Treatment, follow-up and microbiota in acute diverticulitis

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ABSTRACT

Background
Disease-specific variations in intestinal microbiome composition have been found for a number of intestinal disorders, but little is known about diverticulitis. The purpose of this study was to compare the fecal microbiota of diverticulitis patients with control subjects from a general gastroenterological practice and to investigate the feasibility of predictive diagnostics based on complex microbiota data.

Methods
Thirty-one patients with computed tomography (CT)-proven left-sided uncomplicated acute diverticulitis were included and compared with 25 control subjects evaluated for a range of gastrointestinal indications. A high-throughput polymerase chain reaction (PCR)-based profiling technique (IS-pro) was performed on DNA isolates from baseline fecal samples. Differences in bacterial phylum abundance and diversity (Shannon index) of the resulting profiles were assessed by conventional statistics. Dissimilarity in microbiome composition was analyzed with principal coordinate analysis (PCoA) based on cosine distance measures. To develop a prediction model for the diagnosis of diverticulitis, we used crossvalidated partial least squares discriminant analysis (PLSDA).

Results
Firmicutes/Bacteroidetes ratios and Proteobacteria load were comparable among patients and controls ($P = 0.20$). The Shannon index indicated a higher diversity in diverticulitis for Proteobacteria ($P < 0.00002$) and all phyla combined ($P = 0.002$). PCoA based on Proteobacteria profiles resulted in visually separate clusters of patients and controls. The diagnostic accuracy of the cross-validated PLS-DA regression model was 84%. The most discriminative species derived largely from the family Enterobacteriaceae.

Conclusion
Diverticulitis patients have a higher diversity of fecal microbiota than controls from a mixed population, with the phylum Proteobacteria defining the difference. The analysis of intestinal microbiota offers a novel way to diagnose diverticulitis.
INTRODUCTION

The human endogenous intestinal microbiota is known to play a fundamental role in health and disease. Functions of the commensal gut flora include protection against direct epithelial cell injury, regulation of host fat storage as one of many metabolic functions, stimulation of intestinal angiogenesis, and influencing the development and function of the gut immune system.

Nucleic acid sequencing methods have undergone tremendous developments and have provided a major advance in the culture-independent analysis of the intestinal microbiota. However, these techniques are typically laborious and expensive for application on small batches of samples, as is common in clinical practice. Profiling techniques are a cheap and reliable alternative. We have recently validated and optimized a specific profiling technique termed IS-pro for human intestinal microbiome analysis. It has proved to be a highly reproducible method suitable for high-throughput profiling of the human intestinal microbiota.

With molecular techniques, it has been shown that the intestine harbors a complex bacterial community that consists largely (>95%) of two bacterial phyla, the Bacteroidetes and the Firmicutes. Molecular genetics research suggests that, at the level of the individual, the colonic microbiota may consist of up to 5,000 different bacterial species. The composition seems to be relatively stable over time and is more or less conserved throughout the colonic tract. Between individuals, however, the composition is highly variable. There are also differences between mucosal and fecal communities. Disease specific variations in the composition of the colonic microbiota have been identified, for example, in inflammatory bowel disease (IBD) and metabolic syndrome. Furthermore, specific bacterial species have been found to infiltrate the epithelium and submucosa in acute appendicitis.

Diverticulitis disease (DD) patients have also been hypothesized to harbor a change in colonic flora that promotes disease and inflammation, due either to altering the immune process or by permitting an abnormal response to potentially harmful bacteria. DD is a common condition in Western countries and is defined as symptomatic disease associated with colonic diverticula. Diverticula are outpocketings of the colonic mucosa and submucosa through weaknesses of muscle layers in the colon wall. Acute diverticulitis develops in 10–25% of individuals with diverticula and imposes an impressive clinical and socioeconomic burden on health care resources. Currently, we lack a clear understanding of the pathophysiologic mechanisms responsible for the progression from diverticulosis to diverticulitis. Theories are now shifting away from the traditional dogma that posits fecalith obstruction of a diverticulum to cause acute diverticulitis towards a view in which microbiota may play a central role. However, neither a diverticulitis-specific microbiome nor a single causative microorganism has yet been found. Characterization
of the colonic microbiota composition is the first step in elucidating their possible role in the etiopathogenesis of DD and its inflammatory complications.

The aim of our study was to characterize the fecal microbiota by means of IS-pro7 in patients with a first episode of uncomplicated acute diverticulitis and compare these to the microbiota of controls. The identification of a diverticulitis specific microbial composition could lead to clinical application of this technique in diagnosing disease. No published data on species composition during a first episode of uncomplicated acute diverticulitis are available yet.

MATERIALS AND METHODS

Study design
This study was ancillary to the “DIABOLO Trial: A multicenter randomized clinical trial investigating the cost-effectiveness of treatment strategies with or without antibiotics for uncomplicated acute diverticulitis”, which was approved by the Medical Ethics Committee, Academic Medical Center, Amsterdam, The Netherlands (no. 2009_233), and registered at ClinicalTrials.gov (no. NCT01111253). We carried out this prospective cohort study in three of the 22 participating centers (one academic and two teaching hospitals), based on practical grounds and logistics.

Subjects
Eligible diverticulitis patients were consecutive trial subjects from the three included centers aged 18 years or older with a first episode of acute left-sided uncomplicated modified Hinchey 1A or 1B diverticulitis demonstrated by computed tomography (CT). Included patients were recruited between August 2011 and September 2012. Informed consent was obtained from these trial subjects.

The control subjects were derived from an existing database of a mixed population of adult patients evaluated in another academic hospital for a range of gastrointestinal complaints, notably with no diagnosis of diverticulitis. Diverticulosis is a common finding at colonoscopy, with a prevalence of DD that increases with age from less than 10 % in people younger than 40 years old to 50–66% in octogenarians. The lifetime risk to develop diverticulitis is less than 25% in these patients. Possibly, a continuum in the microbiota composition exists in patients with diverticulosis and diverticulitis. To incorporate the possibility to distinguish mild diverticulitis from diverticulosis, the control group also included patients with diverticulosis.

The indications for and/or the diagnoses after colonoscopy in the control subjects were as follows: follow-up after polypectomy (n=1), anemia e.c.i. (n=1), benign neoplasm (n=3), malignant neoplasm (n=1), Morbus Crohn (n=4), ulcerative colitis (n=2), indeterminate colitis (n=1), irritable bowel syndrome (n=2), abdominal pain e.c.i. (n=1), surveillance for familial cancer susceptibility (n=3), and diverticulosis (n=6).
Rectal swabs

In the diverticulitis patients, sampling by means of a rectal swab (FLOQSwabs 552C, Copan, Murrieta, CA, USA) was performed at presentation to the emergency ward, prior to starting antibiotics when allocated to this treatment. The control subjects had their rectal swab taken prior to colonoscopy, which was performed to evaluate their gastrointestinal complaints or for other indications. Rectal swabs were inserted into the anal canal, beyond the anal verge (±3 cm). Subsequently, the tips of the swabs were gathered in sterile containers with 1 ml of reduced transport fluid (RTF) medium\(^\text{26}\) and stored at −20 °C within 2 h of collection.

DNA isolation

After thawing of the samples, total DNA extraction was performed on all samples with the NucliSENS® easyMag® automated DNA isolation machine (bioMérieux, Marcy l’Etoile, France). One milliliter of nucliSENS® lysis buffer, containing guanidine thiocyanate, was added to each vial containing a swab tip and the mixture was shaken at 1,400 rpm (Thermomixer comfort, Eppendorf, Hamburg, Germany) for 5 min. Afterwards, all samples were centrifuged for 4 min at 12,000g and added to the easyMag container. DNA extraction was performed on the easyMag machine with the Specific A protocol as described by the manufacturer. DNA was eluted in 110 μl of buffer and stored at 4 °C until use for polymerase chain reaction (PCR) amplification.

IS-profiling of the intestinal microbiota

The amplification of IS-regions was performed with the IS-pro assay (IS-Diagnostics, Amsterdam, the Netherlands). IS-pro involves bacterial species differentiation by the length of the 16S–23S rDNA interspace region with taxonomic classification by phylum-specific fluorescent labeling of PCR primers. Essentially, the IS-pro procedure consists of two multiplex PCRs: a first PCR for the phyla Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, and Verrucomicrobia, and a second PCR for the phylum Proteobacteria.

The assay was performed according to the protocol provided by the manufacturer. Amplifications were carried out on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). After PCR, 5 μl of PCR product was mixed with 19.8 μl of formamide and 0.2 μl of MapMarker 1000 ROX-labeled size marker (BioVentures, Murfreesboro, TN, USA). DNA fragment analysis was performed on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems). The results are presented as color labeled peak profiles (Figure 1).
Figure 1 | Heat map of all profiles sorted and colored by phylum. When profiles are clustered by the total profile, it can be seen that there is a separation between profiles from diverticulitis patients and controls. Proteobacteria profiles can be seen to generally harbor more species in diverticulitis patients than in controls.
Data analysis

Log2 transformation and phylum abundance

All data were pre-processed with the IS-pro proprietary software suite (IS-Diagnostics, Amsterdam, the Netherlands). This process resulted in profiles consisting of a set of 1,071 peaks with a specific length, measured in nucleotides, reflecting the lengths of IS fragments, and a specific height, measured in relative fluorescence units (RFU), reflecting the quantity of PCR product. In order to further analyze the data, we considered each peak in a profile as an operational taxonomic unit (OTU) and its corresponding intensity as its abundance. All intensities were log2 transformed. Log2 transformation of complex profiles compacts the range of variation in peak heights, reducing the dominance of high peaks and including less abundant species of the microbiota in downstream analyses. This results in improved consistency of the estimated correlation coefficient, lower impact of inter-run variation, and improved detection of less prominent species. This conversion was used in all downstream analyses, such as calculating within-sample and between-sample microbial diversity. A clustered heat map was made by generating a correlation matrix of all log2 transformed profile data followed by clustering with the unweighted pair group method with arithmetic mean (UPGMA).

Diversity analysis

Diversity was calculated both per phylum and for the overall microbial composition (by pooling all phyla together). The within-sample diversity was calculated as the Shannon index. Dissimilarities between samples, or between-sample diversity, was represented in a dissimilarity matrix that was built using the cosine distance measure. Given two vectors of attributes (two profiles in our case), A and B, the cosine dissimilarity is represented using a dot product and magnitude as:

\[
dissimilarity = 1 - \cos \theta = 1 - \frac{\sum_{i=1}^{n} A_i \times B_i}{\sqrt{\sum_{i=1}^{n} (A_i)^2} \times \sqrt{\sum_{i=1}^{n} (B_i)^2}}
\]

The resulting dissimilarity matrix was summarized and visualized in a low-dimensional space using principal coordinate analysis (PCoA). Diversity analysis was performed using the vegan software package in R.

Partial least squares discriminant analysis (PLS-DA)

A partial least squares discriminant analysis (PLS-DA) regression model was used for the prediction of the clinical status of samples, i.e., whether it belonged to a diverticulitis patient or to a control subject. The PLS-DA model was constructed on the basis of four different datasets: one for each of the three separate phylum groups and one for the overall microbial composition, by pooling all phyla. Only the top 25% most variable predictors were considered in the analysis.
PLS-DA model validation was carried out by a 10-fold cross validation procedure. In practice, the dataset was split into 90% of samples for model construction (i.e., the training set), with the aim to predict the other 10% (i.e., the test set). This procedure was repeated for ten iterations, where each sample served as a test sample exactly once. Accuracy rates, specificity, and sensitivity were computed for the samples that were used as a test set in every iteration, and the model predictive power was further assessed using a receiver operating characteristic (ROC) curve, a function of the true-positive rate (TPR or sensitivity) and false-positive rate (FPR or 1 – specificity).

PLS-DA provides a quantitative estimate of the discriminatory power of each descriptor by means of variable importance for the projection (VIP) parameters. VIP values rank the descriptors by their ability to discriminate different groups and are, therefore, considered an appropriate quantitative statistical parameter. We used the VIP criterion to rank the different OTUs based on their contribution to the response variable (clinical status, i.e., diverticulitis: yes or no) and PLS components. The OTUs with the highest contribution (VIP score > 1.2) were translated to the most likely bacterial species by comparison to a database consisting of > 1,500 bacterial species and their associated IS lengths. Finally, to assess whether prediction of the clinical status would be feasible with a set of specific qPCRs, we performed the same PLS-DA validation as mentioned above for a subset of the ten most discriminative OTUs (the ten OTUs with the highest VIP values).

PLS-DA analysis was performed using the DiscriMiner package in R (version 2.15.2). All data visualizations were performed with the Spotfire software package (TIBCO, Palo Alto, CA, USA).

Ethics
This study has been approved by the appropriate ethics committee and has, therefore, been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All persons gave their informed consent prior to their inclusion in the study.

RESULTS

Patient characteristics
Thirty-one patients diagnosed with a first episode of uncomplicated acute diverticulitis were included, of which 20 were males and 11 were females, with a mean age of 58 years (95% confidence interval (CI): 54–62). In the control group, a total of 25 subjects were included, comprising 12 males and 13 females, with a mean age of 53 years (95% CI: 47–59). The patients’ characteristics are listed in Table 1.
Table 1 | Demographic and baseline characteristics of diverticulitis patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Diverticulitis patients (N=31)</th>
<th>Control subjects (N=25)</th>
</tr>
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<tbody>
<tr>
<td>Sex [M]</td>
<td>20 (64.5%)</td>
<td>12 (48%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.8 (53.6-62.0)</td>
<td>52.6 (46.6-58.6)</td>
</tr>
<tr>
<td>ASA (I:II)</td>
<td>18 (58.1%) :13 (41.9%)</td>
<td>UK</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1 (25.7-28.5)</td>
<td>26.7 (24.0-29.6)</td>
</tr>
<tr>
<td>Duration of complaints† (days)</td>
<td>2 (1-3)</td>
<td>UK</td>
</tr>
<tr>
<td>Restricted oral intake</td>
<td>11 (35.5%)</td>
<td>UK</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>3 (9.7%)</td>
<td>UK</td>
</tr>
<tr>
<td>Temperature* (°C)</td>
<td>37.1 (36.8-37.4)</td>
<td>UK</td>
</tr>
<tr>
<td>CRP† (mg/dl)</td>
<td>89 (47.9-131.0)</td>
<td>UK</td>
</tr>
<tr>
<td>WBC* (11x10⁹/L)</td>
<td>11.9 (10.7-13.1)</td>
<td>UK</td>
</tr>
</tbody>
</table>

Abbreviations: ASA, American Society of Anesthesiologists (physical status classification); BMI, body mass index; UK, unknown; CRP, C-reactive protein; WBC, white blood cell (count).
*Data are means with 95% confidence intervals (CI);
†Data are medians with interquartile ranges.

Bacterial phylum abundance and profile clustering

The Firmicutes to Bacteroidetes ratio is commonly used to describe and characterize a dysbiosis of the gut microbiota. Since these two phyla are being amplified in the same PCR reaction, we could compare their relative abundance between patients and controls. The phylogenetic characterization of samples from control subjects revealed that Bacteroidetes represented 51% and Firmicutes 49% of the total abundance in the Firmicutes/Bacteroidetes PCR. Exactly the same proportions were found for the patient group. The total load of bacteria of the Proteobacteria phylum was relatively similar between patients and controls (10.2±1.9 log2 RFU and 10.1±2.0 log2 RFU for patients and controls, respectively; P = 0.20, Mann–Whitney U-test). A heatmap was generated from all IS-profiles separated by phylum. IS-profiles showed a general separation of samples from diverticulitis patients and controls when clustering was performed on the total profiles (Figure 1).
Microbial diversity and composition in diverticulitis patients versus controls

While the diversity of the phyla *Bacteroidetes* or *Firmicutes* did not differ between patients and controls, the Shannon index indicated that the diversity of the *Proteobacteria* phylum was significantly higher in patients compared to controls (2.6 [IQR: 1.07] and 3.2 [IQR: 0.5] for controls and patients respectively; $P < 0.00002$, Mann–Whitney U-test), which also affected the difference in diversity measured when considering all phyla together (3.9 [IQR: 0.3] and 4.1 [IQR: 0.3] for controls and patients, respectively; $P < 0.002$, Mann–Whitney U-test) (Figure 2).

PCoA did not segregate diverticulitis patients and controls into different groups for the phyla *Bacteroidetes* and *Firmicutes*. However, patients could be clustered separately from controls in a three-dimensional space based on their *Proteobacteria* profiles (Figure 3).
Figure 3 | Principle coordinate analysis (PCoA) scatterplot to express the between-sample diversity displays the clustering of diverticulitis patients separate from control subjects for the phylum Proteobacteria. Three samples that were wrongly classified by partial least squares discriminant analysis (PLS-DA) are encircled.

**Discriminative ability of PLS-DA**

The use of an unsupervised approach for classification (PCoA) already demonstrated the diagnostic potential of Proteobacteria profiles in predicting the health status of a given patient. This potential was born out in a supervised analysis, using PLS-DA, known to be suitable for high dimensional data.\textsuperscript{28,30,31} The PLS-DA model used 268 OTUs, representing the 25% most variable OTUs, as predictors and the clinical status of the samples (i.e., diverticulitis: yes or no) as the response variable. In order to quantify the discriminative ability of the model, we first considered the full datasets (three individual phylum datasets and one composed of all phyla). Taking the Bacteroidetes or Firmicutes data as input resulted in low predictive accuracy rates (55 and 53% for Bacteroidetes and Firmicutes, respectively; data not shown). Taking the Proteobacteria data as input resulted in a predictive accuracy rate of 95% (Figure 4). Three out of 56 samples were misclassified: one control and two patients, whose samples are the encircled ones in the...
PCoA scatterplot (Figure 3). The resulting specificity was thus calculated to be 96%, with a sensitivity of 94%. Taking the combined dataset, composed of all three phyla, as input resulted in an accuracy rate of 96% with two misclassified controls, corresponding to a specificity of 92% and 100% sensitivity. The misclassified controls were two subjects with diverticulosis. The most discriminative OTUs were found to derive largely from the family Enterobacteriaceae (Table 2).

Figure 4 | The PLS-DA scores plot for the phylum Proteobacteria shows a clear differentiation between diverticulitis patients and control subjects.
**Table 2** | Most discriminative OTUs based on a Variable Importance for Projection value > 1.2

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>Klebsiella varicola</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>Providencia stuartii</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>Desulfovibrio sp.</td>
<td>Desulfovibrionaceae</td>
</tr>
<tr>
<td>Xanthomonas sp.</td>
<td>Xanthomonadaceae</td>
</tr>
<tr>
<td>Stenotrophomonas</td>
<td>Xanthomonadaceae</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Pseudomonadaceae</td>
</tr>
<tr>
<td>Burkholderia sp.</td>
<td>Burkholderiaceae</td>
</tr>
<tr>
<td>Aggregatibacter actinomycetemcomitans</td>
<td>Pasteurellaceae</td>
</tr>
<tr>
<td>Unknown Proteobacteria species*</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

*Eleven types of unknown Proteobacteria species were identified

**Prediction of diverticulitis using the PLS-DA model**

The predictive ability of the model was assessed by crossvalidation. The prediction results were pooled together, which enabled us to estimate the performance of the model by means of the predictive power. Taking account of the Bacteroidetes or the Firmicutes phylum only resulted in a low predictive accuracy. For both the Bacteroidetes and the Firmicutes phyla, the cross-validated accuracy rate was 51 %. Considering only the Proteobacteria phylum, we reached a cross-validated accuracy rate of 80 %. Six controls and five patients were wrongly classified, which resulted in a specificity of 76 % and a sensitivity of 84 %. When we combined the three phyla, we could reach a diagnostic accuracy rate of 84 % (specificity = 80 %; sensitivity = 87 %). Figure 5 summarizes the predictive power of the PLS-DA model by means of ROC curves. To evaluate whether a set of specific qPCRs might be able to attain similar predictive power, we performed PLS-DA analysis on a subset of the ten most discriminative OTUs. Here, a specificity of 71 % and a sensitivity of 77 % was reached.
DISCUSSION

The results of our study suggest that the fecal microbiota diversity of patients with a first episode of acute uncomplicated left-sided diverticulitis differs significantly from control subjects from a general gastroenterological practice, with the Proteobacteria phylum mainly defining this difference. Furthermore, predictive diagnostics based on complex microbiota data seems feasible for diagnosing diverticulitis, with a diagnostic accuracy rate of 84%. The most discriminative species derived from the family Enterobacteriaceae. An approach based on a limited set of specific qPCRs is unlikely to attain the same diagnostic accuracy as IS-pro.

Several studies have identified characteristics of the intestinal microbiota that may be associated with disease, but clinical diagnostic tools based on microbiome analysis still need to be developed. Whereas most studies into microbiota composition in health and disease identified correlations, here, we demonstrate an approach in which microbiota composition may be used as a clinical predictor. By employing a supervised algorithm in combination with cross-validation, we show how microbiota analysis may move towards prediction instead of correlation. PLS regression provides a dimension reduction strategy in situations where a set of response variables needs to be related to a set of predictor variables. It is considered a supervised learning method, since it uses the dependent (clinical status in this study) as well as the independent variables (OTUs) to construct variable selection and importance ranking. PLS-DA refers to the particular case where the response variable is a set of binary variables describing the categories of a categorical variable, e.g., disease states. This model is commonly used in the field of chemo-metrics and in the analysis of microarray expression data, as it is especially suited to deal with...
a much larger number of predictors than observations and with multicollinearity. In this study, we encountered similar challenges; the number of OTUs is much larger than the number of samples, and some of them are highly correlated. Due to the properties mentioned above, we found that this approach is also very appropriate to apply to IS-pro data. The VIP criterion was previously used in PLS-DA microarray analyses to assess which genes were useful to discriminate between different groups.

Specific shifts in the phylum *Proteobacteria*—other than general measures like diversity—have not previously been found to be associated with disease. This might be caused by the fact that *Proteobacteria* generally have a low relative abundance in the intestinal microbiota. Because almost all current approaches to analyze the intestinal microbiota use universal bacterial amplification as a starting point, low abundant phyla such as the *Proteobacteria* remain relatively underexplored, as other, more prevalent taxa will dominate the PCR reaction and following analyses. In contrast, the IS-pro molecular technique comprises two separate phylum specific PCR reactions: one for the amplification of *Bacteroidetes/Firmicutes* and another for the specific amplification of *Proteobacteria*. While the separation of the different phyla in two PCRs prevents us from addressing all three phyla together when presenting their relative abundances—consequently hampering direct comparisons of abundances—it does allow us to zoom in and analyze the *Proteobacteria* community composition in depth.

Brook and Frazier retrospectively studied the aerobic and anaerobic microbiology of 110 specimens from the peritoneal cavity after intestinal perforation and in 22 specimens from abdominal abscesses of patients with complicated diverticulitis. With conventional culture techniques, they identified *E. coli* and *Streptococcus spp.* as the predominant aerobic and facultative bacteria. The most frequently isolated anaerobes were *Bacteroides spp.* (*B. fragilis* group), *Peptostreptococcus, Clostridium*, and *Fusobacterium spp.* The only study to date using PCR-based sequencing of the microbiota in diverticulitis patients was conducted by Gueimonde et al. They identified a significantly higher occurrence of *Bifidobacterium longum* and *Bifidobacterium animalis* in patients with diverticulitis, and their overall conclusion was that aberrances in mucosa-associated microbiota are present in different intestinal diseases. However, in their study, only nine diverticulitis patients were included. Resected mucosal samples were compared with those of 21 colon cancer patients and four inflammatory bowel disease patients, but no healthy controls. Surprisingly, they looked only at the genus *Bifidobacterium* and did not analyze the entire profile; they stated that they used the bifidobacterial microbiota as an indicator of alterations in the mucosal colonization pattern. The bifidobacterial microbiota however, is known to constitute only a small fraction of the intestinal microbial composition in adults.

Currently, antibiotics are often used in the conservative treatment of uncomplicated diverticulitis, despite the lack of sound evidence. Cyclic administration of rifaximin has been proven to be effective in reducing symptoms and complications and possibly prevents recurrence in patients after complicated diverticulitis. Relatively new therapies,
such as probiotic therapy, are proposed as well for the management of DD. Indeed, a few small open-label studies already show promising results.\textsuperscript{44-47} Considering that antibiotic and probiotic treatments are regularly prescribed to DD patients, it is striking that relatively few studies have been performed to improve our understanding of the composition of the colonic microbiota. The pathophysiology of diverticulitis was assumed to be clear and well understood, but, actually, astonishingly little is known about the causal factors for this disease. Our understanding of the effect of changes in microbiota abundance, diversity, and composition is limited. Our study, therefore, is a first step in further elucidating the etiopathogenesis of DD and its inflammatory complications.

Since a clinical diagnosis of diverticulitis cannot be made with a high certainty without imaging\textsuperscript{48}, it seems appropriate to evaluate a test intended for making a specific clinical diagnosis against a patient group with variable clinical presentation. By taking a cross-section of patients in a general gastroenterological practice instead of a healthy control group, the specificity of the prediction becomes more meaningful.

This study has some limitations. First, we have data on only a small study group. As a result, we are not able to estimate and optimize predictive ability robustly. The performance of a predictive tool is prone to be overestimated in its own study cohort. For diagnostics by microbiome to be applied in daily practice, a study like this one should be externally validated and followed by a larger study to confirm the results and calculate sensitivity and specificity more robustly. Second, as a consequence of a small sample size, we were not able to firmly compare diverticulitis patients with subjects with diverticulosis. It has been hypothesized that DD patients have a changed colonic microbiome. From an etiopathogenetic point of view, it would be informative to know to what extent the microbiome in diverticulosis resembles the microbiome in diverticulitis or health. Indeed, the two controls that were misclassified were subjects with diverticulosis. This seems to underline a shift in microbiota related to DD. It would be interesting to further investigate whether there is a gradual shift in microbiota composition from patients with diverticulosis towards diverticulitis. Such a phenomenon should be investigated in a larger study group. Further, it should be noted that species identification was done by in silico comparison of fragment lengths. While this technique generally gives consistent results, identification is not definitive.

The present study demonstrated that the diagnosis of diverticulitis can be done by microbiome analysis with relatively good accuracy. More generally, this study illustrates a proof of concept of how diagnostics based on complex microbiota data in a broader sense may be applied. This could lead to the use of fecal microbiota as a diagnostic tool for diverticulitis, with possible patient stratification directing a personalized treatment strategy, whether or not to prescribe antibiotics, the type of antibiotic, and even to monitor disease course. Clinical application as a diagnostic tool could reduce the need for imaging to diagnose diverticulitis. Clinical applicability needs to be confirmed in a larger study.
Acknowledgments
We thank all the physicians and nurses in the participating centers (Academic Medical Center, Amsterdam; Meander Medical Center, Amersfoort; and Kennemer Gasthuis Hospital, Haarlem) for the accrual of patients and performing rectal swabs. We thank Malieka Degen for performing DNA isolation and the IS-pro procedure on all fecal samples.
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