The Scent of Colorectal Cancer: Detection by Volatile Organic Compound Analysis

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Q3 **The overall metabolic state of an individual is reflected by emitted volatile organic compounds (VOCs), which are gaseous carbon-based chemicals. In this review, we will describe the potential of VOCs as fully noninvasive markers for the detection of neoplastic lesions of the colon. VOCs are detected by our sensory olfactory nerves and form the molecular basis for our sense of smell. As such, we emit our own individual odor fingerprint or so-called smellprint. This may change over time in response to any alteration in metabolism such as modifications caused by gastrointestinal infection, inflammation, external factors such as medication and diet, or development of neoplastic disease such as colorectal cancer. This means that analysis of VOCs can provide a fully noninvasive metabolomics biomarker profile that could be used as a diagnostic tool. Thus far, canine scent detection, gas chromatography-mass spectrometry, and electronic nose technologies allow for discrimination between patients with and without colorectal cancer and also its precursor (advanced adenoma) with promising accuracy. The challenge for future research is to identify specific biomarkers driving these signals. This enables the development of primed sensors tailored toward accurate identification of volatiles specific to colorectal cancer and adenomas. Such a technique may allow noninvasive monitoring of response to therapy and could revolutionize screening practices for colorectal cancer and potentially many other gastrointestinal diseases.**

Keywords: Colorectal Cancer; Adenoma; Screening; Volatile Organic Compounds; Electronic Nose; Canine Scent; Flatography.

The molecular basis for our sense of smell lies in the detection of volatile organic compounds (VOCs) by sensory olfactory nerves. Each individual produces VOCs as waste products of their metabolism. This volatile profile or smellprint changes over time and reflects any alteration in metabolism as caused by infection, inflammation, neoplastic disease, and external factors such as medication, diet, or changes in microbiome composition. Therefore, these volatiles may serve as potent biomarkers for health and disease.[1] Analysis of human VOCs can technically be performed in exhaled breath, urine, sweat, skin, vaginal secretions, and feces (Table 1). In several benign gastrointestinal diseases such as irritable bowel syndrome, infectious colitis, and inflammatory bowel disease, VOCs have been studied by various techniques in an attempt to unravel the underlying pathophysiology and discover possible biomarkers for detection of disease. An overview of key studies is depicted in Table 2. With respect to analysis of VOCs for detection of colorectal cancer (CRC), only limited data are available, despite its clinical potential of being an inexpensive and noninvasive screening tool. Here we provide an overview of the current evidence supporting the clinical use of VOCs as biomarkers and elaborate on its potential application in future screening for CRC and adenomas.

Methods of Volatile Organic Compound Analysis

In 1971 Nobel Prize laureate Linus Pauling was the first to identify several hundred organic volatiles in extremely low concentrations (parts per billion/parts per trillion) in exhaled breath.[2] Nowadays, several techniques are available to identify these VOCs. First, identification of individual chemical compounds in a VOC mixture can be done by chemical analytical techniques such as gas chromatography linked to mass spectrometry (GC-MS). Specific volatiles in a gaseous mixture can be identified by GC-MS by separating them on the basis of their physical and chemical properties. This technique allows researchers to link individual volatiles to specific pathophysiological changes.

Alternatively, analysis of VOC mixtures can be done by pattern recognition sensor arrays to create specific smellprints or VOC profiles representing the total VOC mixture.[3] This technique most closely resembles mammalian olfaction and is therefore dubbed an electronic nose (eNose).[4] Although this latter technique does not allow identification of individual VOCs, it does circumvent the need for expensive and laborious testing

Abbreviations used in this paper: CRC, colorectal cancer; eNose, electronic nose; GC-MS, gas chromatography-mass spectrometry; VOC, volatile organic compound.

1542-3565/$36.00
http://dx.doi.org/10.1016/j.cgh.2014.05.005

Table 1. VOCs Emanating From Healthy Individuals

	Number of known VOCs
Breath	872
Saliva	359
Blood	154
Breast milk	256
Skin secretion	532
Urine	279
Feces	381

Adapted From Reference 13

by providing real-time (desktop) classification of patients. This makes this technique well-suited for daily clinical practice where knowledge of the specific VOCs is not mandatory. In addition, because the eNose is a high-throughput technique, it holds particular promise for population screening programs. Figure 1 shows an example of a scatter plot that is based on the analysis of fecal VOCs by an eNose.

Metabolic Origins of Volatile Organic Compounds in Colorectal Cancer

Potential biomarkers for CRC screening originate both at the tumor site and systemically. First, VOCs will be produced directly by the tumor because these cells have an aberrant metabolism (protein and gene alterations) related to their unchecked growth. Furthermore, this is usually paralleled by necrosis of both body and tumor cells. Both processes are likely to produce VOCs that are not present, at least in these concentrations, in healthy individuals. These processes will also affect molecules that dissolve into the bloodstream at the tumor site. Some of these may subsequently be detectable as exhaled biomarkers.[5] Furthermore, evidence has linked progression of CRC to changes in the resident gut microbiome.[6,7] Because local VOCs emitting from feces largely reflect the microbiota composition, these volatiles are obviously candidate biomarkers for detection of CRC.

A second source of exhaled biomarkers related to CRC likely arises from the systemic effects of CRC such as increased oxidative stress, increased catabolism, and immune activation. These effects are likely to be paralleled by alterations of produced VOCs in tissues other than the primary disease site.[8] Volatiles related to CRC have both local and systemic origins, indicating that breath and feces are the primary specimens of interest.

Findings

Thus far, analysis of VOCs for the detection of CRC and its benign precursors has been investigated by canine olfaction and by analysis of exhaled and fecal VOCs. We will provide an overview of the literature for each of these approaches.

Canine Olfaction

After canines were reported to smell skin cancer, bladder cancer (in urine), lung cancer (in breath), breast cancer (in breath), and ovarian cancer (in tissue), the only study on the accuracy of canines to detect CRC in breath samples and watery stool samples was published in 2011.[9] When compared with a diagnosis of CRC made by colonoscopy, a trained Labrador retriever was able to discriminate patients with CRC from healthy controls with high sensitivity (0.91) and specificity (0.99), which was even higher in watery stool samples (sensitivity, 0.97; specificity, 0.99). The diagnostic accuracy of canine scent detection proved to be superior to that of fecal occult blood testing (sensitivity, 0.70; specificity, 0.85).

Although canine olfaction has clearly demonstrated the potential of VOCs for diagnosis of CRC, there are obvious obstacles limiting the use of canine scent detection in clinical practice because training is costly and time-consuming and performance variability remains high. Future development of cancer-specific VOC-based electronic sensors is therefore expected to outweigh canine judgment.

Table 2. Studies on VOCs in Benign Gastrointestinal Diseases

Disease	Sample	Technique	Reference
Inflammatory bowel disease	Feces	GC-MS	14, 15
	Urine	eNose	16
		Field asymmetric ion mobility spectrometer	
Nonalcoholic fatty liver disease	Feces	GC-MS	17
	Breath	Selective ion flow tube mass spectrometry	18
Clostridium difficile	Feces and hospitalized patients	Canine nose	19
Rotavirus	Feces	Human nose	20
Necrotizing enterocolitis	Feces	GC-MS	21
Irritable bowel syndrome	Feces	GC sensor	22