Differentiation Between Pediatric Irritable Bowel Syndrome and Inflammatory Bowel Disease Based on Fecal Scent: Proof of Principle Study

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Background: The diagnostic work-up of pediatric irritable bowel syndrome (IBS) and functional abdominal pain–not otherwise specified (FAP-NOS) commonly includes invasive tests for discrimination from inflammatory bowel disease (IBD). As this carries a high burden on patients, an ongoing need exists for development of noninvasive diagnostic biomarkers for IBS and FAP-NOS. Several studies have shown microbiota alterations in IBS/FAP, which are considered to be reflected by fecal volatile organic compounds (VOCs). The object of the study was to evaluate whether pediatric IBS/FAP-NOS could be discriminated from IBD and healthy controls by fecal VOC analysis.

Methods: IBS/FAP-NOS was diagnosed according to the ROME IV criteria, and *de novo* IBD patients and healthy controls (HCs) aged 4 to 17 years were matched on age and sex. Fecal VOCs were analyzed by means of field asymmetric ion mobility spectrometry.

Results: Fecal VOCs of 15 IBS/FAP-NOS, 30 IBD (15 ulcerative colitis, 15 Crohn's disease) patients and 30 HCs were analyzed and compared. Differentiation between IBS/FAP-NOS and IBD was feasible with high accuracy (area under the curve [AUC], 0.94; 95% confidence interval [CI], 0.88–1; $P < 0.00001$). IBS/FAP-NOS profiles could not be differentiated from HCs (AUC, 0.59; 95% CI, 0.41–0.77; $P = 0.167$), whereas IBD profiles could with high accuracy (AUC, 0.96; 95% CI, 0.93–1; $P < 0.00001$).

Conclusion: Pediatric IBS/FAP-NOS could be differentiated from IBD by fecal VOC analysis with high accuracy, but not from healthy controls. The latter finding limits the potential of fecal VOCs to serve as a diagnostic biomarker for IBS/FAP-NOS. However, VOC could possibly serve as additional noninvasive biomarker to differentiate IBS/FAP-NOS from IBD.

Key Words: inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), biomarkers, metabolome, volatile organic compounds (VOCs)

INTRODUCTION

Irritable bowel syndrome (IBS) and functional abdominal pain–not otherwise specified (FAP-NOS) are functional gastrointestinal disorders in children, with a worldwide prevalence of about 13%, often lasting for more than 5 years after the diagnosis has been established.[1] As biochemical diagnostic biomarkers are yet not available, the diagnosis relies on the symptom-based ROME IV criteria.[2] To fulfill 1 of the different ROME IV criteria, the symptoms must not be explained by another medical condition after appropriate evaluation. Differentiation between IBS and somatic disorders like inflammatory bowel disease (IBD) can be difficult. To exclude

Received for publications December 19, 2017; Editorial Decision March 8, 2018.

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Conflicts of interest: S. Bosch has nothing to declare. N. van Gaal has nothing to declare. R. Zuurbier has nothing to declare. J. Covington has nothing to declare. A. Wicaksono has nothing to declare. M. Biezeveld has nothing to declare. M. A. Benninga has nothing to declare. C. J. J. Mulder has served as a principal investigator for TEVA Pharma BV. He has received a research grant from TEVA Pharma BV. N. K. H. 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 an unrestricted research grant from Dr. Falk and Takeda. T. G. J. de Meij served on the advisory board of Danone.

Supported by: No funding sources declared.

Author contributions: T. G. J. de Meij is the guarantor of this article. N. van Gaal and R. Zuurbier collected the fecal samples. R. Zuurbier prepared the samples and performed VOC analysis. J. Covington and A. Wicaksono analyzed the results. S. Bosch drafted the first version of the manuscript. N. van Gaal, R. Zuurbier, J. Covington, R. Savage, M. Biezeveld, M. Benninga, C. J. J. Mulder, N. K. H. de Boer, and T. G. J. de Meij reviewed the manuscript for important intellectual content. S. Bosch finalized the manuscript. All authors agreed to the final version of the manuscript.

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doi: 10.1093/ibd/izy151
Published online 18 May 2018

somatic diseases, the diagnostic work-up may include colonoscopy, which carries a high burden on patients and leads to high costs and risk of complications.[3, 4] Currently, fecal calprotectin (FCP) is the most commonly used noninvasive diagnostic biomarker to discriminate between IBS/FAP-NOS and IBD, which is characterized by a high sensitivity for mucosal inflammation (0.98; 95% confidence interval [CI], 0.95–0.99) but limited specificity (0.68; 95% CI, 0.50–0.86).[5] Therefore, the search for an accurate, noninvasive biomarker to differentiate between functional gastrointestinal disorders and IBD remains warranted.

Alterations of the intestinal microbiota have been described in IBS/FAP-NOS patients.[6] However, the described results are contradictory, and a specific microbial signature has not yet been defined. Furthermore, microbiota analysis is not easily applicable as a noninvasive biomarker in clinical practice, as the analysis is complex, time-consuming, and expensive.[7] Assessment of volatile organic compound (VOC) composition, which is considered to reflect microbiota composition and function, is a novel field in metabolomics.[8] VOC has shown potential to serve as a diagnostic biomarker for a broad range of gastrointestinal diseases, in particular those linked to microbial dysbiosis, for example, *Clostridium difficile* infection, IBD, colorectal cancer, and necrotizing enterocolitis.[8–11] Field asymmetric ion mobility spectrometry (FAIMS) is an easy-to-use, pattern-based technique to assess VOC profiles, characterized by high reproducibility and relatively low costs, and therefore has potential as a point-of-care tool.[12]

We hypothesized that pediatric IBS/FAP-NOS and IBD could be differentiated based on differences in fecal VOC profiles. The aim of this study was to investigate whether fecal VOC patterns, analyzed by FAIMS, could serve as a biomarker to differentiate IBS/FAP from IBD and from healthy controls in a pediatric population.

METHODS

Study Design

This case-control study was performed at the outpatient clinics of the pediatric (gastroenterology) departments of 2 tertiary centers, VU University Medical Center and Emma Children's Hospital, Academic Medical Centre (AMC), and 1 general hospital, OLVG Oost (all centers located in Amsterdam, the Netherlands). The study was performed between December 2013 and December 2016.

Study Participants

Three subgroups were defined:

IBS and FAP-NOS

Children aged 4 to 17 years visiting the outpatient clinic in 1 of the 3 hospitals between August 2016 and December 2016 and fulfilling the ROME IV criteria for IBS or FAP-NOS were eligible to participate.[2] During clinical appointment, patients were asked to participate in this study. Patients, for whom informed consent was obtained, were provided a stool container and a questionnaire on abdominal symptoms and defecation pattern, including consistency of stool using the Bristol stool chart, medication use, and medical history. Exclusion criteria were the use of anti-/probiotics or immunosuppressive therapy 3 months before inclusion, immunocompromised disease (ie, leukemia, human immunodeficiency virus), diagnosis of a gastrointestinal disease, proven infectious colitis in the month before presentation (determined by positive stool culture for *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Campylobacter* spp., *Clostridium* spp. toxins, or parasites in stools), and a history of gastrointestinal surgery (except appendectomy). From all IBS and FAP-NOS patients included in this study, fecal calprotectin levels were assessed to exclude IBD.

Inflammatory bowel disease

Participants aged 4 to 17 years were extracted from an existing cohort consisting of *de novo* treatment-naïve pediatric IBD patients (59 Crohn's disease [CD], 40 ulcerative colitis [UC]), included at the VU University medical center and the Emma Children's Hospital (AMC) between December 2013 and October 2015 for study of diagnostic fecal biomarkers. All participants were instructed to collect a fecal sample before bowel cleansing, ileocolonoscopy, and esophagogastroduodenoscopy. The diagnosis of IBD was made according to the revised diagnostic Porto-criteria for pediatric IBD, including endoscopic, histologic, and radiologic findings by means of magnetic resonance enteroclysis (MRE).[13] Localization and behavior of disease were classified according to the Paris classification.[14] Clinical activity was determined at study inclusion based on the Physician Global Assessment (PGA score), levels of fecal calprotectin (FCP >250 ug/g was considered active disease), and C-reactive protein (CRP). Exclusion criteria were similar to the IBS/FAP-NOS group, except for exclusion when diagnosed with IBD.

Healthy controls

Children aged 4 to 17 years attending elementary and high schools in the province North-Holland, the Netherlands, were instructed to collect a fecal sample. Similar to the IBS/FAP-NOS group, all participants completed a questionnaire containing similar items. Exclusion criteria were functional gastrointestinal disorders according to the ROME IV criteria, diagnosis with a gastrointestinal or immunocompromised disease, history of gastrointestinal surgery (except appendectomy), or the use of pro- or antibiotics 3 months before inclusion.

Matching Procedure

A total of 15 IBS/FAP-NOS patients (9 IBS, 6 FAP-NOS) were strictly matched to 15 UC, 15 CD, and 30 health controls (HCs), based on age and sex. For this, the following procedure was performed. First, from the 99 IBD patients (59

CD, 40 UC) of the existing cohort, all of the eligible subjects were strictly matched to IBS/FAP-NOS patients. Then, IBD patients were randomly included from the matched groups in a 1:1:1 ratio (IBS/FAP-NOS to UC to CD). After this, 30 HCs recruited for this study were matched to the IBS/FAP-NOS group in a 2:1 ratio.

Sample Collection

Patients were instructed to collect a fresh fecal sample in a stool container (Stuhlgefäß 10 mL, Frickenhausen, Germany) and store the sample in the refrigerator at home directly after bowel movement. The samples were transported to the hospital by 1 of the researchers, using cool elements and a cool bag. Here, samples were directly stored at –20ºC until further handling.

Sample Analysis

Fecal volatile organic compounds analysis was performed using FAIMS (Lonestar, Owlstone, Ltd.), according to the protocol described in an earlier study by Bomers et al.[9] In short, fecal samples were thawed to room temperature 10 minutes before VOC analysis. A mixture of 0.5 g fecal sample and 3.5 mL tap water was manually shaken to homogenize the sample. Compressed air (0.1 MPa) was used as carrier gas to transfer the sample headspace into the FAIMS device. The Lonestar was set up in a pressurized configuration with a flow rate of 2 L/min. The temperatures were set at 35°C for the sample holder, 70°C for the lid, and 100°C for the filter region. After the procedure, the air in the Lonestar was refreshed by analyzing the headspace of 10 mL tap water.[15] The dispersion field passed through 51 equal settings between 0% and 100% (in the ratio of high electric field to low electric field). The compensation voltage was set between +6V and –6V in 512 steps for each dispersion field.[9] Each fecal sample was analyzed 3 times sequentially, producing 3 matrices in 540 seconds. For the statistical analysis, only the third matrix was used for optimal diagnostic potential.[12]

Statistical Analysis

The demographic data of each group (IBS/FAP-NOS, UC, CD, and HC) were compared using the Kruskal-Wallis H test with addition of the Wilcoxon rank-sum test for continuous data. The Fisher exact test was performed for dichotomous data using IBM SPSS, version 22.

Each FAIMS datum consists of the 52,224 data points in a 2D matrix. A preprocessing method was first performed on each datum by applying 2D discrete wavelet transform. This step aims to decompose the data and extract subtle chemical signals hidden within a much larger signal. A 10-fold cross-validation was then applied, where feature selection and classifier training were performed on 90% of the data (training set), and class predictions were produced from 10% of the data (test set). A Wilcoxon rank-sum test as feature selection was used to calculate *P* values in the training set to identify which features were best for disease prediction. From this, 44 statistically important features were used. Four classification algorithms were applied, Sparse logistic regression, random forest, Gaussian process, and support vector machine. A receiver operating characteristic curve was created to predict the area under curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and *P* values.

Ethical Considerations

This study was approved by the Medical Ethical Review Committee (METc) of the VU University Medical Centre under file number 2015.393 and by the local medical ethical committees of the other 2 participating centers. Written informed consent was obtained from all parents, and from the child in case of age over 12 years.

RESULTS

Baseline Characteristics

Baseline characteristics and disease specifics of the study subjects are displayed in Table 1. There were no significant differences in age, sex, and BMI between the IBS/FAP-NOS, IBD, and HC subgroups. Levels of FCP were below 250ug/g in the IBS/FAP-NOS group, with the exception of 1 patient (476 ug/g), in whom it normalized after repeating the measurement, whereas the IBD group had a median FCP level (interquartile range [IQR]) of 1237 (580–1885) ug/g. At study inclusion, the majority of IBS/FAP-NOS patients had experienced abdominal symptoms for more than a year, with frequencies varying from once a week to daily. All of the children in the HC group were asymptomatic. Fecal frequency was higher in the IBS/FAP-NOS group compared with the HC group, although this was not significant. In addition, no differences in fecal consistency based on the Bristol Stool Chart and way of delivery were found between IBS/FAP-NOS and HC.

IBS/FAP-NOS vs IBD

The results of the VOC analysis by the FAIMS technique are shown in Table 2. For each analysis, the best performing of the 4 different applied classification models is shown. A complete overview of the data generated by the 4 classification models is given in Supplementary Tables 1–4. Fecal VOCs of IBS/FAP-NOS patients differed from IBD patients (AUC, 0.94; 95% CI, 0.88–1; 1, 0.87, 0.79, 1, 0.00000002613; sensitivity, specificity, PPV, NPV, *P* value, respectively). Corresponding receiver operating characteristic (ROC) curves are visualized in Figure 1. An overview of the complete outcome of the 4 performed classifiers is displayed in Supplementary Tables 1 and 2. In addition, there were significant differences between the VOC profiles of IBS/FAP-NOS patients and both UC and CD subgroups (Table 2; Supplementary Tables 1–4). A complete overview of the data generated by the 4 classification models is given in Supplementary Tables 1–4.

TABLE 1: Baseline Characteristics

	Crohn's Disease (n = 15)	Ulcerative Colitis (n = 15)	IBS/FAP-NOS (n = 15 [9/6])	Control (n = 30)
Male sex, No. (%)	9 (60)	8 (53)	8 (53)	15 (50)
Age, median (IQR) (minimum–maximum), y	12.8 (5.0) (5.9–17.9)	11.8 (7.8) (3.2–17.8)	12.9 (8.4) (4.4–18.1)	12.7 (8.1) (4.1–17.9)
Storage time, median (IQR) (minimum–maximum), mo	31.7 (25.3)[a] (8.2–54.5)	45.1 (36.2)[a] (15.0–59.4)	0.6 (0.6)[a] (0.2–2.9)	1.4 (0.3)[a] (0.5–4.5)
BMI, median (IQR)	NA	NA	16.7 (5)	17.0 (3)
Bristol stool chart, No. (%)	NA	NA		
Type 2			2 (14)[b]	4 (14)[b]
Type 3			5 (36)	19 (66)
Type 4			4 (29)	5 (17)
Type 5			3 (21)	1 (3)
Stool frequency, No. (%)	NA	NA		
2 times/wk or less			2 (14)[b]	1 (4)[b]
3–6 times/wk			1 (7)	9 (33)
Once/d			5 (36)	14 (44)
2–3 times/d			5 (36)	4 (15)
4 times/d or more			1 (7)	1 (4)
Way of delivery	NA	NA		
Cesarean section, No. (%)			3 (23)[c]	2 (7)[b]
Natural, No. (%)			10 (77)	27 (93)
Frequency of symptoms (IBS/FAP), No. (%)	NA	NA		
None			0 (0)	30 (100)
Once/wk			4 (27)	0 (0)
2 to 4 times/wk			10 (66)	0 (0)
Every day			1 (7)	0 (0)
Duration of symptoms, No. (%)				NA
>1 y	0 (0)[b]	1 (7)	10 (67)	
2 to 12 mo	11 (73)	7 (47)	3 (20)	
≤2 mo	3 (13)	7 (47)	2 (13)	
Physician Global Assessment				
Quiescent	1	0	NA	NA
Mild	0	3	NA	NA
Moderate	5	5	NA	NA
Severe	9	7	NA	NA
Fecal calprotectin, median (IQR), µg/g	1214 (627–1860)	1260 (401–1950)	22 (4.8–133)	NA
CRP, median (IQR), mg/L	21 (7–68)	4 (<2.5–7)	NA	NA
Crohn's disease localization[d]				
Ileal (L1)	0	NA	NA	NA
Colonic (L2)	6	NA	NA	NA
Ileocolonic (L3)	9	NA	NA	NA
Proximal disease (L4)	5	NA	NA	NA
Crohn's disease behavior[d]				
B1 (NSNP)	11	NA	NA	NA
B1p (NSNP+p)	2	NA	NA	NA
B2 (S)	0	NA	NA	NA
B2p (S + p)	0	NA	NA	NA
B3 (P)	0	NA	NA	NA
B3p (P + p)	2	NA	NA	NA

(Continued)

TABLE 1: Continued

	Crohn's Disease (n = 15)	Ulcerative Colitis (n = 15)	IBS/FAP-NOS (n = 15 [9/6])	Control (n = 30)
Ulcerative colitis[d]				
Proctitis (E1)	NA	3	NA	NA
Left-sided (E2)	NA	2	NA	NA
Extensive (E3)	NA	10	NA	NA

All values were obtained at study inclusion. Localization of IBD was obtained by ileocolonoscopy and esophagogastroduodenoscopy before treatment initiation and MR enteroclysis.
Abbreviations: NA, not applicable; NSNP, nonstricturing nonpenetrating; S, stricturing; P, penetrating; p, perianal disease.
[a]Significant differences between all subgroups ($P < 0.001$), analyzed using Wilcoxon rank-sum tests.
[b]Missing data from 1 subject.
[c]Missing data from 2 subjects.
[d]Based on Paris classification for inflammatory bowel disease.[14]

IBS/FAP-NOS vs HC

Children diagnosed with IBS/FAP could not be discriminated from HCs (AUC, 0.59; 95% CI, 0.41–0.77; $P = 0.6, 0.63, 0.45, 0.76, 0.1667$, sensitivity, specificity, PPV, NPV, respectively) (Table 2, Fig. 1; Supplementary Tables 1–4).

IBD vs HC

Patients with IBD could be distinguished from HCs (AUC, 0.96; 95% CI, 0.9–1; $P = 0.93, 0.97, 0.97, 0.94, 0.0000000003962$, sensitivity, specificity, PPV, NVP, respectively) (Table 2, Fig. 1; Supplementary Tables 1–4). Both IBD subtypes UC and CD could each be differentiated from HCs (Table 2; Supplementary Tables 1–4). Differentiation between CD and UC was not possible based on fecal VOC profiles (AUC, 0.67; 95% CI, 0.47–0.88; $P = 0.6, 0.8, 0.75, 0.67, 0.05799$, sensitivity, specificity, PPV, NPV, respectively) (Table 2; Supplementary Tables 1–4).

IBS vs FAP

Patient with IBS could not be discriminated from patients with FAP-NOS (AUC, 0.76; 95% CI, 0.44–1; $P = 1, 0.6, 0.83, 1, 0.9504$, sensitivity, specificity, PPV, NPV, respectively) (Table 2; Supplementary Tables 1–4).

Duration of Sample Storage

Duration of storage of the collected fecal samples did not differ between IBS/FAP-NOS and HCs. IBD samples were stored for a significantly longer period compared with both other subgroups (medium in months: CD, 31.7; UC, 45.1; IBS/FAP, 0.6; HC, 1.4; $P < 0.001$).

DISCUSSION

In this multicenter case-control study, we observed that fecal VOC profiles could differentiate between pediatric IBS/FAP-NOS patients and children with new-onset,

TABLE 2: Performance Characteristics for the Discrimination of Irritable Bowel Syndrome, Functional Abdominal Pain–Not Otherwise Specified, Inflammatory Bowel Disease, and Healthy Controls by Fecal VOC Analysis

	AUC (95% CI)	Sensitivity	Specificity	PPV	NPV	*P*
IBS/FAP-NOS vs IBD	0.94 (0.88–1)	1	0.87	0.79	1	0.00000002613
IBS/FAP-NOS vs CD	0.87 (0.73–0.1)	0.93	0.82	0.82	0.92	0.0001617
IBS/FAP-NOS vs UC	0.96 (0.91–1)	1	0.8	0.83	1	0.000007501
IBS/FAP-NOS vs HC	0.59 (0.41–0.77)	0.6	0.63	0.45	0.76	0.1667
IBS vs FAP-NOS	0.76 (0.44–1)	1	0.6	0.83	1	0.9504
IBD vs HC	0.96 (0.93–1)	0.93	0.97	0.97	0.94	0.0000000003982
UC vs HC	0.98 (0.94–1)	0.93	0.97	0.93	0.97	0.0000000005654
CD vs HC	0.95 (0.88–1)	0.93	0.93	0.88	0.97	0.0000001636
CD vs UC	0.67 (0.47–0.88)	0.6	0.8	0.75	0.67	0.05799

Sensitivities, specificities, *P* values, and AUCs are reported for the respective optimum cut-points.

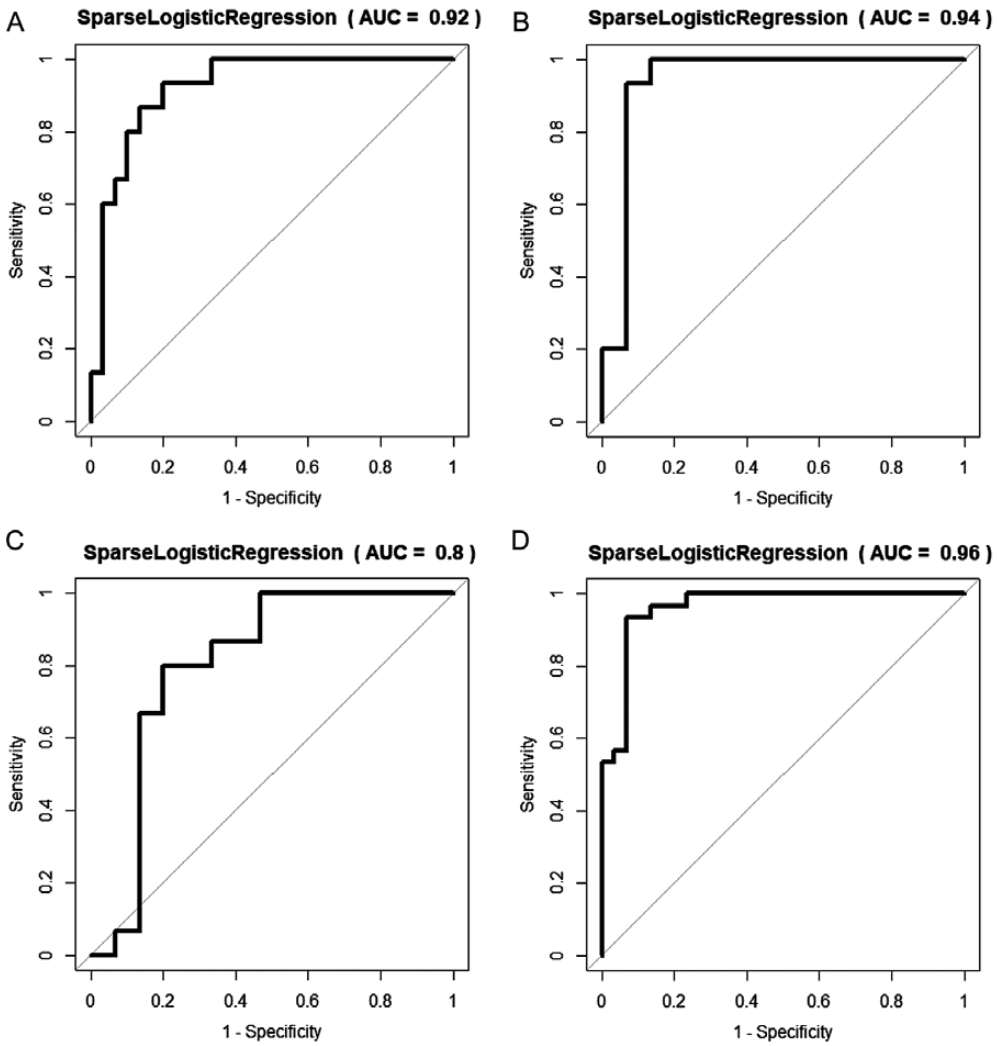

FIGURE 1. Receiver operating characteristics for irritable bowel syndrome/functional abdominal pain–not otherwise specified vs inflammatory bowel disease, ulcerative colitis, and Crohn's disease and IBD vs healthy controls. AUCs are reported for the Sparse logistic regression analyses.

treatment-naïve IBD with high accuracy, but not from HCs. Furthermore, we have validated previous study results indicating that IBD and HC could be discriminated by VOC composition with high accuracy.

Studies on the potential of fecal VOC profiling to discriminate pediatric IBS/FAP-NOS from IBD have not yet been performed. Ahmed et al. compared the fecal VOC profiles of 30 adult diarrhea-predominant IBS (IBS-D) patients, with 62 active CD, 48 active UC, and 109 healthy subjects using gas chromatography–mass spectrometry (GC-MS).[16] In that study, IBS-D could be discriminated from IBD based on 44 significantly different levels of metabolites. Specifically, increased levels of 35 metabolites, mostly consisting of esters from short-chain fatty acids and (derivates of) cyclohexanecarboxylic acid, were seen in the IBS-D group, whereas only 6 metabolites (aldehydes and ketones) were increased in CD, and only 3 (1-propanol, 2-methyl, undecane, methoxy-phenyl oxine) in UC. All of these metabolites were used to construct a discriminatory model with high diagnostic accuracy (AUC IBS-D vs CD, 0.97; AUC IBS-D vs UC, 0.96; $P = 0.001$). This diagnostic accuracy is comparable to that observed in our study. In addition, in the study by Ahmed and colleagues, significantly increased levels of 48 fecal metabolites were identified in adult IBS-D patients compared with HCs (28 increased in IBS-D, of which 22 were esters, 20 increased in HC with no specific pattern, and all weak associations) and were used for a discriminatory model as well (AUC, 0.92; $P < 0.05$). In the present study, however, VOC profiles of IBS/FAP-NOS were not significantly different compared with VOC profiles of HCs. This difference could possibly be explained by our relatively small sample size. Another explanation could be our heterogeneous IBS/FAP-NOS group, in which subjects experienced a variety of symptoms (diarrhea, abdominal pain, bloating, constipation), whereas Ahmed et al. solely included patients with diarrhea-predominant IBS type.

However, we observed no significant differences in VOC profiles between the 2 subgroups IBS and FAP-NOS. In addition, the diagnostic accuracy could differ due to the fact that GC-MS and FAIMS analyze metabolite signals based on different techniques.[17] However, as the diagnostic accuracy to differentiate between IBS/FAP-NOS and IBD is highly similar between these studies, we believe this had minimal influence on our study outcomes.

In a study performed by Walton et al., differences in fecal VOC composition between adult IBS (n = 26), active CD (n = 22), active UC (n = 20), and HC (n = 19) were assessed by means of GC-MS. Increased levels of metabolites (especially propanoic and butanoic acids and products from amino acid fermentation) were found in all disease groups, but were only significantly elevated in CD patients.[18] Unfortunately, no AUC values were provided, which complicates comparison with our study. The authors did report considerable overlap of volatile compound levels between the different subgroups and a wide dynamic range in all groups including the controls.

Volatile organic compounds are considered to reflect (changes in) microbiota composition and function.[8] In a recent study, the gut microbiota composition of patients with IBS (n = 30) and IBD (60 UC, 50 CD) was compared with that of HCs (n = 50) using DNA sequencing.[19] Here, progressive increases in abundance of species belonging to the phyla *Proteobacteria* and *Firmicutes* were detected from HC to IBS to IBD, whereas *Bacteriodetes* representation was gradually reduced along this spectrum. The fact that differences in the microbiota composition between IBS and HC were shown in this study, whereas we did not find these differences based on VOC pattern, contradicts the above-mentioned hypothesis. However, not all microbial changes might reflect in corresponding alterations of VOC composition. Furthermore, VOC composition is not only influenced by the gut microbiota but also by systemic metabolic processes and exogenous VOCs from diet and medication.[20] Despite these facts, our results are in line with the finding that microbial differences between IBD and HC are more pronounced than between IBS and HC.

Until now, pediatric studies on fecal VOCs as a noninvasive biomarker for IBD have focused on the discrimination between IBD patients and healthy subjects, showing high accuracy to discriminate between the 2 groups.[10, 21] This high diagnostic value was found again in the current study. In the previous studies, however, children with abdominal symptoms were not included, limiting reliable exploration of the specificity of VOC analysis to discriminate IBD from an intention-to-diagnose population. As differentiation between IBS/FAP-NOS and active IBD is often challenging in daily practice, a strength of this study was that a pediatric IBS/FAP-NOS group was included. In addition, potential bias by colonic lavage, colonoscopy, and medication on VOC composition was circumvented in IBD patients, as we only included *de novo* treatment-naïve IBD patients. Another strength is the participation of 3 medical centers, 2 tertiary hospitals, and 1 general hospital. Furthermore, the performance characteristics of VOC analysis were assessed using supervised learning models, which are suitable for high-dimensional, complex data sets as they allow for reduction of dimensionality. These classifiers have previously been shown effective in studies involving human microbiota.[22] We have provided a complete overview of results of all applied learning models, as it is not known yet which model is most useful for fecal VOC analysis. There were also several limitations. First, the researchers were not blinded for both VOC and data analysis. Second, the IBS/FAP-NOS group represents a heterogeneous population, although no significant differences in VOC profiles were observed between these 2 subgroups. We therefore believe that the heterogeneity of this group has not significantly influenced study outcomes. Another limitation is that we have not taken potential influence of medication and diet on fecal VOC outcome into account, which could possibly have influenced outcome.[23, 24] In addition, it is known that fecal calprotectine has a lower accuracy in CD patients with isolated proximal or ileal CD. It may be possible that these patients can be distinguished based on VOC patterns, as microbiota changes may differ compared with HC and colonic CD and UC, and VOCs are considered to reflect microbiota composition and function. Based on this study, it is hard to draw conclusions about the application of VOC pattern–based diagnostics for specific groups because we did not include CD patients with isolated proximal or ileal disease. Lastly, the potential influence of sample storage time on metabolic degradation of VOCs has not yet been studied. It could be hypothesized that storage duration influences VOC outcome by metabolic degradation, even in a frozen state. As storage time of the IBD samples differed from that of the HC/IBS/FAP-NOS samples, this may possibly have affected outcome. However, the diagnostic accuracy to differentiate between IBD and HC is similar to our earlier studies, in which samples with comparable storage duration were used.[10] We therefore believe that metabolite degradation has had no substantial influence on the presented results. Furthermore, it is important to point out that we used a pattern-recognition method in this study, rather than identification of individual volatiles. We have chosen to use specifically the FAIMS method because this device is an easy-to-use tool that could be suitable for clinical implementation. Analyses using tools that can detect VOCs on an individual level are expensive and time-consuming, and can therefore not be suited to daily practice.[12]

Our findings indicate that fecal VOC analysis may have the potential to serve as a noninvasive biomarker to discriminate IBS/FAP-NOS from IBD, with a higher specificity (87%) compared with the currently used FCP (specificity 68%), but not IBS/FAP-NOS from healthy state. Combination of biomarkers like FCP and fecal VOCs could possibly lower the rate of unnecessary colonoscopies in the diagnostic process of IBS/FAP-NOS patients. This was, however, a proof-of-principle study meant to explore the diagnostic value of fecal

VOCs in IBS/FAP-NOS patients. Whether this technique sufficiently contributes to this diagnostic process needs to be elucidated in a larger "intention-to-diagnose" cohort, in which patients with suspected IBD should be divided into case and control groups based on FCP, combined with upper and lower endoscopy findings and radiography (like MR enteroclysis). In addition, discrimination between IBS/FAP-NOS-like symptoms and active disease in the course of IBD patients who present with nonspecific abdominal pain may be challenging in clinical practice because of the limited specificity of FCP. Whether VOC analysis could serve as an additional biomarker in this specific population needs to be evaluated in future studies including IBD and non-IBD patients with high FCP levels.

In conclusion, we have shown that patients with IBS/FAP-NOS could be distinguished from IBD patients with a high diagnostic accuracy, but not from HCs, by fecal VOC analysis using FAIMS technology. This signifies its potential role as an additional noninvasive biomarker in the diagnostic work-up to discriminate (pediatric) functional gastrointestinal disorders from IBD.

SUPPLEMENTARY DATA

Supplementary data are available at *Inflammatory Bowel Diseases* online.

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