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REVIEW – CLINICAL ONCOLOGY

Fecal volatile organic compounds for early detection of colorectal cancer: where are we now?

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Abstract

Introduction The fecal volatolome, which is composed of fecal volatile organic compounds (VOCs), seems to hold potential as non-invasive biomarker for the detection of colorectal cancer (CRC) and its precursor lesions advanced adenomas (AA). The potential of the fecal volatolome has been subject of various studies using either chemical analytical or pattern-recognition techniques. The available literature on the potential of the fecal volatolome as CRC and AA biomarker was reviewed.

Methods A systematic literature search was conducted in PubMed, Embase, the Cochrane Library, Google Scholar and ResearchGate using the following keywords: Colorectal Cancer, Advanced Adenoma, Volatile Organic Compound, Metabolome, Gas Chromatrography–Mass Spectrometry, Selected-Ion Flow-Tube Mass Spectrometry, eNose, and Fecal Biomarkers.

Results Eighty-eight titles or abstracts were identified from the search, of which 11 papers describing the potential of the fecal volatolome for CRC detection were selected. In these studies, different techniques were used for the headspace analyses of fecal VOCs, limiting the possibility to compare outcomes. Increased levels of amino acids and short chain fatty acids, and decreased levels of bile acids and polyol alcohols in the gas phase of feces were observed repeatedly. All selected papers reported high diagnostic value for the detection of both CRC and AA based on fecal VOCs.

Conclusion Based on the included studies, fecal VOC analyses seem promising for future screening of CRC and AA, with potentially improved test performances allowing for earlier detection of AA and CRC and consequently earlier initiation of treatment, possibly reducing morbidity and mortality rates next to lower rates of (unnecessary) colonoscopies.

Keywords Colorectal carcinoma · Advanced adenoma · Volatile organic compounds · Biomarker · Screening

Abbreviations

AA	Advanced adenoma
AUC	Area under the curve
CRC	Colorectal cancer
eNose	Electronic Nose
FIT	Fecal immunochemical testing
FOBT	Fecal occult blood test
GC	Gas chromatography
GC–MS	Gas chromatography–mass spectrometry
GC–MSD	Gas chromatography–mass selective detector
GC–SCD	Gas chromatography–sulfur chemiluminescence detector
GC–TCD	Gas chromatography–thermal conductivity detector
HC	Healthy control
SCFA	Short chain fatty acid
SIFT-MS	Selected ion flow tube linked to mass spectrometry
VOCs	Volatile organic compounds

Novelty and impact: This systematic review provides an overview of studies on the diagnostic accuracy of volatile organic compounds as novel noninvasive biomarkers for the detection of colorectal carcinoma and its precursor lesions (advanced adenomas).

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Introduction

Colorectal cancer (CRC) is the third most common malignancy with an incidence rate of 40.7 per 100,000 in the US, and has the highest cancer-related mortality rate in the industrialized world (Ferlay et al. 2010; Jemal et al. 2009; Siegel et al. 2017). The 5-year survival rate for colon cancer and rectum cancer are 64.4% and 66.6%, respectively (Siegel et al. 2017). Early detection and treatment are critical factors in the course and prognosis of CRC, as the survival rate decreases with disease progression (Winawer et al. 2003). Most CRC develops from advanced adenomas (AA), and early detection and removal of these adenomas has been found to decrease CRC incidence and mortality (Atkin et al. 1992; Leslie et al. 2002). The most widely used screening modalities for CRC and its neoplastic precursors are fecal immunochemical testing (FIT) and endoscopic evaluation of the colon. Although this screening program has led to a decrease in mortality, the performance of this test is suboptimal, with a sensitivity and specificity for CRC of 56–89% and 94–97%, respectively, depending on used cut-off values (Nakama et al. 2001). This results in a substantial number of false negative tests, and as a consequence missed diagnosis of colorectal cancer in 11–44% of the cases. In addition, 3–6% of the healthy participants undergoing population based screening still receive false positive test results, which leads to the performance of unneeded colonoscopies. These colonoscopies carry a high burden on patients and create a small risk of complications (e.g. bleeding or perforation) (Nakama et al. 2001). Because of these limitations, a clear unmet need exists for a more accurate and non-invasive test to select high-risk individuals who need to undergo colonoscopy.

The use of gas molecules as noninvasive disease biomarkers stems from a long history of medicine in which Hippocrates characterized the distinct smell of melena as early as 400 years BC, and patients with diabetes were described as have urine with a smell of rotten apples in ancient Chinese medicine (Buljubasic 2015). Nowadays, gaseous molecules are analyzed using highly sensitive techniques, resulting in smellprints comprised of over a thousand different gaseous molecules, referred to as volatile organic compounds (VOCs), or the 'volatolome'. These VOCs are produced during metabolic processes such as inflammation, cancer degeneration and necrosis and can be measured in all conceivable bodily excrements including breath, urine and feces dependent on their volatility and temperature of the sample. The fecal volatolome is also believed to reflect alterations in gut-microbiota by a change in VOCs created during gut-microbiota interactions (Boots et al. 2014). Multiple studies have focused on the use of the fecal volatolome as biomarker for CRC and AA with promising results (Bishop et al. 2017; Weir et al. 2013). In this systematic review we have aimed to summarize the available literature on fecal volatolome analysis for the differentiation between CRC, AA and controls, and philosophize about the clinical implications for improved screening on colorectal cancer.

Methods

An electronic literature search was performed systematically, using the electronic database of the National Center for Biotechnology Information (PubMed), Embase, Cochrane Library, Google Scholar and ResearchGate to collect publications before June 2018. The following search terms for colorectal cancer and advanced adenoma, including synonyms and closely related words, were used as index terms and/or free words: 'colorectal carcinoma', 'colon cancer', 'rectal cancer', 'colorectal neoplasia', 'colorectal tumor', 'advanced adenoma', 'high-risk adenoma'. These terms were combined with index terms and/or free words for VOC analyses ('gas chromatography–mass spectrometry', 'ion mobility spectrometry', 'selected-ion flow-tube mass-spectrometry', 'electronic nose', 'volatile organic compounds', 'volatolome', 'gas molecules', 'metabolome') and fecal biomarkers ('feces', 'fecal marker', 'fecal biomarker'). After the search, the collected literature was screened on title and abstract by two authors independently, and included in this study after selection by both of the authors (SB and NKHDB). The reference lists of identified papers were checked on additional studies missed during the original search. Full-text articles, abstracts and posters were only included if they focused on the fecal VOC composition, excluding other bodily fluids and metabolites. Non-original articles, reviews, duplicates and articles in other languages than English or Dutch were excluded from this review.

Results

Eighty-six titles or abstracts were identified from the primary electronic search and reference lists after the literature search was performed in PubMed, Embase and the Cochrane Library and two articles were found using Google Scholar and ResearchGate (Fig. 1). Of the identified records, 77 publications did not meet the criteria for inclusion for various reasons; a total of 11 publications could be included in this systematic review. Reasons for exclusion were the use of mucosal biopsies and other material rather than feces, newly described methods without statistics provided, replies on papers or editorial comments and papers not relevant to the topic. Included literature consisted of nine full-texts original

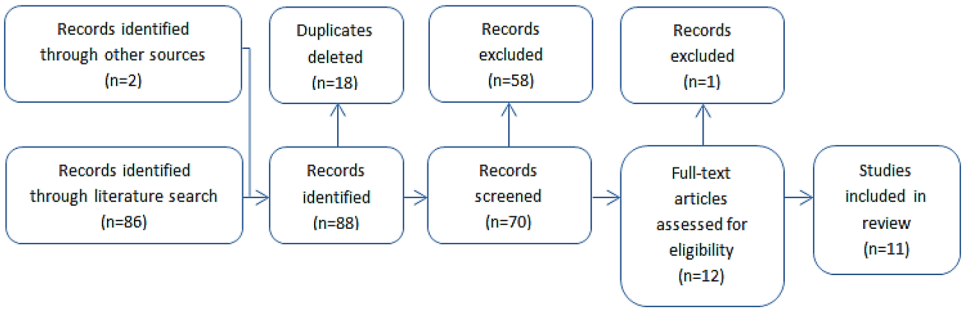

Fig. 1 PRISMA flow diagram

articles, one abstract and one poster presentation (Table 1). In six studies, gas chromatography (GC) was used for VOC identification, of which three used GC combined with mass spectrometry (GC–MS). Two studies made use of a self-made VOC sensor: SCENTA1. Other studies made use of selected-ion flow-tube mass spectrometry (SIFT-MS), electronic Nose (eNose) and dogs for scent detection.

Volatolome analytical techniques

Analytical methods can be separated into chemical analytical techniques and pattern-recognition technology. In chemical analytical techniques, alterations in the presence and concentrations of specific molecules can be detected, whereas pattern-recognition technologies focus on the discrimination of VOC profiles by differences in sensor resistance to specific VOCs. In this section of this review an overview of the used analytical methods for fecal VOC analyses for the detection of CRC is given. In Table 2, technical characteristics per analytical method are summarized.

Chemical analytical techniques

Gas chromatography–mass spectrometry This method has been proved to successfully analyze the VOC composition of various bodily fluids and is considered the gold standard for volatolome analyses (Garner et al. 2007). Coupling chromatography to spectrometry allows for separation and quantification of individual VOCs. These analytes are transported through the chromatography column in a carrier gas and interact with the column surface. VOCs with different chemical characteristics have specific interactions with the column, resulting in a distinct transportation time for sets of VOCs with comparable characteristics. VOCs are then ionized and pushed into the second (spectrometry) column, consisting of an electric field with varying voltage. The transportation time of the analytes is influenced by their electric charge, resulting in a distinct transportation time for sets of VOCs with comparable masses (Hites 1997). By combining separation on chemical characteristics and mass, the VOC composition can be measured very accurately which allows for biomarker recognition.

Selected ion flow tube-mass spectrometry Another technique used for fecal volatolome analyses for the detection of CRC is selected ion flow tube-mass spectrometry (SIFT-MS). This technique allows for real-time measurements and is, therefore, faster compared to the gold standard. Mass spectrometry is coupled to a selected ion flow tube, in which VOCs are ionized by precursor ions (H_3O^+, NO^+ and O_2^+) in a defined order (Smith and Spanel 2005). The precursor ions are generated using a microwave discharge and their order for the analysis is chosen using quadrupole mass spectrometry. Before the sample is injected, the selected precursor ions are introduced in the flow tube using helium as carrier gas. The precursor ions and VOCs interact in the flow tube and enter a second quadruple mass spectrometer where the product ions and precursors are separated. Real-time data analyses is done by scanning a specific spectrum of mass-to-charge-ratios defined by the user. The absolute concentration can be calculated from the ratios using the precursor and product ion signals. This technique provides less detailed information on VOC composition compared to GC–MS, since VOCs remain less separated using this analysis, but allows for real-time measurements, has lower maintenance costs and does not require specialized personnel.

Pattern-recognition techniques

eNose technology There are many sorts of different electronic nose (eNose) devices, which all have in common that VOC analysis is based on pattern-recognition. In eNose technology, VOCs are presented to an array of sensors made from specific material. Analytes interact with the individual sensors based on their chemical characteristics. This sensor-interaction is analyzed with different techniques dependent on which eNose is used (e.g., conducting-polymer sensors, electrochemical sensors, metal oxide sensors). The differences in sensor-interaction create a pattern which is called the VOC 'smellprint', and can be analyzed with pattern recognition algorithms to identify specific diseases (Buijck et al. 2016). The main benefits of eNose technology are measurement speed and low costs and although disease-specific patterns can be recognized, individual VOC biomark-

Table 1 An overview of study design, number of patients included, analytical techniques, identified compounds and test specifics of the 11 selected papers

Author (year)	Study design	Number of inclusions	Analytical technique	Identified compounds[a]	Chemical classes	AUC	Sensitivity	Specificity	*p* value	Article type
Chemical analytical techniques										
Weir et al. (2013)	Case–control study	Global metabolome 9 CRC 10 HC	GC–MS	↑ Alanine, glutamate, glycine, aspartic acid, leucine, lysine, proline, serine, threonine, valine, phenylalanine, benzeneacetic acid, propionic acid, myristic acid, pantothenic acid, cholesterol derivate	Amino acids Carbocylic acid, SCFA, saturated fatty acid Vitamin B5 Steroid	–	–	–	All *p* values < 0.05 to < 0.0001	Full-text original article
				↓ Oleic acid, linoleic acid, elaidic acid, glycerol, monooleoylglycerol, ursodeoxycholic acid	Unsaturated fatty acids, polyol and polyol derivate, bile acid					
		Targeted SCFAs 9 CRC 11 HC	GC–MS	↑ Acetic acid, valeric acid, isobutyric acid, isovaleric acid	SCFAs					
				↓ Butyric acid						
Batty et al. (2015)	Case–control study	62 FOBT+: 31 AA 31 HC	SIFT-MS	↑ Hydrogensulphide Dimethylsulphide Dimethyldisulphide *m/z* 90 (unknown)	Sulphides	–	72% AA	78% AA	–	Full-text original article

Table 1 (continued)

Author (year)	Study design	Number of inclusions	Analytical technique	Identified compounds[a]	Chemical classes	AUC	Sensitivity	Specificity	*p* value	Article type
Bond et al. (2016b)	Prospective case–control cohort	21 CRC 56 Adenoma 60 HC	GC–MS	No specific VOCs described due to potential future IP	–	0.82 CRC	87.9%	84.6%	< 0.0001 CRC	Abstract
Wang et al. (2017)	Case–control study	15 CRC	GC–MS	↑ Acetic acid, valeric acid, butyric acid and isovaleric acid, Glutamic acid, glycine, aspartic acid, leucine, glycerin, proline, serine, valine, phenylalanine, phenylacetic acid, cholesterol derivates	SCFAs, amino acids, steroids	–	–	–	All *p* values < 0.05 to < 0.0001	Full-text original article
		12 HC		↓ Isobutyric, oleic acid, elaidic acid, linoleic acid Glycerin, monoacyl glycerol, myristic acid, ursodeoxycholic acid, pantothenic acid	SCFA, unsaturated fatty acids, polyhydric alcohols, saturated fatty acid, bile acid, vitamin B					
Ishibe and Takeshita (2017)	Prospective case–control study	30 CRC	GC–SCD	↑ Methyl mercaptan (MM)	Sulphide	0.78	–	–	$p = 0.036$ MM $p = 0.008$ H_2	Full-text original article
		26 HC	GC–TCD	↓ Hydrogen (H_2)	Hydrogen					

Table 1 (continued)

Author (year)	Study design	Number of inclusions	Analytical technique	Identified compounds[a]	Chemical classes	AUC	Sensitivity	Specificity	*p* value	Article type
Song et al. (2018)	Prospective case–control study	26 CRC	GC–MS (for long chain fatty acids)	↑ Oleic acid, Linoleic acid only in male subjects	Mono and polyunsaturated fatty acids	–	–	–	Only significant increases in male subjects ($p < 0.04$ to $p < 0.01$)	Full-text original article
		28 HC	GC–MSD (for SCFAs)	–	–					
Pattern-recognition techniques										
Sonoda et al. (2011)	Prospective case–control study	37 CRC 148 HC	Canine scent judgement	–	–	–	97%	99%	–	Full-text original article
De Meij et al. (2014)	Case–control study	40 CRC 60 AA 57 HC	eNose (Cyranose® 320)	Pattern-recognition	–	0.92 CRC 0.79 AA 0.82 AA vs CRC	85% CRC 62% AA 75% AA vs CRC	87% CRC 86% AA 73% AA vs CRC	< 0.001 CRC < 0.001 AA < 0.001 AA vs. CRC	Full-text original article
Bond et al. (2016a)	Prospective case–control study	18 CRC 18 AA 67 Adenomas 78 HC	GC + metal oxide gas sensor	Pattern recognition based on sensor resistance	–		75% CRC 100% AA 100% CRC vs AA	66.7% CRC 100% AA 100% CRC vs AA	–	Poster
Zonta et al. (2017a)	Case–control cohort	6 CRC 10 HC	SCENT A1	Pattern-recognition	–	–	–	–	–	Full-text original article
Zonta et al. (2017b)	Not clear from text	Feasibility: 86 CRC 71 CRC	SCENT A1	Pattern-recognition	–	–	95% overall	95% overall	–	Full-text original article
	Prospective case–control cohort	Validation 6 CRC + AA 20 low risk adenomas 60 HC	SCENT A1	Pattern-recognition	–	–	–	–	–	

CRC colorectal carcinoma, *AA* advanced adenoma, *HC* health control, *GC–MS* gas chromatography–mass spectrometry, *GC* gas chromatography, *SIFT-MS* selected ion flow tube-mass spectrometry, *eNose* electronic Nose, *SCD* sulfur chemiluminescence detector, *TCD* thermal conductivity detector, *MSD* mass selective detector, *SCFA* short chain fatty acid

[a]Changes in metabolite levels are noted as measured in CRC/AA samples

Table 2 Overview of technical characteristics per volatolome analyses method

	GC–MS	SIFT-MS	Cyranose 320®	SCENT A1
Accuracy	Very good separation of analytes using two techniques to separate based on different characteristics	Good separation but some discrimination of VOCs is lost since there is no chromatography column	High accuracy, however, no conclusions about specific volatiles can be drawn	High accuracy, however, no conclusions about specific volatiles can be drawn
Stability	Needs calibration daily	Very stable, no calibration required	Easily influenced by environment	No information
Speed	10–45 min per sample	A few seconds–minutes per sample	Six minutes per sample including air blank to clean machine	Average measurement time of 1 h per sample
Costs	Expensive Mainly because of costs for technical operators, maintenance and sample preparation	Expensive Machine is double the price of GC–MS, however, less extra expenses because less maintenance and sample preparation	Inexpensive	No information
Usability/practicality	Technical operators only	Technical and non-technical operators	Very easy to use for non-operators	No information
Maintenance	High maintenance	Low maintenance	Low maintenance	Sensor renewal needed every 2 years
Sample preparation	Requires preparation and preconcentration	No preparation required	No preparation required	No preparation required

GC–MS gas chromatography–mass spectrometry, *SIFT-MS* selected-ion flow-tube mass-spectrometry, *eNose* electronic Nose

ers cannot be identified on a molecular level. Furthermore, sensor drift over time may hamper reproducibility.

SCENT A1 A new eNose device for fecal VOC analyses which has solely been described by one research group so far, is the patented SCENT A1 (Zonta et al. 2017a, b). This is a device made of an array of five chemo resistive sensors which are capable of changing their resistance once in contact with specific VOCs, and can detect tumor biomarkers in low concentrations. The sensor material used in this device are $SmFeO_3$ (Iron and samarium oxides), ST25 + Au (tin and titanium), ST20 (tin and titanium oxide 20%), TiTav (titanium, tantalum and vanadium oxides) and In_2O_3 (indium oxide). These sensors have been selected after thorough testing of 20 sensors composed of different material. The array of five sensors with the best diagnostic accuracy to differentiate between CRC and HC was selected for further development and validation (Zonta et al. 2017a, b). Information on practicality, sensor stability and costs are lacking.

Study characteristics and outcomes

Chemical analytical techniques

Gas chromatography–mass spectrometry The first study to explore the use of the fecal volatolome for the detection of CRC using GC–MS was conducted by Weir et al. (2013). Stool samples were collected from 10 CRC patients prior to colon resection surgery and 11 healthy controls (HC). Global volatile metabolite profiling was performed on fecal samples of 9 CRC and 10 HC, and targeted short chain fatty acid (SCFA) profiling on 9 CRC and 11 HC,. The global volatolome of CRC patients showed increased levels of 11 amino acids (alanine, glutamate, glycine, aspartic acid, leucine, lysine, proline, serine, threonine, valine and phenylalanine), one carboxylic acid (benzeneacetic acid), one SCFA (propionic acid) and one saturated fatty acid (myristic acid), one vitamin B5 derivate (pantothenic acid) and one steroid (cholesterol derivate). In addition, three unsaturated fatty acids (oleic, linoleic, and elaidic acid) as well as polyol and its derivates (glycerol and monooleoylglycerol), and one bile acid (ursodeoxycholic acid) were decreased. The SCFA profiles of CRC patients showed increased levels of acetic acid, valeric acid, isobutyric acid, isovaleric acid and decreased levels of butyric acid compared to profiles of HC. This study did not report any outcomes on accuracy, specificity and sensitivity for one (or a combination) of these metabolites as CRC biomarker.

Another study applying GC–MS was reported by Bond et al. (2016a, b), who analyzed fecal samples from symptomatic patients and patients referred after they were tested positive during the UK Bowel Cancer Screening Program. A total of 20 CRC patients and 60 HC were included. Four

discriminating compounds were reported, of which compound A showed an area under the curve (AUC) of 0.76 ($P < 0.0001$), and the combination of A and B increased the AUC to 0.82. After a tenfold cross validation, an AUC of 0.82 with a sensitivity of 87.9% and a specificity of 84.6% was found. During further logistic regression analyses a combination of three compounds (A, X and Y) was found, of which the AUC was 0.86 ($P < 0.0001$). Unfortunately, no specific VOCs were named in this abstract, due to potential future intellectual property.

Wang et al. (2017) performed a study on the volatolome for CRC detection using GC–MS analyses on fecal samples of 15 CRC patients and 12 HC. The levels of SCFAs acetic acid, valeric acid, butyric acid and isovaleric acid were increased in CRC patients, whereas isobutyric acid was decreased in this group. There was no difference in the level of propionic acid between CRC and HC. Furthermore, levels of 9 amino acids (glutamic acid, glycine, aspartic acid, leucine, glycerin, proline, serine, valine, phenylalanine), phenylacetic acid and cholesterol derivates were significantly increased in the CRC group compared to HC, and the unsaturated fatty acids (oleic acid, elaidic acid and linoleic acid), two types of glycerin (glycerin and monoacyl glycerol), one saturated fatty acid (myristic acid), one vitamin B5 derivate (pantothenic acid) and one bile acid (ursodeoxycholic acid) were decreased.

In the most recent study on the use of GC–MS for fecal volatolome analyses, samples from 26 newly diagnosed CRC patients were compared to 28 healthy individuals (Song et al. 2018). Analyses of long-chain fatty acids using GC–MS was combined with the analyses of SCFAs using GC coupled to a mass selective detector (GC–MSD). In contrast to the previous studies, no significant differences were found in the levels of both these short and long-chain fatty acids when comparing CRC to HC. Interestingly, sub-analyses by gender did reveal an increase in fecal concentrations of both oleic acid and linoleic acids in male CRC patients whereas in previous studies decreased concentrations of these fatty acids were observed.

Other chemical analytical techniques

In 2015, selected ion flow tube linked to mass spectrometry (SIFT-MS) was used for the identification of individual fecal VOCs by Batty et al. (2015), who included 62 patients with a positive fecal occult blood test (FOBT) resulting in a subsequent performed colonoscopy. During endoscopy, at least one advanced adenoma (referred to as high grade adenoma) or adenocarcinoma was found in 31 of these participants (high-risk group), while no abnormalities were observed in the residual of the participants (low risk group). In this article it is not noted how many patients included in the 'high-risk' group had CRC or AA. Four analytes (*m/z* 35 and *m/z* 90 using H_3O^+ as precursor ion, *m/z* 62 and *m/z* 94 using NO^+ as precursor ion) were found to be significantly increased in the high-risk group. Possible identities of these analytes were hydrogen sulphide, dimethyl sulphide and dimethyl disulphide. The compound with mass-to-charge ratio 90 remained unknown. Based on the whole dataset created by analyses of all three precursors, the researchers found an overall classification accuracy of 75%, with a specificity of 78% and a sensitivity of 72% for 'high-risk' patients vs healthy controls.

Another study was conducted by Ishibe who included 30 CRC patients and 26 healthy volunteers. A gas sampling apparatus was built into a toilet which collected gas components during defecation. Fecal gas was analyzed on methyl mercaptan (MM) and hydrogen sulphide (H_2S) using gas chromatography coupled to a sulfur chemiluminescence detector (GC–SCD). Next hydrogen (H_2), methane (CH_4) and carbon dioxide (CO_2) were analyzed by gas chromatography coupled to a thermal conductivity detector (GC–TCD). Levels of MM were found significantly increased in CRC patients, whereas levels of H_2 were significantly decreased compared to HC. Using a logistic regression analyses, a discriminant formula was calculated with values of AUC, sensitivity, specificity and accuracy of 0.78, 90%, 57.7% and 75%, respectively.

Pattern recognition techniques

Canine scent Dogs are renowned for their excellent sense of smell. Their olfactory capacities have been mainly used for hunting and protection of property, but trained dogs are also able to accurately detect drugs and explosives at borders, airplane fields or in prisons. For disease detection, multiple studies have focused on the use of dogs for various carcinomas (e.g., prostate cancer, ovarian cancer, bladder cancer), all showing high accuracy to detect diseases (Horvath et al. 2008; Taverna et al. 2015; Willis et al. 2004). A canine trial with a Labrador retriever aimed at identifying CRC was performed in 2013 and demonstrated a remarkable diagnostic accuracy. The sensitivity and specificity of fecal samples was high (97% and 99%, respectively) (Sonoda et al. 2011). The correctness proved to be better than FOBT.

Electronic Nose technology

Cyranose In 2014, the VOC profiles, of fecal samples of 40 CRC patients, 60 patients with advanced adenomas (described as polyps sized > 1.0 cm or exposing villous features or high-grade dysplasia) and 57 healthy controls were analyzed and compared using an eNose (Cyranose 320®) (de Meij et al. 2014). The Cyranose 320® is a portable machine consisting of an array of 32 nanocomposite carbon sensors. The interaction of specific VOCs with the sensor

material causes swelling of the sensors, which induces a change in the electrical resistance. This change is measured and creates a specific 'smellprint'. Fecal VOC profiles of CRC patients could be discriminated from controls based on their smellprint with a sensitivity of 85% and a specificity of 87% (AUC 0.92). Patients with advanced adenomas could be discriminated from controls with a sensitivity of 62% and specificity of 86% (AUC 0.79). Differentiation between CRC and AA was possible with a sensitivity and specificity of 75% and 73%, respectively (AUC 0.82).

SCENT A1 This fairly new eNose apparatus to discriminate CRC from HC has been described in two trials by the same research group. First, a total of 20 sensors composed of different material were tested in different arrays of 5 units, to find the best performing array for the detection of CRC (Zonta et al. 2017a). Fecal samples of six CRC patients and ten HC were used, of which all CRC patients and nine out of ten HCs were classified correctly using the best performing array. No statistics were described. In their following publication, a feasibility and validation study for this sensor array was performed (Zonta et al. 2017b). In the feasibility study, the best performing array for the detection of CRC was again chosen after testing different combinations of sensors on a total of 157 samples in the laboratory (86 CRC, 71HC). An overall accuracy, sensitivity and specificity of 95% was computed when considering all the samples tested with all the sensor combinations tried. Then, the best performing array was validated in a new prospective case–control cohort including fecal occult blood test (FOBT) positive patients. A total of 6 CRC/AA patients, 20 low-risk adenoma patients and 60 healthy controls were included. Using this sensor-based eNose, all CRC and AA patients were classified correctly. Healthy controls were classified correctly in 58 out of 60 cases, 1 was classified in the low-risk adenoma group and 1 was classified in the CRC and AA group. No values on sensitivity or specificity were given in this section of the paper.

Gas chromatography coupled to metal oxide gas sensor GC coupled to a metal oxide gas sensor has been described in an abstract by Bond (2016a, b) and is similar to eNose technology, in which resistance patterns are identified by computer algorithms. In their study performed in 2016, the VOC patterns of 18 CRC and 18 AA patients were compared to 78 healthy controls with a positive bowel screening. Advanced adenoma was described as any polyp sized > 1.0 cm. High accuracy values of 83.3% and 87.5%, were observed for the discrimination of CRC and AA patients compared to HC, respectively. Differentiation between CRC and AA patients was feasible with accuracy, sensitivity and specificity levels of 100%.

Discussion

Analysis of the 11 selected papers demonstrated that the composition of the fecal volatolome is different in the presence of CRC and AA compared to a healthy state. In addition, the clinical value of the fecal volatolome as colonic neoplasia biomarker has been demonstrated in all published studies, with sensitivity values ranging between 75–97% for CRC and 62–100% for AA, and specificity values between 66.7–99% for CRC and 78–100% for AA. The metabolic 'smellprint' was analyzed using multiple techniques, which limits the possibility of a detailed comparison of the papers.

Three of the studies selected for this review used GC–MS, of which two investigated the global metabolic profile and one investigated only short-chain and long-chain fatty acids. Although research groups were small, ranging from 9 to 26 CRC cases, there are some interesting similarities in potential biomarkers found. For example, increased levels of similar amino acids (glycine, leucine, proline, serine and phenylalanine) were found in both studies evaluating the global metabolite profile, which could be caused by various reasons. First, an increase of the mucin-degrading bacteria *Bacteroides* and *Akkermansia muciniphila* in CRC patients has been observed in various studies (Baxter et al. 2014; Weir et al. 2013). These bacterial strains are known to break down mucin, which consists of glycoproteins built from amino acids. Enrichment of these mucin-degrading strains can cause an accumulation of free amino acids. In addition, both mucin degradation and colon cancer itself have been linked to intestinal inflammation (Ganesh et al. 2013; Janakiram and Rao 2014; Maeda et al. 2011). Inflammation of the colon can cause a reduction in the absorption of amino acids, amongst other nutrients. Last, autophagy is known to be activated in colorectal cancer cells, leading to the release of free amino acids (Sato et al. 2007).

Other similarities were the increased levels of the SCFAs valeric acid, isovaleric acid and acetic acid. Dissimilation of leucine by anaerobe bacteria results in the production of isovaleric acid, whereas valine dissimilation results in the production of isobutyric acid (Britz and Wilkinson 1982; Dickinson et al. 1998). Both these amino acids have been found increased in stool of CRC patients. Therefore, the increase in isobutyric and isovaleric acid may be explained by the fact that they are final products of bacterial amino acid metabolism. Acetic acid may be used by colonic bacteria to produce butyric acid. Low levels of butyric acid-producing bacteria have been found in CRC patients in multiple studies (Wang et al. 2017; Weir et al. 2013). High levels of acetic acid may be caused by a decrease in bacterial consumption. Another explanation

given by Weir is the degradation of butyric acid to acetic acid in the colon (Weir et al. 2013). Although these SCFAs may seem promising biomarker for CRC, it has to be noted that differences in levels of SCFAs were not shown by Song et al. (2018), who specifically focused on that section of the volatolome using the same analytical platform.

In addition, the role of butyric acid is of importance. Butyric acid is a widely studied microbial metabolite, which is notorious for its prevention against colorectal cancer (Bishop et al. 2017; Williams et al. 2003; Xu et al. 2018). Previous studies have shown decreased levels of butyric acid-producing bacterial strains in fecal samples of CRC patients (Wang et al. 2017; Weir et al. 2013). It could be hypothesized that levels of butyric acid may also decrease in feces of CRC patients, and that this compound may hold potential as CRC biomarker. Results of the publications included in this review are, however, inconclusive on this particular metabolite. One study has indeed shown decreased levels of butyric acid in fecal samples of CRC patients, whereas the other two studies focusing on SCFAs have either shown increased levels or no differences. These differences could at least partly be explained by differences in sampling methods, storage conditions and machine settings, underlining the need for the implementation of standardized protocols in fecal VOC analyses. Interestingly, the contradicting studies both found lower levels of butyric acid-producing bacterial strains in the fecal samples of their participants.

Last, decreased levels of polyhydric alcohols (e.g., glycerol) and one bile acid (ursodeoxycholic acid) were found. Ursodeoxycholic acid is a secondary bile acid produced by colonic bacteria, and is considered to have a chemopreventive effect on colorectal cancer cells by inhibiting tumor development (Serfaty et al. 2010). Polyhydric alcohols are used by human cancer cells, which possess a well-known transport system for increased glycerol absorption (Fujimoto et al. 2006). Uptake of these alcohols could explain the decreased levels in stool of CRC patients.

In colon cancer screening programs, the costs/quality ratio of biomarker detection is of great importance. Although there have been several studies published on the use of dogs for disease detection, all showing very high sensitivity, no studies have ever compared the accuracy of dogs and analytical techniques in a systematic manner. However, implementation of canine olfaction in daily practice is obviously hampered by obstacles as training is costly and laborious. It seems even more challenging to train sufficiently enough dogs for (nationwide) CRC screening programs (Teodoro-Morrison et al. 2014). The quality of the GC–MS technique is high, though it is expensive, time-consuming and needs specialized laboratory personnel for its operation and maintenance which limits its use for population based screening or large scale research (Arasaradnam et al. 2014; Buijck et al. 2016; Garner et al. 2007). Less expensive compared to GC–MS is SIFT-MS since it requires less maintenance and sample preparation, but this technique provides less detailed information on VOC composition. It is dependent of the chemical and mass characteristics of potential fecal CRC biomarkers, whether this technique could accurately differentiate between CRC and HC, and thus holds potential for population based screening. The publications on pattern-based techniques all report a high-diagnostic value for the detection of both CRC and AA. The compact size, low costs, user-friendliness and speed of eNose analyses underline their potential for the high-throughput analyses required in population based screening. However, their main limitation is their inability to identify specific VOCs.

To date, VOC tests for the detection of AA and CRC are still far from the use in a public health setting. For this, the number of subjects studied in the current available literature is rather small, and the use of various techniques for VOC analyses in combination with the use of different criteria used for advanced adenomas, hampers reliable comparison between studies. In addition, data on the VOC profiles of non-advanced polyps is lacking. It is well-known that the tendency of polyps to develop into malign neoplasia is dependent of multiple characteristics as epithelial differentiation, size, basis and the presence of villous features. It would be of interest to assess whether these specific characteristics influence the VOC profile and whether there is similarity with the VOC profile of CRC patients. Another challenge to encounter before developing a disease-specific eNose, is the correction for other factors influencing VOC profile. Though for breath analyses it is known that there is only small similarity in VOC profile when measured in a single individual at consecutive moments, information is lacking on the intraindividual stability of fecal VOC profiles (Schmidt and Podmore 2015). In addition, it is known that lifestyle factors as smoking, dietary intake and use of medication have an effect on the fecal VOC composition (de Swart et al. 2018; El Manouni El Hassani et al. 2018; Lange et al. 2016). Future studies should, therefore, take these variables into account by either matching of study participants or calculating correction factors per variable. The next step for developing CRC-specific VOC tests, is to combine measurements with a chemical analytical technique such as GC–MS and a pattern-recognition based technique in a cohort including CRC, AA, non-advanced adenomas and healthy controls, to identify which disease-specific molecules interact with which sensors. This allows for the development of a highly accurate, inexpensive, easy-to-use, CRC-specific eNose. The CRC-specific eNose should then be tested on suitability for clinical implementation in mass screening programs, preferably by comparing the eNose to the presently used screening program test on performance characteristics, user friendliness and costs in a large population study.

In conclusion, an increasing number of studies demonstrate the potential of the fecal volatolome as non-invasive biomarkers for the detection of CRC and advanced adenomas. The use of different techniques limits comparability, however, all studies demonstrated a high-diagnostic value of the fecal volatolome for CRC detection. This holds promise for future screening on CRC and advanced adenoma, with potentially better test performances allowing for earlier detection of AA and CRC and consequently earlier initiation of treatment, possibly reducing morbidity and mortality rates next to lower rates of (unnecessary) colonoscopies. Future studies should focus on validation of previously found fecal VOC biomarkers in a large prospective cohort linked to the population based screening, preferably by combining a chemical analytical technique with pattern-recognition, so CRC- and AA-specific biomarkers can be identified which can be used to develop tailor-made eNose sensors to be used in clinical practice.

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Compliance with ethical standards

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