Volatile organic compounds as new biomarkers for colorectal cancer: a review

Abstract

Analysis of the volatile part of the metabolome (volatile organic compounds, VOC) present in the gas phase of excreted materials is a promising new screening tool for several cancers, including colorectal cancer (CRC). The VOC signature can reflect health status, like a ‘fingerprint’, and can be modified in several diseases. Technical difficulties still limit the widespread use of VOC analysis in the clinical setting, but this approach has already been applied successfully in the diagnosis of CRC. The present study reviews the available data on VOC present in the headspace (the gaseous constituents of a closed space above a liquid or solid) of blood, urine, faeces and breath as a potential screening tool for CRC. A systematic electronic literature search was conducted in PubMed, Scirus and Google using the following keywords: Metabolomic, Volatile Organic Compounds (VOC), Electronic-nose and Colorectal Cancer. Only articles published in English between 2000 and 2015 were selected and these were independently checked by two of the authors. Ten papers describing the reliability of VOC analysis in breath and faeces, blood and urine were selected; all indicated good reliability in detecting CRC. The use of different substrates and different analytical platforms has led to the identification of different patterns of VOC. The reliability of a metabolomic approach as a noninvasive biomarker for use in CRC screening is supported by this review despite several limitations due to the number of patients included in each study, the different analytical platforms and biological materials used and different VOC identified.

Introduction

Colorectal cancer (CRC) is one of the commonest tumours and is an important cause of cancer-related mortality [1]. The adenoma–carcinoma sequence has allowed for a mass screening with the aim of decreasing mortality [2]. Colonoscopy is the gold standard for the diagnosis of CRC, although its cost prevents its use for mass screening. Faecal immunochemical blood testing (FIT) is the most widely used noninvasive screening tool, showing fairly good specificity but a high variation in sensitivity (61–91%) and adherence to screening programmes rarely reaches 70% of the target population [3]. Volatile organic compounds (VOC) are present in various excreted biological materials and their analysis offers a possibility for cancer screening.

Analysis of the volatile part of the metabolome, named the ‘volatome’, was initiated in 1971 by the Nobel laureate Pauling et al. [4], who described the presence of about 250 VOC in human breath and urine using gas chromatography–mass spectrometry (GC-MS). In 1989 Williams and Pembroke [5] demonstrated that sniffer dogs could be trained to detect the presence of melanoma, suggesting the idea of a characteristic ‘smellprint’ for any disease. This ‘volatile hypothesis’ was confirmed by other studies that used the canine sense of smell for the diagnosis of bladder cancer [6], lung and breast cancer [7] and CRC [8]. VOC are the final products of cellular metabolism probably produced by the oxidative stress or peroxidation of cell membranes as a consequence of gene or protein alterations in cancer cells. VOC can reflect any metabolic changes in response to inflammation, necrosis, cancer degeneration or alteration of microbiota or can be related to external factors such as environmental pollution, medication and diet [8]. These metabolites are released into the bloodstream, and wherever they are produced in the body will reach the alveoli or renal tubules, where they are excreted. VOC released into the gut may be detected in the faeces.

In the last 20 years this approach has been applied in different clinical settings, including cancer. Several studies have demonstrated the high reliability of a metabolomic approach for detecting and discriminating patients with lung [9], prostate [10], breast [11], skin [12], gastric and oesophageal [13,14] and thyroid [15] cancer from controls. The present review aims to evaluate the potential role of VOC analysis as a mass screening tool for CRC.

Method

A systematic electronic literature search was conducted by accessing the United States National Library of Medicine database (Medline–PubMed), Scirus (for scientific information) and the Google search engine. The search was supplemented by further scanning of the reference lists obtained from retrieved articles in order to identify
any relevant additional study material missed on the initial surveys. The selected literature was screened by two authors (MDL and FP) independently and then cross-matched. Any duplicate nonoriginal articles such as editorials and reviews were excluded.

The search terms ‘Metabolomic’, ‘Volatile Organic Compounds (VOC)’, ‘Electronic-nose’ and ‘Colorectal Cancer’ were cross-checked and the risk of bias in individual studies was eliminated by selecting only papers dealing with VOC excluding other metabolites and only screening for primary CRC. All the eligible studies on VOC in CRC were included irrespective of the outcome. Only original articles published in the English language and appearing in peer-reviewed journals between January 2000 and June 2015 were considered.

Results

Ninety-six titles or abstracts were identified from the electronic search and reference lists in the preliminary review. According to the PRISMA flow diagram (Fig. 1) 84 did not meet the criteria for inclusion. After removal of duplicates and screening for relevant titles, 10 articles were submitted for a full-text analysis and included in this systematic review. Three were related to VOC identified in urine [16–18], four in exhaled breath [8,19–21], one in blood [22] and two in faeces [23,24] using different analytical platforms.

Analytical platforms

Several types of technologies using different analytical platforms have been applied to the analysis of VOC for CRC.

Gas chromatography–mass spectrometry

This is the gold standard hybrid analytical platform employed successfully for the chemical characterization of VOC biomarkers. This method was used in seven [8,16,18–22] of the 10 studies. GC-MS allows the
physical separation of the volatile molecules in samples and the subsequent identification of their nature and quantification, permitting identification of the exact composition of the sample analysed. Giving qualitative and quantitative information about sample composition, this analytical technique allows the identification of the metabolic profile characteristic of CRC patients compared with healthy controls.

Despite the advantage of its high efficiency, GC-MS has a high cost and low manageability, which limit its use in routine application for mass screening. The technique requires three different operative steps including breath collection, sample preconcentration and analysis. Different sample preconcentration methods have been employed. In four studies [8,16,21,22] solid phase microextraction (SPME) was used. In this technique equilibrium is established between the sample and the headspace above and analytes from the sample are absorbed by a polymer-coated fused fibre. They are then desorbed from the fibre to a chromatography column. In two studies a multi-bed sorbent cartridge technique was adopted which focused on breath monitoring [19] and in one study [18] a Combi-PAL ITEX automated preconcentration system was coupled to GC-MS for urine analysis.

In a paper on urinary VOC [18] another innovative technique, field asymmetric ion mobility spectrometry (FAIMS), was used. This technique achieves separation of chemical compounds according to their different ionized chemical mobilities in an electric field, working at atmospheric pressure and room temperature, unlike GC-MS. After ionization, the neutral molecules of the sample become ions of various sizes and types. By applying an asymmetric high-voltage waveform, different movements of these molecules within the high electric field can be measured, and a consequent separation of the complex mixture will be observed. This technology has the advantage of supplying a physical separation of the compounds and has better manageability than GC-MS, but the absence of a reference database limits the ability to obtain reliable qualitative and quantitative information about the VOC contained in the samples. These compounds, therefore, can be recognized and quantified only if their nature is already known.

**Selected ion flow tube mass spectrometry (SIFT-MS)**

This technique was employed in a study which focused on the monitoring of VOC from faecal samples [23]. SIFT-MS is a quantitative MS technique that allows the real-time measurement of concentrations of trace gaseous molecules in humid air samples. It is based on chemical ionization induced by precursor ions H3O+, NO+ and O2+ generated in a microwave discharge and selected by a quadrupole mass filter. The selected ion is injected into a fast flowing helium carrier gas down a flow tube. The sample is then introduced into the flow tube and the precursor ion reacts with the trace gases and VOC in the sample. The precursor and product ions in the carrier gas are separated in a second quadrupole mass spectrometer and counted in a detector. Data may be obtained through scanning a spectrum at a user-defined range of mass-to-charge ratio (m/z) values and absolute concentrations of trace compounds can be calculated in real time from the ratio of the precursor and product ion signal ratios without the need for sample preparation or calibration with standard mixtures.

**Electronic-nose (e-nose) technology**

This was used in four of the studies [8,17,20,24] and is based on an array of sensors able to generate an electrical response in the presence of a chemical class of VOC, giving a qualitative nonspecific response. An e-nose does not enable a single VOC to be recognized, but as in human olfaction, it detects the presence of a combination of different chemical classes, such as alkanes, alcohols and aromatic compounds. E-nose technology is faster and easier to perform than GC-MS and can be trained using pattern recognition to identify a particular state. At present there are different kinds of commercial and custom e-noses, containing an array of 6–32 sensors.

**Study characteristics**

All the studies considered were prospective, comparative, observational case–control studies with relatively small samples.

**Volatile organic compounds in the urine headspace**

The high concentration of volatile metabolites in the urine makes it an attractive target for VOC analysis. Silva et al. [16] evaluated differences in the urinary VOC profile of cancer patients and controls using dynamic headspace microextraction (DHS-SPME) in combination with GC-MS. The cancer group included 14 cases of leukaemia, 12 CRC patients and seven lymphoma patients; this group was compared with 21 healthy controls. Fifteen VOC were identified that differed between CRC and controls, although the authors concluded that CRC patients were better characterized by the presence of 1,4,5-trimethylnaphthalene, 2,7-dimethylquinoline and 2-methyl-3-phenyl-2-propenal (Table 1). This study was the first application of VOC analysis of urinary samples from CRC patients, although it is nonspecific for CRC and evaluated three other types of cancers that are very different from each other.
<table>
<thead>
<tr>
<th>Analytical platform</th>
<th>Patients</th>
<th>Biomarkers</th>
<th>Chemical classes</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breath</td>
<td></td>
<td></td>
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<tr>
<td>Peng (2010) [8]</td>
<td>26 CRC</td>
<td>1,1’-(1-Butenylidene)-bis-benzene, 1,3-dimethylbenzene, 2-Amino-5-isopropyl-8-methyl-1-azulenecarbonitrile, 1-Iodononane, [(1,1-Dimethylethyl)thio] acetic acid, 4-(4-Propylecyclohexyl)-4’-cyano[1,1’-biphenyl]-4-yl ester benzoic acid</td>
<td>Aromatic compound, Aromatic compound, Amino derivative, Haloalkane, Acid</td>
<td>&lt; 30%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>GC-MS</td>
<td>22 HC</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Altomare (2013) [19]</td>
<td>37 CRC</td>
<td>41 HC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC-MS</td>
<td></td>
<td>Nonanal, Decanal, 4-Methyl-2-pentanone, 2-Methylbutane, 4-Methyloctane, 4-Methylundecane, 2-Methylpentane, 3-Methylpentane, Methylcyclopentane, Cyclohexane, Methylcyclohexane, Trimethyldecane-1,2-pentadiene, 1,3-Dimethylbenzene, 1,4-Dimethylbenzene</td>
<td>Aldehyde, Aldehyde, Ketone, Alkane, Alkane, Alkane, Alkane, Cycloalkane, Cycloalkane, Alkene, Aromatic compound</td>
<td>86%</td>
<td>83%</td>
<td>–</td>
</tr>
<tr>
<td>GC-MS</td>
<td></td>
<td>Cyclohexanone, Dodecane, 2,2-Dimethyldecane, 4-Ethyl-1-octyn-3-ol, Trans-2-dodecen-1-ol, Cyclooctylmethanol, Ethylaniline</td>
<td>Ketone, Alkane, Alcohol, Alcohol, Alcohol, Aromatic amine derivative</td>
<td></td>
<td></td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>
Table 1 (Continued).

<table>
<thead>
<tr>
<th>Analytical platform</th>
<th>Patients</th>
<th>Biomarkers</th>
<th>Chemical classes</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amal</strong> (2015) [20]</td>
<td>GC-MS e-nose</td>
<td>65 CRC</td>
<td>3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate</td>
<td>Ester</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 advanced aden.</td>
<td>6-t-Butyl-2,2,9,9-tertaremethyl-3,5-decadien-7-yne</td>
<td>Alkyne</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 nonadvanced aden.</td>
<td>Ethylacetate</td>
<td>Ketone</td>
<td>For e-nose: 85% (CRC vs HC)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>122 HC</td>
<td>Ethanol</td>
<td>Alcohol</td>
<td>For e-nose: 94% (CRC vs HC)</td>
<td>–</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td>GC-MS</td>
<td>16 CRC</td>
<td>Acetone</td>
<td>For e-nose: 94% (CRC vs HC)</td>
<td>–</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Wang (2014) [22]</td>
<td></td>
<td>20 HC</td>
<td>Ethylacetate</td>
<td>Ester</td>
<td>88% (CRC vs HC)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethylhexanol</td>
<td>Alcohol</td>
<td>94% (CRC vs aden.)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6-t-Butyl-2,2,9,9-tertaremethyl-3,5-decadien-7-yne</td>
<td>Alkyne</td>
<td>94% (control vs aden.)</td>
<td>–</td>
</tr>
<tr>
<td><strong>Faeces</strong></td>
<td>Cyranose® 320</td>
<td>40 CRC</td>
<td>Ethylacetate</td>
<td>Carboxylic acid ester</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>de Meij (2014) [24]</td>
<td></td>
<td>60 advanced aden.</td>
<td>Ethylhexanol</td>
<td>Alcohol</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57 HC</td>
<td>6-t-Butyl-2,2,9,9-tertaremethyl-3,5-decadien-7-yne</td>
<td>Alkyne</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,1,4,4-Tetramethyl-2,5-dimethylene-cyclohexane</td>
<td>Cycloalkane</td>
<td>85% for CRC</td>
<td>&lt; 0.001 for CRC</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td>DHS-SPME/</td>
<td>12 CRC</td>
<td>Hydrogen sulphide</td>
<td>Sulphur compound</td>
<td>87% for CRC</td>
<td>&lt; 0.001 for CRC</td>
</tr>
<tr>
<td>Batty (2015) [23]</td>
<td>GC-MS</td>
<td>21HC</td>
<td>Dimethylsulphide</td>
<td>Sulphur compound</td>
<td>62% for adenomas</td>
<td>&lt; 0.001 for adenomas</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dimethyldisulphide</td>
<td>Sulphur compound</td>
<td>86% for adenomas</td>
<td>–</td>
</tr>
<tr>
<td><strong>Silva</strong></td>
<td></td>
<td></td>
<td>2-Methyl-3-phenyl-2-propenal; 1,2,4-Trimethylbenzene</td>
<td>Aldehyde</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(2011) [16]</td>
<td></td>
<td></td>
<td>Aldehyde</td>
<td>Aldehyde</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aldehyde</td>
<td>Aldehyde</td>
<td>–</td>
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</tbody>
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VOC as biomarkers for colorectal cancer

M. Di Lena et al.
Table 1 (Continued).

<table>
<thead>
<tr>
<th>Analytical platform</th>
<th>Patients</th>
<th>Biomarkers</th>
<th>Chemical classes</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Westernbrink (2015) [17]</td>
<td>e-nose 39 CRC</td>
<td>p-Cymene, 1,2-Dihydro-1,1,6-trimethyl-naphthalene, 1,4,5-Trimethylnaphthalene, Anisole, 1-Octanol, 4-Methylphenol, γ-Terpinene, Bomylene, Dimethyl disulphide, 2-Methoxythiophene, 2,7-Dimethylquinoline</td>
<td>Aromatic compound, Aromatic compound, Aromatic compound, Aromatic ether, Alcohol, Aromatic alcohol, Terpene, Terpene, Sulphur compound, Thiophene derivative, Quinoline derivative</td>
<td>78%</td>
<td>79%</td>
<td>-</td>
</tr>
<tr>
<td>Arasaradnam (2014) [18]</td>
<td>FAIMS/GC-MS 83 CRC</td>
<td>Acetaldehyde (or ethylene oxide or oxalic acid), Acetone, 2-Pentanone (or 3-methyl-2-butanol or 2,3-butanedione), 4-Heptanone (or 3-heptanone or 2,4-dimethyl-3-pentanone), Dimethyl diazene (or cyclobutylamine or oxepane), Acetyloxime pyridine carboxaldehyde (or hydrocinnamoylbenzene-ethanamine or styrene or dimethylthiourea), Allylisothiocyanate (or isothiocyanato-cyclopropane or 2-cyanoacetamide)</td>
<td>Aldehyde, Ketone, Ketone, Ketone, Nitrogen compound, Pyridine derivative</td>
<td>88%</td>
<td>60%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>50 HC</td>
<td></td>
<td></td>
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</tbody>
</table>
Two further studies [17,18] were conducted at the University Hospital Coventry and Warwickshire (UK). These studies considered that since for patients admitted to a CRC screening programme collecting urine can be more acceptable than collecting faeces compliance could be improved. The first of the studies evaluated urine samples obtained from 83 CRC patients and 50 healthy controls using a FAIMS instrument and a Combi-PAL ITEX automated preconcentrator system (CTC Analytics AG, Switzerland) coupled to a GC-MS. This showed a sensitivity of 88% and a specificity of 60% in discriminating between the two groups [18].

The VOC identified by GC-MS are shown in Table 1. In the second study [17] the same authors developed a dedicated e-nose, the WOLF system, made up of 13 sensors (10 amperometric electrochemical sensors, two nondispersive infrared optical devices and one photoionization detector), testing its discriminatory power on the headspace of 92 urine samples including 39 CRC patients, 35 patients with irritable bowel syndrome (IBS) and 18 healthy controls. This new technology showed a sensitivity of 78% and specificity of 79% in detecting CRC patients compared with the other two groups, confirming the presence of a metabolomic derangement identifiable in the urine samples of CRC patients and the efficiency of urine as a potential good biological material for screening.

Volatile organic compounds in the faecal headspace

The first report of the analysis of faecal samples was by de Meij et al. [24]. They used the Cyranose® 320 (Smiths Detections, Pasadena, California, USA) to monitor 40 CRC patients, 60 patients with advanced colonic adenomas and 57 healthy controls. A FIT was performed on the faecal samples of 35 CRC, 46 advanced adenomas and 52 controls from the same cohort. The Cyranose® 320, a commercial e-nose based on an array of 32 nanocomposite organic polymeric sensors, showed a sensitivity and a specificity of 85% and 87%, in the comparison of CRC patients and controls. A sensitivity of 62% and a specificity of 86% was found when comparing advanced adenoma patients with controls and a sensitivity of 75% and a specificity of 73% was obtained for the comparison of patients with CRC and advanced adenoma. FIT used in this study was an automated OC-sensor test, showing a specificity of 100% in the three groups and a sensitivity of 63% for CRC and 7% for advanced adenoma. Batty et al. [23] studied 31 faecal samples from normal controls and 31 from a group of high-risk patients with a high grade adenoma or adenocarcinoma, stratified after colonoscopy performed on the basis of positivity on a faecal occult blood test. SIFT-MS analysis was performed.
using each of the three precursor ions available (H$_3$O$^+$, NO$_3^-$, O$_2^-$). Four ions were identified that were statistically different in the two groups monitored (m/z 35, m/z 90, m/z 62, m/z 94) most likely derived from hydrogen sulphide, dimethylsulphide and dimethyldisulphide (the fourth being unknown). The technique showed a specificity of 78% and a sensitivity of 72%.

Volatile organic compounds in the blood headspace
The metabolomic approach has been applied in blood/serum samples from CRC patients, showing different altered metabolic pathways compared with healthy subjects [25,26], but only one study analysed the volatile part of the metabolome in the complex matrix of blood/serum [22]. Based on the hypothesis that VOC are first released into the bloodstream and then reach the alveoli and are exhaled, Wang et al. [22] investigated the VOC present in blood samples from 16 CRC patients (eight colon cancer and eight rectal cancer) and 20 healthy controls using SPME-GC-MS. They found three VOC to be present in significantly lower amounts (phenylmethylcarbamate, ethylhexanol and 6-t-butyl-2,2,9,9-tetramethyl-3,5-decadien-7-yne) and one in a significantly higher amount (1,1,4,4-tetramethyl-2,5-dimethylene-cyclohexane) (P < 0.05) in CRC patients compared with healthy subjects.

Volatile organic compounds in the breath
Peng et al. [8] published the first report of metabolomic breath analysis in CRC patients using a tailor-made nanosensor array based on organically functionalized gold nanoparticles (GNPs) and a SPME-GC-MS analysis to identify suitable representative VOC to distinguish CRC from other types of cancer. Breath samples were collected from 26 CRC patients, 30 lung cancer patients, 22 breast cancer patients, 18 prostate cancer patients and 22 healthy controls. The GNP array showed high discriminant ability in identifying CRC patients from healthy controls, while using the six VOC identified (see Table 1) by GC-MS in CRC patients the sensitivity dropped to about 30%.

In 2013 our group analysed breath samples from 37 CRC patients and 41 healthy controls with thermal desorption (TD)-GC-MS, and we were able to identify a pattern of 15 VOC (see Table 1) showing a sensitivity of 86%, a specificity of 83% and an accuracy of 85% in discriminating between the two groups, with an area under the receiver operating characteristic curve (AUC) of 0.85. The discriminant power of the exhaled VOC pattern was then validated in a blind phase on a further series of 25 subjects showing an accuracy of 76% [19].

A further study on breath analysis was published by Wang et al. [21], who analysed breath samples from 20 CRC patients and 20 healthy controls using SPME-GC-MS. Nine VOC were significantly increased in exhaled breath from CRC patients (P < 0.05) and another one was significantly reduced compared with healthy volunteers (P < 0.05) (see Table 1). Recently Amal et al. [20] published the results of an analysis of exhaled breath from 65 patients with CRC, 22 with adenoma and 122 healthy controls. GC-MS and an e-nose made from cross-reactive nanoarrays in combination were used for the analysis. GC-MS revealed four compounds that were significantly different in the CRC and control groups. In particular, acetone and ethyl-acetate were higher in the CRC group, while ethanol and 4-methyl-octane were higher in the control group. The performance of the sensors in discriminating CRC and control groups resulted in a sensitivity of 85%, a specificity of 94% and an accuracy of 91%. The use of nanoarray technology resulted in good discrimination between the CRC and adenoma groups, the adenoma and control groups and between advanced and nonadvanced adenoma, even if the small number of adenoma patients made the results unreliable. The authors evaluated the role of different confounding factors such as age, gender, current smoking and fasting of at least 12 h, revealing any influence on sampling and the information contained in exhaled air.

Discussion

Analysis of the 10 studies reviewed shows that a derangement of metabolomics related to the presence of CRC really exists and that it could be detected by VOC analysis as a cancer fingerprint, showing fair reliability, with a sensitivity ranging between 30% and 94% and a specificity of 60–94%. This metabolomic ‘signal’ can be differently evaluated according to the analytical platform used and the matrix analysed, but the lack of a standardized experimental procedure makes an overview of the results difficult.

All the experimental projects discussed in this review were pilot studies containing small numbers of patients (ranging from 12 to 83) in which different tools, analytical platforms or experimental procedures were employed, resulting in noncomparable patterns of metabolites or electrical signals, although the most frequent chemical classes of metabolites identified were alkanes, aromatic compounds and alcohols (Table 1).

Two [17,18] of the studies reviewed were conducted by the same authors using different analytical platforms (FAIMS, SPME-GC-MS and the WOLF system) to analyse urine, leading to results which were not comparable. The same was true of the two studies conducted by Wang et al. [21,22] in which different biological samples, including exhaled breath and blood from CRC.
patients, were examined using the same technique (SPME-GC-MS). The two VOC patterns were not completely matched, probably due to the different characteristics of the samples. Interestingly, these authors found that 6-t-butyl-2,2,9,9-tetramethyl-3,5-decadien-7-yne was present at lower levels in breath and blood samples [21].

The four studies aiming to detect VOC in breath samples suggested different patterns of potential biomarkers which did not fit each other, with the exception of 1,3-dimethylbenzene, identified by both Peng et al. [8] and Altomare et al. [19], and of 4-methyloctane, considered a potential biomarker by Altomare et al. [19] and by Amal et al. [20], probably because of the different procedures employed in collection and analysis of the samples [8,19–21]. So far only one study has focused on the monitoring of VOC from blood samples [22] and two from faeces [23,24], and both studies had small sample sizes. These studies should therefore be considered as only preliminary. Furthermore, the two studies of faeces were carried out using different techniques (Cyanose® 320 and SIFT-MS) so that the results are not comparable.

Initials publications on the use of e-noses for diagnosis reported exciting results with the possibility of developing a faster, more reliable instrument for cancer screening. The reliability of the four sensor arrays tested on CRC patients seems to be good, showing a sensitivity ranging from 78 to 94% and a specificity of 79-94% [8,17,20,24]. Although the application of e-noses seems to receive support from the scientific community, it must be pointed out that e-noses cannot identify any specific CRC biomarker. It would be very interesting to interface GC-MS analysis and e-nose technology to establish a dedicated e-nose equipped with sensors able to detect CRC-specific compounds or classes of VOC identified by GC-MS.

Another debate concerning the metabolomic approach to CRC screening is the type of biological sample chosen for VOC analysis. The choice of which biological matrix to analyse is crucial for increasing patients’ compliance and adherence to the screening programme, one of the keys to success for a new screening tool. In this respect, faecal samples represent the worse choice, as demonstrated by the insufficient compliance obtained using the FIT. Blood collection is invasive compared with urine and breath collection. Furthermore, urine, faeces and blood need to be processed, stored within a few hours of collection, then defrosted and warmed-up to obtain the headspace for analysis. Breath testing therefore is the most promising choice owing to its gaseous state, with no need for processing before analysis, easy non-invasive collection and low cost.

The idea of a breath test as a screening tool for CRC is old, and was first proposed in 1977 by Haines et al. [27], who evaluated the presence of higher methane concentrations in the breath of CRC patients. This hypothesis was then rejected by consecutive studies [28,29] and remained neglected for 30 years. Analysis of the VOC content breath has reawakened this idea; however, it is not focused on the monitoring of a single marker but allows the simultaneous identification of several compounds. Our group [30] has demonstrated that the pattern of exhaled VOC in CRC patients is changed after removal of the cancer, suggesting that the profile before surgery is strictly linked to cancer metabolism, confirming a close relationship between tumour metabolism and exhaled VOC. In this study, 11 VOC were selected which were consistently involved in the discriminatory models, even in our previous study [19], and which could represent the core ‘breath print’ of CRC patients, suggesting, if confirmed in a larger cohort, the use of the VOC pattern not only for screening but also for the secondary prevention of CRC.

For any mass screening programme the cost of the procedure is of great importance, and VOC analysis using GCMS equipment is neither cheap nor widely available. The collection of exhaled breath is probably cheaper than for urine or faeces but the true cost of these procedures has never been exactly determined. To fill the gap between the laboratory and clinical practice there is a need to develop different online analysers such as dedicated e-noses or quartz-enhanced photoacoustic spectroscopy sensors [31].

VOC analysis is a very promising new screening tool for CRC, although it will require much effort to standardize all the phases of analysis. The small number of patients in each study, the different analytical platforms and the biological material used probably account for the different VOC patterns, meaning that the results of different studies are not comparable. Biochemical studies aimed at defining the hitherto unknown molecular pathways implicated in VOC production in CRC should be encouraged. This review underlines the presence of a ‘metabolomic signal’ in the excreta of patients with CRC that could be used for screening and diagnosis of CRC.
References


