Microbiota and Flatography in Pediatric Gastrointestinal Disease

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2017

document version
Publisher's PDF, also known as Version of record

CHAPTER 10

Faecal gas analysis by electronic nose as novel, non-invasive method for assessment of active and quiescent paediatric inflammatory bowel disease: proof of principle study

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J Crohns Colitis. 2014 Sep 22. pii: S1873-9946(14)00285-2
doi: 10.1016/j.crohns.2014.09.004

ABSTRACT

Background and aims

Inflammatory bowel disease (IBD) and its two phenotypes ulcerative colitis (UC) and Crohn's disease (CD) are essentially assessed by endoscopy, both in initial diagnostic work-up as during follow-up. This carries a high burden, especially on paediatric patients. Faecal volatile organic compounds (VOCs) are considered potential non-invasive biomarkers for intestinal diseases linked to gut microbiota alterations. We hypothesized that faecal VOC analysis by electronic nose allows discrimination of children with CD, UC and controls during active disease and remission.

Methods

Faecal VOC patterns of children with newly diagnosed IBD and controls were studied by an electronic nose (Cyranose 320®), at baseline and upon achieving remission at 6-weeks follow-up. Disease activity was assessed by global physician's assessment, substantiated by serum C-reactive protein and faecal calprotectin. Internally cross-validated receiver-operator-characteristic curves and corresponding sensitivity and specificity for detection of IBD were calculated.

Results

Faecal VOC profiles of patients with UC (26) and CD (29) differed from controls (28); in active disease (AUC ± 95% CI, p-value, sensitivity, specificity: 1.00 ± 0.00; $p < 0.001$, 100%, 100%) and (0.85 ± 0.05, $p < 0.001$, 86%, 67%) and in clinical remission (0.94 ± 0.06, $p < 0.001$, 94%, 94%) and (0.94 ± 0.06, $p < 0.001$, 94%, 94%), respectively. Furthermore, CD-patients differed from UC-patients during active disease (0.96 ± 0.03; $p < 0.001$, 97%, 92%), and upon achieving clinical remission (0.81 ± 0.08, $p = 0.002$, 88%, 72%).

Conclusion

Faecal VOC analysis allowed discrimination of paediatric patients with IBD from controls, both during active disease and remission. It therefore has potential as non-invasive test, in both diagnostic work-up and assessment of disease activity in IBD.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic, relapsing inflammatory condition of the intestinal tract, compromising two main phenotypes; ulcerative colitis (UC) and Crohn's disease (CD). IBD typically develops in the teens or young adulthood and incidence has increased over the last decades, while age at presentation has shown a downward trend.[1-4] The aetiology of IBD is likely a complex interplay between genetic risk factors and environmental factors such as a disturbance of normal gut microbiota composition.[5,6] To date, endoscopic procedures remain essential in both initial diagnostic work-up as well as in follow-up of disease activity in established IBD. These invasive procedures carry a high burden on patients, especially so on children, since endoscopy in this population is performed under general anaesthesia and usually requires children to be admitted for intensive bowel preparation by nasogastric tube. Therefore, the search for novel, non-invasive biomarkers to monitor disease activity in (paediatric) IBD remains warranted. Various biomarkers, such as C-reactive protein and, more recently, faecal biomarkers like calprotectin and lactoferrin have been evaluated for this purpose.[7-10] In particular faecal calprotectin has shown high sensitivity for the detection of mucosal inflammation, but has only limited specificity for IBD, especially in children.[11] Children with IBD and their parents often report that flare-up of disease coincides with a characteristic smell of stool and flatus. The odour of faeces is caused by volatile organic compounds (VOC), being gaseous carbon-based molecules, mainly produced by the intestinal microbiota, which could therefore hypothetically serve as non-invasive biomarker for disease activity in IBD.[12,13] So far, faecal VOC analysis in IBD patients has exclusively been done in a limited number of studies on adults, all using gas chromatography and mass spectroscopy (GC-MS) which allows identification of individual chemical compounds.[12,14,15] Whilst these studies support the notion that faecal VOC may be of value as biomarkers for IBD, their implementation in clinical practice is hampered because GC-MS is an expensive, time-consuming technique requiring highly trained personnel.[16] In contrast, electronic nose technology allows high-throughput analysis of the complete spectrum of VOC by means of a user-friendly, handheld device. Such electronic nose (eNose) systems utilize an array of cross-reactive sensors that have a competitive interaction with the entire VOC mixture.[17] Likewise, single VOC can interact with multiple sensors. The polymers swell in response to this interaction, inducing a change in electrical resistance. The resulting pattern of firing sensors, or smellprint, is characteristic of the composite mixture of volatiles and can be compared with previously established profiles by pattern recognition algorithms. This technique does not identify individual chemical compounds

but can discriminate groups through probabilistic recognition of VOC-profiles.[17] The stages of this recognition process are in this way similar to human olfaction. We hypothesized that an eNose can discriminate between VOCs from the headspace of faeces of children with CD, UC and healthy controls. We studied this by comparing faecal VOC of children with newly diagnosed IBD, both during active disease and upon achieving clinical remission.

MATERIALS AND METHODS

Subjects and design

This case-control study included subjects between April 2010 and January 2013 from the tertiary hospitals VU University medical centre and Academic Medical Centre in Amsterdam, the Netherlands. Children were included if aged below 18 years and presenting with newly diagnosed, untreated UC or CD, based on the current diagnostic Porto-criteria for paediatric IBD.[18] Localization and behaviour of disease were classified according to the Paris Classification, retrospectively for those included prior to publication of this classification.[19] Exclusion criteria were proven infectious colitis during the last 3 months prior to inclusion, use of antibiotics, steroids or immunosuppressive therapy, all within the last 3 months prior to inclusion, a previous diagnosis of IBD or a diagnosis of IBD undetermined. Patients undergoing diagnostic ileocolonoscopy and esophagogastroduodenoscopy under suspicion of IBD, were instructed to collect a faecal sample prior to bowel preparation in a provided container, and store this in the freezing compartment of the common refrigerator at home. At time of diagnosis, disease activity was assessed by Physician Global Assessment (PGA) on a 4 point scale: severe, moderate, mild and quiescent.[20] All CD patients underwent a MRI enteroclysis to assess small bowel involvement. Additionally C-reactive protein (CRP) and faecal calprotectin (FC) levels were determined. If a diagnosis of UC or CD was established, patients were requested to collect a second faecal sample after 6 weeks. At this time assessment of PGA and CRP levels were repeated.

Patients were defined to be in clinical remission if PGA at 6 weeks was scored as quiescent. According to standard care guidelines, all children diagnosed with CD were primarily offered 6 weeks exclusive enteral nutrition (EEN) for the induction of remission. In case of reluctance, unresponsiveness, or intolerance to EEN, corticosteroids were prescribed instead (starting dose based on body weight, maximum of daily 40 mg orally followed by a regular tapering strategy). Thiopurines were routinely prescribed as maintenance immunosuppressive therapy

to all children with CD, started during EEN therapy. Children with UC were prescribed aminosalycilates for induction of remission, dependent on disease severity, combined with corticosteroids, and also as maintenance (mono)therapy. Controls were healthy children from primary schools in the Dutch provinces of Noord Holland and Overijssel, meeting similar exclusion criteria as the study group. Information on defecation pattern and stool consistency was obtained by means of a questionnaire. Each healthy subject was instructed to collect two faecal samples, with a one week interval. Both samples of the healthy controls were compared to VOC-profiles of the children with IBD, to decrease the risk of a type 1 error.

The study was approved by the University's Ethics Committee.

Faecal volatile biomarker analysis

After delivery to the hospital, faecal samples were stored at -20°C until they were randomly analysed by electronic nose. Faecal gas analysis was performed following the procedures as described in detail in a previous study on VOC-analysis by electronic nose.[21] In short, approximately 2 grams of faeces were thawed in a sealed container placed in a 37°C stove for one hour allowing the headspace to fill with faecal VOCs. The container was subsequently connected to the eNose via a closed-loop system preventing dilution of the faecal VOCs with ambient air. Firstly, a baseline reference signal was created by connection of a VOC-filter (A1, North Safety, NL) to the e-nose. Subsequently the headspace was sampled during sixty seconds, to establish a stable reference sensor signal. Following each sample analysis, sensors were purged with VOC-filtered air to flush away the left VOCs and establish a stable baseline for subsequent measurements. A Cyranose 320 e-nose® (Smiths Detections, Pasadena, CA, USA) was used for faecal VOC analysisThe Cyranose® 320 utilizes the NoseChip® array, comprising thirty-two polymer nanocomposite carbon sensors and advanced pattern recognition algorithms to recognize selected chemical compounds present in a gaseous sample.

Validation measurements

In order to evaluate potential effects on measured VOC-profiles of thawing and refreezing, of differences in temperature of faecal samples, two validation sessions were performed. Effects of thawing and refreezing on faecal VOC profiles were assessed by comparing intra-individual profiles of 5 healthy volunteers. VOC analysis was performed after each cycle of thawing (to room temperature) and refreezing (at -20°C), in total 4 cycles were done.

Effect of temperature on faecal VOC profiles was assessed by measuring VOCs of faecal samples from the same 5 volunteers after heating them to different temperatures (15°C, 20°C, 25°C, 30°C and 35°C).

Data analysis and sample size

A formal sample size estimation was not possible due to lack of previous studies differentiating faecal volatiles of IBD and controls. Based on the effects size of a previous study describing faecal biomarkers for colorectal carcinoma and advanced adenomas, we determined that a sample size of 14 in each group would have 80% power to detect a difference with a 0.05 two-sided significance level.[20]

Data were analysed using SPSS statistics 22 (IBM). Demographic data were compared by chi-square test or independent t-test where appropriate. The variance of the raw sensor data was recombined into a set of principal components (PC) by principal component analysis (PCA). An independent t-test was performed to assess the discriminative potential of these PC with respect to the study groups. Selected PC were subsequently used in a internally cross-validated canonical discriminant analysis based on the leave on out method. The resulting cross-validated probabilities were used to construct a Receiver Operator Characteristic (ROC) curve providing sensitivity, specificity, area under the curve and positive and negative predictive values for the algorithm. As additional validation we determined our p-value by creating 1000 random distributions of cases and controls. The factors discriminating between the original cases and controls were used to discriminate these random distributions. The percentage of these random distributions reaching a similar or greater accuracy than that found with our primary classification (Crohn's Disease/ Ulcerative Colitis/ Healthy Controls) can be interpreted as our validated p-value. Sensor outcomes of the validation studies were compared by Mixed Model analysis.

RESULTS

Patient population

A total of 83 children (29 CD, 26 UC, 28 age-matched controls) met the inclusion criteria and were included, together collecting 153 faecal samples for VOC analysis by electronic nose. Patient characteristics are depicted in Table 10.1.

Table 10.1 Subject characteristics

	Healthy controls	Inflammatory Bowel Disease	
	(n = 28)	Ulcerative Colitis (n = 26)	Crohn's Disease (n = 29)
Age, yr (Median [IQR])	10.1 [6.9]	13.6 [4.6]	13.8 [1.9]
Sex, % ♂	38	65	58
Physician Global Assessment			
Quiescent		0	0
Mild		2	1
Moderate		6	9
Severe		18	19
Location and behavior			
Ulcerative Colitis			
Proctitis	N.A.	2	
Left-sided colitis	N.A.	4	
Extensive	N.A.	20	
Crohn's Disease			
Ileal (L1)	N.A.		1
Colonic (L2)	N.A.		9
Ileocolonic (L3)	N.A.		19
Esophagogastric disease (L4a)	N.A.		6
Jejunal/proximal ileal (L4b)	N.A.		2
Non-stricture or penetrate (B1)	N.A.		25
Stricturing (B2)	N.A.		2
Penetrating (B3)	N.A.		2
Calprotectin (ug/g) (Median[IQR])			
Baseline	N.A.	1272 [1625]	1545 [845]
CRP (mg/l) (Median[IQR])			
Baseline	N.A.	5.5 [43]	34 [46]
Remission at 6 weeks*	N.A.	3 [1]	3 [10]
Medication			
Induction of remission			
Exclusive Enteral Nutrition	N.A.	0	27
Corticosteroids	N.A.	16	2
Aminosalycilates	N.A.	29	0
Maintenance			
Thiopurines	N.A.	0	28
Aminosalycilates	N.A.	26	0

Values were obtained at inclusion. Localization of disease was determined by ileocolonoscopy and esophagogastroduodenoscopy prior to treatment initiation and MR enteroclysis.
* Only for cases in clinical remission.
IQR = Inter-quartile range; N.A. = not applicable.

Clinical remission of active disease was achieved at six weeks follow-up in 20/26 (77%) and 17/29 (59%) children, suffering from CU and CD respectively. Of six UC patients not in clinical remission, three children showed no clinical improvement at all and three showed improvement of 2 points on the PGA scale, at six weeks follow up. Of twelve CD children not achieving clinical remission at six weeks follow-up, three children had similar PGA scores compared to baseline, seven children improved 1 point and two children 2 points on the PGA scale respectively.

During the study period, none of the children underwent intestinal surgery.

The control group consisted of 28 healthy subjects, 19 out of 28 provided a second faecal sample after one week. None of these healthy individuals started any medication or reported a change in general health situation during the week between collection of both samples. None of the healthy controls had diarrhoea in the last 3 months prior to inclusion.

Volatile biomarker analysis

Validation

The freezing and thawing of faecal samples up to 4 times did not influence the sensor outcome (p-values ranging from 0.31 to 0.96). Likewise, there were no significant changes in sensor response when faecal samples were measured at temperatures between 15°C and 35°C (p-values ranging from 0.36 till 0.84).

Outcome

Using internal cross-validated canonical discriminant analysis, faecal VOC profiles of patients with UC differed from healthy controls, both during presentation with active disease (AUC ± 95% CI, p-value, sensitivity, specificity: 1.00 ± 0.00; $p < 0.001$, 100%, 100%), and in clinical remission (0.94 ± 0.06; $p < 0.001$, 94%, 94%) (Figures 10.1, 10.2). Likewise, subjects with CD could be discriminated from healthy controls both during active disease (0.85 ± 0.05 $p < 0.001$, 86%, 67%) and in clinical remission (0.94 ± 0.06 $p < 0.001$, 94%, 94%) (Figure 10.3). Furthermore, patients with CD differed from subjects with UC, both during active disease (0.96 ± 0.03; $p < 0.001$, 97%, 92%) and in clinical remission (0.81 ± 0.08, $p = 0.002$, 88%, 72%) (Figure 10.4). Details for these algorithms can be found in Table 10.2.

Using the second stool sample from controls for comparison reproduced these outcomes. An exception was the discrimination of healthy controls from patients with CD in remission,

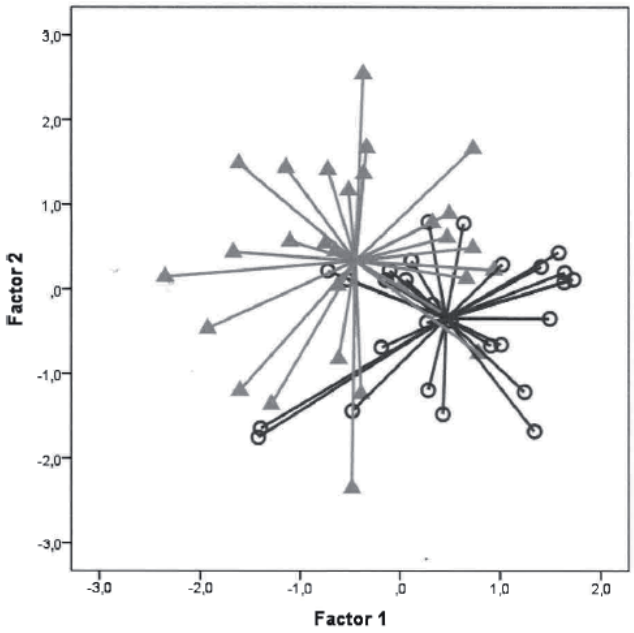

Figure 10.1 Scatterplot for the discrimination of patients with ulcerative colitis at presentation (full triangles) and healthy controls (empty circles), by electronic nose.
Axes depict two orthogonal linear recombination of the original 32 sensor data by means of principal component analysis.

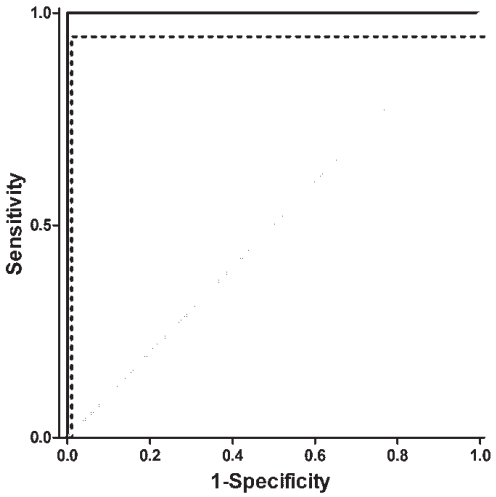

Figure 10.2 Receiver operator characteristics curve for the discrimination of ulcerative colitis and healthy controls at first presentation (solid line) and upon achieving remission (dashed line), by electronic nose.
The areas under the curve ± 95% confidence interval (AUC ± 95% CI) with associated p-value were: first presentation 1.00 ± 0.00, < 0.001; remission 0.94 ± 0.05, < 0.001.

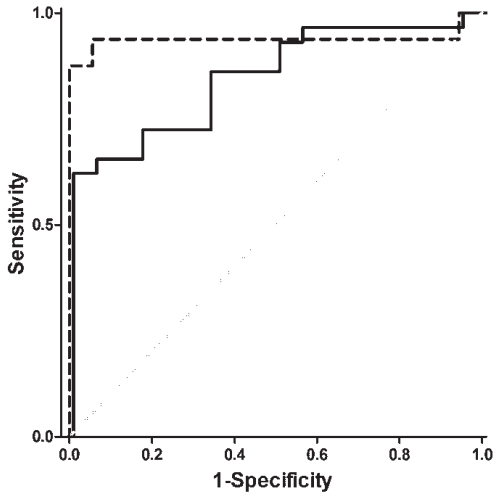

Figure 10.3 Receiver operator characteristics curve for the discrimination of Crohn's disease and healthy controls at first presentation (solid line) and upon achieving remission (dashed line), by electronic nose.
The areas under the curve ± 95% confidence interval (AUC ± 95% CI) with associated p-value were: first presentation 0.85 ± 0.05, < 0.001; remission 0.94 ± 0.06, < 0.001.

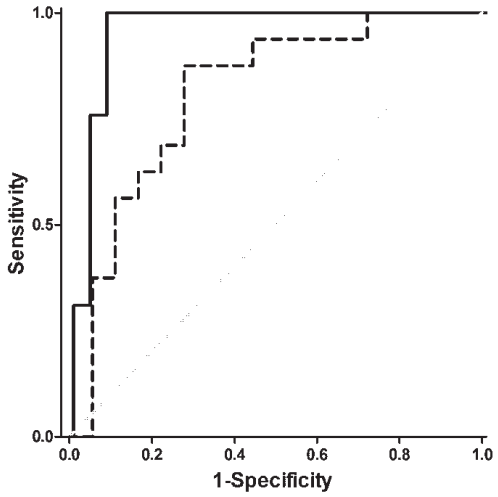

Figure 10.4 Receiver operator characteristics curve for the discrimination of ulcerative colitis and Crohn's disease at first presentation (solid line) and upon achieving remission (dashed line), by electronic nose.
The areas under the curve ± 95% confidence interval (AUC ± 95% CI) with associated p-value were: first presentation 0.96 ± 0.03, < 0.001; remission 0.81 ± 0.08, 0.004.

Table 10.2 Performance characteristics for the discrimination of Ulcerative Colitis, Crohn's Disease and Healthy Controls by fecal VOC analysis

	AUC ± 95% CI	p-value	Sensitivity	Specificity	+ LR	- LR
Distinguishing Ulcerative Colitis from healthy controls						
Active disease	1.00 ± 0.00	< 0.001	100	100	N.A	N.A.
Remission	0.94 ± 0.05	< 0.001	94.4	94.4	16.86	0.06
Distinguishing Crohn's Disease from healthy controls						
Active disease	0.85 ± 0.05	< 0.001	86.2	66.7	2.59	0.21
Remission	0.94 ± 0.06	< 0.001	93.8	94.4	16.75	0.07
Distinguishing Ulcerative Colitis from Crohn's Disease						
Active disease	0.96 ± 0.03	< 0.001	96.6	92.0	12.08	0.04
Remission	0.81 ± 0.08	0.002	87.5	72.2	16.86	0.06

Sensitivities, specificities, positive and negative likelihood ratios are reported for the respective optimum cut-points. Remission was defined as a physician global assessment value of 0. Abbreviations: AUC ± 95% CI = Area Under the Curve with 95% Confidence Interval; + LR = positive likelihood ratio; - LR = negative likelihood ratio.

which decreased significantly to (0.75 ± 0.06; p = 0.005) albeit differences in VOC-profiles remained significant. Details of analysis with the second stool sample of controls are included in Supplemental Table S10.1.

Post-hoc analysis was performed to gain insight whether VOC-profiles associated with disease activity in CD and UC. We did so in two ways. Firstly, we compared intra-individual VOC-course, from active disease upon achieving remission at six weeks follow-up (cases not meeting remission criteria were excluded from this analysis) We found that patients with CU (n = 20) did not and patients with CD (n = 18) did have a significant change in faecal volatile profile; p = 0.085 and p = 0.03 respectively. Interpretation of these findings is however complex as we also observed a significant change over six weeks time in faecal volatiles of healthy controls; p < 0.001. Secondly, we aimed to compare VOC-profiles of patients whom did achieve remission at six weeks follow-up with those who did not. For UC, the subgroup of 6 cases not achieving remission was considered too small for valid statistical analysis. For CD, we found these two groups could not be discriminated (0.68 ± 0.10 p < 0.104, 58.3%, 56.3%). Additional explorative analysis on samples of 12 CD patients not achieving remission revealed that patients with PGA score 2 at six weeks follow-up more closely resembled those in remission, as compared to children with PGA-scores 3 or 4 at this time-point. Numbers were, however, too small to make any definitive statements.

For both children with UC and CD, CRP levels at baseline and six weeks follow-up, and FC baseline levels are depicted in Supplemental Figures S10.1 and S10.2, respectively. Baseline CRP and FCP could not differentiate between CU and CD (0.65 ± 0.08, p = 0.064. 71.4%, 60.0%) and (0.53 ± 0.08, p = 0.67, 53.6%, 52.0%) respectively. These values did not improve by combining both tests (0.60 ± 0.08, p = 0.21, 77.8%, 47.2%). Furthermore, CRP level at six weeks follow-up could also not differentiate between CU and CD (0.67 ± 0.10, p = 0.88, 64.7%, 82.4%).

DISCUSSION

In the present study, we showed that faecal volatile biomarker profiles of UC and CD could be differentiated from healthy controls, both during active disease as well as in clinical remission. Furthermore, UC and CD could be distinguished from each other, both at primary presentation of disease and upon achieving clinical remission.

The assessment of disease activity in IBD by analysis of VOC profiles in the headspace of faeces has been performed in few studies so far, all employing GC-MS.[12,14,15] The first study compared faecal VOC profiles of 18 UC patients, showing a decreased number of different VOCs in UC, but with high numbers of alkenes and very low numbers of nitrogen-containing compounds, as compared to controls.[12] In a recent study, VOC profiles of diarrhoea-predominant irritable bowel syndrome (IBS-D) could well be distinguished from active CD, UC and healthy controls with a sensitivity of 94%, 96% and 90%; and a specificity of 82%, 80% and 80%, respectively.[14] Elevated concentrations of esters and alcohol derivates of short-chain fatty acids and indole were detected in patients with CD as compared to UC-patients and controls, but no statistically significant differences were observed between UC and controls. Just after two weeks of treatment, VOC profiles of CD-patients closely resembled those of healthy subjects.[15]

This is the first study that assessed the potential of the electronic nose to discriminate between faecal volatile biomarkers of children with IBD and healthy controls, both at time of first presentation, before any medical treatment was commenced, and in clinical remission.

Presented results were comparable with the reported sensitivity of 97.8% for the non-invasive faecal biomarker calprotectin (FC) in a recent meta-analysis on the diagnostic accuracy of FC in children undergoing primary investigation for suspected IBD.[22] In the present study, however, specificity of faecal gas analysis for active IBD was higher, as compared to 68%

specificity of FC for IBD.[22] FC reflects mucosal inflammation and does therefore not allow for proper discrimination between different phenotypes of IBD and also between IBD and other causes of colitis, like infectious colitis. Presented results showed that VOC profiling by eNose technology could firmly distinguish UC and CD. Since faecal VOC composition in infectious colitis is likely to be different from IBD, secondary to alterations in intestinal microbiota, it could be hypothesized that the eNose method might also be useful for non-invasive diagnostics of different types of colitis.

Observed differences in VOC profiles as measured by eNose can in part be explained by volatiles identified by GC-MS in previous studies on faecal VOCs in IBD. Since gut microbiota produces most of faecal volatiles, it is likely that a substantially part of these and other differentiating volatiles originated from differences in faecal microbiota composition between paediatric patients with CD and UC, as reported in previous microbiological studies.[5,12,23] This may probably also explain why analysis of faecal VOCs provided greater accuracy with respect to differentiation of subjects with active IBD and healthy controls, when compared to reported test characteristics of breath and urine volatiles in terms of specificity.[24-28]

In contrast to the study performed by Walton on VOC, our data suggested that discrimination of patients and controls reached a similar accuracy whether or not subjects were in clinical remission.[15] This apparent discrepancy may be explained by the fact that Walton and co-workers selected their differentiating compounds (alcohol derivatives and short chain fatty acids) in comparison of diseased and healthy subjects. Their observation that these selected volatile compounds moved towards profiles as found in healthy individuals supported the assumption that composition of VOCs reflect disease activity. However, different VOCs than the selected compounds might still differentiate between IBD patients in clinical remission and healthy controls. Because the eNose assesses the entire composite mixture of VOCs, rather than individual volatiles as with GC-MS, this technique was likely to also detect VOCs outside this selection. Observed differences in VOC profiles between children in clinical remission and controls may, however, also have been generated by the prescribed therapeutic agents, directly or by manipulation of the gut microbiota.[29,30]

We observed fluctuations in VOC-profiles of healthy controls over a six-weeks period, possibly reflecting alterations in intestinal microbiota metabolism due to day-to-day variability in dietary intake next to volatiles deriving from ingested food. Despite these physiological fluctuations, controls could consistently be discriminated from patients with IBD irrespective of the disease activity in the IBD patients and of the chosen control sample.

Strikingly, faecal volatiles of patients with IBD changed less than those of controls over six weeks time, despite the change in disease activity and start of medication. Furthermore, patients with CD, but not CU, showed a significant change in faecal volatile profile over six weeks time, from active disease towards remission state. These findings correspond with observations done in a study on microbiota profiling in paediatric IBD, describing significant changes in microbial composition between children with active and inactive CD over time, but not in CU.[5]

Those CD patients whom did not achieve remission could not be discriminated from those who did. This may in part be explained by the clinical heterogeneity of patients not in remission at six weeks follow-up (1 patient PGA score 4; 5 PGA score 3; 6 PGA score 2), in combination with the relatively low numbers. Explorative analysis revealed that VOC profiles of patients not in remission and with lower PGA scores more closely resembled those in remission than those with higher PGA scores, although numbers were too small to draw firm conclusions. Based on these findings one could speculate that a core volatile profile relates to the presence of IBD, irrespective of disease activity. This may help to explain a lower VOC variability between different disease states and may explain the discrimination of cases and controls both during active disease and remission. Studying this hypothesis will require longer follow-up and detailed analysis of the responsible volatile compounds through chemical analytical techniques.

Strength of the current study is the fact that patients with newly diagnosed IBD were studied prospectively, both at their first presentation and over time. Therefore, we were able to assess faecal volatiles profiles at diagnosis, before interference of therapeutic agents, dietary or lifestyle interventions. Besides these implicit strengths our study also carries flaws. The sample size did not allow external validation. To deal with this limitation, we used strict internal validation by the leave one out method. Presented results need to be externally confirmed in future studies, preferably performed in an age-matched intention to diagnose population adhering to international guidelines.[31] Another limitation is that FC levels were only available at baseline but not at six weeks follow-up to substantiate PGA scores in the assessment of disease activity.

Our results suggest that analysis of faecal volatile biomarkers by electronic nose is a promising technique for non-invasive diagnosis of IBD. Because of the apparently high specificity for newly diagnosed IBD, this method may help to limit the needed number of endoscopies in a subset of children suspected for IBD, which is not feasible by FC.[32]

Furthermore, this technique potentially allows disease monitoring, especially in CD, since significant changes in VOC profiles were observed over time in this subgroup, from active disease towards remission. Because of the ease of use and low costs of eNose analysis, these techniques may even be used as a at-home or desktop tool in the future.

In conclusion, faecal gas analysis by electronic nose allowed discrimination of children with newly diagnosed ulcerative colitis, Crohn's disease and healthy controls, both at first presentation of (active) disease and after achieving remission. As such, this technique seems to hold promise as novel, non-invasive technique for the diagnosis and, potentially, monitoring of disease-activity in paediatric IBD, once properly validated in an "intention-to-diagnose" population. Non-invasive diagnostics based on faecal volatile biomarkers may simplify IBD diagnostics, and opens perspectives of (frequent) home-monitoring of disease activity.

Acknowledgements

This study was in part financially supported by an unrestricted grant from Falks Benelux BV.

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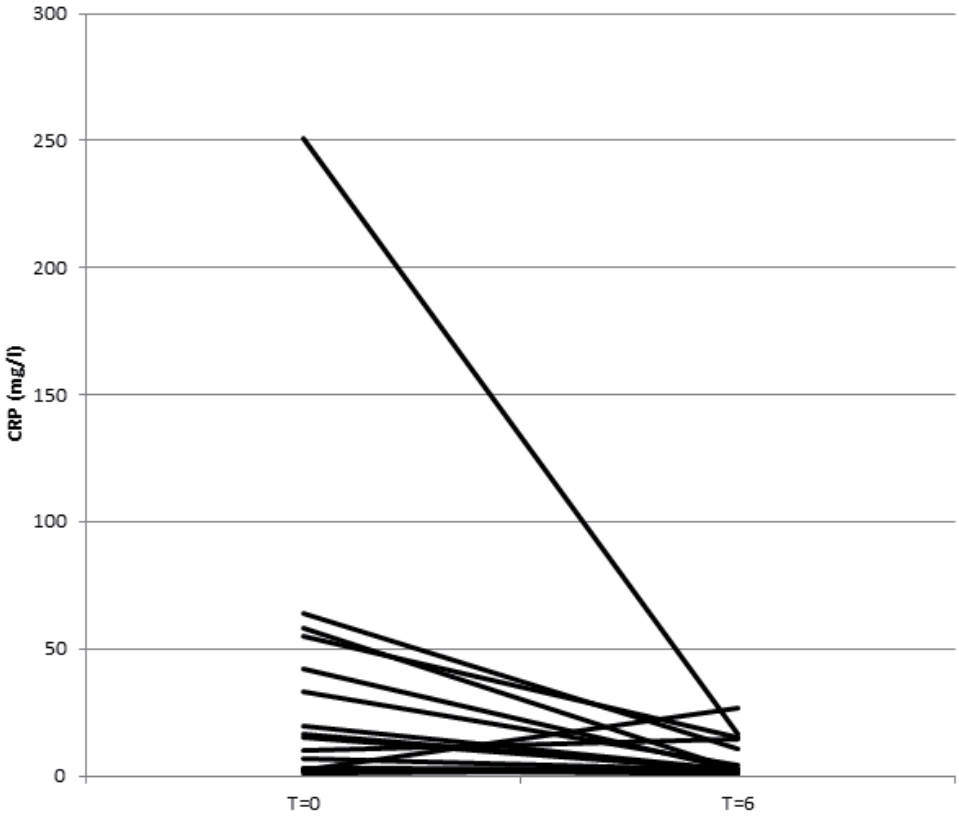
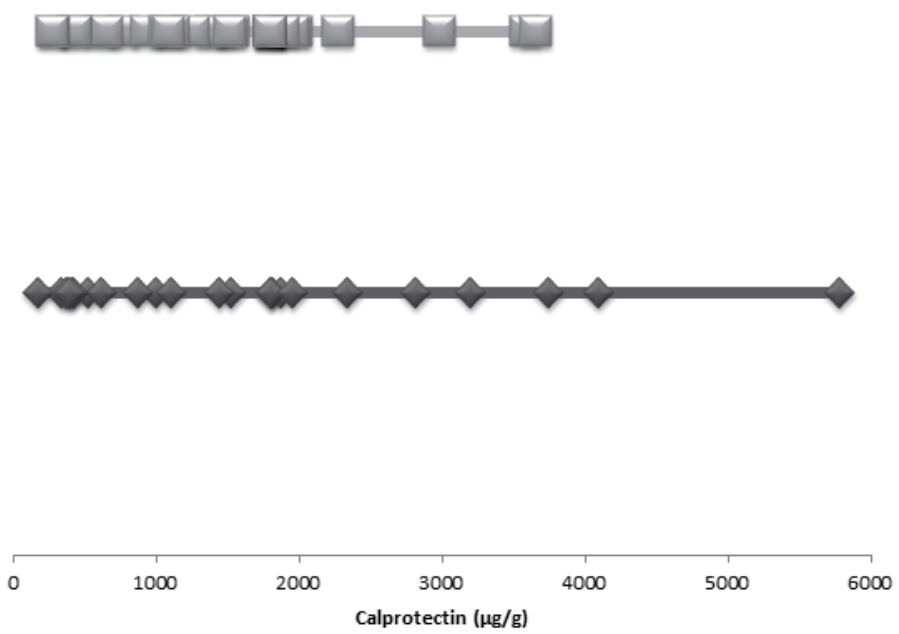
SUPPLEMENTAL MATERIAL



Supplemental Figure S10.1 Intra-individual course of C-reactive protein (CRP) levels (mg/l) in IBD cases, from active disease at presentation (T0) to six weeks follow-up.
Majority of patients showed a decrease in CRP levels over time, although baseline levels were only modestly elevated at T0: 16/26 CU and 12/29 CD patients had values < 10 mg/l at this timepoint.



Supplemental Figure S10.2 Faecal calprotectin (FC) levels (ug/g) at presentation for CD (upper) and CU patients, showing a substantial overlap.
Based on FC levels, CU and CD patients could not be distinguished.

Supplemental Table S10.1 Performance characteristics for the discrimination of Ulcerative Colitis, Crohn's Disease and Healthy Controls second sample by faecal VOC analysis

	AUC ± 95% CI	p-value	Sensitivity	Specificity	+ LR	- LR
Distinguishing Ulcerative Colitis from healthy controls						
Active disease	1.00 ± 0.00	< 0.001	100	100	N.A	N.A.
Remission	0.98 ± 0.01	< 0.001	94.4	89.3	8.82	0.06
Distinguishing Crohn's Disease from healthy controls						
Active disease	0.83 ± 0.05	< 0.001	86.2	64.3	2.41	0.21
Remission	0.75 ± 0.08	0.005	75.0	60.7	1.91	0.41

Sensitivities, specificities, positive and negative likelihood ratios are reported for the respective optimum cut-points. Remission was defined as a physician global assessment value of 0. AUC ± 95% CI = Area Under the Curve with 95% Confidence Interval; + LR = positive likelihood ratio; - LR = negative likelihood ratio.