Early detection and follow-up of colorectal neoplasia based on faecal volatile organic compounds

Journal:	*United European Gastroenterology Journal*
Manuscript ID	UEG-19-0459
Manuscript Type:	Original Article
Date Submitted by the Author:	13-Sep-2019
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Keywords:	Colorectal Cancer, Screening, Volatile organic compounds, Surveillance, Advanced adenoma
Abstract:	Background: Early detection and removal of colorectal cancer (CRC) and advanced adenomas (AA) decreases incidence and mortality. Objective: We aimed to evaluate potential of faecal volatile organic compounds (VOC) for colorectal adenomas detection and follow-up using advanced electronic nose technology. Methods: This was a prospective multi-centre case-control cohort including two district hospitals and one tertiary referral hospital. Patients undergoing colonoscopy were instructed to collect a faecal sample prior to bowel cleansing and were included when CRC, AA, large adenomas (LA; 0.5-1.0cm), small adenomas (SA; 0.1-0.5cm) or no endoscopic abnormalities (healthy controls; HC) were observed. Patients undergoing

	polypectomy and HC were asked for a second sample after three months. Faecal VOCs were measured with gas chromatography-ion mobility spectrometry. Random Forest, Support Vector Machine, Gaussian Process and Neural Net classification were used to evaluate accuracy. Results: In total, 14 CRC, 64 AA, 69 LA, 127 SA and 227 HC were included. Second sample was collected by 32 polypectomy patients and 32 HC. Faecal VOCs discriminated CRC and adenomas from HC (AUC(95%): CRC vs HC 0.96(0.89-1); AA vs HC 0.96(0.93-1); LA vs HC 0.96(0.92-0.99); SA vs HC 0.96(0.94-0.99)). There were no significant differences between CRC and adenoma groups. Patients with adenomas and HC were discriminated prior to polypectomy, whereas three months after polypectomy VOC profiles were similar (T0 adenoma vs HC 0.98(0.95-1); T1 adenoma vs HC 0.55(0.40-0.69)). Conclusion: Faecal VOC profiles may be useful for early CRC and adenoma detection, and timing of polyp surveillance as polypectomy led to a normalization of VOC profile.

Early detection and follow-up of colorectal neoplasia based on faecal volatile organic compounds

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Key words: Volatile organic compounds, eNose, colorectal cancer, advanced adenoma, screening, surveillance

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Running head: The faecal scent of colonic neoplasia

Acknowledgement of research support The department of Gastroenterology and Hepatology received an unrestricted grant from TAKEDA and the Dr. C. J. Vaillant Fonds for this study.

Author contributions de Boer, de Meij and Bosch designed the study protocol and experiment. Bot, Savelkoul, Bosch, Stokkers, Kuijvenhoven and van der Hulst included the participants and collected the samples. Bot, Savelkoul and Bosch prepared the samples. Bosch performed the experiments. Bot, Savelkoul and Bosch created the database. Wicaksono and Covington analysed the data. Bot and Bosch drafted a first version of the manuscript. Savelkoul, de Boer, de Meij, Stokkers, van der Hulst and Covington critically reviewed the manuscript.

Potential competing conflicts S Bosch has nothing to declare. R Bot has nothing to declare. A Wicaksono has nothing to declare. E Savelkoul has nothing to declare. R vd Hulst has nothing to declare. J Kuijvenhoven has served as a consultant for Janssen Pharmaceuticals. P Stokkers has nothing to declare. E Daulton has nothing to declare. JA Covington has nothing to declare. TGJ de Meij has served as a speaker for Mead Johnson. He has received a (unrestricted) research grant from Danone. NKH de Boer has served as a speaker for AbbVie and MSD. He has served as consultant and principal investigator for TEVA Pharma BV and Takeda. He has received a (unrestricted) research grant from Dr. Falk and Takeda.

Abbreviations:

CRC = Colorectal cancer; AA = advanced adenoma; VOC = volatile organic compound; LA = large adenoma; SA = small adenoma; HC = healthy control; FIT = faecal immunoglobulin test; GC-IMS = Gas chromatography – ion mobility spectrometry; eNose = electronic Nose; METc = Medical Ethical Review Committee; ROC = receiver operator characteristics.

Word count: 2995

Abstract (words: 251)

Background: Early detection and removal of colorectal cancer (CRC) and advanced adenomas (AA) decreases incidence and mortality.

Objective: We aimed to evaluate potential of faecal volatile organic compounds (VOC) for colorectal adenomas detection and follow-up using advanced electronic nose technology.

Methods: This was a prospective multi-centre case-control cohort including two district hospitals and one tertiary referral hospital. Patients undergoing colonoscopy were instructed to collect a faecal sample prior to bowel cleansing and were included when CRC, AA, large adenomas (LA; 0.5-1.0cm), small adenomas (SA; 0.1-0.5cm) or no endoscopic abnormalities (healthy controls; HC) were observed. Patients undergoing polypectomy and HC were asked for a second sample after three months. Faecal VOCs were measured with gas chromatography-ion mobility spectrometry. Random Forest, Support Vector Machine, Gaussian Process and Neural Net classification were used to evaluate accuracy.

Results: In total, 14 CRC, 64 AA, 69 LA, 127 SA and 227 HC were included. Second sample was collected by 32 polypectomy patients and 32 HC. Faecal VOCs discriminated CRC and adenomas from HC (AUC(95%): CRC vs HC 0.96(0.89-1); AA vs HC 0.96(0.93-1); LA vs HC 0.96(0.92-0.99); SA vs HC 0.96(0.94-0.99)). There were no significant differences between CRC and adenoma groups. Patients with adenomas and HC were discriminated prior to polypectomy, whereas three months after polypectomy VOC profiles were similar (T0 adenoma vs HC 0.98(0.95-1); T1 adenoma vs HC 0.55(0.40-0.69)).

Conclusion: Faecal VOC profiles may be useful for early CRC and adenoma detection, and timing of polyp surveillance as polypectomy led to a normalization of VOC profile.

Established knowledge

- Fecal volatile organic compounds have previously been shown to hold potential for the detection of colorectal cancer

What is new?

- Faecal volatile organic compounds hold potential to detect polyps, as well as advanced adenomas and colorectal cancer.
- There are no differences in fecal scent profiles between the different types of adenomas and colorectal cancer
- Prior to polypectomy, there is a difference in scent profile between patients with polyps and healthy controls, whereas after polypectomy these profiles are similar.

Introduction

Colorectal cancer (CRC) is one of the three malignancies with the highest incidence in the industrialized world, with a 5-year survival rate of 64.4% and 66.6% for colon and rectum cancer, respectively[1]. Majority of CRC originates from dysplastic adenomatous polyps, so-called advanced adenomas (AA)[2]. Early detection and removal of these precancerous polyps is essential for improvement of CRC course and prognosis, illustrated by a gradually decrease of survival rates with increasing cancer stage[3]. Faecal immunochemical test (FIT) is widely used for population-based screening but lacks sensitivity, indicated by the missed diagnosis of CRC in 1-47% and AA in 43-61% of the tests[4]. In addition, specificity is suboptimal, as approximately 7% of the performed tests provide false-positive results leading to the performance of unneeded colonoscopies. This emphasizes the need for improvement of CRC/AA bowel screening tools.

Endoscopic assessment is advised after FIT positivity, but also remains key for surveillance after removal of polyps (polypectomy) and following CRC treatment[3], as these patients remain at increased risk for development or recurrence of dysplastic lesions. Thus far, no non-invasive biomarkers have been validated for this purpose.

Analysis of the faecal volatolome is a novel approach within the field of biomarker exploration. The faecal volatolome consists of volatile organic compounds (VOCs), which are carbon-bound molecules. Composition of the faecal volatolome is considered to reflect metabolic processes in the human body, like inflammation, necrosis, change in dietary intake, gut microbial dysbiosis and cancer growth[5,6]. Several studies have focused on the application of the faecal volatolome as a biomarker for detection of CRC and AA, with promising results. However, none of the available studies have included patients with (low-risk) adenomas to explore the specificity of VOC analysis. Aim of this study was to assess the potential of faecal VOC as non-invasive biomarker to detect colonic neoplasia and precursor lesions. In addition, we aimed to explore its potential for secondary non-invasive surveillance following polypectomy.

Materials and methods

Study design

This multi-centre prospective case-control study was performed between February 2015 and November 2017 at outpatient clinics of Gastroenterology and Hepatology departments in one tertiary referral hospital (Amsterdam UMC, location VUmc) and two district hospitals (OLVG West, Amsterdam and Spaarne Gasthuis, Hoofddorp and Haarlem), all located in The Netherlands. This study was approved by the Medical Ethical Review Committee (METc) of Amsterdam UMC (2014.404), and by local METcs of OLVG West and Spaarne Gasthuis. Written informed consent was obtained from all participants.

Study participants and sample collection

Detection of colorectal adenomas and cancer

All patients aged ≥18 years with a scheduled colonoscopy were asked to participate in this study, irrespective of endoscopy indication. Patients were divided into five subgroups based on observations during endoscopy, combined with histology reports in case biopsies or polypectomies were performed: (a) CRC, histologically confirmed carcinoma of the colon or rectum; (b) Advanced adenoma (AA), according to the European Society of Gastrointestinal Endoscopy (ESGE), that is, characterized by polyps ≥1cm in diameter, or with villous histology, or high grade dysplasia; (c) Large adenoma (LA), adenomas sized 0.5-1.0cm in diameter without villous histology or high grade dysplasia; (d) Small adenoma (SA), adenomas sized <0.5cm in diameter without villous histology or high grade dysplasia; (e) Healthy controls (HC), characterized by no abnormalities observed during endoscopy (excluding small anal fibroma, haemorrhoids and/or diverticula), and in case of mucosal biopsies, no histopathological abnormalities[7]. Participants were asked to collect a faecal sample (Stuhlgefäß 10ml, Frickenhausen, Germany) prior to bowel preparation, store the sample in their own freezer at home within one hour following bowel movement and bring it to the hospital on the day of their endoscopic assessment. Samples were stored at -24°C directly upon arrival at the hospital. Participants were asked to complete a questionnaire on the day of sample collection, which included items on age, gender, body mass index (BMI), smoking habits, comorbidity and medication use. Exclusion criteria were presence of a known underlying gastrointestinal disease, incomplete endoscopic assessment due to various reasons (e.g. inadequate bowel

cleansing, pain) and/or inability to collect or store sufficient faecal sample mass to perform VOC analysis.

Monitoring of patients post-polypectomy

Between May and November 2017, patients who underwent a successful polypectomy during endoscopy were asked to participate in the follow-up part of this study. Participants were excluded from this group in case of incomplete removal of polyps. Remaining polypectomy patients were randomly matched to HC in a 1:1 ratio. All patients included in the second part of this study were asked to collect a follow-up faecal sample and complete a second questionnaire (same procedure as the first sample and questionnaire). These samples and questionnaires were collected by one of the researchers and transported to the hospital on dry ice where they were stored at -24°C upon arrival.

Endoscopic and histologic evaluation

Endoscopy reports and histologic outcome of mucosal biopsies and/or polypectomy were checked using electronic patient files. These outcomes were used as standard reference for localization and total number of removed adenomas in this study. Endoscopies were either performed or supervised by trained gastroenterologists. Histopathological reports were used as the standard reference for size, differentiation grade (e.g. hyperplasia, dysplasia), villous histology and type of CRC in this study. Mucosal biopsies were noted as sized 0.2 cm. The presence of sessile and/or serrated characteristics was noted for all non-advanced adenomas. When multiple polyps were present, classification was based on the most advanced or largest lesion.

Sample preparation and faecal volatile organic compound analysis

One frozen subsample of 500 mg per participant was weighted and transferred into glass vials (20ml headspace vial, Thames Restek, Saunderton, UK). Samples were transported to the University of Warwick (Coventry, UK) for faecal VOC analysis. Gas chromatography-ion mobility spectrometer (GC-IMS, FlavourSpec®, G.A.S., Dortmund, Germany) was used to measure the faecal VOC patterns. In GC-IMS, analytes are pre-separated by retention time in a GC column (SE54 column) before entering the ion mobility spectrometer (IMS). Within the IMS system, soft chemical-ionization is performed using a low-radiation tritium (H3) source, subsequently

creating reactant ions with injected gas. Ionized VOCs travel against flow of an inert drift gas. Drift time of each substance is determined by ions mass and geometrical structure due to interactions with the drift gas. In general, the larger the molecule, the more it loses its momentum and thus, the longer it takes to travel along the tube. Resulting ion current is measured as a function of time[8]. Prior to analysis, the samples were heated to 80°C for 8 minutes. After this, GC was performed at 40°C using nitrogen 99·9% (3·5 bar) as carrier gas and IMS was performed at 45°C using nitrogen 99·9% as drift gas. Flow rates were set at 150ml/min (0·364 kPa) (IMS), and 20 ml/minute (34,175 kPa) for 6 minutes (GC). A schematic overview of the setup is depicted in *Figure 1.*

Statistical analysis

Using IBM SPSS Statistics (version 22), demographic data of each group were calculated and compared. One way ANOVA or Kruskall Wallis tests were used to compute differences in baseline demographics between groups. For the secondary part of this study, t-tests, Fisher's exact test, chi-square and Mann-Whitney U tests were used to compute differences between groups. Raw GC-IMS data were pre-processed to crop areas that contained chemical information. A threshold was applied to remove background noise. Then, a correction for instrumental disturbances was performed by baseline correction, where reactive ion peaks were aligned on all samples. Data were split into three sets, 70% for training and validation and 30% as a test set. Wilcoxon rank-sum test was used to find the 20, 50 and 100 most discriminatory features and subsequently Sparse Logistic Regression, Random Forest, Gaussian Process, Support Vector Machine and Neural Net classification were used to provide statistical results. In the case of small subgroups of interest, subsets of HC were chosen randomly to avoid skewed analyses.

Results

Baseline characteristics

In total, 1039 patients collected a faecal sample prior to colonoscopy of which samples from 14 CRC patients, 64 AA, 69 LA and 127 SA were included in this study. 227 HC were included as they did not have any mucosal abnormalities during endoscopy. Baseline demographics of all study participants are depicted in *Table 1.* Age differed significantly between groups ($p<0.001$), with HC displaying the lowest mean age (60 ± 11.8) and AA the highest (68.8 ± 6.7). Gender differed significantly between HC, SA, LA and AA ($p<0.0001$) but not between HC and CRC or any of the adenoma groups. There were no significant differences in BMI, smoking status and use of antibiotics between groups.

For the follow-up part of this study, all 32 patients undergoing a complete polypectomy and 32 HC subjects were included and collected a second fecal sample three months after endoscopy. Baseline demographics of the follow-up study are given in *Table 2.* There were no significant differences in BMI, smoking status and use of AB three months prior to sample collected. Gender and age did differ significantly between groups ($p<0.014$, $p<0.001$, respectively).

Faecal volatile organic compound analysis

Results of VOC analyses by means of GC-IMS are shown in *Table 3.* Results from the Random Forest classifier based on the 20 most discriminative features are presented. Data generated based on all five classifiers using the 20, 50 and 100 most discriminative features are given in Supplemental Table 1-3.

Detection of colorectal cancer, advanced adenomas and non-advanced adenomas

Based on faecal VOC profiles, CRC was discriminated from HC with high diagnostic accuracy (AUC ± 95%CI: 0·96(0·89 – 1)(*Table 3, Supplementary table 1-3)*. Likewise, high diagnostic accuracy was observed for discrimination of AA, LA and SA when compared to HC (AA 0·96(0·93 –1); LA 0·96(0·92 – 0·99); SA 0·96(0·94-0·99))(*Table 3, Supplementary tables 1-3)*. There were no significant differences between any of the CRC, AA, LA and SA groups based on faecal VOCs (*Table 3, Supplementary table 1-3)*. Receiver operator characteristic (ROC) curves are constructed in *Figure 2.*

Faecal volatile organic compounds for surveillance after polypectomy

Faecal VOC profiles of patients with adenomas differed significantly from HC before polyp removal (AUC ± 95%CI: 0·98(0·95-1)). Remarkably, there was no difference between the faecal VOC profiles of patients who underwent a polypectomy and HC three months after endoscopic intervention (AUC ± 95%CI: 0·55(0·40-0·66)). There was a highly significant difference in the profiles of patients with adenomas before and after polypectomy, whereas no significant differences were present in faecal VOC profiles of HC before and three months after endoscopy (T0 vs T1 polyps 0·94(0·88-1); T0 vs T1 HC 0·58(0·44-0·73)). ROC-curves for the follow-up study are depicted in *Figure 3.*

Discussion

We observed high diagnostic accuracies for faecal VOC-based differentiation between CRC and HC and between adenomas and HC. Faecal VOC profiles of patients with adenomas converted towards those of HC after polypectomy. VOC profiles of CRC could not be discriminated from different adenoma types. These results demonstrate the potential of faecal VOC analysis for both colonic neoplasia screening and surveillance.

This is the first study to assess faecal VOC profiles for CRC and AA detection, which included patients with non-advanced adenomas as unique control groups. No previous studies have been performed on the potential of focal VOC profiles for surveillance after polypectomy. Surveillance of patients after polypectomy using FIT has been subject of a previous study comparing stool haemoglobin levels with colonoscopy outcomes[9]. A total of 5225 participants completed a first FIT one year after polypectomy, demonstrating sensitivity and specificity values of 27·6% and 94·1% at 40 µg/g, and 51·7% and 86·2% at 10 µg/g for CRC, respectively. For AA, sensitivity and specificity values were 17·0% and 95·1% for 40 µg/g and 33·0% and 88·0% for 10 µg/g, respectively. Replacing colonoscopy with FIT would reduce colonoscopies by 71%, but would lead 30-40% missed CRC cases and 40-70% missed AA cases. Observed discriminative accuracies for CRC and AA detection based on faecal VOC analysis exceed these reported accuracies[4,10]. In addition, accuracy to distinguish patients with adenomas from HC was high prior to polypectomy, whereas intra-individual profiles changed to physiological state three months following polypectomy, indicating the potential of faecal VOCs as biomarker for timing of polyp surveillance, and tight control in high risk populations (e.g. Lynch syndrome).

Few studies have demonstrated the potential to discriminate CRC and AA from HC based on faecal VOC analysis. Current study outcomes are in line with this literature. However, reliable comparison with other studies is restricted due to the small number of included subjects, lack of knowledge on VOC profiles of AA and non-advanced adenomas, and the use of various different techniques and sampling protocols. Four previous studies have focused on detection of CRC using the analytical platform gas chromatography—mass spectrometry (GC-MS), which is considered the gold standard for the detection of specific metabolites [11-14]. In the most recent publication,

a group of adenomatous polyps patients (n=56) was included in addition to CRC (n=21) and no neoplasia as HC (n=60). Multiple differences in metabolite levels were found. Highest diagnostic accuracy was found for the combination of Propan-2-ol and 3-methylbutanioc acid discriminating CRC samples from polyps and HC (AUC 0.82). These differences specifically provided discrimination between CRC patients and other groups, whereas in the current study, VOC profiles differed between CRC and adenoma groups compared to HC. Possible explanations for this are the differences in faecal VOC analysis technique, and in inclusion criteria per subgroup. In the publication by Bond et. al., polyp characteristics are not reported, which hampers reliable comparison to our study groups. All other studies using GC-MS reported relatively small groups of subjects, ranging from n=9-26 CRC and n=10-60 HC. There were interesting similarities in study outcomes (e.g. increased levels of amino acids and short-chain fatty acids and decreased levels of polyhydric alcohols and bile acid), though, none of these metabolite levels were consequently altered. In one previous publication, VOC profiles of 40 CRC, 60 AA and 57 endoscopy controlled HC were compared using pattern-recognition (eNose, Cyranose 320 ®)[15]. Based on faecal VOC patterns, CRC and AA were discriminated from HC with sensitivity of 85% and 62%, and specificity of 87% and 86%, respectively. These test characteristics were below characteristics found in the current study, however, using the Cyranose 320®, subgroups of CRC and AA were discriminated with moderate accuracy (sensitivity 75% and specificity 73%). A possibility for this apparent discrepancy is the number of CRC patients included in the study by de Meij *et al.*, increasing power to find differences between groups. Another possibility is the instrumental difference between the Cyranose 320® and the GC-IMS system. The Cyranose 320® uses an array of nanocomposite sensors, whereas the GC-IMS system is using drift time. Although GC-IMS is more sensitive and repeatable, it cannot measure molecules with low proton affinities. Should differentiation between CRC and AA be based on these type of molecules they could not have been detected in the current study.

Alterations in faecal VOC patterns represent metabolic shifts that may be explained by various mechanisms (e.g. alterations in dietary intake, microbial dysbiosis, inflammatory processes, cancer degeneration). In a recent study, metabolic waste was retrieved from benign cells, colon cancer cells and breast cancer cells that were grown *in vitro*. It was observed that dogs were able to differentiate cancer cells from

benign cells, but not the cell waste of breast from colon cancer, implying that both cancers phenotypes seem to share a common smell print[16]. This may also apply for different phenotypes of adenomas; adenoma and eventually CRC degeneration are possibly based on a shared (metabolic) pathway, explaining the similarities in VOC patterns observed in the current study. Apart from excretion of metabolic end-products, our findings may be explained by the presence of intestinal dysbiosis. Faecal microbiota has important function in protection against invading pathogens and strong evidence exists for the association between microbial dysbiosis and polyp-associated tissue and colonic neoplasia[17]. Causality remains unclear; it is unknown whether this phenomenon is triggered or maintained by carcinogenesis. Intriguingly, in the present study it was found that faecal VOC profiles of patients three months after polypectomy were altered to normalcy following this intervention, suggesting that the alleged faecal microbial dysbiosis returns to physiological state after polyp removal.

Design of this large, multi-centre prospective cohort contributed to generalizability of the outcomes. Bias by colonic abnormalities was avoided by including an endoscopy controlled HC subgroup. This study also had some limitations. Most importantly, CRC subgroup was relatively small which may have contributed to the inability to discriminate CRC and adenoma profiles. In addition, even though most variables expected to influence faecal VOC profiles were evenly present in the subgroups (i.e. smoking status, BMI and use of antibiotics), gender and age did differ between groups. Data on the influence of age and gender on faecal VOC profiles are lacking and we were therefore not able to exclude this possible bias.

Future research aimed at creating a disease-specific faecal VOC algorithms for CRC and adenoma detection may focus either on pattern-recognition or on metabolite specific analytical platforms. The use of pattern-recognition may be favourable for this purpose since this allows for fast, easy-to-perform, high-throughput and low-cost analyses, underlining its suitability for application in clinical practice. Using machine learning, algorithms may be built for the detection of CRC and its precursor lesions, which improve continuously with increasing numbers of samples measured. Once the algorithm reaches satisfactory accuracy, the device may be implemented not only for population based screening but also for intra-individual surveillance after polyp or CRC

removal. For surveillance, the device should be validated coupled to endoscopy outcomes in the surveillance program. Last, our data have raised the hypothesis that faecal VOCs associated with CRC and AA may be a consequence rather than the cause of adenoma presence. It would be interesting from a pathophysiological and therapeutic point of view to unravel the underlying entities causing the faecal VOC differences pre- and post-polypectomy.

In conclusion, because of its high sensitivity, this study highlights the potential faecal VOC analysis for population based screening. Additionally, intra-individual faecal VOC profiles of patients with adenomas altered towards a physiological state following polypectomy, emphasizing its potential for intra-individual surveillance and timing of endoscopy.

References

1. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. *Eur J Cancer.* 2010;46(4):765-781.
2. Ballinger AB, Anggiansah C. Colorectal cancer. *BMJ.* 2007;335(7622):715-718.
3. Winawer S, Fletcher R, Rex D, et al. Colorectal cancer screening and surveillance: clinical guidelines and rationale-Update based on new evidence. *Gastroenterology.* 2003;124(2):544-560.
4. Katsoula A, Paschos P, Haidich AB, Tsapas A, Giouleme O. Diagnostic Accuracy of Fecal Immunochemical Test in Patients at Increased Risk for Colorectal Cancer: A Meta-analysis. *JAMA Intern Med.* 2017;177(8):1110-1118.
5. Boots AW, Smolinska A, van Berkel JJ, et al. Identification of microorganisms based on headspace analysis of volatile organic compounds by gas chromatography-mass spectrometry. *J Breath Res.* 2014;8(2):027106.
6. Shirasu M, Touhara K. The scent of disease: volatile organic compounds of the human body related to disease and disorder. *J Biochem.* 2011;150(3):257-266.
7. Rex DK, Kahi CJ, Levin B, et al. Guidelines for colonoscopy surveillance after cancer resection: a consensus update by the American Cancer Society and US Multi-Society Task Force on Colorectal Cancer. *CA Cancer J Clin.* 2006;56(3):160-167; quiz 185-166.
8. Eiceman GA KZ. *Ion Mobility Spectrometry, 2nd edition.* 6000 Broken Sound Parkway NW, Suite 300, USA: T&F Informa; 2005.
9. Cross AJ, Wooldrage K, Robbins EC, et al. Faecal immunochemical tests (FIT) versus colonoscopy for surveillance after screening and polypectomy: a diagnostic accuracy and cost-effectiveness study. *Gut.* 2018.
10. Rozen P, Levi Z, Hazazi R, et al. Identification of colorectal adenomas by a quantitative immunochemical faecal occult blood screening test depends on adenoma characteristics, development threshold used and number of tests performed. *Aliment Pharmacol Ther.* 2009;29(8):906-917.
11. Eun Mi Song J-SB, Sun Mi Lee, Hyun Ju Yoo, Su Jung Kim, Sun-Ho Lee, Kiju Chang, Sung Wook Hwang, Dong-Hoon Yang, Jin-Yong Jeong. Fecal fatty acid profiling as a potential new screening biomarker in patients with colorectal cancer. *Digestive Diseases and Sciences.* 2018(63):1229-1236.
12. Weir TL, Manter DK, Sheflin AM, Barnett BA, Heuberger AL, Ryan EP. Stool microbiome and metabolome differences between colorectal cancer patients and healthy adults. *PLoS One.* 2013;8(8):e70803.
13. Wang X, Wang J, Rao B, Deng L. Gut flora profiling and fecal metabolite composition of colorectal cancer patients and healthy individuals. *Exp Ther Med.* 2017;13(6):2848-2854.
14. Bond A, Greenwood R, Lewis S, et al. Volatile organic compounds emitted from faeces as a biomarker for colorectal cancer. *Aliment Pharmacol Ther.* 2019.
15. de Meij TG, Larbi IB, van der Schee MP, et al. Electronic nose can discriminate colorectal carcinoma and advanced adenomas by fecal volatile biomarker analysis: proof of principle study. *Int J Cancer.* 2014;134(5):1132-1138.
16. Seo IS, Lee HG, Koo B, et al. Cross detection for odor of metabolic waste between breast and colorectal cancer using canine olfaction. *PLoS One.* 2018;13(2):e0192629.
17. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nature Reviews Microbiology.* 2014;12:661.

Tables

Table 1. Demographics of all study participants

		Colorectal Cancer (n=14)	Advanced adenomas (n=64)	Large adenomas (n=69)	Small adenomas (n=127)	Healthy controls (n=227)
Age (mean, ± s·d·)		66·6 ± 8·7	68·8 ± 6·7	68·7 ± 7·2	63·7 ± 10·0	60 ± 11·8
Gender, f (n, [%])		6 [42·9]	17 [26·3]	21 [30·4]	44 [34·6]	129 [56·8]
BMI* (mean, ± s·d·)		25·9 ± 5·3	27·0 ± 4·1	26·6 ± 3·9	27·3 ± 6·5	26·6 ± 7·0
ABx 3 months prior to inclusion (n, [%])		0 [0]	6 [9·4]	3 [4·3]	21 [16·5]	31 [13·7]
Smoking Status (n, [%])						
	Active	2 [14·3]	9 [14·1]	15 [21·7]	23 [18·1]	37 [16·3]
	Quit	10 [71·4]	37 [57·8]	36 [52·2]	64 [50·4]	92 [40·5]
	Never	2 [14·3]	18 [28·1]	17 [24·6]	40 [31·5]	98 [43·2]
Size largest adenoma (mean, ± s·d·)		0·7 ± 0·63	1·4 ± 0·64	0·7 ± 0·26	0·4 ± 0·32	NA
Localization of Largest adenoma† (n, [%])						
	Caecum	0 [0]	4 [6·3]	7 [10·1]	12 [9·4]	NA
	Colon Ascendens	1 [7·1]	8 [12·5]	12 [17·4]	32 [25·2]	NA
	Flexura Hepatica	1 [7·1]	2 [3·1]	0 [0]	1 [0·8]	NA
	Colon Transversum	0 [0]	3 [4·7]	15 [21·7]	18 [14·2]	NA
	Flexura Lienalis	0 [0]	1 [1·6]	0 [0]	1 [0·8]	NA
	Colon Descendens	1 [7·1]	4 [6·3]	6 [8·7]	9 [7·1]	NA
	Sigmoid	5 [35·7]	30 [46·9]	19 [27·5]	24 [18·9]	NA
	Rectosigmoid	2 [14·3]	3 [4·7]	1 [1·4]	4 [3·1]	NA
	Rectum	2 [14·3]	6 [9·4]	6 [8·7]	14 [11·0]	NA
	Terminal ileum	1 [7·1]	0 [0]	0 [0]	1 [0·8]	NA
CRC type (n, [%])						
	Adenocarcinoma	12	NA	NA	NA	NA
	Neuroendocrine	2	NA	NA	NA	NA

AA characteristics (largest adenoma) (n, [%])						
	≥ 10 mm	NA	54 [84·4]	NA	NA	NA
	Villous histology	NA	31 [48·4]	NA	NA	NA
	HGD	NA	6 [9·4]	NA	NA	NA
Polyp characteristics (largest adenoma) (n, [%])						
	No dysplasia	NA	1 [1·6]	9 [13·0]	9 [7·1]	NA
	Hyperplasia	NA	2 [3·1]	4 [5·8]	14 [11·0]	NA
	LGD	NA	55 [85·9]	55 [79·7]	100 [78·7]	NA
	Sessile/serrated	NA	NA	8 [11·6]	10 [7·9]	NA
Total number adenomas removed (n, [%])						
	1	6 [42·8]	11 [17·2]	18 [26·1]	59 [46·5]	NA
	2	1 [7·1]	14 [21·9]	20 [29·0]	34 [26·8]	NA
	3	3 [21·4]	11 [17·2]	14 [20·3]	14 [11·0]	NA
	4-5	2 [14·3]	14 [21·9]	8 [11·6]	15 [11·8]	NA
	6-10	1 [7·1]	13 [20·3]	9 [13·0]	4 [3·2]	NA
	>10	0 [0]	1 [1·6]	0 [0]	0 [0]	NA

Table 1. Baseline characteristics. Abbreviations: CRC, colorectal cancer; AA, advanced adenoma, LA, large adenomas; SA, small adenomas; HC, healthy controls; BMI, body mass index; NA, not applicable; HGD: high grade dysplasia; LGD: low-grade dysplasia; AB: antibiotics. *Insufficient documentation of 2CRC, 6AA, 6LA, 10SA; 11 HC.

Table 2. Demographics of participants included in follow-up study

		Polypectomy group (n=32)	Healthy controls (n=32)
Age (mean, ±SD)		71·0 ± 5·9	60·5 ± 11·3
Gender (n females, %)		5 [15·6]	15 [46·9]
BMI (mean, ±SD)		26·8 ± 4·2	26·3 ± 3·4
Smoking status (n, %)			
	Active	6 [18·8]	4 [12·5]
	Quit	18 [56·3]	19 [59·4]
	Never	8 [25·0]	9 [28·1]
Indication for endoscopic assessment (n, %)			
	Positive FIT	10 [31·3]	4 [12·5]
	Rectal blood loss	4 [12·5]	1 [3·1]
	Change in bowel habits	2 [6·3]	3 [9·4]
	Surveillance	5 [15·6]	3 [9·4]
	Abdominal Pain	2 [6·3]	11 [34·4]
	Diarrhea	3 [9·4]	1 [3·1]
	Weight Loss	1 [3·1]	0 [0]
	Anaemia	0 [0]	2 [6·3]
	Constipation	0 [0]	2 [6·3]
	Family history CRC+	1 [3·1]	8 [25]
	Monitoring previous diverticulitis/abscess	0 [0]	2 [6·3]
	Other	4 [12·5]	5 [15·7]
ABx 3 months prior to inclusion		2 [6·3]	6 [18·8]
ABx 3 months prior to second sample		1 [3·1]	3 [9·4]
Size adenoma (mean, ±SD)		1·1 ± 0·5	NA
Localization of adenoma (n, %)			
	Caecum	3 [9·4]	NA
	Colon Ascendens	6 [18·8]	NA

	Flexura Hepatica	0 [0]	NA
	Colon Transversum	2 [6·3]	NA
	Flexura Lienalis	1 [3·1]	NA
	Colon Descendens	2 [6·3]	NA
	Sigmoid	11 [34·4]	NA
	Rectosigmoid	1 [3·1]	NA
	Rectum	4 [12·5]	NA
	Ileocecal valve	1 [3·1]	NA
Adenoma characteristics (largest adenoma) (n, [%])			
	≥ 10 mm	13 [40·6]	NA
	Villous histology	9 [28·1]	NA
	HGD	0 [0]	NA
	No dysplasia	3 [9·4]	NA
	Hyperplasia	0 [0]	NA
	LGD	29 [90·6]	NA
	Sessile/serrated	3 [9·4]	NA
Total number adenomas removed (n, [%])			
	1	7 [21·9]	NA
	2	11 [34·4]	NA
	3	7 [21·9]	NA
	4-5	3 [9·4]	NA
	6-10	4 [12·6]	NA

Table 2. Baseline characteristics of participants in polypectomy follow-up study. Abbreviations: HC, healthy controls; BMI, body mass index; ABx: antibiotics.

Table 3. Differences between all subgroups of colorectal neoplasia, polyps and healthy controls based on faecal volatile organic compounds

Comparison	AUC (95% CI)	Sensitivity	Specificity	PPV	NPV	P-value
Colorectal cancer vs healthy controls	0·961 (0·891-1)	1	1	0·889	1	<0·001
Advanced adenoma vs healthy controls	0·964 (0·932-0·996)	0·969	0·938	0·939	0·968	<0·001
Large adenomas vs healthy controls	0·957 (0·923-0·992)	0·985	0·912	0·918	0·984	<0·001
Small adenomas vs healthy controls	0·964 (0·940-0·987)	0·960	0·929	0·931	0·959	<0·001
Colorectal cancer vs advanced adenomas	0·541 (0·382-0·700)	0·984	0·188	0·829	0·75	0·294
Colorectal cancer vs large adenomas	0·41 (0·31 - 0·51)	0·06	0·96	0·25	0·81	0·920
Colorectal cancer vs small adenomas	0·413 (0·379-0·446)	1	0	0·113	NA	0·965
Advanced adenomas vs large adenomas	0·53 (0·43 - 0·63)	0·75	0·36	0·55	0·58	0·278
Advanced adenomas vs small adenomas	0·578 (0·495-0·662)	0·719	0·444	0·397	0·757	0·039
T0 Healthy controls vs T0 pre-polypectomy	0·982 (0·946-1)	1	0·967	0·970	1	<0·001
T1 Healthy controls vs T1 post-polypectomy	0·548 (0·404-0·691)	0·906	0·25	0·547	0·828	0·256
T0 vs T1 pre- and post-polypectomy	0·936 (0·875-0·996)	0·906	0·906	0·906	0·906	<0·001
T0 vs T1 Healthy controls	0·581 (0·436-0·726)	0·938	0·267	0·577	0·8	0·139

Table 3. Outcomes obtained using Random Forest analyses based on 20 most discriminating features. Abbreviations: AUC, area under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; CRC, colorectal cancer; AA advanced adenoma; LA, large adenomas; SA, small adenomas; LGD, low-grade dysplasia; HC healthy controls.

Figure Legends

Figure 1. Gas Chromatography Ion Mobility Spectrometry

Figure 1. Samples are heated to 80°C for 8 minutes prior to analyses. Headspace of sample is injected into GC-IMS. VOCs enter the GC column where they are separated based on their interaction with carrier gas. VOCs with similar retention times enter the IMS column simultaneously and are ionized using a soft-chemical ionization. Ionized VOCs travel against flow of inert gas. Drift time of VOCs is determined by ion's mass and geometrical structure. The resulting ion current is measured by electrometer as function of time.

Figure 2. Receiver operator characteristic curves for the comparison between colorectal cancer, advanced adenoma, large adenoma and small adenoma and healthy controls

Figure 2. Receiver operator characteristics curves. Abbreviations: CRC, colorectal cancer; AA, advanced adenomas; LA, large adenoma; SA, small adenoma; HC, healthy controls; AUC, area under the curve.

Figure 3. Receiver operator characteristic curves for the polypectomy follow-up

Figure 3. Receiver operator characteristics curves. Abbreviations: AUC, area under the curve; HC, healthy controls.

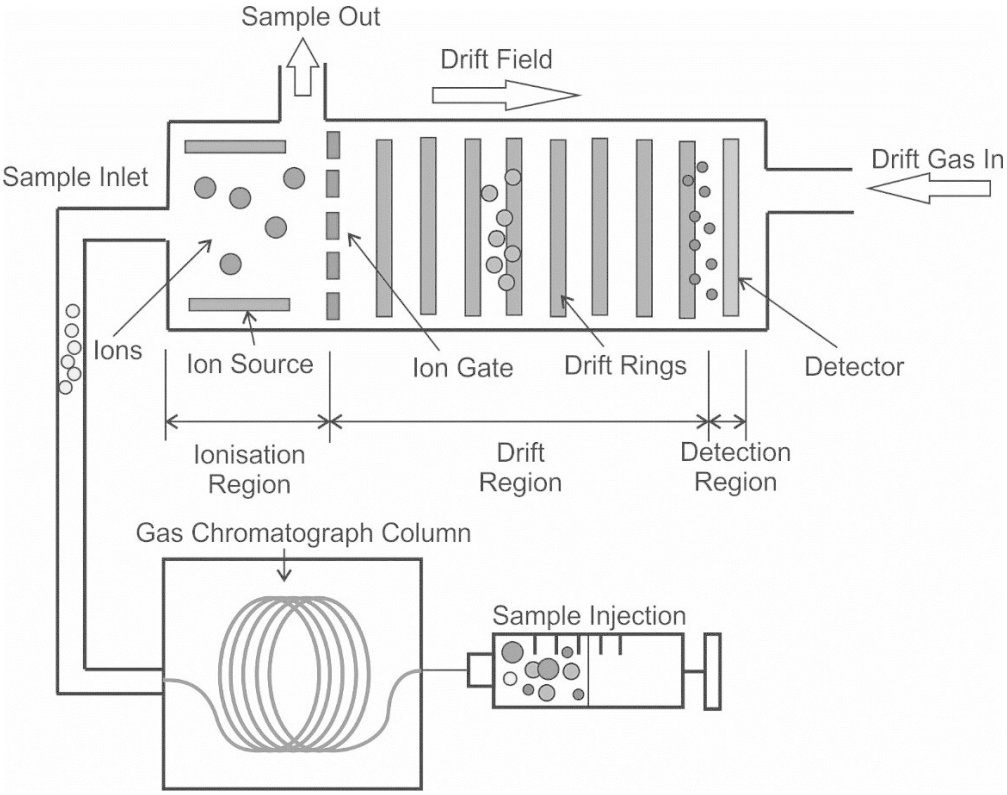

Figure 1. Samples are heated to 80°C for 8 minutes prior to analyses. Headspace of sample is injected into GC-IMS. VOCs enter the GC column where they are separated based on their interaction with carrier gas. VOCs with similar retention times enter the IMS column simultaneously and are ionized using a soft-chemical ionization. Ionized VOCs travel against flow of inert gas. Drift time of VOCs is determined by ion's mass and geometrical structure. The resulting ion current is measured by electrometer as function of time.

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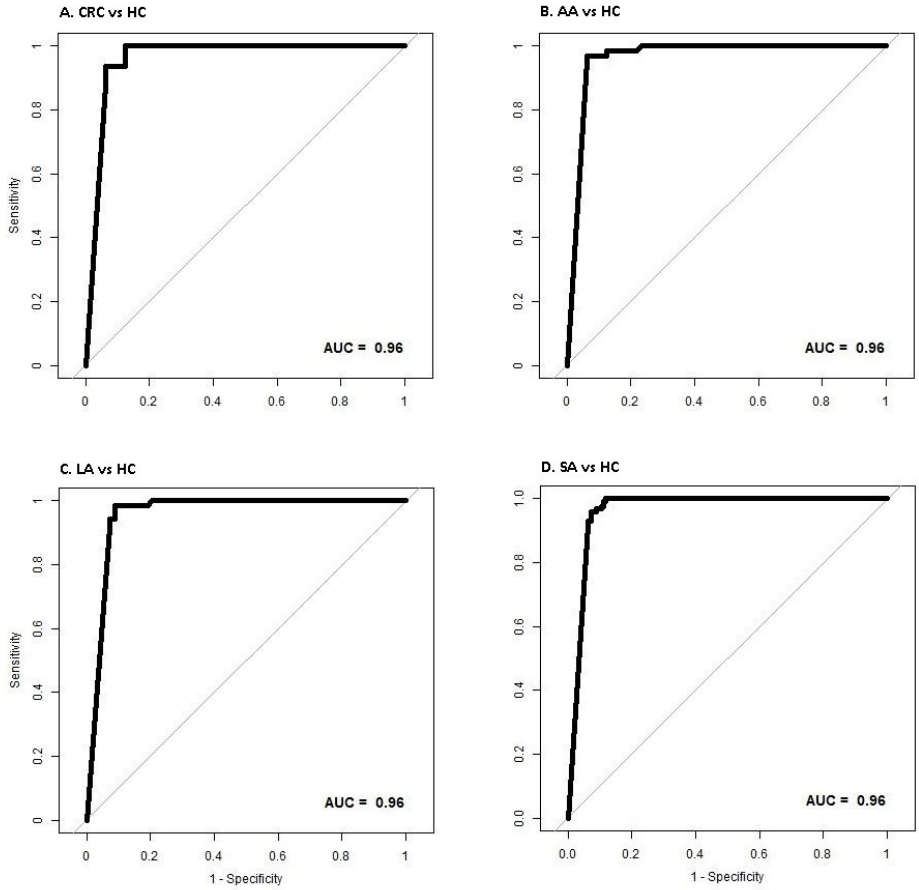

Figure 2. Receiver operator characteristic curves for the comparison between colorectal cancer, advanced adenoma, large adenoma and small adenoma and healthy controls
Figure 2. Receiver operator characteristics curves. Abbreviations: CRC, colorectal cancer; AA, advanced adenomas; LA, large adenoma; SA, small adenoma; HC, healthy controls; AUC, area under the curve.

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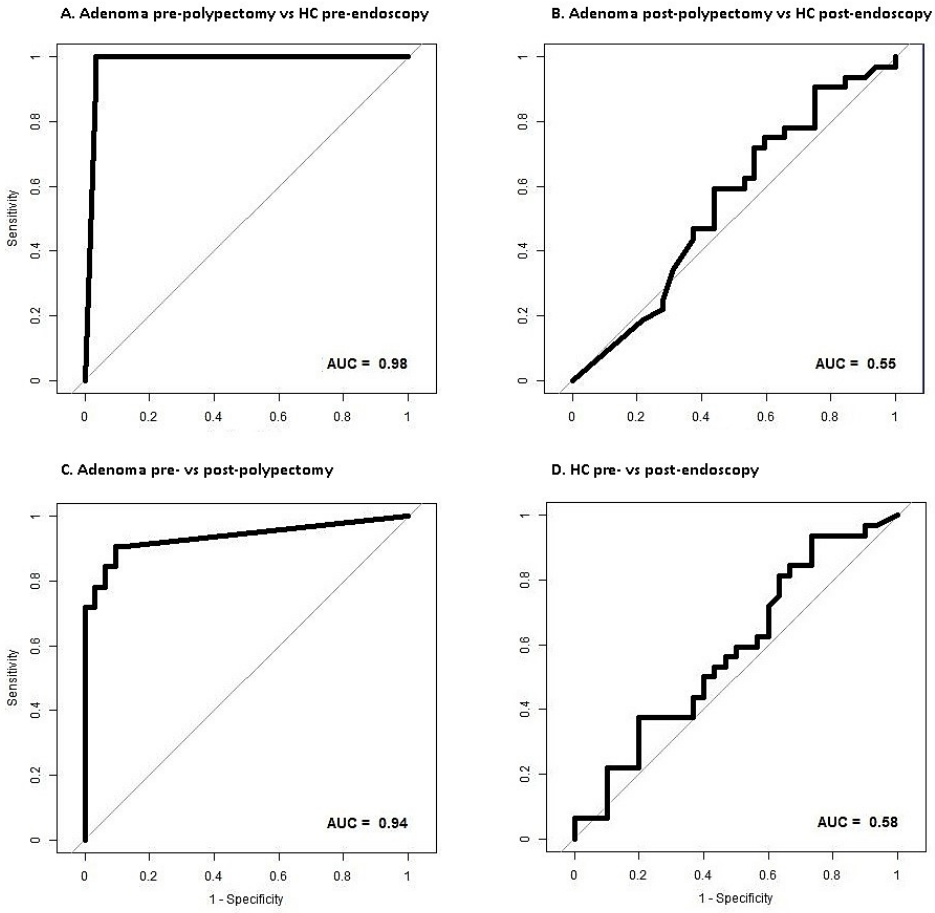

Figure 3. Receiver operator characteristic curves for the polypectomy follow-up
Figure 3. Receiver operator characteristics curves. Abbreviations: AUC, area under the curve; HC, healthy controls.

255x245mm (95 x 95 DPI)

Supplemental Table 1. Overview of the data generated using all five classifiers based on the 20 most discriminative features

Classifier	AUC (95% CI)	Sensitivity	Specificity	PPV	NPV	p-value
CRC versus HC						
Sparse logistic regression	0·949 (0·863-1)	1	1	0·889	1	<0·001
RandomForest	0·961 (0·891-1)	1	1	0·889	1	<0·001
GaussianProcess	0·938 (0·836-1)	1	1	0·842	1	<0·001
SupportVectorMachine	0·945 (0·851-1)	1	1	0·889	1	<0·001
NeuralNet	0·906 (0·775-1)	1	1	0·889	1	<0·001
AA versus HC						
Sparse logistic regression	0·927 (0·869-0·985)	0·969	0·906	0·912	0·967	<0·001
RandomForest	0·964 (0·932-0·996)	0·969	0·938	0·939	0·968	<0·001
GaussianProcess	0·949 (0·902-0·996)	0·953	0·938	0·938	0·952	<0·001
SupportVectorMachine	0·953 (0·907-1)	0·984	0·922	0·926	0·983	<0·001
NeuralNet	0·949 (0·901-0·998)	1	0·906	0·914	1	<0·001
Large non-AA polyps (0·5-1·0 cm) versus HC						
Sparse logistic regression	0·947 (0·903-0·991)	0·971	0·926	0·930	0·970	<0·001
RandomForest	0·957 (0·923-0·992)	0·985	0·912	0·918	0·984	<0·001
GaussianProcess	0·953 (0·912-0·994)	0·985	0·912	0·918	0·984	<0·001
SupportVectorMachine	0·936 (0·886-0·987)	0·985	0·912	0·918	0·984	<0·001
NeuralNet	0·955 (0·917-0·992)	0·985	0·990	0·905	0·984	<0·001
Small non-AA polyps (0·1-0·5 cm) versus HC						
Sparse logistic regression	0·929 (0·890-0·969)	0·976	0·905	0·911	0·974	<0·001
RandomForest	0·964 (0·940-0·987)	0·960	0·929	0·931	0·959	<0·001
GaussianProcess	0·949 (0·916-0·982)	1	0·889	0·9	1	<0·001
SupportVectorMachine	0·944 (0·909-0·979)	0·968	0·921	0·924	0·967	<0·001
NeuralNet	0·934 (0·895-0·972)	0·984	0·905	0·912	0·983	<0·001
CRC versus AA						
Sparse logistic regression	0·616 (0·465-0·768)	0·531	0·75	0·895	0·286	0·077
RandomForest	0·541 (0·382-0·700)	0·984	0·188	0·829	0·75	0·294
GaussianProcess	0·438 (0·273-0·604)	0·936	0·125	0·811	0·333	0·226
SupportVectorMachine	0·444 (0·275-0·613)	0·875	0·188	0·812	0·273	0·756
NeuralNet	0·604 (0·465-0·742)	0·516	0·75	0·892	0·279	0·102
CRC versus Large non-AA polyps (0·5-1·0 cm)						
Sparse logistic regression	0·46 (0·31 - 0·61)	0·81	0·24	0·2	0·84	0·704
RandomForest	0·41 (0·31 - 0·51)	0·06	0·96	0·25	0·81	0·920
GaussianProcess	0·59 (0·44 - 0·74)	0·94	0·28	0·23	0·95	0·865
SupportVectorMachine	0·67 (0·53 - 0·80)	0·81	0·54	0·30	0·93	0·981
NeuralNet	0·59 (0·44 - 0·74)	0·94	0·26	0·23	0·95	0·857
CRC versus Small non-AA polyps (0·1-0·5 cm)						
Sparse logistic regression	0·647 (0·540-0·753)	1	0·373	0·168	1	0·972
RandomForest	0·413 (0·379-0·446)	1	0	0·113	NA	0·965
GaussianProcess	0·640 (0·516-0·764)	1	0·278	0·150	1	0·966
SupportVectorMachine	0·618 (0·490-0·746)	0·938	0·349	0·155	0·978	0·939
NeuralNet	0·652 (0·554-0·770)	0·875	0·476	0·175	0·968	0·973
AA versus Large non-AA polyps (0·5-1·0 cm)						
Sparse logistic regression	0·51 (0·41 - 0·61)	0·79	0·33	0·56	0·6	0·397
RandomForest	0·53 (0·43 - 0·63)	0·75	0·36	0·55	0·58	0·278
GaussianProcess	0·52 (0·42 - 0·62)	0·90	0·22	0·55	0·67	0·323
SupportVectorMachine	0·52 (0·42 - 0·62)	0·54	0·56	0·57	0·54	0·338
NeuralNet	0·47 (0·37 - 0·57)	0·56	0·5	0·54	0·52	0·729
AA versus Small non-AA polyps (0·5-1·0 cm)						
Sparse logistic regression	0·558 (0·471-0·644)	0·391	0·722	0·412	0·7	0·098
RandomForest	0·578 (0·495-0·662)	0·719	0·444	0·397	0·757	0·039
GaussianProcess	0·554 (0·468-0·640)	0·672	0·484	0·398	0·744	0·114
SupportVectorMachine	0·527 (0·437-0·616)	0·313	0·778	0·417	0·690	0·276

NeuralNet	0·554 (0·467-0·641)	0·703	0·460	0·398	0·753	0·112
T0 Healthy versus T0 Polyp						
Sparse logistic regression	0·969 (0·907-1)	1	0·967	0·970	1	<0·001
RandomForest	0·982 (0·946-1)	1	0·967	0·970	1	<0·001
GaussianProcess	0·969 (0·907-1)	1	0·967	0·970	1	<0·001
SupportVectorMachine	0·969 (0·907-1)	1	0·967	0·970	1	<0·001
NeuralNet	0·966 (0·904-1)	0·969	0·967	0·969	0·967	<0·001
T1 Healthy versus T1 Polyp						
Sparse logistic regression	0·581 (0·438-0·723)	0·281	0·968	0·818	0·566	0·135
RandomForest	0·548 (0·404-0·691)	0·906	0·25	0·547	0·828	0·256
GaussianProcess	0·547 (0·401-0·693)	0·25	0·969	0·889	0·564	0·259
SupportVectorMachine	0·487 (0·342-0·632)	0·656	0·438	0·538	0·56	0·575
NeuralNet	0·667 (0·530-0·804)	0·469	0·906	0·833	0·630	0·011
T0 Polyp versus T1 Polyp						
Sparse logistic regression	0·944 (0·889-0·998)	0·906	0·875	0·879	0·903	<0·001
RandomForest	0·936 (0·875-0·996)	0·906	0·906	0·906	0·906	<0·001
GaussianProcess	0·932 (0·866-0·998)	0·906	0·875	0·879	0·903	<0·001
SupportVectorMachine	0·898 (0·811-0·985)	0·875	0·906	0·903	0·879	<0·001
NeuralNet	0·951 (0·889-1)	0·906	0·969	0·967	0·912	<0·001
T0 Healthy versus T1 Healthy						
Sparse logistic regression	0·634 (0·495-0·774)	0·531	0·733	0·68	0·595	0·035
RandomForest	0·581 (0·436-0·726)	0·938	0·267	0·577	0·8	0·139
GaussianProcess	0·534 (0·387-0·681)	0·938	0·233	0·566	0·778	0·324
SupportVectorMachine	0·474 (0·326-0·622)	0·531	0·567	0·567	0·531	0·365
NeuralNet	0·627 (0·486-0·769)	0·406	0·867	0·765	0·578	0·043

Supplemental Table 1. Overview of the data generated using all five classifiers based on the 20 most discriminative features. Abbreviations: CRC, colorectal cancer; AA, advanced adenomas; LGD, low grade dysplasia; HC, healthy controls; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.

Supplemental Table 2. Overview of the data generated using all five classifiers based on the 50 most discriminative features

Classifier	AUC (95% CI)	Sensitivity	Specificity	PPV	NPV	p-value
CRC versus HC						
Sparse logistic regression	0·965 (0·901-1)	1	0·875	0·889	1	<0·001
RandomForest	0·989 (0·965-1)	1	0·875	0·889	1	<0·001
GaussianProcess	0·965 (0·906-1)	1	0·875	0·889	1	<0·001
SupportVectorMachine	0·984 (0·955-1)	1	0·875	0·889	1	<0·001
NeuralNet	0·930 (0·823-1)	0·938	0·938	0·938	0·938	<0·001
AA versus HC						
Sparse logistic regression	0·908 (0·849-0·967)	0·969	0·860	0·873	0·965	<0·001
RandomForest	0·959 (0·923-0·994)	0·953	0·938	0·938	0·952	<0·001
GaussianProcess	0·947 (0·9-0·995)	0·953	0·938	0·938	0·952	<0·001
SupportVectorMachine	0·941 (0·889-0·993)	0·953	0·938	0·938	0·952	<0·001
NeuralNet	0·920 (0·861-0·979)	0·953	0·938	0·938	0·952	<0·001
Large non-AA polyps (0·5-1·0 cm) versus HC						
Sparse logistic regression	0·956 (0·915-0·996)	0·985	0·926	0·931	0·984	<0·001
RandomForest	0·970 (0·942-0·999)	0·985	0·912	0·918	0·984	<0·001
GaussianProcess	0·964 (0·929-1)	0·970	0·941	0·943	0·970	<0·001
SupportVectorMachine	0·956 (0·913-0·998)	0·985	0·926	0·931	0·984	<0·001
NeuralNet	0·938 (0·888-0·988)	0·985	0·912	0·918	0·984	<0·001
Small non-AA polyps (0·1-0·5 cm) versus HC						
Sparse logistic regression	0·944 (0·909-0·980)	1	0·897	0·906	1	<0·001
RandomForest	0·970 (0·949-0·991)	1	0·889	0·9	1	<0·001
GaussianProcess	0·956 (0·925-0·987)	0·984	0·913	0·919	0·983	<0·001
SupportVectorMachine	0·951 (0·918-0·984)	0·968	0·929	0·931	0·967	<0·001
NeuralNet	0·939 (0·903-0·975)	0·984	0·913	0·919	0·983	<0·001
CRC versus AA						
Sparse logistic regression	0·608 (0·469-0·748)	0·406	0·875	0·929	0·270	0·916
RandomForest	0·536 (0·378-0·693)	1	0·188	0·831	1	0·319
GaussianProcess	0·477 (0·313-0·640)	0·969	0·125	0·816	0·5	0·389
SupportVectorMachine	0·587 (0·414-0·760)	0·906	0·313	0·841	0·455	0·560
NeuralNet	0·541 (0·378-0·704)	0·406	0·75	0·867	0·24	0·309
CRC versus Large non-AA polyps (0·5-1·0 cm)						
Sparse logistic regression	0·520 (0·364-0·677)	0·438	0·647	0·226	0·830	0·601
RandomForest	0·432 (0·321-0·544)	0·063	0·971	0·333	0·815	0·855
GaussianProcess	0·546 (0·398-0·694)	0·875	0·309	0·230	0·913	0·718
SupportVectorMachine	0·582 (0·419-0·745)	0·563	0·603	0·25	0·854	0·846
NeuralNet	0·521 (0·372-0·670)	0·875	0·265	0·219	0·9	0·604
CRC versus Small non-AA polyps (0·1-0·5 cm)						
Sparse logistic regression	0·670 (0·548-0·792)	0·938	0·484	0·188	0·984	0·987
RandomForest	0·400 (0·328-0·472)	1	0	0·113	NA	0·959
GaussianProcess	0·578 (0·444-0·712)	0·938	0·349	0·155	0·978	0·847
SupportVectorMachine	0·429 (0·282-0·575)	0·625	0·421	0·120	0·898	0·824
NeuralNet	0·476 (0·345-0·607)	0·938	0·206	0·130	0·963	0·622
AA versus Large non-AA polyps (0·5-1·0 cm)						
Sparse logistic regression	0·54 (0·44 - 0·64)	0·85	0·30	0·56	0·66	0·202
RandomForest	0·57 (0·48 - 0·67)	0·76	0·42	0·58	0·63	0·072
GaussianProcess	0·58 (0·49 - 0·68)	0·34	0·80	0·64	0·53	0·050
SupportVectorMachine	0·58 (0·49 - 0·68)	0·60	0·58	0·60	0·58	0·047
NeuralNet	0·55 (0·45 - 0·65)	0·72	0·42	0·57	0·59	0·167
T0 Healthy versus T0 Polyp						
Sparse logistic regression	0·969 (0·907-1)	1	0·967	0·970	1	<0·001
RandomForest	0·981 (0·943-1)	1	0·967	0·970	1	<0·001
GaussianProcess	0·969 (0·907-1)	1	0·967	0·970	1	<0·001
SupportVectorMachine	0·969 (0·907-1)	1	0·967	0·970	1	<0·001

NeuralNet	0·945 (0·869-1)	0·969	0·967	0·969	0·967	<0·001
T1 Healthy versus T1 Polyp						
Sparse logistic regression	0·566 (0·420-0·706)	0·875	0·406	0·596	0·765	0·182
RandomForest	0·562 (0·418-0·706)	0·906	0·281	0·558	0·75	0·200
GaussianProcess	0·533 (0·389-0·678)	0·688	0·469	0·564	0·6	0·326
SupportVectorMachine	0·606 (0·463-0·750)	0·813	0·5	0·619	0·727	0·072
NeuralNet	0·579 (0·435-0·722)	0·75	0·531	0·615	0·68	0·141
T0 Polyp versus T1 Polyp						
Sparse logistic regression	0·961 (0·917-1)	0·938	0·906	0·910	0·935	<0·001
RandomForest	0·967 (0·924-1)	0·969	0·875	0·886	0·966	<0·001
GaussianProcess	0·958 (0·902-1)	0·969	0·906	0·912	0·967	<0·001
SupportVectorMachine	0·926 (0·844-1)	0·938	0·938	0·938	0·938	<0·001
NeuralNet	0·964 (0·905-1)	0·938	0·969	0·968	0·940	<0·001
T0 Healthy versus T1 Healthy						
Sparse logistic regression	0·756 (0·635-0·878)	0·469	0·967	0·938	0·630	0·000
RandomForest	0·680 (0·546-0·815)	0·563	0·733	0·692	0·611	0·008
GaussianProcess	0·599 (0·456-0·742)	0·688	0·567	0·629	0·630	0·092
SupportVectorMachine	0·621 (0·480-0·762)	0·406	0·867	0·765	0·578	0·052
NeuralNet	0·725 (0·591-0·860)	0·719	0·833	0·821	0·735	0·001

Supplemental Table 3. Overview of the data generated using all five classifiers based on the 50 most discriminative features. Abbreviations: CRC, colorectal cancer; AA, advanced adenomas; LGD, low grade dysplasia; HC, healthy controls; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.

Supplemental Table 3. Overview of the data generated using all five classifiers based on the 100 most discriminative features

Classifier	AUC (95% CI)	Sensitivity	Specificity	PPV	NPV	p-value
CRC versus HC						
Sparse logistic regression	0·973 (927-1)	1	0·875	0·889	1	<0·001
RandomForest	0·988 (0·965-1)	1	0·875	0·889	1	<0·001
GaussianProcess	0·965 (0·910-1)	1	0·875	0·889	1	<0·001
SupportVectorMachine	0·992 (0·974-1)	1	0·938	0·941	1	<0·001
NeuralNet	1 (1-1)	1	1	1	1	<0·001
AA versus HC						
Sparse logistic regression	0·929 (0·875-0·984)	0·984	0·875	0·887	0·982	<0·001
RandomForest	0·956 (0·917-0·994)	0·953	0·938	0·938	0·952	<0·001
GaussianProcess	0·941 (0·889-0·993)	0·953	0·938	0·938	0·952	<0·001
SupportVectorMachine	0·939 (0·887-0·992)	0·953	0·938	0·938	0·952	<0·001
NeuralNet	0·915 (0·853-0·977)	0·953	0·938	0·938	0·952	<0·001
Large non-AA polyps (0·5-1·0 cm) versus HC						
Sparse logistic regression	0·925 (0·870-0·980)	0·985	0·912	0·918	0·984	<0·001
RandomForest	0·961 (0·926-0·995)	0·985	0·912	0·918	0·984	<0·001
GaussianProcess	0·958 (0·916-1)	0·985	0·941	0·944	0·985	<0·001
SupportVectorMachine	0·939 (0·887-0·992)	0·985	0·941	0·944	0·985	<0·001
NeuralNet	0·950 (0·904-0·995)	0·985	0·926	0·931	0·984	<0·001
Small non-AA polyps (0·1-0·5 cm) versus HC						
Sparse logistic regression	0·954 (0·921-0·987)	0·984	0·913	0·919	0·983	<0·001
RandomForest	0·973 (0·953-0·994)	0·952	0·952	0·952	0·952	<0·001
GaussianProcess	0·964 (0·935-0·992)	0·913	0·920	1		<0·001
SupportVectorMachine	0·961 (0·931-0·991)	0·976	0·944	0·946	0·975	<0·001
NeuralNet	0·944 (0·908-0·979)	0·984	0·929	0·932	0·983	<0·001
CRC versus AA						
Sparse logistic regression	0·583 (0·417-0·750)	0·484	0·688	0·861	0·25	0·153
RandomForest	0·577 (0·420-0·734)	0·578	0·625	0·860	0·270	0·167
GaussianProcess	0·480 (0·321-0·640)	0·875	0·188	0·812	0·272	0·407
SupportVectorMachine	0·497 (0·323-0·671)	0·875	0·25	0·824	0·333	0·517
NeuralNet	0·614 (0·453-0·775)	0·625	0·688	0·888	0·314	0·081
CRC versus Large non-AA polyps (0·5-1·0 cm)						
Sparse logistic regression	0·495 (0·333-0·656)	0·438	0·647	0·226	0·830	0·530
RandomForest	0·447 (0·329-0·564)	0·063	0·985	0·5	0·817	0·792
GaussianProcess	0·545 (0·393-0·697)	0·938	0·221	0·221	0·938	0·714
SupportVectorMachine	0·576 (0·410-0·742)	0·313	0·824	0·294	0·836	0·830
NeuralNet	0·460 (0·303-0·617)	0·5	0·574	0·216	0·830	0·692
CRC versus Small non-AA polyps (0·1-0·5 cm)						
Sparse logistic regression	0·667 (0·527-0·808)	0·688	0·667	0·208	0·944	0·986
RandomForest	0·435 (0·324-0·545)	0·188	0·825	0·12	0·889	0·852
GaussianProcess	0·460 (0·318-0·601)	0·875	0·198	0·122	0·926	0·704
SupportVectorMachine	0·560 (0·386-0·834)	0·5	0·683	0·167	0·915	0·783
NeuralNet	0·497 (0·340-0·655)	0·563	0·587	0·148	0·914	0·515
AA versus Large non-AA polyps (0·5-1·0 cm)						
Sparse logistic regression	0·457 (0·309-0·605)	0·813	0·235	0·2	0·842	0·704
RandomForest	0·413 (0·313-0·513)	0·063	0·956	0·25	0·813	0·920
GaussianProcess	0·588 (0·441-0·736)	0·938	0·279	0·234	0·95	0·865
SupportVectorMachine	0·666 (0·533-0·800)	0·813	0·544	0·295	0·925	0·981
NeuralNet	0·585 (0·435-0·736)	0·938	0·265	0·231	0·947	0·857
AA versus Small non-AA polyps (0·5-1·0 cm)						
Sparse logistic regression	0·613 (0·531-0·694)	0·800	0·452	0·425	0·814	0·006
RandomForest	0·566 (0·472-0·659)	0·328	0·841	0·512	0·711	0·070
GaussianProcess	0·606 (0·522-0·689)	0·719	0·492	0·418	0·775	0·009
SupportVectorMachine	0·566 (0·479-0·653)	0·438	0·698	0·424	0·710	0·068

NeuralNet	0·541 (0·455-0·626)	0·578	0·595	0·420	0·735	0·181
T0 Healthy versus T0 Polyp						
Sparse logistic regression	0·969 (0·907-1)	1	0·967	0·970	1	<0·001
RandomForest	0·979 (0·938-1)	1	0·967	0·970	1	<0·001
GaussianProcess	0·969 (0·907-1)	1	0·967	0·970	1	<0·001
SupportVectorMachine	0·969 (0·907-1)	1	0·967	0·970	1	<0·001
NeuralNet	0·949 (0·877-1)	0·969	0·967	0·969	0·967	<0·001
T1 Healthy versus T1 Polyp						
Sparse logistic regression	0·560 (0·415-0·704)	0·875	0·313	0·56	0·714	0·208
RandomForest	0·547 (0·403-0·692)	0·906	0·25	0·547	0·727	0·260
GaussianProcess	0·559 (0·415-0·703)	0·719	0·469	0·575	0·625	0·210
SupportVectorMachine	0·588 (0·444-0·733)	0·813	0·469	0·605	0·714	0·113
NeuralNet	0·551 (0·406-0·697)	0·563	0·626	0·621	0·6	0·243
T0 Polyp versus T1 Polyp						
Sparse logistic regression	0·983 (0·959-1)	1	0·906	0·914	1	<0·001
RandomForest	0·982 (0·959-1)	1	0·844	0·865	1	<0·001
GaussianProcess	0·979 (0·951-1)	1	0·875	0·889	1	<0·001
SupportVectorMachine	0·937 (0·854-1)	0·969	0·906	0·812	0·967	<0·001
NeuralNet	0·989 (0·969-1)	0·969	0·969	0·969	0·969	<0·001
T0 Healthy versus T1 Healthy						
Sparse logistic regression	0·804 (0·690-0·918)	0·719	0·9	0·885	0·75	<0·001
RandomForest	0·851 (0·627-0·874)	0·594	0·867	0·826	0·667	<0·001
GaussianProcess	0·707 (0·573-0·838)	0·531	0·833	0·773	0·625	0·002
SupportVectorMachine	0·668 (0·532-0·803)	0·594	0·733	0·704	0·629	0·002
NeuralNet	0·773 (0·651-0·894)	0·594	0·933	0·905	0·683	<0·001

Supplemental Table 3. Overview of the data generated using all five classifiers based on the 100 most discriminative features. Abbreviations: CRC, colorectal cancer; AA, advanced adenomas; LGD, low grade dysplasia; HC, healthy controls; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.