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1 **The fecal scent of inflammatory bowel disease: detection and monitoring of**
2 **IBD based on volatile organic compound analysis**

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2 S Bosch has nothing to declare. D Wintjens has nothing to declare. A Wicaksono has nothing to declare.

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21 de Boer, de Meij and Bosch designed the study protocol and experiment. Wintjens, Bosch, van der Hulst,
22 Kuijvenhoven and Stokkers collected the samples. Wintjens and Bosch prepared the samples for analyses.
23 Daulton and Bosch run the sample measurements. Daulton and Covington developed the sample test method.
24 Wicaksono and Covington analyzed the data. Bosch drafted a first version of the manuscript. Wintjens,
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30

1 **Abstract** (words: 380)

2 **Background** The gold standard to diagnose and monitor inflammatory bowel disease (IBD) remains
3 endoscopic assessment, which is invasive and costly. Fecal calprotectin (FCP) is the most commonly
4 used non-invasive biomarker to assess IBD but has limited specificity. The aim of the current study
5 was to evaluate the potential of fecal volatile organic compounds (VOC) analysis to diagnose IBD and
6 to identify disease exacerbation.

7 **Methods** IBD patients who visited the outpatient clinic of one of the two tertiary hospitals were
8 eligible to participate, independently of disease activity. All patients collected fecal samples prior to
9 their scheduled consult. Healthy controls (HC) were patients without mucosal abnormalities
10 observed during colonoscopy. Active disease was defined as FCP ≥ 250 mg/g, remission as FCP < 100
11 mg/g combined with Harvey Bradshaw Index < 4 points for Crohn's disease (CD) or Simple Clinical
12 Colitis Activity Index < 3 points for ulcerative colitis (UC). VOCs were measured from the headspace of
13 fecal samples using gas chromatography-ion mobility spectrometry. Data were split into two sets,
14 70% for training and validation and 30% as a test set. A Wilcoxon rank-sum test was used to find e
15 th20, 50 and 100 most discriminatory features. Random Forest, Support Vector Machine, Gaussian
16 Process and Neural Net classification were used to provide statistical results.

17 **Results** A total of 280 IBD patients and 227 HC provided 292 CD, 197 UC and 227 HC fecal samples. Of
18 IBD samples, 107 and 84 were CD active (CDa) and remission (CDr) samples, and 80 and 63 were UC
19 active (UCa) and remission (UCr) samples, respectively. Based on VOC profiles, UC and CD could be
20 discriminated from HC with high accuracy (AUC (95%CI), p-values: UCa vs HC 0.96(0.94-.99),
21 $p < 0.0001$; UCr vs HC 0.95(0.93-0.98), $p < 0.0001$; CDa vs HC 0.96(0.94-0.99), $p < 0.0001$; CDr vs HC
22 0.95(0.93-0.98), $p < 0.0001$). A small difference was observed between fecal VOC profiles of UC and
23 CD (0.55(0.50-0.6), $p = 0.03$). There was no significant difference between active disease state and
24 remission (UCa vs UCr 0.63(0.44-0.82), $p = 0.082$; CDa vs CDr 0.52(0.39-0.65), $p = 0.64$).

25 **Conclusion** Based on fecal VOC analysis, IBD would be discriminated from HC both during active
26 disease state and remission, though there was no difference between active disease and remission.
27 These characteristics imply that fecal VOC analysis seems to hold potential as non-invasive
28 biomarkers for IBD disease detection, but not to monitor disease activity in patients with established
29 diagnosis of IBD.

30

1 **Introduction**

2 Crohn's disease (CD) and ulcerative colitis (UC) are chronic gastrointestinal diseases characterized by
3 periods of relapse and remission, and are together referred to as inflammatory bowel disease (IBD).
4 Over the past few decades, a worldwide increase in incidence has been observed with mean annual
5 rates ranging from 14.0 per 100.000 persons in Western Europe to 21.3 per 100.000 persons in
6 Australia in 2011[1, 2]. The gold standard to diagnose and monitor mucosal inflammation in IBD
7 patients is ileocolonoscopy substantiated by histological assessment of biopsy specimens and/or
8 radiology. This diagnostic workup is invasive, expensive and carries risks of complications. The use of
9 non-invasive biomarkers for IBD evaluation is therefore preferred. Fecal calprotectin (FCP) is the
10 most commonly used non-invasive biomarker to assess and monitor IBD. This test is characterized by
11 a high sensitivity for mucosal inflammation (0.98, 95%CI 0.95-0.99), but limited specificity for IBD
12 (0.81-0.91 for adults, and 0.68 for children) [3]. For example, FCP elevations have been observed in
13 patients with infectious diarrhea, celiac disease, rheumatoid arthritis and gastrointestinal
14 malignancies[4-7]. Therefore, the search for a more specific non-invasive IBD biomarker remains
15 warranted.

16 Volatile organic compounds (VOCs) are gaseous carbon-bound chemicals that include hydrocarbons,
17 alcohols, aldehydes, ketones, esters and organic acids. Fecal VOCs are thought to represent both
18 metabolic processes in the human body and the interaction between gut microbiota and host[8].
19 These molecular end-products can be found in all bodily excrements dependent on their volatility
20 and sample temperature. Great potential of VOC profiles as noninvasive biomarkers have been
21 described for various gastrointestinal diseases[9-11]. The detection of pediatric IBD using fecal VOC
22 patterns has also been subject of various studies, with promising results, but the literature on its
23 potential in adults is limited[12-14]. The aim of the current study is to validate the potential of fecal
24 VOC patterns to detect IBD and to assess their potential to identify disease exacerbation in adults.
25

1 **Methods**

2 Study design

3 This study was performed at the outpatient clinics of the gastroenterology and hepatology
4 department in two tertiary referral hospitals (Amsterdam UMC, location VUmc, Amsterdam and
5 Maastricht University Medical Center (MUMC+) in Maastricht), and two district hospitals (OLVG West
6 in Amsterdam and Spaarne Gasthuis (SG), location Hoofddorp and Haarlem) all located in The
7 Netherlands. This study was approved by the Medical Ethical Review Committee (METc) of the
8 Amsterdam UMC, location VUmc under file number 2016.135, by the METc of the MUMC+ under
9 filenumber NL24572.018.08, and by the local medical ethical committee of the OLVG West and
10 Spaarne Gasthuis. Written informed consent was obtained from all study participants. Once sample
11 collection was complete, all samples were shipped to the School of Engineering, University of
12 Warwick (Coventry, UK) for VOC analysis.

13 Study participants

14 *Inflammatory bowel disease patients*

15 All patients aged 18 years or older with an established diagnosis of IBD based on clinical, endoscopic,
16 histological and/or radiological criteria and with a scheduled consult at the outpatient clinic of one of
17 the two tertiary referral hospitals, and independently on disease activity, were asked to participate in
18 this study[15]. Patients were asked to collect a fecal sample and to complete a questionnaire on the
19 same day, which included information on age, gender, BMI, smoking status, abdominal symptoms,
20 medication use, dietary habits, comorbidity and questions on clinical disease activity based on the
21 Harvey Bradshaw Index (HBI) for CD patients and the Simple Clinical Colitis Activity Index (SCCAI) for
22 UC patients [16, 17]. Active disease was defined as an FCP level of ≥ 250 mg/g. Remission was defined
23 as FCP < 100 mg/g combined with a HBI < 4 points or SCCAI < 3 points. All IBD patients were included in
24 the primary statistical analysis assessing the diagnostic potential of fecal VOCs to differentiate
25 between IBD and HC. Only IBD patients with clearly defined disease activity based on FCP and
26 HBI/SCCAI levels were included in the secondary analyses aiming to assess the difference in fecal VOC
27 pattern between active disease and remission. Demographic and clinical data (including Montreal
28 classification and history of bowel surgery) were obtained from electronic patient files[18].

29 *Healthy controls*

30 All patients aged 18 years and older with a scheduled colonoscopy at the Amsterdam UMC, OLVG
31 West and Spaarne Gasthuis were asked to participate in this study regardless of their endoscopy
32 indication. They were also asked to complete a questionnaire on age, gender, BMI, smoking status,
33 abdominal symptoms, bowel movements, dietary intake, comorbidity and medication use. Patients

1 without endoscopic abnormalities observed during endoscopy were included in this study as healthy
2 controls (except asymptomatic external haemorrhoids, asymptomatic diverticula and/or small anal
3 fibromas). In case of mucosal biopsies to exclude microscopic alterations, histologic reports were
4 checked and subjects were only included as HC if no histologic abnormalities were detected by the
5 pathologist. Exclusion criteria to be included as HC were mucosal abnormalities observed during
6 endoscopy, a history of bowel diseases (e.g. celiac disease, IBD, CRC), failure to perform a complete
7 colonoscopy because of various reasons (e.g. inadequate bowel cleansing, pain) and/or the collection
8 of insufficient fecal sample mass prior to endoscopy to perform VOC analysis.

9 Sample collection

10 *Inflammatory bowel disease*

11 *Amsterdam University Medical Centers*

12 Between February 2015 and November 2017, IBD patients were asked to collect two fecal samples
13 (Stuhlgefäß 10ml, Frickenhausen, Germany) from the same bowel movement prior to the consult:
14 one for FCP levels and one for VOC analysis. The sample for FCP measurement was sent to the
15 hospital by mail directly after collection. The sample for VOC analysis was stored in their own freezer
16 within one hour following collection and transported to the hospital in cooled condition using ice
17 packs and/or ice cubes on the day of their consultation. The samples were stored at -24°C directly
18 upon arrival at the hospital until further handling.

19 *Maastricht University Medical Center*

20 Between October 2009 and December 2010 patients were asked to collect stool from one bowel
21 movement on the day of their consult and bring it fresh to the hospital. This stool sample was stored
22 in the fridge (4 °C) directly upon arrival at the hospital. From this bowel movement, two samples
23 were prepared on the day of arrival. One for fecal calprotectin measurements (using ELISA) and one
24 for research purposes. The second sample was stored in the freezer at -80°C on the day of delivery
25 until further handling.

26 *Healthy controls*

27 Between February 2015 and November 2017, patients from the Amsterdam UMC, OLVG West and
28 SG collected a fecal sample in a container (Stuhlgefäß 10ml, Frickenhausen, Germany) prior to bowel
29 cleansing and endoscopic assessment. They were asked to store their sample in their own freezer
30 within one hour after collection. These samples were transported to the hospital in cooled condition
31 using ice packs and/or ice cubes on the day of their endoscopy. The samples were stored at -24°C
32 directly upon arrival at the hospital.

1 Sample preparation and analyses of volatile organic compounds

2 The samples were then shipped to the University of Warwick on dri-ice for preparation. From the
3 original samples, one subsample of 0.5 g per participant was weighted on a calibrated scale (Mettler
4 Toledo, AT 261 Delta Range, Ohio, United States), transferred into a glass vial (20ml headspace vial,
5 Thames Restek, Saunderton, UK), labelled and re-stored in a -24°C freezer until further handling. The
6 amount of sample was chosen to provide an optimum ratio of VOCs to the sample headspace, as
7 validated by our research team in a previous sampling method study for VOC pattern-recognition
8 using field asymmetric ion mobility spectrometry (FAIMS)[19].

9 Fecal volatile organic compound analysis

10 Fecal samples were analysed using gas chromatography coupled to an ion mobility spectrometer
11 (GC-IMS, FlavourSpec®, G.A.S., Dortmund, Germany). The instrument is formed on a GC column,
12 coupled with a drift tube IMS. The GC provides pre-separation of the complex mixture of chemicals
13 found in the headspace, before detection by IMS. Within the IMS, volatile organic compounds are
14 ionized, in this case, by means of soft chemical-ionization initiated by a low-radiation tritium (H3)
15 source, creating reactant ions with the gas atmosphere. The ionized VOCs travel at atmospheric
16 pressure against the flow of an inert drift gas. In general, larger molecule are struck more times than
17 smaller molecules, losing momentum and thus, taking longer to travel along the tube. The drift time
18 of each substance is therefore determined by the ions mass and geometrical structure. The resulting
19 ion current is measured by an electrometer as a function of time[20]. During this study, GC-IMS was
20 connected to an automatic sampling system with chiller allowing processing of a batch of 32 samples
21 kept in cooled condition (4 °C) until start of the analyses. The samples were heated to 80 °C during
22 the 8 minutes prior to analyses. Then, a syringe transports the headspace from the vial into the
23 injector port of the instrument and into the GC column. The experiments were performed at 40 °C
24 using nitrogen 99.9% (3.5 bar) as carrier gas and the IMS was performed at 45 °C using nitrogen as
25 drift gas. Flow rates were set at 150ml/min (0.364 kPa) (IMS), and at 20 ml/minute (34.175 kPa) for 6
26 minutes (GC). A schematic overview of the setup is depicted in Figure 1.

27 Statistical analysis

28 Prior to the statistical analyses, the data was pre-processed to only crop areas that contain chemical
29 information, then a threshold is applied to remove background noise and finally corrected for
30 instrumental disturbances by baseline correction. This reduces the data points per sample from
31 around 11 million to a more manageable 100,000. Data were split into three sets, 70% for training
32 and validation and 30% as test set. Wilcoxon rank-sum test was used to find the 20, 50 and 100 most
33 discriminatory features and Sparse Logistic Regression, Random Forest, Gaussian Process, Support

1 Vector Machine and Neural Net classification were used to provide statistical results from the 30%
2 test set based on the 70% training set.

3 **Results**

4 Baseline characteristics

5 A total of 280 IBD patients (164 CD patients, 112 UC patients, 4 IBD-undetermined) were included in
6 this study. Sample collection of IBD patients is depicted in *Figure 2*. In total, 495 fecal IBD samples
7 (292 CD, 197 UC, 6 IBD-U) were collected during the follow-up period of this study. Of these, 107
8 were active CD, 84 were CD in remission, 80 were active UC and 63 were UC in remission according
9 to the previously mentioned criteria. The number of samples collected per individual varied as 159
10 patients provided one sample, 65 patients were sampled twice, 34 patients collected three samples,
11 10 patients collected four samples, 10 patients collected five samples, and two participants provided
12 six and eight samples. Samples of these IBD patients were compared to 227 HCs who all collected a
13 single sample. Baseline demographics of all study participants are given in *Table 1*. There was no
14 significant difference in gender between CD and UC patients compared to HC. Mean age of the IBD
15 group was 46.1 (± 29.8) compared to 60.6 (± 11.8) for HC. Mean FCP levels for active disease and
16 remission were 664.6 mg/g and 29.9 mg/g for CD and 1108.5 mg/g and 39.5 mg/g for UC,
17 respectively (*Table 2*).

18 Fecal volatile organic compound analysis

19 The results of the VOC analysis by means of GC-IMS are shown in *Table 3*. For every comparison, the
20 results from the Sparse Logistic Regression classification based on the 100 most discriminative
21 features are presented. A complete overview of the data generated using all five classifiers based on
22 the 20, 50 and 100 most discriminative features are given in Supplemental Table 1-3.

23 Inflammatory bowel disease versus healthy controls

24 IBD patients could be discriminated from HC with a high diagnostic accuracy (AUC \pm 95%CI,
25 sensitivity, specificity, PPV, NPV, P-values; 0.96 (0.92 – 0.99), 0.97, 0.92, 0.98, 0.87, <0.0001)(*Table 3*,
26 *Supplementary table 1-3*). Likewise, high diagnostic accuracy was found for the detection of CD
27 during active state and remission (0.96 (0.94 – 0.99), 1, 0.92, 0.74, 1, <0.0001 for active CD; 0.95
28 (0.93 – 0.98), 1, 0.90, 0.67, 1, <0.0001 for CD in remission) (*Table 3*, *Supplementary tables 1-3*). This
29 was similar for the detection of UC both during active state and remission (0.96 (0.94 – 0.99), 1, 0.92,
30 0.74, 1, <0.0001 for UC_a; 0.95 (0.93 – 0.98), 1, 0.88, 0.52, 1, <0.0001 for UC_r) (*Table 3*, *Supplementary*
31 *tables 1-3*). Corresponding Receiver Operating Characteristic (ROC) curves are visualized in *Figure 3a-*
32 *d*.

1 *Crohn's disease versus ulcerative colitis*

2 Fecal VOC patterns of CD and UC differed statistically significant, though the diagnostic accuracy was
3 very low (0.55 (0.50-0.60), 0.17, 0.96, 0.90, 0.36, 0.03) (Table 3, Figure 3h). Furthermore, there was
4 no difference between UC and CD when comparing active disease and remission subgroups
5 separately (Table 3).

6 *Active disease versus remission*

7 There was a slight significant difference in fecal VOC patterns between active IBD (UC and CD
8 combined) and remission (0.59 (0.51-0.67), 0.21, 0.96, 0.90, 0.39, 0.019) (Figure 5). However,
9 comparing active and remission state of CD and UC subgroups separately, this significance was not
10 found (CD active vs CD in remission 0.52 (0.39-0.65), 0.72, 0.43, 0.71, 0.45, 0.645; UC active vs UC in
11 remission 0.63(0.44-0.82), 0.67, 0.57, 0.79, 0.42, 0.08) (Table 3, Figure 3i).

1 **Discussion**

2 We demonstrated high diagnostic accuracies for the detection of adults with IBD, UC and CD based
3 on fecal VOC profiles using GC-IMS, both during active disease state and remission. Furthermore,
4 VOC profiles of the phenotypes CD and UC, and of IBD in active disease state and remission, differed
5 statistically, but not clinically significant.

6 The presented results are comparable to the reported sensitivity of the currently used non-invasive
7 biomarker FCP (0.98, 95%CI 0.95-0.99) to discriminate between IBD and HC[3]. Additionally, in the
8 present study, the specificity of fecal VOC patterns for IBD both during active disease and remission
9 was higher compared to reported values for AUC (0.81-0.91) for FCP in adults. It is known that FCP is
10 a biomarker for mucosal inflammation and is therefore sensitive to detect active IBD, whereas fecal
11 VOCs seem highly sensitive and specific for both active disease state and remission. This underlines
12 its diagnostic potential in clinical practice.

13 The potential of fecal VOC profiles to discriminate CD from HC is in concordance with the existing
14 literature. To the best of our knowledge, in only one study this potential has previously been
15 assessed in an adult population, including fecal samples of 117 CD, 100 UC and 109 HC analyzed by
16 gas chromatography – mass spectrometry (GC-MS). Although AUC values were not provided in that
17 article, active CD and HC could be separated excellently based on three unique metabolites;
18 Differences between active CD and HC were (amongst several others) mainly based on increased
19 levels of alcohols, ketones and aldehydes. Though, in contrast to our findings, study results of Ahmed
20 et. al did not allow to discriminate between UC patients compared to HC. In addition, they did find
21 separation of UC and CD profiles both during active disease and remission. Other studies have been
22 performed to assess the diagnostic potential of fecal VOCs for IBD using pattern-based techniques in
23 pediatric cohorts, such as an electronic nose (eNose) instruments (CD 29, UC 26, HC 28) and field
24 asymmetric ion mobility spectrometry (FAIMS) (23 CD, 13 UC, 24 HC)([12, 13]. The study using eNose
25 technology demonstrated similar accuracies to the current study for the detection of IBD and was
26 also able to distinguish UC from CD, whereas the study using FAIMS was only able to separate CD
27 from HC with high accuracy. Comparing UC to HC and CD, only moderate separation was found (AUC
28 of 0.74 and 0.67, respectively). In the current study, active IBD was discriminated from remission
29 with a very weak accuracy and there was no difference between the active and inactive subgroups of
30 CD as well as UC. The existing literature on the differentiation between active and inactive IBD based
31 on VOC profiles is both scarce and contradictory. Only one study so far has assessed IBD activity
32 based on fecal VOC profiles [21]. In that study, active and inactive CD could be separated significantly
33 based on fecal VOC profiles, though with some overlap, whereas active and inactive UC did not differ.

1 The separation of CD patients was mainly based on aldehyde levels (e.g. heptanal, propanal,
2 benzeneacetaldehyde). This accuracy was similar to a study on VOC profiles comparing 135 breath
3 samples of CD in remission with 140 breath samples active CD using GC-MS, where an AUC of 0.98
4 was measured [22]. Though, this accuracy was based on different metabolite alterations (i.e.
5 elevation of 2,2,4-trimethylpentane, 1-butoxy-2-propanol, heptadecane and decrease of isoprene
6 and acetone). In addition, a high diagnostic accuracy to differentiate between 62 active and 70
7 inactive UC breath VOC profiles was found by the same research group (AUC 0.94), based on
8 increased levels of 2,4-dimethylpentane and methylcyclopentane and decreased levels of octane,
9 acetic acid and m-cymene[23]. Urinary VOC profiles have also been found to discriminate between
10 active IBD (24 CD, 24 UC) and remission (4 CD, 4 UC) using the pattern-recognition technique FAIMS,
11 with moderate accuracies (AUC CD 0.66, UC 0.74)[24].

12 Alterations in the fecal VOC patterns may be explained by alterations in metabolic processes, like the
13 secretion of inflammatory end products in the colon or alterations in dietary intake, by microbial
14 dysbiosis or a combination of all the above. In a recent study on canine olfaction, in vitro breast
15 cancer and colon cancer were grown and it was observed that dogs were able to differentiate
16 between the metabolic waste retrieved from these cancer cells and from benign cells, but not
17 between the cell waste of breast and colon cancer, implying that both cancers share a common smell
18 sprint[25]. The same might apply for inflammatory diseases like Crohn's disease and ulcerative colitis
19 of which it may be hypothesized that the VOC patterns of CD and UC patients are based on a shared
20 (metabolic) reaction, explaining the similarities in VOC patterns observed in the current study. This
21 might also mean that fecal VOC patterns may not be sufficiently specific to differentiate between IBD
22 and other causes of mucosal inflammation (such as infectious colitis).

23 The discrimination between IBD and HC as well as inability to discriminate between active and
24 quiescent disease in the current study may partly be explained by one of the main sources of fecal
25 VOCs: the gut microbiota. The fecal microbiota of a healthy individual consists of over 400 different
26 species which play an important role in the defense against invading organisms. There is a large inter-
27 individual diversity in the fecal microbiota composition of healthy individuals [26]. Nonetheless, the
28 microbiome of an individual is remarkably stable, suggesting the presence of a core microbial
29 community, which is dependent of host factors [27, 28]. In multiple studies, this microbial stability
30 has been found greater in healthy individuals compared to IBD patients of which the microbial
31 composition is defined by more deviations over time and a decrease in diversity, specifically in
32 abundance of *Firmicutes* in CD patients and a decrease in butyrate-producing bacteria in UC patients
33 (*F. Prausnitzii*, *R. Hominis*) [29-34]. This may well explain the high diagnostic accuracy to discriminate
34 between IBD patients and HC based on fecal VOC profiles in the current study. Remarkably, the

1 variability of microbial composition in IBD patients does not well correlate with disease activity.
2 Inflammation has not been found directly associated with significant deviations from the healthy
3 microbial core and fluctuations in microbiota composition have been observed during clinical
4 remission as well, which has hampered the identification of microbial changes related to the
5 presence of flare-ups [29, 30, 35]. These findings may explain the inability to discriminate between
6 active disease state and remission based on fecal VOCs as observed in the current study. Another
7 explanation for the inability to discriminate between active IBD disease state and remission is the
8 medication use in both groups that may have masked differences in VOC profiles.

9 The dissimilarities with other studies considering differentiation between active and remission
10 disease state may also be due to the use of different techniques to analyze VOC profiles. In the
11 current study, we made use of pattern-recognition, since this is a fast and relatively cheap manner to
12 analyze fecal VOCs and is therefore highly adequate for clinical implementation. A downside of this
13 technique is the inability to identify specific metabolites. Differences between IBD and HC based on
14 fecal VOCs have previously been demonstrated due to an altered composition of esters, short chain
15 fatty acids (SCFAs) and cyclohexanecarboxylic acid, of which the first group is believed to be
16 associated with bacterial dysbiosis[36]. The differences between active disease state and remission in
17 fecal as well as breath VOC profiles originate from a different group of metabolites, mainly aldehydes
18 and ketones [22, 23, 37]. These metabolites play a role in inflammatory processes as they are the
19 metabolic products of tissue damage and oxidative stress, and may therefore be the result of a more
20 general host-response to inflammation rather than an IBD specific metabolic alteration. It is possible
21 that the GC-IMS column we used has been sensitive to the range of metabolites differentiating IBD
22 from HC, but not to the metabolites produced in inflammatory processes.

23

24 Strength of this study was the large sample size, which allowed for the creation of a training set for
25 the machine learning classifiers, an internal and an external validation set. In addition, this was a
26 prospective multicenter cohort which made use of endoscopy controlled healthy individuals. The
27 large scale of the cohort has contributed to the generalizability and the endoscopy controlled HC
28 group excluded bias by other colonic abnormalities. A limitation of this study was the use of clinical
29 activity indices and FCP for defining disease activity in the current study, instead of using the gold
30 standard: endoscopic assessment. Because of its invasiveness, it was not ethically feasible to ask of
31 study participants to undergo this investigation without immediate clinical indication. Although we
32 used the second best marker next to clinical activity scores, FCP has a low specificity, which might
33 have led to the inclusion of IBD patients with non-IBD mucosal inflammation in the active disease
34 state group. Furthermore, sample age might have been of influence on study outcomes, especially on
35 the IBD samples collected in the period of 2009-2010[19]. However, our results from a post hoc

1 analyses comparing IBD samples to HC samples collected in a similar time period did not influence
2 diagnostic accuracy found in this study and we therefore believe that this has not influenced our
3 results significantly. Last, although we have designed this study using pattern-recognition because of
4 its suitability for clinical implementation, the inability to detect specific metabolites complicated the
5 comparison to other literature.

6
7 Future research should focus on the validation of specific metabolites within the fecal VOC pattern
8 allowing to differentiate between IBD and HC, preferably including subgroups of patients with non-
9 IBD induced mucosal inflammation for a reliable assessment of its specificity. Using these specific
10 metabolites, an easy to use, disease-specific sensor may be built into a so called 'smart toilet',
11 enabling fast and accurate IBD detection. Additionally, to assess the potential of fecal VOCs for IBD
12 monitoring, the potential differences in VOC patterns between active IBD and remission should be
13 further studied in an endoscopy-controlled cohort with standardized follow-up moments, ensuring
14 the sole inclusion of patients with active disease and remission, based on mucosal appearance and
15 histology findings. Third, it would be interesting to compare the fecal metabolite composition and
16 microbiota in IBD simultaneously in a multi-omics approach, exploring the origin of the fecal VOC
17 pattern alterations.

18
19 In conclusion, our results suggest that fecal VOC pattern analysis is a promising technique for non-
20 invasive diagnosis of IBD. Because of its high specificity, this new technique may be beneficial to both
21 patients and health care costs by lowering the number of (unnecessary) invasive endoscopies
22 currently needed to diagnose IBD in patients with a high FCP value. Since fecal VOC patterns did not
23 allow for differentiation between disease activity state, its potential for monitoring intra-individual
24 course of IBD may be hampered and should be assessed in a future study enrolling an endoscopy
25 controlled cohort.

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