THE FAECAL SCENT OF PAEDIATRIC IRRITABLE BOWEL SYNDROME AND FUNCTIONAL ABDOMINAL PAIN DIFFERS FROM ACTIVE INFLAMMATORY BOWEL DISEASE BUT NOT FROM HEALTHY STATE: PROOF OF PRINCIPLE STUDY

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TGJ de Meij was the guarantor of this article.

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R Zuurbier prepared the samples and performed VOC analysis.

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Non-standard abbreviations

VOC = volatile organic compound

FAIMS = field asymmetric ion mobility spectrometry

eNose = electronic nose

GC-MS = gas chromatography–mass spectrometry

Abstract (256 words)

Background The diagnostic work-up of paediatric functional gastrointestinal disorders, including irritable bowel syndrome (IBS) and functional abdominal pain – not otherwise specified (FAP-NOS), and discrimination from organic conditions, like inflammatory bowel disease (IBD), commonly includes invasive tests carrying a high burden on patients**.** Therefore, there is an ongoing need to develop non-invasive diagnostic biomarkers for IBS and FAP-NOS. The aim of this study was to evaluate whether paediatric IBS/FAP-NOS could be discriminated from IBD and healthy controls by faecal VOCs (volatile organic compound) analysis.

Methods In this multicentre case-control study, IBS/FAP-NOS patients according to the ROME IV criteria, with age and sex matching *de novo* IBD patients aged 4 to 17 years, were recruited from outpatient clinics of three hospitals in Amsterdam, The Netherlands. Healthy controls (HC) were children without gastrointestinal symptoms. Faecal VOCs were analysed by means of field asymmetric ion mobility spectrometry (FAIMS, Lonestar®, Owlstone, UK).

Results Faecal VOCs of 15 IBS/FAP-NOS, 30 IBD (15UC, 15CD) patients and 15 HC were analysed and compared. Differentiation between IBS/FAP-NOS and IBD was feasible with high accuracy (AUC ± 95%CI, sensitivity, specificity; PPV; NPV; P-values; 0.94 (0.88-1), 1, 0.87, 0.79, 1, 0.00000002613). IBS/FAP patients could not be discriminated HC (AUC ± 95%CI, sensitivity, specificity, PPV, NPV, P-values; (0.59 (0.41-0.77), 0.6, 0.3, 0.45, 0.76, 0.1667).

Conclusion Paediatric IBS/FAP-NOS could be differentiated from IBD by faecal VOC analysis with high accuracy, but not from controls. The latter finding limits the potential of faecal VOCs to serve as diagnostic biomarker for IBS/FAP-NOS. However, it could possibly serve as additional non-invasive biomarker to discriminate between IBS/FAP-NOS and IBD.

Introduction

Irritable bowel syndrome (IBS) and functional abdominal pain – not otherwise specified (FAP-NOS) are gastrointestinal disorders in children. It has a worldwide prevalence of about 13% and often lasts for more than five years after the diagnosis has been established (1). Since biochemical diagnostic biomarkers are yet not available, diagnosis relies on the symptom-based ROME IV criteria (2). The ROME IV criteria include that the symptoms cannot 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 somatic diseases, the diagnostic work-up may include colonoscopy, which carries a high burden on patients, leads to high costs and risk of complications (3, 4). Currently, faecal calprotectin (FCP) is the most commonly used non-invasive diagnostic biomarker to discriminate between IBS/FAP-NOS and IBD, which is characterized by a high sensitivity for mucosal inflammation (0.98, 95%CI 0.95-0.99), but limited specificity (0.68, 95% CI 0.50-0.86) (5). Therefore, the search for an accurate, non-invasive biomarker to differentiate between IBD and functional disorders like IBS/FAP-NOS remains.

Alterations of the intestinal microbiota have been described in IBS/FAP-NOS patients (6). Yet, microbiota analysis is not desirable as a non-invasive biomarker test in clinical practice, as the analysis is complex, time-consuming and expensive (7). Assessment of volatile organic compound (VOC) composition, which are considered to reflect microbiota composition and function, is an upcoming field in metabolomics (8). VOC analysis (i.e. detecting the odours than emanate from a biological sample) has shown potential to serve as a diagnostic biomarker for a broad range of gastrointestinal diseases, in particular those linked to microbial dysbiosis, e.g. *Clostridium difficile* infection, IBD, colorectal cancer and necrotizing

enterocolitis (8-11). There are several techniques to analyse VOCs from simple gas sensor arrays to high-end analytical istruments such as gas chromatography/mass spectrometry. A more modern technique finding favour in many fields is Field asymmetric ion mobility spectrometry (FAIMS), which measures the mobility of ions in high electric fields. It is characterized by high reproducibility, relatively low costs and minimal sensor drift (12). Differentiation between IBS, active IBD and healthy controls has been subject of a study on adults, showing high sensitivity (CD 94%, UC 96%, HC 90%) and specificity (CD 82%, UC 80%, HC 80%) (13).

We hypothesized that paediatric IBD and IBS/FAP-NOS could be distinguished based on differences in faecal VOC composition. The aim of this study was to investigate whether faecal VOC patterns, analysed by FAIMS, could serve as biomarker to distinguish IBS/FAP from IBD and from healthy controls, in a paediatric population.

Methods

Study design

This case-control study was performed at the outpatient clinics of the paediatric (gastroenterology) departments of the tertiary centres VU university medical centre, Emma Children's Hospital, and OLVG Oost (all centres located in Amsterdam, the Netherlands).The study was performed between December 2013 and December 2016.

Study participants

Three subgroups were defined:

1) Inflammatory bowel disease

Participants aged 4 to 17 years were extracted from an existing cohort consisting of *de novo* treatment-naïve paediatric IBD patients (59 CD, 40 UC), included at the VU University medical centre and the Emma Children's Hospital (AMC) between December 2013 and October 2015 for a study on diagnostic biomarkers. The selection procedure is explained in the Matching procedure section. All participants were instructed to collect a faecal sample prior to bowel cleansing and colonoscopy. The diagnosis of IBD was made according to the revised diagnostic Porto-criteria for paediatric IBD, including endoscopic, histologic and radiologic findings(14). Localisation and behaviour of disease were classified according to the Paris Classification(15). Clinical activity was determined at study inclusion based on the Physician Global Assessment (PGA-score), levels of faecal calprotectin (FCP) and C-reactive protein (CRP). Exclusion criteria were the use of anti-/probiotics or immunosuppressive therapy three months prior to inclusion, immunocompromised disease (i.e. leukaemia, human immunodeficiency virus), diagnosis of another 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).

2) IBS and FAP-NOS

Children aged 4 to 17 years visiting the outpatient clinic in one of the three hospitals between August and December 2016, and fulfilling the ROME IV criteria for IBS or FAP-NOS were eligible to participate(2). All subjects completed a questionnaire on abdominal symptoms, defecation pattern based on Bristol stool chart scores, medication use and medical history. Exclusion criteria were similar to the IBD subgroup.

3) Healthy controls

Children aged 4 to 17 years visiting elementary and high schools in the province North-Holland, The Netherlands, were instructed to collect a faecal 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 three months prior to inclusion.

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 other two participating centres. Written informed consent was obtained from all parents, and from the child in case of age over twelve years.

Matching procedure

A total of 15 IBS/FAP-NOS patients (9 IBS, 6 FAP-NOS) were strictly matched to 15UC, 15CD and 30HC based on age and gender. 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 HC recruited for this study were matched to the IBS/FAP-NOS group in a 1:2 ratio.

Sample collection

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

Sample analysis

Faecal volatile organic compounds analysis was performed using FAIMS (Lonestar, Owlstone Ltd., UK), according to the protocol as described in an earlier study by Bomers et.al. (9). In short, faecal samples were thawed to room temperature ten minutes prior to VOC analysis. A mixture of 0.5g faecal sample and 3.5mL tap water was manually shaken to homogenize the sample. Compressed air (0.1MPa) was used as carrier gas to transfer the sample headspace (the air above the sample) into the FAIMS instrument. The Lonestar was set up in a pressurised configuration with a flow rate of 2L/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 analysing the headspace of 3.5mL tap water(16). The dispersion field passed through 51 equal settings between 0% and 100% (in the ratio of the 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 faecal sample was analysed three times sequentially, producing three matrices in 540s. For the statistical analysis, only the third matrix was used as we have previously shown that this approach gives the optimum diagnostic potential(12).

Statistical analysis

The demographic data of each group (IBS/FAP-NOS, UC, CD and HC) was compared using the Kruskal-Wallis-H test with the addition of the Wilcoxon-rank-sum test for continuous data. The Fisher's exact tests was performed for dichotomous data using IBM SPSS version 22. For FAIMS analysis, each sample consisted of 52224 data point in a 2D matrix. A pre-processing method was first performed to each sample data by applying a 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 was performed to 90% of data (training set) and class predictions produced from 10% of data (test set). A Wilcoxon rank sum test as feature selection was used to calculate p-values in training set to identify which features are optimum for disease prediction. Four classification algorithms were applied, Sparse Logistic Regression, Random Forest, Gaussian Process, and Support Vector Machine. A receiver operator characteristic curve was created to predict area under curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and p-values.

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. In addition, no differences in faecal consistency based on the Bristol Stool Chart, faecal frequency and way of delivery were found between IBS/FAP-NOS and HC.

IBS/FAP-NOS versus IBD

The results of the VOC analysis by FAIMS technique are shown in *Table 2*. For each analysis, the best performing of the four different applied classification models is shown. A complete overview of the data generated by the four classification models is given in supplemental Table 1-4. Faecal VOCs of IBS/FAP patients differed from IBD patients (AUC ± 95%CI, sensitivity, specificity, PPV, NPV, P-values; 0.94 (0.88-1), 0.87, 0.79, 1, 0.00000002613). Corresponding Receiver Operating Characteristic (ROC)-curves are visualised in *Figure 1*. An overview of the complete outcome of the four performed classifiers is displayed in *supplementary tables 1-2*. In addition, there were significant differences between VOC profiles of IBS/FAP-NOS patients and both UC and CD subgroups (*table 2, Supp table 1-4).*

IBS/FAP-NOS versus HC

Children diagnosed with IBS/FAP could not be discriminated from HC (AUC ± 95%CI, sensitivity, specificity, PPV, NPV, P-values; (0.59 (0.41-0.77), 0.6, 0.3, 0.45, 0.76, 0.1667) (*Table 2, Supp table 1-4, Figure 1*).

IBD versus HC

Patients with IBD could be distinguished from HC (AUC ± 95%CI, sensitivity, specificity, PPV, NVP, P-values; 0.96 (0.9-1), 0.93, 0.97, 0.97, 0.94, 0.0000000003962) (*Table 2, Supp table 1-4, Figure 1*). Both IBD subtypes UC and CD could each be differentiated from HC (*Table 2, Supp table 1-4)*.

IBS versus FAP

Patients with IBS could not be discriminated from patients with FAP-NOS (AUC ± 95%CI, sensitivity, specificity, PPV, NPV, P-values; (0.76 (0.44-1), 1, 0.6, 0.83, 1, 0.9504) (*Table 2, Supp table 1-4)*.

Duration of sample storage

Duration of storage of the collected faecal samples did not differ between IBS/FAP-NOS and HC. IBD samples were stored for a significantly longer period compared to 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 multicentre case-control study, we observed that faecal VOC profiles could discriminate paediatric IBS/FAP-NOS patients from children with new onset, treatment naïve IBD with high accuracy, but not from HC. Furthermore, we validate earlier study results that IBD and HC could be discriminated with high accuracy.

Studies on the potential of faecal VOC profiling to discriminate between paediatric IBS/FAP-NOS and IBD have not yet been performed. Ahmed et. al. compared faecal VOC profiles of 30 adult diarrhoea-predominant IBS (IBS-D) patients, with 62 active CD, 48 active UC and 109 healthy subjects using gas chromatography-mass spectrometry (GC-MS)(13). In that study, IBS-D could be discriminated from IBD, based on 60 statistically significant different metabolites. These metabolites were used to construct a discriminatory model with high diagnostic accuracy (AUC IBS-D vs CD 0.97; IBS-D vs UC 0.96; p=0.001). This diagnostic accuracy is similar to that observed in our study. In addition, significantly increased levels of 28 faecal metabolites were identified in IBS-D patients compared to HC and were used for a discriminatory model as well (AUC 0.92; $p<0.05$). In the present study, however, IBS/FAP-NOS could not be discriminated from HC. 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 could experience a variety of symptoms (diarrhoea, abdominal pain, bloating, constipation), whereas Ahmed. et. al. solely included patients with diarrhoea-predominant IBS type. Though, we observed no significant differences in VOC profiles between the two subgroups IBS and FAP-NOS. In addition, the diagnostic accuracy could differ due to the fact that GC-MS is a more sensitive technique compared to FAIMS(17). However, since the diagnostic accuracy to differentiate between IBD and IBS/FAP-NOS is

very similar between these studies, we believe this had minimal influence on our study outcomes.

In a study performed by Walton et. al., differences in faecal 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 and were found in eight metabolites, displaying gradually increased levels from HC to IBS to IBD (CD>UC)(18). Unfortunately, no AUC values are given in the article, which complicates comparison with our study. However, the authors did report considerable overlap of 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, gut microbiota composition of patients with IBS (n=30) and IBD (60 UC, 50 CD) were compared to HC (n=50) using DNA sequencing(19). Here, progressive increase 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 be reflected 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 like from diet and medication (20). Despite these facts, our results are in line with the finding that microbial differences between IBD and HC are larger than IBS and HC.

Until now, paediatric studies on faecal VOCs as non-invasive IBD biomarker have focussed on the discrimination between IBD patients and healthy subjects, lacking a reliable exploration of the specificity to discriminate IBD from IBS by VOC analysis. Main strength of this study was that a paediatric IBS/FAP-NOS group was included, allowing for assessment of the diagnostic accuracy in an intention-to-diagnose design. In addition, potential bias by colonic lavage, colonoscopy and medication on VOC composition was circumvented in IBD patients, since we only included *de novo* treatment-naïve IBD patients. Another strength is the participation of three medical centres, two referral hospitals and one district hospital. Furthermore, the performance characteristics of VOC analysis were assessed using supervised learning models, which are suitable for high-dimensional data as they reduce dimensionality. These classifiers have previously been shown effective in studies on the human microbiota(21). We have decided to provide a complete overview of all learning models applies in this study, as it is not known which model is most useful for VOC analysis. There were also several limitations. As noted previously, the IBS/FAP-NOS group represents a heterogeneous population, although no significant differences in VOC profiles were observed between these two subgroups. We therefore believe that the heterogeneity of this group has not significantly influenced study outcomes. Another limitation was that we have not taken potential influence of medication and diet on faecal VOC outcome into account, which could possibly have influenced the result(22, 23). 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 frozen state. Since 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 presented results.

Our findings implicate that faecal VOC analysis may serve as non-invasive biomarker to discriminate IBS from IBD, with a higher specificity (87%) compared to the currently used FCP (specificity 68%), but not IBS from healthy state. To discriminate between IBS-like symptoms and active disease in the course of IBD patients with nonspecific abdominal pain may be challenging in clinical practice, by limited specificity of FCP. Whether VOC analysis could serve as an additional biomarker in this specific population needs to be evaluated in future studies. Combination of the biomarkers FCP and faecal 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 to explore the diagnostic value of faecal 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 conclusion, we have shown that patients with IBD could be distinguished from IBS/FAP-NOS and from HC with a high diagnostic accuracy based on faecal VOC analysis using FAIMS technology. This signifies its potential role as additional non-invasive biomarker in the diagnostic work-up of IBD to discriminate from functional gastrointestinal disorders.

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1 **Table 1. Baseline characteristics**

	Crohn's disease (n=15)	Ulcerative colitis (n=15)	IBS/FAP-NOS (n=15 [9/6])	Control (n=30)
Sex, male (n [%])	9 [60]	8 [53]	8 [53]	15 [50]
Age (median [IQR]), years (minimum-maximum)	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]), months (minimum-maximum)	31.7 [25.3]¥ (8.2 – 54.5)	45.1 [36.2]¥ (15.0 – 59.4)	0,6 [0.6]¥ (0.2 – 2.9)	1.4 [0.3]¥ (0.5 – 4.5)
BMI (median [IQR])	NA	NA	16.7 [5]	17.0 [3]
Bristol stool chart (n [%]) Type 2 Type 3 Type 4 Type 5	NA	NA	 2 [14]* 5 [36] 4 [29] 3 [21]	 4 [14]* 19 [66] 5 [17] 1 [3]
Stool frequency (n [%]) 2 times a week or less 3-6 times a week Once a day 2-3 times a day 4 times a day or more	NA	NA	 2 [14]* 1 [7] 5 [36] 5 [36] 1 [7]	 1 [4]* 9 [33] 14 [44] 4 [15] 1 [4]
Way of delivery Caesarean section (n [%]) Natural (n [%])	NA	NA	 3 [23]** 10 [77]	 2 [7]* 27 [93]
IBS/FAP	NA	NA	33	NA
Frequency of symptoms (IBS/FAP) (n [%]) Once a week 2 to 4 times a week Every day	NA	NA	 4 [27] 10 [66] 1 [7]	NA
Duration of symptoms ((n [%]) Over a year 2 to 12 months ≤2 months	 0 [0]* 11 [73] 3 [13]	 1 [7] 7 [47] 7 [47]	 10 [67] 3 [20] 2 [13]	NA
Physician Global Assessment				
Quiescent	1	0	NA	NA
Mild	0	3	NA	NA
Moderate	5	5	NA	NA
Severe	9	7	NA	NA
Faecal calprotectin (µg/g) (median[IQR])	1214 [627-1860]	1260 [401-1950]	NA	NA
CRP (mg/l) (median[IQR])	21 [7-68]	4 [<2.5 – 7]	NA	NA
Crohn's disease localisation[1]				

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 behaviour[1]				
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
Ulcerative Colitis[1]				
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: IQR, interquartile range; NA, not applicable; NSNP, non-stricturing non-penetrating; S, stricturing; P, penetrating; p, peri-anal disease. [1]Based on Paris classification for inflammatory bowel disease(15).

* Missing data from one subject. ** Missing data from two subjects. ¥ Significant differences between all subgroups $p<0.001$, analysed using Wilcoxon-rank-sum tests.

Table 2. Performance characteristics for the discrimination of irritable bowel syndrome, functional abdominal pain-not otherwise specified, inflammatory bowel disease and healthy controls by faecal 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

Table 2. Sensitivities, specificities, p-values and AUCs are reported for the respective optimum cut-points.. Abbreviations: AUC, area under the curve; PPV: positive predictive value; NPV: negative predictive value; IBS: Irritable bowel syndrome; FAP-NOS: functional abdominal pain-not otherwise specified; IBD: Inflammatory bowel disease; UC: ulcerative colitis; CD: Crohn's disease; HC: Healthy controls.

Figure 1. Receiver operating characteristics for irritable bowel syndrome/functional abdominal pain-not otherwise specified versus inflammatory bowel disease, ulcerative colitis and Crohn's disease and IBD versus healthy controls.

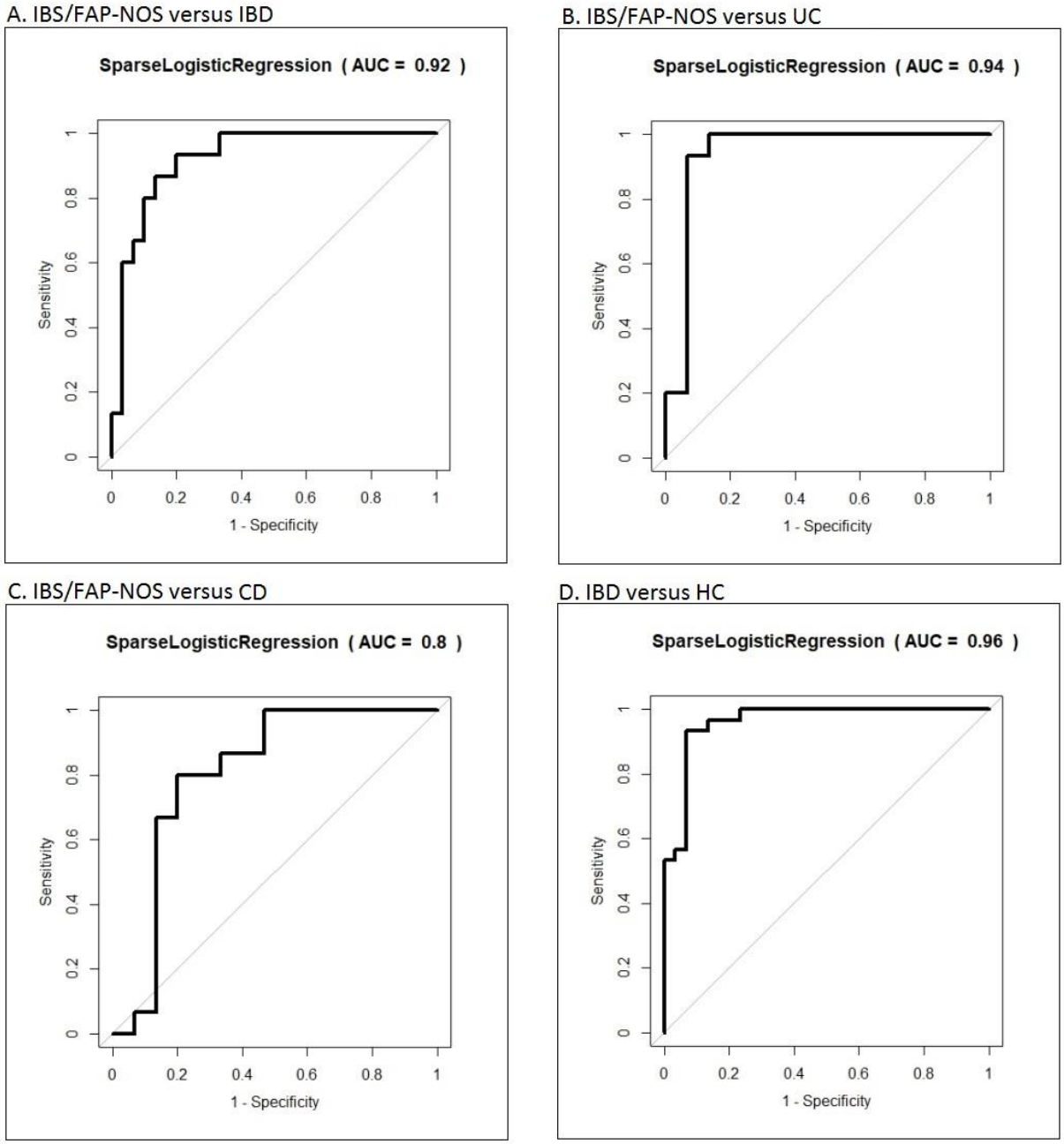

Figure 1. AUCs are reported for the Sparse logistic regression analyses. Abbreviations: AUC, area under the curve; IBS: Irritable bowel syndrome; FAP-NOS: functional abdominal pain-not otherwise specified; IBD: Inflammatory bowel disease; UC: ulcerative colitis; CD: Crohn's disease; HC: Healthy controls.