feces(g):H20(mL). Furthermore, no differences were seen when fresh fecal samples were compared to fecal samples which underwent three freeze (−20 °C)–thaw (up to room temperature) cycles. This study underlines the importance of drafting a standardized protocol in both research setting and clinical usage, since VOC profile composition as measured by eNose device is significantly influenced by numerous variables.

Both validation studies emphasize the influence of fecal mass, temperature and freeze–thaw cycles on VOC outcome, illustrating the need for standardization in studies on fecal VOC analysis.

6. Summary, Future Perspectives and Conclusions

Over the past two decades, the potential of VOCs as biomarker in a wide variety of clinical conditions have raised considerable interest in the medical field. This is well illustrated by the increasing number of clinical studies addressing this matter. However, in contrast to other industries [79], widespread application of VOCs in daily clinical practice has still not been realized. Before a VOC-based medical tool can be developed and eventually implemented in clinical practice, several obstacles need to be addressed.

First, for each clinical condition, the substrate of choice needs to be selected. In this particular review, we focus on studies using VOCs derived from feces. Fecal VOCs provide us with valuable information about resident gut microbiota composition, and both local and systemic (patho)physiological processes. Consequently, fecal VOCs harbor great potential as diagnostic and monitoring tool in both intra- and extra-intestinal clinical conditions. Major advantage of fecal VOCs is that stools can be collected in a noninvasive manner and they have logistic advances over for example exhaled breath, since transport and storage is relatively easy. However, analysis of fecal VOCs also harbors several important limitations. Despite an increasing acceptance of fecal sampling for medical purposes, (collection of) feces is still considered a taboo in large parts of the world [80,81], potentially resulting in decreased sampling compliance. Another limitation is the fact that stools cannot be obtained on request at set times. However, in such situations, the use of rectal swabs could be an acceptable alternative [78].

Another item on the research agenda is the selection of optimal analytical techniques. In the majority of the currently reviewed studies, eNose devices are used in which discrimination between subpopulations is based on pattern recognition algorithms. Although such devices allow for fast and inexpensive measurements, resulting in relatively easy interpretable outcomes, they are limited by their inability to identify individual VOCs present in a gaseous mixture. Furthermore, since each eNose device has a different operating mechanism, comparison of studies using different devices is

hampered, and eNose sensors are vulnerable for sensor drift. On the other end of the spectrum are chemical analytical techniques such as SIFT-MS and GC-MS. Although they allow for the identification of individual VOCs, application in daily clinical practice is not feasible due to their relatively expensive and time consuming measurements in combination with complex (statistical) analyses. We hypothesize that the development of a medical device, implementable in clinical practice and allowing for accurate disease detection based on VOC profile, can be achieved in two different ways. The first method is by collecting a large amount of healthy and diseased fecal VOC profiles, analyzed by means of an eNose. Subsequently, newly obtained fecal VOCs profiles are compared with the profiles already available (VOC cloud), determining a possible diagnosis. Connecting the eNose to a self-learning algorithm will optimize eNose accuracy with each new sample analyzed. A clear example of such a self-learning eNose is the SpiroNose. Being connected to an artificial intelligence application named BreathCloud, it is currently used to detect pulmonary diseases based on the VOCs present in exhaled breath [82]. The second method includes identification of disease-specific volatiles using chemical analytical techniques. With this information, a tailored eNose may be developed, specifically designed to accurately detect the presence or absence of key volatiles and consequently associated clinical condition. To date, several studies have attempted to identify discriminating volatiles using chemical analytical techniques. Recently, metal oxide-based gas sensors (MOS) are increasingly studied in its application for VOC analysis. One study in particular demonstrated that VOCs known to be abundant in exhaled breath of CRC patients, could be detected using MOS technology [83]. Application of MOS technology on fecal samples might be the next step in the development of tailor-made disease specific sensors. Although majority of the available studies demonstrate promising results, only a small subset of research groups aimed to validate obtained outcomes in an external cohort. In addition, obtained outcomes are rarely reproduced by other study groups, resulting in a wide variety of volatiles considered to be disease specific. Inter-study variation in study and sampling protocols currently impedes the ability to make reliable comparisons between studies. Therefore, before VOCs could live up to their potential as clinical biomarker, protocols regarding sampling conditions (sampling, transport, and storage) and analytical techniques need to be standardized. This is illustrated by the observation that a broad range of different sampling conditions significantly influence fecal VOC outcome, as described previously. Use of strict protocols on methodology will permit inter-study comparisons, rapidly increasing knowledge about fecal VOCs in both healthy and diseased individuals. In addition, being the waste-product of metabolic processes, detected VOCs may allow for unraveling pathophysiological mechanisms underlying a clinical condition. Next to identification of pathophysiological VOCs, it is first key to determine the spectrum of VOCs still considered to reflect a healthy state

This leads us to the third hurdle needed to be taken before VOCs could be implemented as clinical biomarker in daily practice. To date, a total of 1840 VOCs have been identified as volatiles deriving from a healthy human body, of which 381 individual VOCs are linked to feces [84]. Presumably, factors including age, gender, body weight, gut microbiota composition, and life style, as well as variation in sampling techniques, all may significantly influence VOC composition. Again, this underlines the need for widespread standardization of sampling techniques and the protocol-driven use of analytical devices, allowing for inter-study comparison.

To conclude, a continuously increasing number of studies have attempted to discriminate diseased from healthy individuals based on their fecal VOC profile. Although the majority of these studies demonstrate promising results, outcomes are yet rarely validated or reproduced. Several key issues need to be addressed before (fecal) VOC analysis could live up to its potential as diagnostic or monitoring tool. Especially efforts concerning universal standardization of fecal sampling and methodology, ultimately allowing for inter-study comparison, should receive priority.

Conflicts of Interest: The authors declare no conflicts of interest.

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