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Detection of gas components as a novel diagnostic method for colorectal cancer

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Abstract

Background: The fecal occult blood test (FOBT) is widely accepted as the most economic and non-invasive screening method for colorectal cancer (CRC). However, the FOBT is inconvenient because it requires a fecal sample and shows limited accuracy. Alternatively, we hypothesized that fecal gas compounds from bowel movements may be a non-invasive biomarker for CRC.

Methods: Gas compounds were collected from the bowel movements of 30 patients with CRC and from 26 healthy controls. The patient group comprised 17 males and 13 females, and the average age was 68 years. Additionally, 22 patients had colon cancer, and eight patients had rectal cancer. Gas compounds were analyzed using gas chromatography and compared with those from healthy controls.

Results: In the gas analysis, methyl mercaptan was significantly higher in the CRC group than in the control group. Hydrogen was significantly lower in the CRC group than in the control group and was correlated with tumor depth and advanced disease stage. Sensitivity, specificity, and accuracy of detection by a discriminant formula were 90%, 57.7%, and 75%, respectively.

Conclusion: Gas compounds from defecation constitute a promising, novel non-invasive approach for CRC screening. (UMIN000028256)

KEYWORDS
accuracy, colorectal cancer, diagnosis, gas

1 | INTRODUCTION

Colorectal cancer (CRC) is the second most common cancer in both genders in Western countries, with more than 70% of such cases involving patients over 65 years of age.[1] In Japan, CRC was ranked the second and fourth most common type of cancer in 2015 in women and men, respectively.[2]

The fecal occult blood test (FOBT) is widely accepted as the most economic and non-invasive screening method for CRC and, because many patients with positive FOBT results subsequently undergo a colonoscopy, there is a reduced incidence and mortality of CRC.[3–5] Moreover, the sensitivity of the FOBT for CRC is high, ranging from 73.3% to 88.9%.[6,7] However, the FOBT detects blood in the stool that is not always associated with CRC, with the positive

predictive value (PPV) of the FOBT for CRC being 10.6%-12.0%.[8,9] Therefore, an easier, more convenient and more effective method for CRC screening must be developed.

Recently, several authors reported a relationship between cancer and odor gas components. Seaman et al reported that odorous gas dimethyl trisulfide is associated with fungating wounds in skin and breast cancer,[10] and several authors reported that detection dogs can detect skin cancer.[11,12] The first analysis of volatile organic compounds (VOC) was reported in 1971 by Pauling et al,[13] who identified several hundred VOC in human breath and urine by using gas chromatography-mass spectrometry. Over the course of a quarter-century, several authors reported the association of VOC with lung cancer,[14] breast cancer,[15] skin cancer,[16] and prostate cancer.[17] Regarding CRC, several studies have shown the reliability of VOC in detecting CRC in different substances, including urine,[18–20] exhaled breath,[21,22] blood,[23] and feces.[24,25] However, sample collection was inconvenient in these studies. Therefore, the development of a more convenient method is necessary.

In the present study, we examined gas components in bowel movements from patients with CRC using a novel method and evaluated the diagnostic value of this method for CRC.

2 | METHODS

2.1 | Patients

This was a prospective observational study at Yokohama City University Hospital and Yokohama City University Medical Center. Inclusion criteria were age 20 years or older, histologically proven adenocarcinoma, any clinical stage, and elective operation for CRC.

Exclusion criteria were concurrent malignancy in another organ, inflammatory bowel disease, infectious enteritis, bowel obstruction or the inability to tolerate surgery under general anesthesia. A total of 30 patients with CRC who were scheduled for surgery were enrolled in this study from July 2014 to April 2015. Gas samples were collected from the patients before starting preoperative bowel preparation. Twenty-six healthy adult volunteers were enrolled as a control group.

This study was approved by the Yokohama City University Ethics Committee and was conducted in accordance with the Ethical Guidelines for Medical and Health Research Involving Human Subjects (IRB number B140703028), and all patients provided informed consent before any study procedure was carried out. This study is registered on the University Hospital Medical Information Network (UMIN) Center (UMIN000028256).

2.2 | Gas analysis

2.2.1 | Apparatus for gas sampling

As shown in Figure 1, a gas sampling apparatus was placed in a toilet in the hospital ward. Defecation gas samples from patients were collected from the toilet into a 25-L sampling bag (500 mm × 700 mm). The sampling bag was made of fluorinated vinyl resin (Tedlar bag, DuPont, Wilmington, WA, USA), and a Teflon flexible pipe was connected to a fan and the Tedlar bag. A commercial gas sensor (semiconductor gas sensor, TGS2600; FIGARO Engineering, Osaka, Japan) was arranged at the rear of the fan, and a stopper was used at the junction between the Tedlar bag and Teflon flexible pipe.
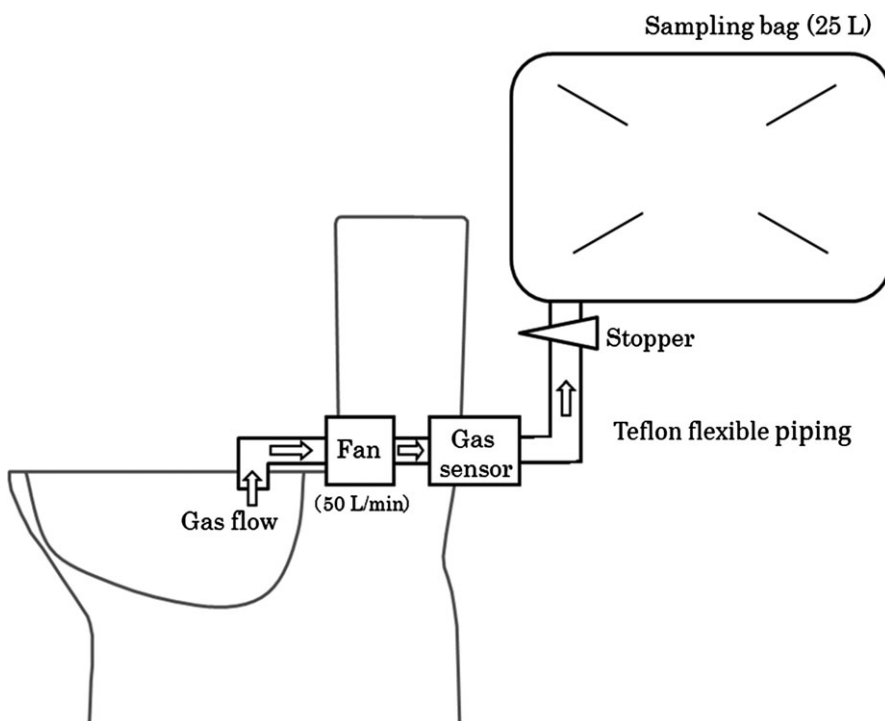

FIGURE 1 Gas sampling system

2.2.2 | Collecting gas components during defecation

Gas components were collected during defecation as follows: (i) Air in the toilet was aspirated with a fan (50 L/min) and passed through a commercial gas sensor. (ii) The stopper automatically opened immediately after the gas sensor detected gas components. (iii) The stopper closed after 30 seconds. (iv) The Tedlar bag was sealed with a large clip. To measure background gas, air in the toilet was aspirated when the toilet was not in use.

2.2.3 | Measurement of methyl mercaptan and hydrogen sulfide concentrations

In the defecation gas samples, we measured the content of sulfur-containing compounds methyl mercaptan (MM) and hydrogen sulfide (H_2S). Volatile sulfur-containing compounds were analyzed by gas chromatography using a Sulfur Chemiluminescence Detector (GC/SCD) (Agilent Technologies, Santa Clara, CA, USA.). Briefly, gases collected in the Tedlar bag were aspirated into a cold-trapping tube, injected into the gas chromatography system by an Entech 7100A Preconcentrator (Entech Instruments Inc., Simi Valley, CA, USA) and then analyzed.

2.2.4 | Measurement of hydrogen, carbon dioxide, and methane concentrations

Hydrogen (H_2), methane (CH_4) and carbon dioxide (CO_2) were analyzed by gas chromatography using a thermal conductivity detector (GC/TCD) (Agilent 490 Micro GC; Agilent Technologies). Then, gases collected in the Tedlar bag were injected into the gas chromatography system and analyzed.

2.2.5 | Calculation of gas amounts during defecation

Concentrations of background gases were ignored because the concentration of background gas (except for CO_2) was sufficiently lower than the concentration of gas during defecation. The amount of gas during defecation was calculated by multiplying the gas concentration and the aspirated gas volume.

2.3 | Statistical analysis

When samples were recorded more than once, mean and standard deviation (SD) were calculated and used for subsequent analyses. Patient characteristics are expressed as numbers and percentages or means ± SD as appropriate. A _t_ test was used to evaluate the significance of the differences in continuous variables. Using a multivariate logistic regression model, we developed a formula to discriminate between CRC patients and healthy volunteers by using gas data from study participants. A formula was calculated by discriminant analysis using factors that had a significant difference by multivariate analysis. All analyses were carried out using IBM SPSS,

version 21 (SPSS Inc., Chicago, IL, USA) and SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

3 | RESULTS

3.1 | Patient characteristics

Characteristics of the CRC group are shown in Table 1, and 17 males and 13 females were included, with the average age being 68 years. Twenty-two patients had colon cancer, and eight patients had rectal cancer. Although the most common tumor site was the rectum (n = 8), other primary tumors were located in the sigmoid colon (n = 7), cecum (n = 6), rectosigmoid colon (n = 5), transverse colon (n = 2), ascending colon (n = 1), and descending colon (n = 1). Mean tumor size was 3.8 cm. There were nine cases of stage I disease, 12 stage II disease, six stage III disease, and three stage IV disease. The control group included 22 males and four females, and the mean age was 37 years.

3.2 | Gas compound measurements obtained from the sampling apparatus

For both groups, gas compound measurements obtained from the sampling apparatus are shown in Figure 2. Mean amount of MM in the defecation gas was 4.25 μg in the CRC group and 1.62 μg in the control group. The amount of MM was significantly higher in the

TABLE 1 Characteristics of colorectal cancer patients

Factor		N = 30 (%)
Gender	Male	17 (56.7)
	Female	13 (43.3)
Age (years)	mean ± SD	68 ± 11
Tumor location	Cecum	6 (20)
	Ascending	1 (3.3)
	Transverse	2 (6.7)
	Descending	1 (3.3)
	Sigmoid	7 (23.3)
	Rectosigmoid	5 (16.7)
	Rectum	8 (26.7)
Tumor size (mm)	Mean ± SD	38 ± 24
Tumor depth	T1	7 (23.3)
	T2	4 (13.4)
	T3	14 (46.6)
	T4	5 (16.7)
LN involvement	N0	21 (70)
	N1	7 (23.3)
	N2	2 (6.7)
Stage (UICC)	I	9 (30)
	II	12 (40)
	III	6 (20)
	IV	3 (10)

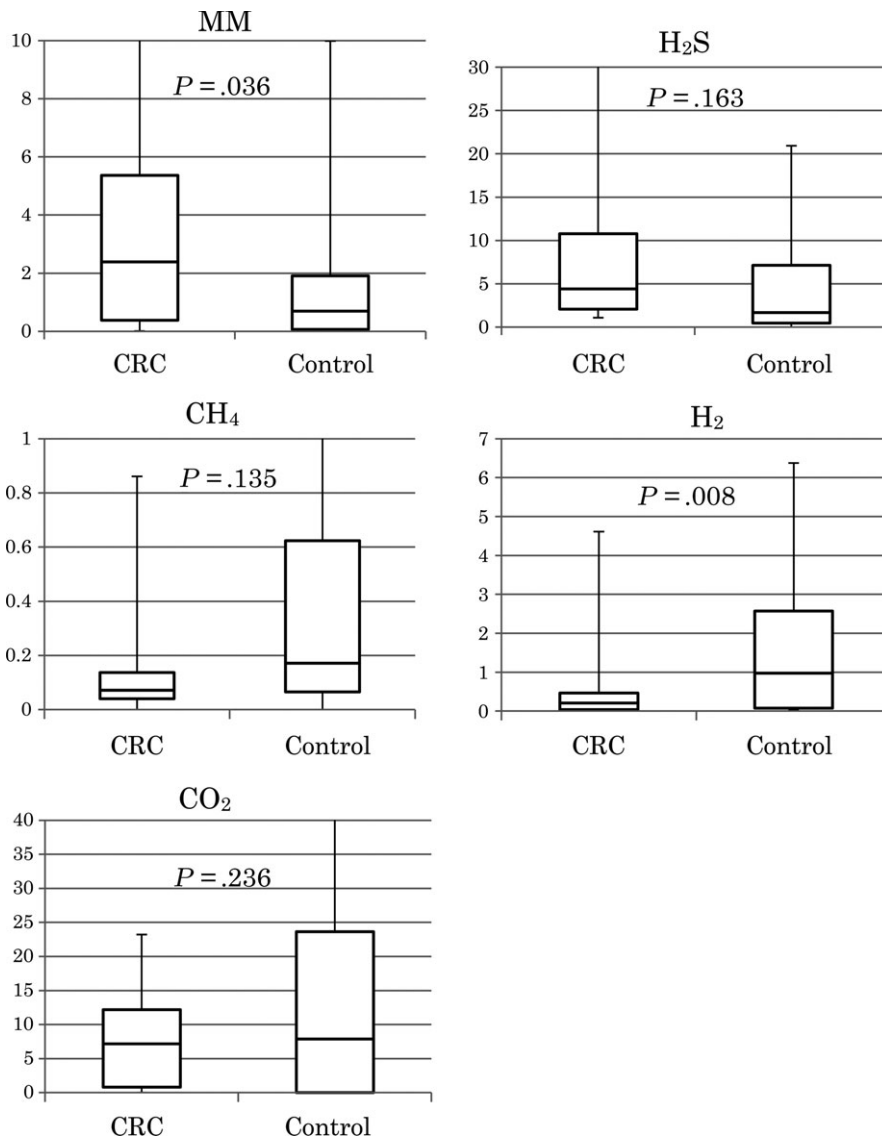LN, lymph node; UICC, Union for International Cancer Control.

FIGURE 2 Comparison between gas compounds in the CRC and control groups (box plot). CH_4, methane; CO_2, carbon dioxide; CRC, colorectal cancer; H_2, hydrogen; H_2S, hydrogen sulfide; MM, methyl mercaptan

CRC group than in the control group ($P = .036$). However, although the mean amount of H_2S was 12.36 µg in the CRC group and 4.74 µg in the control group, there was no significant difference between the groups ($P = .163$). The mean amount of H_2 was 0.49 mg in the CRC group and 1.55 mg in the control group, resulting in a significantly lower amount of H_2 in the CRC than in the control group ($P = .008$). Mean amounts of CH_4 and CO_2 were not significantly different between the groups.

The control group was younger than the CRC group. To consider the effect of age, we compared the findings from the younger and older healthy controls and found that there were no significant differences in the amount of any gas compound between the two groups (data not shown).

3.3 | Correlations between gas compound amounts and clinicopathological status of CRC patients

Correlations between gas compound amounts and clinicopathological status of CRC patients are shown in Table 2. The amount of H_2 was significantly lower in patients with T3/4 tumors and advanced-stage disease than that in other CRC patients. For the other gas compounds, no significant correlations were observed with the clinicopathological status of CRC patients.

3.4 | Logistic regression analysis and discriminant analysis

Multivariate logistic regression analysis was carried out to identify CRC patients (Table 3), and a total of 56 data sets (30 CRC patients and 26 controls) were used. When there were two or more samples, an average value was used for logistic regression analysis.

The discriminant formula is as follows: $0.137 \times$ MM (mean) $-0.582 \times H_2$ (mean) $+ 0.137$

(positive: more than 0)

The receiver operating characteristic (ROC) curve of the discriminant formula is shown in Figure 3. Sensitivity, specificity, negative predictive value (NPV), PPV, and accuracy of the discriminant formula were 90%, 57.7%, 83.3%, 75%, and 75%, respectively.

TABLE 2 Correlations between gas compound amounts and clinicopathological status of CRC patients

Factor	N	MM (µg) Mean (SD)	P-value	H$_2$S (µg) Mean (SD)	P-value	CH$_4$ (mg) Mean (SD)	P-value	CO$_2$ (mg) Mean (SD)	P-value	H$_2$ (mg) Mean (SD)	P-value
Gender											
M	17	5.66 (7.32)	.134	17.75 (34.96)	.214	0.67 (1.33)	.134	9.19 (7.96)	.703	0.26 (0.34)	.144
F	13	2.41 (2.18)		5.30 (4.42)		0.10 (0.09)		10.33 (8.13)		0.80 (1.44)	
Location											
Colon	22	3.14 (3.64)	.113	7.65 (8.59)	.113	0.51 (1.19)	.485	8.75 (7.66)	.296	0.44 (0.97)	.664
Rectum	8	7.28 (9.35)		25.3 (50.0)		0.18 (0.22)		12.2 (8.54)		0.63 (1.11)	
CEA (mg/dL)											
<5	22	4.51 (6.58)	.695	13.62 (31.14)	.678	0.53 (1.19)	.338	11.24 (8.16)	.073	0.55 (1.14)	.587
≥5	8	3.54 (3.29)		8.89 (7.99)		0.12 (0.18)		5.40 (5.56)		0.33 (0.40)	
Tumor size (mm)											
<40	15	4.24 (7.36)	.991	14.92 (37.37)	.610	0.20 (0.25)	.239	10.06 (7.61)	.799	0.59 (1.15)	.611
≥40	15	4.26 (4.08)		9.79 (9.23)		0.64 (1.42)		9.30 (8.46)		0.40 (0.84)	
Tumor depth (mm)											
<T3	11	3.87 (8.32)	.793	17.5 (43.6)	.426	0.23 (0.29)	.437	13.2 (7.83)	.059	0.99 (1.51)	.035
≥T3	19	4.46 (4.06)		9.32 (8.73)		0.53 (1.27)		7.62 (7.38)		0.21 (0.29)	
Lymph node metastasis											
N	21	4.44 (6.43)	.79	14.7 (31.7)	.776	0.56 (1.21)	.259	11.4 (8.10)	.66	0.62 (1.16)	.215
N+	9	3.80 (4.49)		6.70 (6.81)		0.09 (0.07)		5.64 (6.06)		0.14 (0.16)	
UICC Stage											
0-I	9	4.66 (9.09)	.802	21.3 (47.9)	.236	0.25 (0.31)	.562	13.1 (8.38)	.119	1.12 (1.65)	.020
II-IV	21	4.07 (4.05)		8.49 (8.69)		0.49 (1.21)		8.20 (7.41)		0.22 (0.28)	

CEA, carcinoembryonic antigen; CH$_4$, methane; CO$_2$, carbon dioxide; CRC, colorectal cancer; H$_2$, hydrogen; H$_2$S, hydrogen sulfide; MM, methyl mercaptan; UICC, Union for International Cancer Control.

**TABLE 3** Multivariate logistic regression analysis

Factor	Hazard ratio	95% CI	P-value
MM	1.224	0.978-1.532	.078
H$_2$	0.557	0.325-0.952	.032

H$_2$, hydrogen; MM, methyl mercaptan.

4 | DISCUSSION

To our knowledge, our study is the first to analyze fecal gas compounds from CRC patients using an extremely non-invasive technique. Our data show that the amount of gas compounds obtained during defecation was significantly different between CRC patients and controls.

The first analysis of VOC was reported in 1971 by Pauling et al,[13] who identified several hundred VOC in human breath and urine by using gas chromatography-mass spectrometry. Moreover, several authors have shown the usefulness of VOC for detecting CRC in different substances. These studies showed fair reliability, with sensitivities ranging from 30% to 94% and specificities ranging from 60% to 94%. In our study, the sensitivity was 90%, the
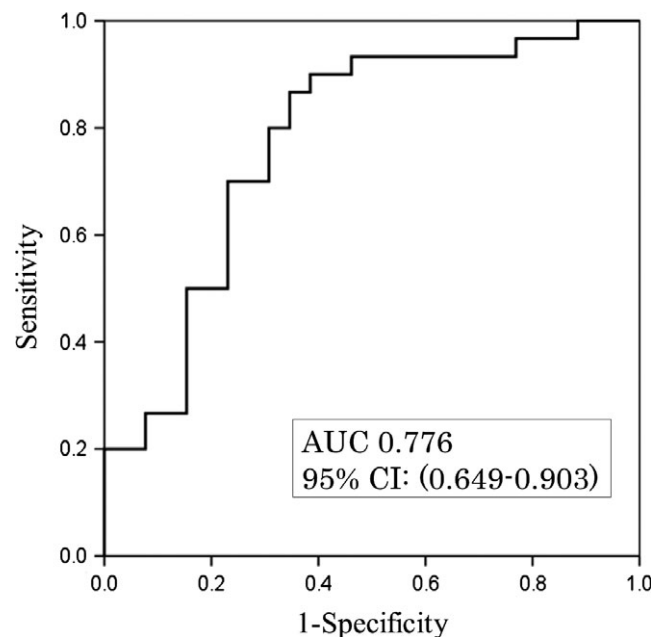
FIGURE 3 Receiver operating characteristic curve of the discriminant formula

AUC 0.776
95% CI: (0.649-0.903)

specificity was 57.7%, and the accuracy was 75%. In contrast, the sensitivity and specificity of FOBT in previous studies were 73.3%–88.9% and 95.8%–97.6%.[6,7,26] However, the analysis of VOC is still not as useful as FOBT in CRC screening.

In the present study, MM concentration was higher in the CRC group than in the control group. MM is a sulfur-containing gas, and Yamagishi et al showed that sulfur-containing gas can be produced by reacting sulfur-containing amino acids with glucose or lactic acid. Thus, it can be presumed that an accumulation of lactic acid and glucose results in the production of sulfur-containing gas in tumor tissues.[27] It is well known that lactic acid is produced by cancer cells by the Warburg effect.[28] Another hypothesis for the mechanism of increasing sulfur-containing gas is related to colonic flora. Recently, _Fusobacterium_ in the colonic flora was implicated in periodontitis, inflammatory bowel disease and CRC.[29] By genomic analysis, Kostic et al showed that _Fusobacterium_ sequences were enriched in colorectal carcinoma.[30] Nakano et al[31] reported that _Fusobacterium nucleatum_ is one of the most potent producers of MM from L-methionine by L-methionine-α-deamino-γ-mercaptomethane-lyase.

In the present study, amounts of CH_4 were not significantly different between the groups. CH_4 is produced by methanogen, which is common in wetlands, where it is responsible for marsh gas, and in the digestive tracts of animals such as ruminants and humans. A previous study[32] showed that patients with CRC produced significantly more CH_4 than healthy controls. However, other studies could not confirm an association between CH_4 and colon cancer by breath methane analysis[33] or animal experiments.[32] Moreover, although the mechanism involved in the increased CH_4 in CRC is unclear, tumors may increase CH_4 by obstructing the bowel.[32]

We analyzed the concentrations of H_2 and CO_2 relative to the pH of the intestinal environment. In the CRC group, the amount of H_2 was significantly lower and the concentration of CO_2 was lower (not significant) than in the control group. Ohigashi et al reported that CRC patients had different intestinal environments, including alterations in microbial flora, decreased short-chain fatty acids, and increased pH. They also showed that organic acids, particularly acetic, propionic, butyric and valeric acids, were decreased in the CRC group.[35] Carbohydrates in the intestine are fermented by bacteria to form organic acids and gases including CO_2 and H_2. When organic acids decrease, the pH increases, and the concentration of CO_2 decreases.[36] Therefore, we speculated that the concentrations of H_2 and CO_2 were lower in the CRC group.

The FOBT is now a standard screening method for CRC.[3–5] However, our method of collecting gas compounds during defecation is more convenient because stool collection is not necessary. Nevertheless, cost-effectiveness is one of the most important factors for medical check-up, and although the cost of gas compound analysis by gas chromatography is expensive, approximately 30 000 yen per analysis, FOBT costs approximately 2000 yen. However, if toilets are equipped with a gas sensor in the future, gas compounds can be detected without using gas chromatography. This will allow more people to be routinely screened, and early detection of CRC may increase.

The first limitation of the present study was its small sample size and, as this was a cross-sectional study of a CRC group and a control group, the second limitation was that the predictive value of the gas compound for screening an unselected population is not yet known. Moreover, we could not compare data from the gas compound analysis and FOBT because we did not have data from FOBT of patients and healthy volunteers. The third limitation was that the reason for the differences in gas compounds between CRC patients and healthy controls was unclear. Gas composition is probably affected by meals and, unfortunately, information regarding the food of CRC patients and controls was not accessible. Thus, further study of the association between consumed food, colonic flora and gas compound production is warranted. Furthermore, a large-scale trial comparing our method with the FOBT, which is a standard screening method, is necessary to confirm the usefulness of our method for CRC screening.

In conclusion, our study showed that analyzing gas compounds from defecation might provide a complete and non-invasive metabolomics biomarker profile that could be used as a diagnostic tool.

DISCLOSURE

Conflicts of Interest: Authors declare no conflicts of interest for this article.

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