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Gut microbiota profiles and the role of anti-CdtB and anti-vinculin antibodies in patients with functional gastrointestinal disorders (FGID)

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Abstract

Background: Distinct faecal microbiota profiles are reported to be associated with various subtypes of IBS. Circulating antibodies to cytolethal distending toxin B (CdtB) and vinculin are proposed as biomarkers to identify post-infectious IBS. The aim of our study was to analyse serum levels of anti-CdtB and anti-vinculin antibodies in patients with different functional gastrointestinal disorders (FGID) and their correlation with the composition of faecal microbiome.

Methods: The study cohort comprised 65 prospectively recruited individuals: 15 with diarrhoea-type-IBS (IBS-D), 13 with constipation-type-IBS (IBS-C), 15 with functional dyspepsia (FD) and 22 healthy controls. FGID subgroups were defined according to Rome III criteria. Serum levels of anti-CdtB and anti-vinculin antibodies were measured by ELISA. Faecal microbiome composition analysis and assessment of dysbiosis were performed by GA-map® Dysbiosis Test.

Results: Positivity rate either for anti-CdtB or anti-vinculin antibodies was higher in the IBS-C group (76.9%) compared to IBS-D (40.0%), FD (60%) and healthy (63.6%) groups.

Dysbiosis was more frequent in subjects positive for anti-CdtB antibodies and in IBS-C patients, who showed an increased amount of opportunistic/pro-inflammatory bacteria and reduced gut protective bacteria. IBS-C patients showed a high inter-individual variation of bacterial communities compared to other FGID subgroups and healthy individuals, whereas microbial profiles of patients with IBS-D and FD were overlapping with those of healthy controls. No bacteria markers showed significant differences between FGID subgroups and healthy controls.

Conclusion: Neither anti-CdtB/anti-vinculin antibodies nor faecal microbial profiles allowed to discriminate between specific FGID subgroups. Dysbiosis was more frequent in patients presenting with anti-CdtB antibodies and in IBS-C patients.

KEYWORDS
cytolethal distending toxin/ vinculin, dysbiosis, faecal microbiota, functional dyspepsia, irritable bowel syndrome

1 | INTRODUCTION

Irritable bowel syndrome (IBS) and functional dyspepsia (FD) are two distinct but often overlapping gastrointestinal disorders (FGID), that affect up to 20% of the general population.[1] FGID lead to frequent medical consultations and diagnostic examinations with a significant burden on the healthcare system. Diagnosis of IBS and FD is based on the presence of characteristic symptoms, currently defined by the Rome criteria with exclusion of organic disease. Also, the selection of therapy is prioritized according to the leading symptom in FD and IBS as proposed in the Rome consensus.[2]

Many aspects in the aetiology and pathophysiology of IBS are still unclear and symptoms are only partially helpful in guiding the selection of the most appropriate therapy. Therefore, the availability of accurate biomarkers that allow to diagnose and categorize patients with FGID would be very helpful in clinical management. In this context, gut microbial signatures are a promising tool and have been reported to be altered (i.e., dysbiosis) in IBS patients when compared to healthy individuals.[3] Factors that trigger faecal microbial dysbiosis that need to be taken into account include dietary changes, stress, intake of antibiotics and previous infections.[4,5] Dysbiotic changes of microbial communities were shown in various subtypes of IBS. Past episodes of acute gastroenteritis leave persisting alterations of the gut microbiota and promote the development of long-lasting predominantly diarrhoea-type IBS (IBS-D).[6] Based on this notion, a serological tool, measuring circulating anti-CdtB and anti-vinculin antibodies, has been proposed for the discrimination of patients with IBS-D from patients with other conditions of diarrhoea, including inflammatory bowel disease (IBD) and celiac disease, as well as from healthy individuals.[7,8] The diagnostic contribution of cytolethal distending toxin (Cdt), a bacterial toxin produced by most common bacteria causing gastroenteritis, including *Clostridioides difficile*, *Campylobacter jejuni*, *Salmonella*, *Escherichia coli*, and *Shigella*, and vinculin, a host cell adhesion protein that cross-react with anti-CdtB antibodies, had originally been studied in animal models of post-infectious IBS (PI-IBS).[9,10] Also, dyspepsia originating as sequelae from previous gastrointestinal infections has been reported.[11] *Helicobacter pylori* may be considered as a model condition for post-infectious dyspepsia. Successful *H. pylori* eradication leads to relief of dyspeptic symptoms in a subset of patients,[12] while in some patients dyspeptic symptoms recur or persist even after cure of the infectious gastritis. This condition, referred as post-infectious functional dyspepsia, may be considered analogous to post-infectious IBS.

Aims of this explorative study were (a) to determine the prevalence in serum of anti-CdtB and anti-vinculin antibodies, (b) to determine the faecal microbiota structure and a previously validated Dysbiosis Index (DI), and (c) to correlate the serum antibodies with specific microbial signatures and the degree of abnormalities reflected in the DI in different patient subsets with Functional Gastrointestinal Disorders (FGID) including IBS subtypes and FD.

2 | MATERIAL AND METHODS

2.1 | Subjects and specimen collection

Patients with different FGID and healthy controls were prospectively recruited at Magdeburg University Hospital from October 2015 to March 2017. The study cohort comprised a total of 65 individuals including 15 patients with IBS-D, 13 patients with IBS-C, 15 patients with FD and 22 healthy controls. Subjects were recruited within the EMGASTA project (DRKS-ID: DRKS00009737), a large-scale prospective study focussed on research into gut microbiota profiles in healthy individuals and in patients with functional GI diseases. The study was approved by the local ethics committee and government authorities and was conducted in accordance with the current Good Clinical Practice guidelines and the Declaration of Helsinki.[13] All recruited subjects provided their written informed consent to participate in the study.

Patients with IBS were diagnosed according to Rome III criteria after an accurate anamnesis and clinical evaluation.[14] Healthy controls did not report any GI disease, tumour disease, severe metabolic or cardiovascular disease requiring therapy, or neurodegenerative disease. Study participants must not have any antibiotic intake in the 8 weeks prior to inclusion. From each study, participant blood samples for routine laboratory tests and serological analysis were collected. A subset of participants provided also faecal samples for microbiome analysis. This subgroup included 13 patients with IBS-D, 11 with IBS-C, 14 with FD and 15 healthy controls. Faecal samples were collected with a sterile stool sample collection kit (PT26.1; Carl Roth, Karlsruhe, Germany) and kept in the refrigerator at 4°C (for a maximum of 8 h) until transferred to the laboratory. After aliquoting, faecal samples were stored at −80°C until further analysis. All patients with dyspepsia underwent esophagogastroduodenoscopy (EGD) for further

clinical evaluation and diagnosis of structural disorders. In those patients, a standard histopathologic assessment was performed according to the updated Sydney System. Testing of dyspeptic patients for *H. pylori* infection was performed by histology, serology and stool antigen test, as previously described.[13] Concordant negative results in all tests except for *H. pylori* serology were required to exclude *H. pylori* infection.

2.2 | ELISA analysis

Blood samples were obtained from all study participants by venipuncture into a blood collection tube (BD Vacutainer RST, BD, Franklin Lakes, NJ, USA) and then centrifuged at 3050 rpm for 10 min and stored after aliquoting at −80°C. Anti-CdtB and anti-vinculin antibody levels in plasma were measured by a second-generation Enzyme-linked immunosorbent assays (ELISA) as previously described (see also Supplementary Material). Based on previous literature, the cut-offs for positivity were set at OD values of ≥ 1.68 for anti-vinculin and ≥ 2.80 for anti-CdtB antibodies, respectively.[7]

2.3 | Microbiome analysis

To assess gut microbiota composition, we used GA-map® Dysbiosis Test Lx (Genetic Analysis), which is a targeted approach to evaluate gut microbiota profiles.[15] In brief, the test utilizes faecal homogenization, mechanical and enzymatic bacterial cell disruption to isolate and bind total bacterial genomic DNA to magnetic beads. Then, 16S rRNA hypervariable regions V3-V9 are amplified by polymerase chain reaction (PCR). Bacterial DNA labelling is done by single nucleotide extension and hybridized to a complementary DNA strand coupled to beads. Finally, the abundance of bacteria is assessed by the strength of fluorescent signal (probe intensity), detected and measured by a Luminex® L×200™ instrument (Luminex Corporation). In total, the relative abundance of 48 bacteria markers targeting more than 300 bacteria species is measured. The test also algorithmically assesses faecal bacterial abundance and profile in comparison with a reference healthy population. A deviation in the microbiome from normobiosis is summarized in a Dysbiosis Index (DI) 1 to 5. DI of 1–2 indicates normobiosis, 3 indicates mild dysbiosis, and 4–5 indicates severe dysbiosis. In addition, abundance scores within the range of −3 to +3 (where 0 indicates within the normal range) for all 48 bacteria markers are assigned for each sample analysed (see Table S1). Principal component analysis (PCA) was used to explore the variation in microbiota between the groups. The analysis was performed on scaled and log-transformed GA-map® signal strength data in R using the Stats package. Additional information on the microbiome data analysis is provided in Supplementary Material.

2.4 | Statistical analyses

Descriptive statistical analysis was performed using IBM SPSS Statistics 21.0.0 (IBM Corporation) and GraphPad Prism 7. Numerical variables were expressed by mean values ±standard deviation. Chi-square test was used for comparison of categorical data. Shapiro-Wilk test was used to assess the normality of the data. To evaluate statistical differences between two or multiple groups, Mann-Whitney U tests or Kruskal-Wallis tests were performed for non-normally distributed data, respectively, while normally distributed variables were compared across more than two groups by one-way ANOVA and Dunnett's post hoc tests. All tests were carried out two-sided with a level of significance set to 0.05.

Reporting of the study conforms to broad EQUATOR guidelines (Simera et al. January 2010 issue of EJCI).[16]

3 | RESULTS

3.1 | Characteristics of the study cohort

Four patients with dyspepsia were tested *H. pylori*-positive and showed in EGD mild to moderate signs of chronic gastritis without severe structural alterations (i.e., ulcer). No statistically significant differences were found in age distribution and gender proportions between groups. The characteristics of the study population are shown in Table 1.

TABLE 1 Characteristics of the study population. Mean and standard deviation (in brackets) are shown. Anti-CdtB and anti-vinculin antibodies titres are indicated by optical densities (OD)

	Healthy controls ($n = 22$)	IBS-D ($n = 15$)	IBS-C ($n = 13$)	FD ($n = 15$)	p
Age	54.8 (11.2)	43.5 (20.6)	50.6 (18.2)	48.7 (17.6)	ns
Male/Female	10 / 12	5 / 10	3 / 10	7 / 8	ns
Anti-CdtB antibodies (OD)	2.53 (0.74)	2.35 (0.81)	2.72 (0.71)	2.62 (0.66)	ns
Anti-vinculin antibodies (OD)	1.60 (1.01)	0.86 (0.85)	1.26 (0.70)	1.30 (0.84)	ns

Note: p values were calculated with the Kruskal-Wallis test. 'ns' denotes no significant difference. Abbreviations are defined in Materials and Methods.

3.2 | Comparison of circulating antibody titres among groups

The highest mean values of circulating anti-CdtB antibodies titres indicated by optical density (OD) were found in patients with IBS-C (2.72 ± 0.71), followed by patients with FD (2.62 ± 0.66), healthy subjects (2.53 ± 0.74) and patients with IBS-D (2.35 ± 0.81). Anti-vinculin antibodies were higher in healthy individuals (1.60 ± 1.01) and slightly reduced in patients with IBS-D (0.86 ± 0.85), while patients with FD and IBS-C showed similar levels (1.30 ± 0.84 and 1.26 ± 0.70, respectively, Table 1). No significant changes in the mean levels of measured biomarker were found between FGID groups and healthy controls, both for anti-CdtB and for anti-vinculin antibodies ($p = .598$ and $p = .076$, respectively, Figure 1).

According to the cut-off values proposed by Pimentel et al.,[7] anti-CdtB positivity rates were higher in the IBS-C group (53.8%) compared to IBS-D group (33.3%), FD group (40.0%) and healthy controls (40.9%), Figure 2. Individuals positive for anti-vinculin antibodies were found less frequently among patients with IBS-D (13.3%) compared to patients with IBS-C (30.8%), patients with FD (33.3%) and healthy subjects (36.4%). However, no significant differences were observed comparing the positivity rates between the four groups neither for anti-CdtB ($p = .742$) nor for anti-vinculin antibodies ($p = .473$). The IBS-C group showed the highest positivity rate for at least one biomarker with 76.9% of patients being positive either for anti-CdtB or anti-vinculin antibodies (or both), followed by healthy subjects (63.6%), patients with FD (60.0%) and patients with IBS-D (40.0%). Also, this parameter was not statistically different between groups ($p = .243$), Figure 2.

3.3 | Microbiota composition and Dysbiosis Index in patients with different subtypes of FGID and healthy controls

According to the GA-map® Dysbiosis Test Lx, the majority of patients with IBS-C showed a tendentially higher degree of dysbiosis (81.1% of patients with DI >2 vs. 18.9% of patients with DI = 1–2: 18.9%, $p = .068$), whereas the IBS-D, FD and healthy controls groups had lower rates of subjects with dysbiosis (46.2%, 57.1% and 46.7% with DI >2, respectively), Figure 3 & Table S1.

Principal component analysis (PCA) was used to visualize variation in signal data from GA-map® test. In general, we observed a marked inter-individual variation of bacterial communities' composition. Microbial structures from patients with IBS-D and FD were similar to those from healthy controls and covered a mostly overlapping area (Figure 4). No significant differences were observed between dyspeptic patients positive for *H. pylori* and negative ones, neither in faecal microbiota composition nor with regards to DI.

Faecal samples from patients with IBS-C showed a different profile with a larger internal variation and a distinct distribution compared to the other groups. No bacterial markers were found to display significant differences (padj <0.05) between healthy individuals and FGID subgroups. However, Proteobacteria, *Ruminococcus albus & Ruminococcus bromii* and *Phascolarctobacterium sp*. were found to be more abundant individuals with high DI score and in those with antibody positivity.

The OPLS-DA model was used to define differences in faecal bacterial communities between the groups. According to multivariate factor discriminant analysis, the microbiota composition could not discriminate between healthy subjects and IBS or FD patients, as demonstrated by poor fitness ($R^2Y < 0.5$) and limited discriminant predictive ability ($Q^2 < 0.5$) of the model. Notably, whilst the limited fitness of the OPLS-DA, the IBS-C group showed the highest Q^2 value (−0.375) compared to the other groups (Figure S1).

Functional bacterial profile analysis revealed that the majority of IBS-C patients showed a higher degree of imbalance in all the five profiles compared to other FGID groups. In IBS-C patients and in subjects with severe dysbiosis, the abundance of essential butyrate-producing, and gut mucosa protective bacteria were reduced, while pro-inflammatory and opportunistic bacteria abundance were found in increased abundance.

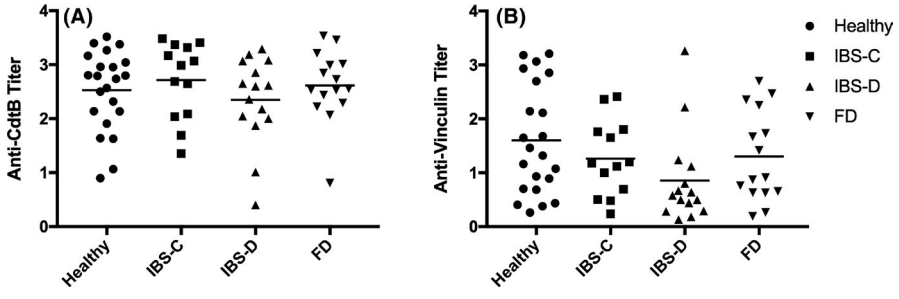

FIGURE 1 Anti-CdtB (A) and anti-vinculin (B) antibodies titres in patients with IBS-D ($n = 15$), patients with IBS-C ($n = 13$), patients with FD ($n = 15$) and in healthy controls ($n = 22$). Data are shown as optical densities (OD) in scatter plots where the horizontal line marks the mean value

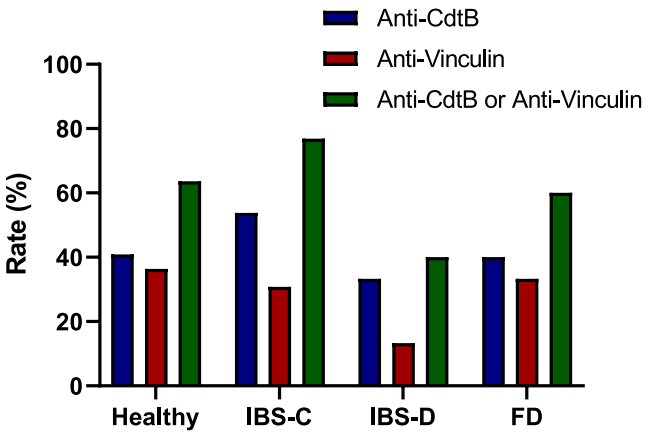

FIGURE 2 Comparison of antibody positivity rates (anti-CdtB, anti-vinculin, anti-CdtB OR anti-vinculin) among the study groups. Comparisons of categorical variables between groups were performed by Pearson's chi-square test. No significant differences were observed in inter-groups comparisons

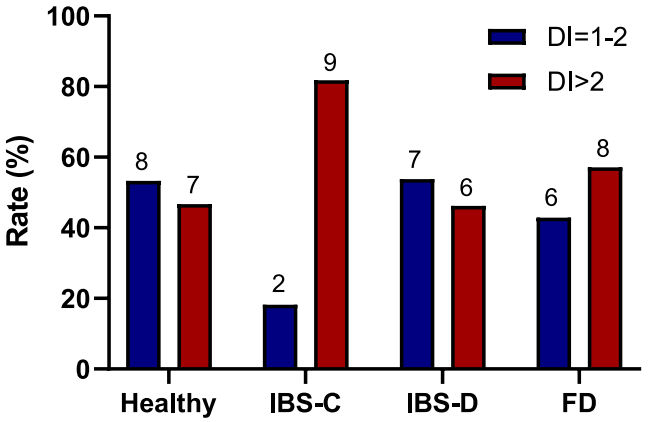

FIGURE 3 Distribution of the Dysbiosis Index (DI) among patients with IBS-C ($n = 11$), patients with IBS-D ($n = 13$), patients with FD ($n = 14$) and in healthy controls ($n = 15$). Dysbiosis was evaluated in faecal samples by the GA-map® Dysbiosis Test. DI is scored between 1 and 5, where a score of 1 and 2 signifies normobiosis (blue) and 3–5 dysbiosis of increasing severity (red). Digits above the columns represent the number of subjects within each column. Comparisons of categorical variables between groups were performed by Pearson's chi-square test. IBS-C patients showed more frequently higher DI ($p = .068$) compared to controls. No other significant differences were observed in inter-groups comparisons

3.4 | Microbiota changes in dysbiotic patients with FIGD positive for anti-CdtB or anti-vinculin antibodies

Dysbiosis was tendentially more common in individuals who were positive for at least one biomarker between anti-CdtB or anti-vinculin antibodies (63% showed DI >2) or for both biomarkers (100% of these subjects presented with DI >2) compared to subjects negative for both antibodies (48% with DI >2, $p = .053$, Figure S2). In the subgroup analysis, a significantly higher proportion of individuals with moderate/severe dysbiosis was observed in patients with high levels of anti-CdtB antibodies compared to subjects negative for anti-CdtB (72.7% vs. 45.1%, $p = .046$), suggesting that the presence of anti-CdtB antibodies may be associated with a more dysbiotic microbiota profile. No significant association was detected between presence of dysbiosis and positivity to anti-vinculin antibodies (Figure 5).

The faecal microbiota composition of individuals positive for anti-CdtB or anti-vinculin antibodies showed a larger variation compared to individuals negative for both antibodies. There was a substantial grade of overlap in the PCA between microbiota profiles of antibody-positive and those of antibody-negative subjects (Figure 6).

The OPLS-DA model could not discriminate the group of antibody positives from that of antibody-negative subject ($R^2Y = .199$, $Q^2 = -0.146$).

However, Proteobacteria, *Ruminococcus albus & R. bromii* and *Phascolarctobacterium* sp. appeared to have a higher effect on the DI score for antibody-positive subjects with high DI (DI >4) than for the remaining subjects. This effect is explained by the DI algorithm, in which the bacteria markers influence differently, in combination with measured signal strength.

4 | DISCUSSION

Gut microbiota is suggested to be a contributing factor to the pathophysiology of FGID and presents with distinct profiles in different subforms of IBS.[17] One such subform is post-infectious IBS which is well documented in a subset of patients following gastrointestinal infections.[18] The assessment and characterization of FGID relies on the patient's history, exclusion of organic pathologies and definition of symptom clusters.[19] No specific biomarkers and specific faecal microbiota profiles to objectively confirm the diagnosis of FGID are available for clinical use at present.

For the purpose of progressing in this field, we assessed the composition of faecal microbiota in patients with FGID and in healthy controls combined with the determination of plasma levels of 2 selected biomarkers. The most notable finding in our study was that severe dysbiosis was most prevalent in patients with IBS-C. Severe dysbiosis was also more frequently detected in individuals positive for circulating anti-CdtB antibodies compared to other groups. However, a discrimination between different subgroups of FGID based on the positivity for anti-CdtB/anti-vinculin antibodies or on the Dysbiosis Index (DI) was not possible.

In our cohort, we were not able to discriminate patients with IBS and different IBS subtypes from healthy controls by the determination of circulating anti-CdtB and anti-vinculin

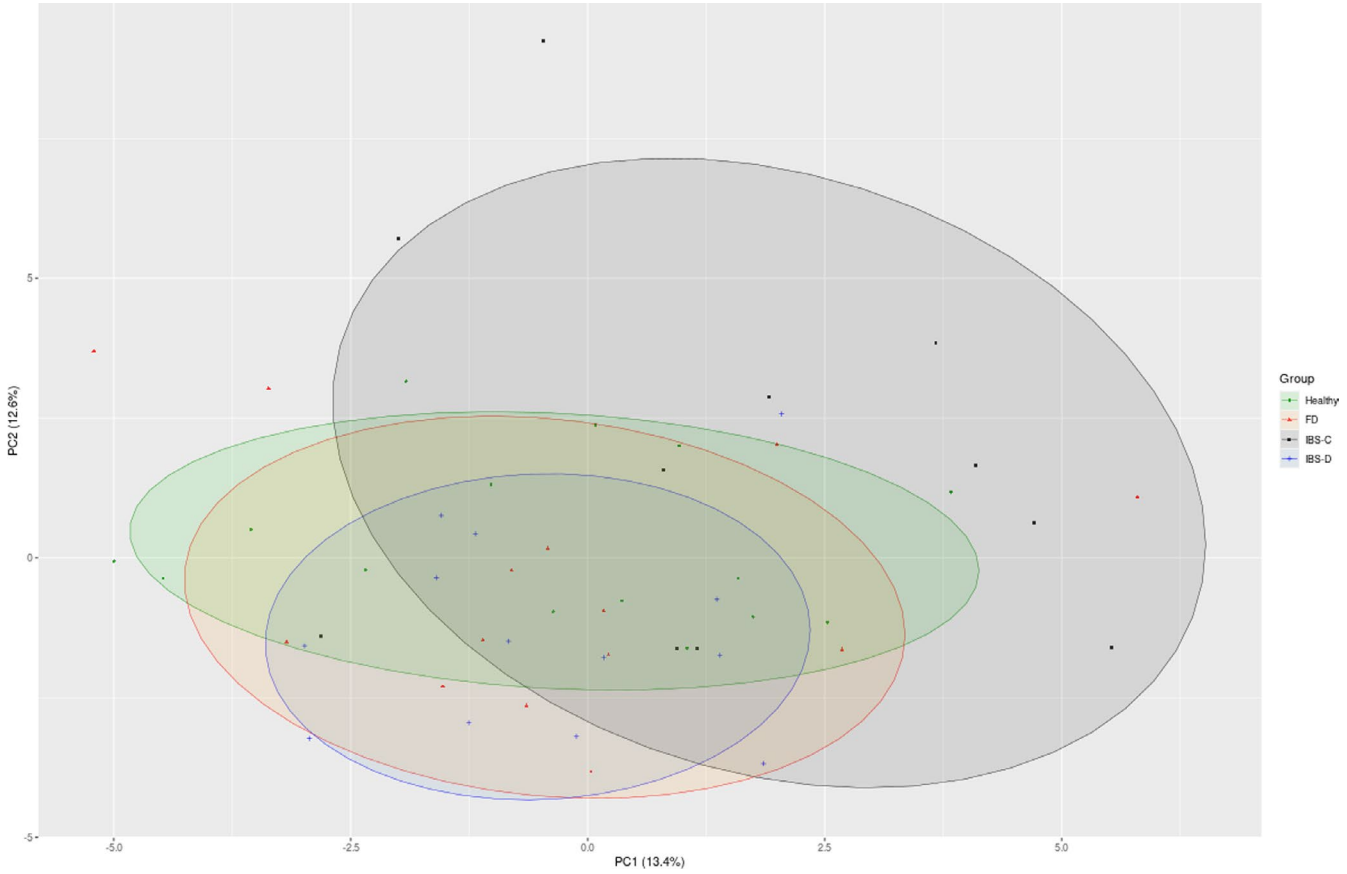

FIGURE 4 PCA score plot showing the differences in global bacterial community structures between the groups (IBS-C ($n = 11$), IBS-D ($n = 13$), FD ($n = 14$) and healthy controls ($n = 15$)). The ellipses cover approximately 80% of the scores for each group

antibodies. This is in contrast with a previous study of Pimentel et al.,[7] that reported increased plasma levels of these antibodies in IBS-D patients compared to IBD patients and healthy subjects. A further study by Rezaie et al.[8] described a gradual increase in the prevalence of anti-CdtB and anti-vinculin positivity rates according to IBS subtypes, being highest in patients with diarrhoea-predominant IBS (IBS-D and IBS-M) and lowest in subjects with constipation (IBS-C) and healthy controls. The most likely explanation for this discrepancy is the selection of our patient cohort in Germany, differing in environment, lifestyle, nutritional habits and 'microbial world' compared to the previously studied patient populations. Gut microbiota structures vary depending on geographic location of the host and on the related differences of environmental factors.[20] In some of previous studies with a positive association of the serological antibacterial antibodies, the group of patients with IBS-D was overrepresented compared to other IBS groups and healthy controls (i.e., 2375 patients in the IBS-D group vs. 25 patients with IBS-M, 30 patients with IBS-C and 43 healthy controls[8]).

A study from Australia reported findings similar to ours.[21] Using two cohorts, one community population-based ($n = 331$) and one outpatient clinic-based ($n = 460$) Talley et al. found no significant differences in levels of both anti-CdtB and anti-vinculin biomarkers between IBS patients and healthy controls, between IBS-D and IBS-C individuals and between IBS and FD groups. Interestingly, authors of that study found that patients with FD and IBS/FD overlap presented with higher levels of anti-CdtB compared to healthy subjects. To further investigate the possible association of anti-CdtB with post-infectious, FD we extended our analysis to a group of patients presenting with dyspepsia. However, we did not detect increased anti-CdtB antibodies levels nor did we find bacterial profiles of patients with FD that were distinct from those of healthy controls.

Analysis of changes in the composition and function of the gut microbiota in patients with IBS compared to normal healthy subjects leave us with inconsistent results. A meta-analysis on 24 studies published up till 2018 highlighted the lack of consistency between findings and the relevant differences in methodologies used for microbiota analysis.[22] Most frequent findings considered being of relevance in IBS were pointing out dysbiotic changes consisting in decreased biodiversity of microbial communities,[23–27] reduction in relative abundancies of uncultured Clostridiales,[28-30] *Faecalibacterium*[23,30] and *Bifidobacterium*[24,29,31] and increase in relative abundancies of potentially harmful bacteria, such as Proteobacteria,[23,30] Enterobacteriaceae (e.g.,

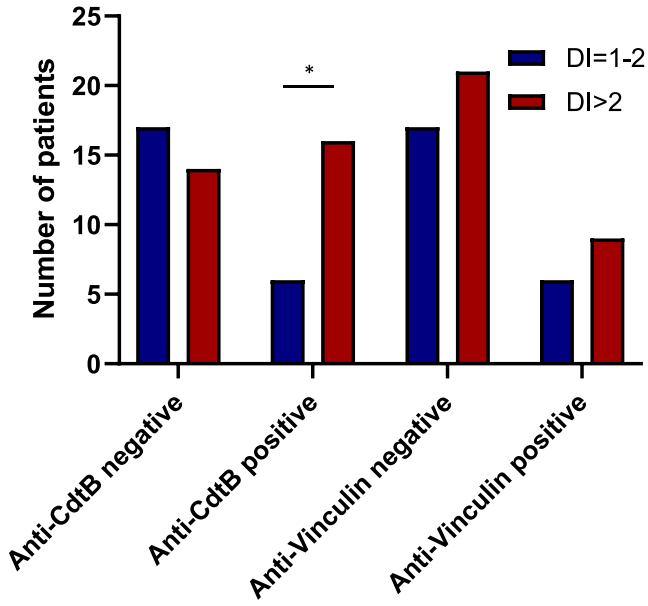

FIGURE 5 Distribution of the Dysbiosis Index (DI) among individuals according to serological status of anti-CdtB and anti-vinculin antibodies. Antibody positivity was defined according to the cut-off values proposed by Pimentel et al.[6] * $p < .05$

Escherichia, *Shigella*, *Campylobacter* and *Salmonella*),[23,32] *Streptococcus*[23,33] and *Bacteroides*[24,31,34] in patients with IBS compared to healthy individuals. None of these previous studies could, however, distinguish different IBS subtypes from healthy controls on the ground of specific microbial signatures both in mucosal and in luminal samples.[22,35] In our study, there was great overlap among IBS groups and healthy controls. Even moderate/severe dysbiosis (DI >2) were found with similar prevalence in both patients and healthy subjects. Only patients with IBS-C showed a trend for higher scores of DI compared to controls, even though this difference did not reach statistical significance, likely due to the small size of the cohort. These findings are in contrast with previous reports, where dysbiosis frequency in IBS cases was around 73% and 16% in healthy subjects,[15] and might be explained by the different inclusion criteria adopted and by the different geographical locations. Dysbiosis Index as general tool for the diagnosis of FGID cannot find clinical application unless regional, cultural, dietetic and lifestyle factors are taken into consideration. This suggests the need for local validation of DI for defined populations.

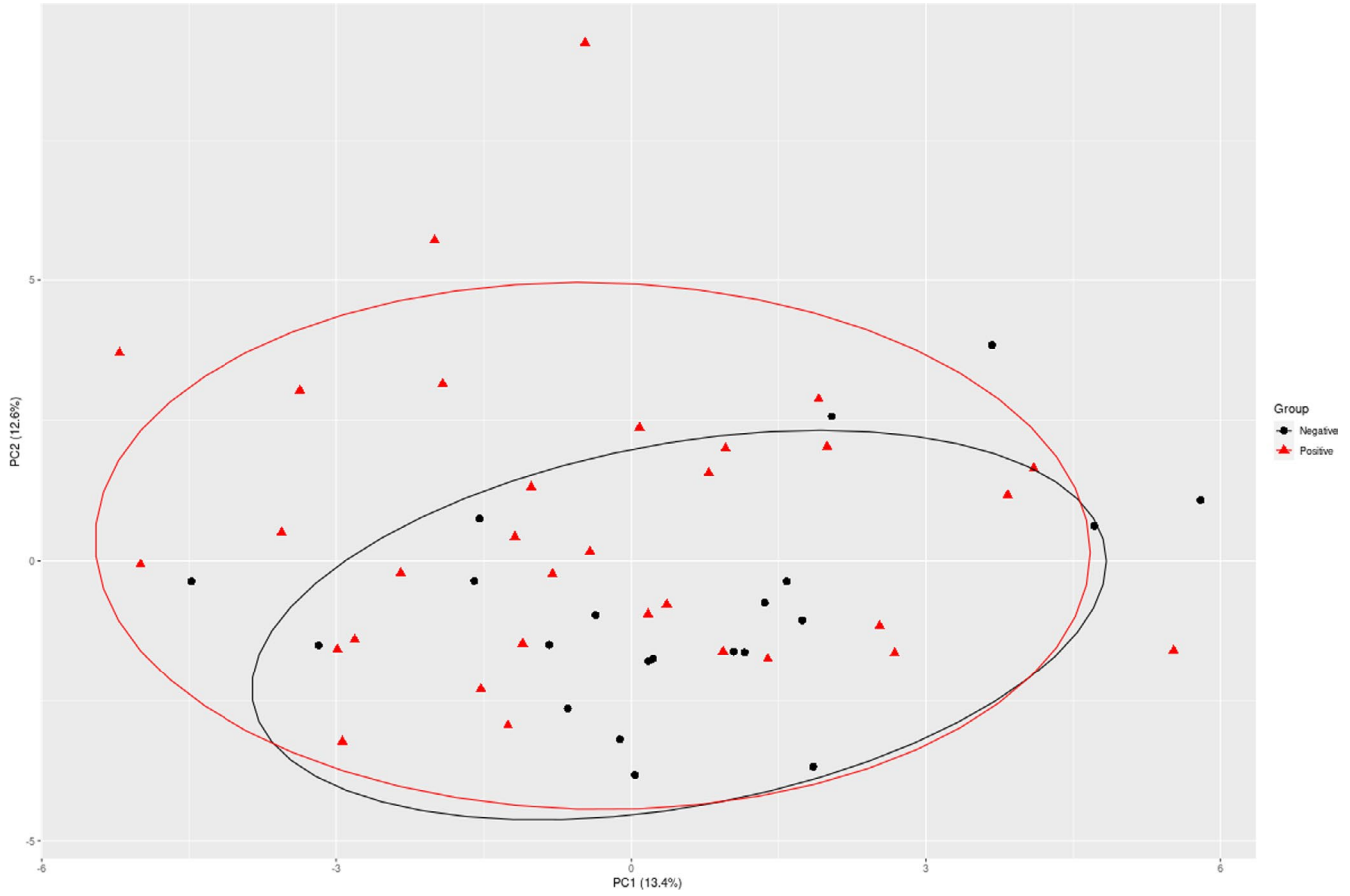

FIGURE 6 PCA score plot showing the differences in global bacterial community structures between individuals positive for anti-CdtB or anti-vinculin antibodies (Ab-positive, $n = 32$) and individuals negative for both antibodies (Ab-negative, $n = 21$)

This is the first study that analysed the microbial profiles of patients with FD and subforms of IBS in their correlation with specific microbial antibodies serum markers. In patients positive for anti-CdtB antibodies, severe dysbiosis was more frequent and bacterial profiles were characterized by impaired functions including essential butyrate production (*Eubacterium* spp., *Faecalibacterium prausnitzii*) and gut mucosa protection (*Akkermansia muciniphila*, *Faecalibacterium prausnitzii*). Similar alterations were reported from other authors.[22] This also goes along with a recent meta-analysis including 15 studies that reports reduction in butyrate and propionate levels in patients with IBS-C.[36] The role of SCFAs in gut barrier protection and gastrointestinal motility is well documented and has important implications with the pathogenesis of IBS.[37] Our results indicate that functional bacterial profiles leading to severe DI were associated with anti-CdtB antibodies and characterized by an increase in relative abundancies of pro-inflammatory and opportunistic bacteria (e.g.,. *Salmonella*, *Escherichia coli*, and *Shigella*). Several of these bacteria are able to produce CdtB and considered to possess a possible trigger function in post-infectious-IBS.[6,38]

There is an important limitation of our study due to the small number of patients in each cohort, which, with the further reduction of numbers for stratification of patients, leads to an underpowered statistical analysis. The small number of IBS cases in our exploratory study, compared with the large cohorts in two previous studies,[7,8] carries the risk of a potential sampling bias. This, independently of the geographical location where our study was carried out, and of similar findings to ours reported from another world region,[21] does not allow to draw definitive conclusions on the role of anti-CdtB and anti-vinculin as biomarkers in IBS. Nevertheless, even though patient numbers were low, our findings indicate high inter-individual variations in each subgroup with IBS. This indication suggests that patterns of microbial signatures, combined with specific antibacterial antibodies in plasma as used in our approach, are not significantly contributing to the allocation of the individual patient to a subgroup of FGID.

Large-scale prospective studies will help to better define the role of dysbiosis following episodes of gastroenteritis in the pathogenesis of FGID. Future studies may also benefit from extending the analysis to other members of the gut microbiota, such as fungi and bacteriophages, that may also play an important role in FGID pathogenesis, as recent evidences have shown.[39,40]

In conclusion, we found a high inter-individual variation of faecal microbiota profiles and selected serum antibodies. This precludes the identification of clear distinctive microbial signatures and the discrimination of FGID subgroups on the basis of bacterial markers. Analysis of faecal microbiota profiles and functions should be therefore at present cautiously interpreted for FGID subgroup definitions. On the horizon, their role appears a promising tool within the personalized medicine approach and deserves to be intensively studied whether it can provide useful information for tailored treatments to modulate the gut microbiota in the individual patient with FGID.

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CONFLICT OF INTEREST

The authors have nothing to disclose. CC, GTK, KHK are employees of Genetic Analysis AS. CC and KHK own stocks and shares in Genetic Analysis AS.

AUTHOR CONTRIBUTIONS

KS, CS and PM performed the study design. KS and PM obtained funding and supervised the study procedures. RV, MS, CS and PM drafted the manuscript. RV, CS and KS recruited suitable subjects, collected samples and registered data. CC, GTK, KHK, MS performed laboratory workup, NGS analysis, bioinformatic and statistical analyses. RV, CS, MS, LM, CC, GTK, KHK, KS and PM interpreted the data. PM is the guarantor of the study. All authors red and approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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