CHAPTER 9

Sniffing out paediatric gastrointestinal diseases: the potential of volatile organic compounds as biomarkers for disease

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ABSTRACT

The diagnostic work-up and follow-up of paediatric functional gastrointestinal disorders and organic conditions usually includes invasive tests, carrying a high burden on patients. There is a place, therefore, for novel, noninvasive disease-specific biomarkers. Volatile organic compounds (VOCs), originating from (patho)physiological metabolic processes in the human body, are excreted as waste products through all conceivable bodily excrements. The spectrum of VOCs harbours a magnificent source of information, with the potential to serve as noninvasive diagnostic biomarkers and to monitor disease activity. VOC analysis has been studied in children and infants with a variety of gastro-intestinal diseases, including inflammatory bowel disease, liver diseases, irritable bowel syndrome, necrotising enterocolitis and infectious diarrhoea. Most of these studies, although limited in sample size, show that patients can be discriminated from controls based on their VOC profiles, underscoring the potential of VOC analysis in diagnosis and follow-up. Currently, however, the application of VOC analysis in clinical practice is limited; substantial challenges, including methodological, biological and analytical problems, still need to be met. In this review we provide an overview of the available literature on the potential of VOCs as biomarkers for paediatric gastro-intestinal diseases. We discuss the available techniques to analyse VOCs and provide topics for VOC-related research, which need to be addressed before VOC diagnostics can be implemented in daily clinical practice.
INTRODUCTION

In Western medicine, the earliest description of a diagnostic test based on odour originates from Hippocrates, who noted in his Aphorisms that the sputum of patients with tuberculosis spreads a characteristic, foul smell when poured upon hot coals.\(^1\) Over the past decades, there has been a rapidly increasing interest in the potential of analysis of the responsible molecules (volatile organic compounds (VOC)) as diagnostic biomarker of diseases, including malignancies, infections and metabolic conditions. This can largely be attributed to the development of nanotechnology-based sensors enabling fast, point-of-care analysis of the VOCs present in complex gaseous mixtures.\(^2\) VOCs are carbon-based chemicals, typically in volatile state at ambient temperature, and originating from physiological and pathophysiological metabolic processes in the human body. They may have a local, systemic or exogenous origin.\(^3\) Exogenous VOCs originate from nonhuman sources and are absorbed by the body, e.g. through skin, lung alveoli or mucosa. Researchers have characterized the VOCs of a broad range of human samples, identifying over 1840 different compounds.\(^4\)

As VOCs are excreted as waste products through all conceivable bodily excrements (e.g. breath, sweat, urine and faeces), they can be analysed by noninvasive techniques.\(^5,6\) The VOC pool harbours a magnificent source of information with the potential to serve as noninvasive diagnostic biomarkers and as markers of disease activity.\(^6,7\) Additionally, VOC analysis may increase understanding of the pathophysiological metabolic pathways underlying diseases, possibly leading to new therapeutic strategies.\(^8\)

Currently, diagnostic work-up and follow-up of paediatric diseases frequently requires a myriad of invasive, time-consuming and expensive tests, carrying a high physical burden on the patients as well as economic consequences for the health system.\(^8,14\) The need for novel, noninvasive, disease-specific biomarkers particularly exists for the paediatric population; the research into paediatric applications of volatile biomarkers is consequently emerging. In this review we provide an overview of the literature regarding the potential of VOCs as biomarkers in paediatric gastro-intestinal diseases. Furthermore, we will discuss the available techniques for the analysis of VOCs and the challenges to be overcome before widespread implementation of VOC-based diagnostics in daily clinical practice is feasible.
ANALYTICAL TECHNIQUES

The available VOC detection techniques can roughly be divided into two categories: 1) chemical analytical techniques; and 2) electronic devices using pattern-based recognition algorithms. A detailed evaluation of all available methods within these two categories would be beyond the scope of this review. We will focus on the devices applied in the reviewed studies.15

Chemical analytical techniques, like gas chromatography-mass spectrometry (GC-MS), can identify VOCs in a complex mixture based on their physiochemical properties. GC-MS is currently the most commonly used technique for VOC analysis.4 Limitations of this technique include the need for intensively trained personnel to perform the complex (statistical) analyses and labour intensiveness, since the samples need to undergo condensation and desorption prior to analysis.16 In contrast, selected ion flow tube-mass spectrometry (SIFT-MS) allows for real-time analysis of selected compounds, does not need pre-analysis preparations, is easier in use and can provide results within seconds.16 SIFT-MS involves ionization of VOCs, that are subsequently detected downstream by quadruple mass spectrometry and an ion-counting system.17 Purchasing analytical devices like SIFT-MS and GC-MS is relatively expensive, > €200,000 and > €150,000, respectively.4 GC-MS is available in many general and academic hospital laboratories, as the technique is widely used for both clinical and research purposes.

At the other end of the spectrum are electronic devices (‘eNose’), with costs < € 40,000, containing an array of different gas sensors and deploying pattern recognition algorithms for the discrimination of VOC combinations.4 Electronic nose technology enables real-time, high-throughput analysis of the complete spectrum of VOCs in complex gaseous mixtures. The VOCs in a given mixture interact with a matrix of eNose sensors, influencing a measurable attribute of each individual sensor, such as electrical resistance or oscillation frequency. Since eNose sensors do not identify individual chemical compounds, the specificity of this approach is generally lower compared to other VOC detection techniques. As they are cheap in purchase and use as well as small and mobile, however, they are suited as point-of-care tools, which is not the case for classical chemical analytical techniques. In addition, they can be learned to recognise different pathologic conditions.6

So while chemical analytical techniques are expensive, but provide robust and reproducible results, eNose devices carry the promise to perform adequate for clinical purposes at...
an affordable price. Recent research efforts have focused on the development of eNose sensors with a coating that specifically interacts with target compounds, or employing miniaturise classic chemical analytical techniques such as (field asymmetric) ion mobility spectrometry. These developments aim at combining optimal sensor accuracy with user friendliness at minimal cost.

VOCS IN PAEDIATRIC GASTROINTESTINAL DISEASES

In the following paragraphs, the available evidence will be discussed regarding the potential of VOC analysis in children and infants with inflammatory bowel disease (IBD), liver disease, irritable bowel syndrome (IBS), necrotising enterocolitis (NEC) and infectious diarrhoea. Table 9.1 provides an overview of available studies.

INFLAMMATORY BOWEL DISEASE

IBD is a chronic, relapsing inflammatory condition of the intestinal tract, the two main phenotypes being ulcerative colitis (UC) and Crohn’s disease (CD). Over the past years, worldwide incidence rates of paediatric IBD have shown an upward trend, whereas the presenting age is decreasing. While there are numerous studies on VOC analysis in adult IBD, so far only two studies have focused on the potential of VOCs in diagnosing and monitoring IBD in the paediatric population.

Patel and colleagues aimed to identify a unique breath VOC pattern allowing to differentiate between paediatric IBD patients and healthy controls by means of SIFT-MS. Exhaled breath samples of 62 paediatric IBD patients (51 CD, 11 UC) were compared with those of 55 healthy controls. Six VOCs differed significantly between the two groups: 1-octene, 1-decene, (E)-2-nonene, 1-nonene, 3-methylhexane and hydrogen sulphide. Assumedly gut microbiome dysbiosis, which has been found in several studies, explains the VOC pattern in the diseased group. For example, it has been shown that hydrogen sulphide is produced by Escherichia coli and Enterococcus faecalis, species that have been observed in higher concentrations in the mucosa of IBD patients. Yet, the origin of the majority of VOCs remains unclear. Furthermore, the study could not discriminate between UC and CD based on exhaled VOCs and no correlation was observed between VOC composition and disease activity. This latter might be due to the small sample size; only 20% of patients had active disease upon inclusion. Another limitation of the study was that none of the
Table 9.1 Overview of the available evidence regarding the potential of volatile organic compounds analysis in paediatric gastrointestinal diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Study (ref.)</th>
<th>Study group cases/controls</th>
<th>Method</th>
<th>Sample medium</th>
<th>Main outcomes</th>
<th>Study groups</th>
<th>VOCs used in ROC analysis</th>
<th>P; AUC (±95% CI or range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBD</td>
<td>de Meij et al (9)</td>
<td>55 (26 UC, 29 CD)/28</td>
<td>eNose</td>
<td>Faeces</td>
<td>UC vs control: - active</td>
<td>Not applicable</td>
<td>P &lt; 0.001; AUC 1.00 (±0.00)</td>
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<td></td>
<td>CD vs control: - active</td>
<td>Not applicable</td>
<td>P &lt; 0.001; AUC 0.94 (±0.05)</td>
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<td></td>
<td></td>
<td></td>
<td>Remission</td>
<td>Not applicable</td>
<td>P &lt; 0.001; AUC 0.85 (±0.05)</td>
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<td></td>
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<td></td>
<td>UC vs CD: - active</td>
<td>Not applicable</td>
<td>P &lt; 0.001; AUC 0.94 (±0.06)</td>
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<td></td>
<td></td>
<td>Remission</td>
<td>Not applicable</td>
<td>P &lt; 0.001; AUC 0.96 (±0.03)</td>
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<td></td>
<td></td>
<td>UC vs CD: - active</td>
<td>Not applicable</td>
<td>P &lt; 0.001; AUC 0.94 (±0.06)</td>
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<td></td>
<td></td>
<td></td>
<td>CD vs control: - active</td>
<td>Not applicable</td>
<td>P &lt; 0.001; AUC 0.96 (±0.06)</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Remission</td>
<td>Not applicable</td>
<td>P = 0.02; AUC 0.77 (±0.21)</td>
</tr>
<tr>
<td></td>
<td>Patel et al (13)</td>
<td>62 (11 UC, 51 CD)/55</td>
<td>SIFT-MS</td>
<td>Breath</td>
<td>IBD vs control</td>
<td>Three different VOCs</td>
<td>P &lt; 0.001; AUC 0.96 (±0.03–0.99)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Garner et al (12)</td>
<td>6/7</td>
<td>GC-MS</td>
<td>Faeces</td>
<td>NEC vs control: - overall, training set</td>
<td>Three different VOCs</td>
<td>P &gt; 0.05</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td>NEC vs control: - overall, training set</td>
<td>Six different VOCs</td>
<td>AUC 0.83</td>
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<td></td>
<td></td>
<td>NEC vs control: - overall, training set</td>
<td>Six different VOCs</td>
<td>AUC 0.78–0.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>de Meij et al (10)</td>
<td>13/14</td>
<td>eNose</td>
<td>Faeces</td>
<td>NEC vs control: - overall, training set</td>
<td>Not applicable</td>
<td>P &lt; 0.001; AUC 0.99 (±0.04)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NEC vs control: - overall, training set</td>
<td>Not applicable</td>
<td>P = 0.02; AUC 0.77 (±0.21)</td>
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</tr>
</tbody>
</table>
### Liver disease

<table>
<thead>
<tr>
<th>Condition</th>
<th>Study</th>
<th>Liver Failure Cases</th>
<th>Stool/Breath Test</th>
<th>Comparison</th>
<th>VOCs Used</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAFLD</td>
<td>Alkhouri et al (8)</td>
<td>37/23</td>
<td>SIFT-MS Breath</td>
<td>-NAFLD vs control; validation set</td>
<td>Four different VOCs</td>
<td>AUC 0.763</td>
</tr>
<tr>
<td>NASH</td>
<td>Okwu et al (19)</td>
<td>22/74</td>
<td>SIFT-MS Breath</td>
<td>-NASH vs control; training set</td>
<td>Two different VOCs</td>
<td>AUC 0.73</td>
</tr>
<tr>
<td>CLD</td>
<td>Eng et al (11)</td>
<td>49/55</td>
<td>SIFT-MS Breath</td>
<td>-CLD vs control; training set</td>
<td>Five different VOCs</td>
<td>AUC 0.97</td>
</tr>
<tr>
<td>Acute diarrhoea</td>
<td>Al-Kateb and Cunliffe (20)</td>
<td>27/26</td>
<td>GC-MS Faeces</td>
<td>Rotavirus infected vs noninfected</td>
<td>Not applicable</td>
<td>'significant'</td>
</tr>
<tr>
<td></td>
<td>Poulton and Tarlow (21)</td>
<td>10/13</td>
<td>Human smell</td>
<td>Rotavirus infected vs noninfected</td>
<td>Not applicable</td>
<td>P &lt; 0.009</td>
</tr>
<tr>
<td></td>
<td>Pashbert et al (22)</td>
<td>38/6</td>
<td>GC-MS Faeces</td>
<td>Rotavirus infected vs noninfected</td>
<td>Not applicable</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>IBS</td>
<td>Patel et al (14)</td>
<td>22/55</td>
<td>SIFT-MS Breath</td>
<td>IBS vs control; training set</td>
<td>Four different VOCs</td>
<td>AUC 0.99</td>
</tr>
</tbody>
</table>

AUC = area under the curve; CD = Crohn disease; CLD = chronic liver disease; eNose = electronic nose; GC-MS = gas chromatography-mass spectrometry; IBD = inflammatory bowel disease; IBS = irritable bowel syndrome; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; NEC = necrotizing enterocolitis; ROC = receiver operating characteristics; SIFT-MS = selected ion flow tube-mass spectrometry; UC = ulcerative colitis; VOCs = volatile organic compounds.
patients were treatment-naïve at inclusion, leaving the possibility open that the exhaled VOC profile was biased by the use of medication.

In a recent study using an eNose device for faecal VOC analysis, newly diagnosed treatment-naïve paediatric IBD patients (29 CD, 26 UC) could be discriminated from 28 matched healthy controls. Interestingly, UC patients could also be discriminated from CD with high accuracy. This was both true for active disease (area under the curve (AUC) = 0.96) and for clinical remission after six weeks follow-up (AUC = 0.81). It is tempting to hypothesise that this method is helpful in cases where diagnosis is unclear, such as IBD-unclassified, leading to improved, personalised care for this subgroup. An interesting observation in this study was that after 6 weeks faecal VOC profiles of CD children had changed from active disease towards clinical remission state, while those from UC children remained unchanged despite the change in disease activity and initiation of medication. Interestingly, these observations correspond with the microbial changes in IBD-subjects with active and inactive disease. Even in healthy children, however, VOC profiles did undergo statistically significant changes over periods as short as one week, illustrating the difficulty of linking VOC changes in IBD to changes in disease activity. As nearly fifty percent of the entire stool VOC composition can be assigned to the diet, both directly by VOCs originating from diet components and indirectly as a result of bacterial metabolism, VOC changes in healthy children may be attributable to the day-to-day variation in dietary intake. This emphasises that information on the diet is pivotal when comparing faecal VOC profiles between cohorts, in order to minimise the risk of type I errors.

Both studies show that VOC profiling holds promise as a noninvasive tool in diagnosing and monitoring paediatric IBD, with faecal, but not exhaled VOCs providing information differentiating UC from CD. Supposedly, faecal samples harbour more local and exogenous (derived from the resident microbiome) VOCs than exhaled breath, which predominantly harbours VOCs of systemic origin.

While presently VOC analysis cannot replace the currently used tools in the diagnostic work-up of IBD, it might improve their performance. The commonly used noninvasive biomarker faecal calprotectin is characterised by high sensitivity for mucosal inflammation (97%), but a relatively low specificity (70%) for paediatric IBD as the cause of this inflammation. Faecal VOC profiling has a reported pooled sensitivity of 86% and specificity of 85% for IBD. Consequently, adding VOC-analysis to faecal calprotectin in the diagnostic work-up of IBD might increase specificity, leading to better selection of the children suspected of IBD who need to undergo further diagnostic procedures such as endoscopy.
LIVER DISEASE

More than 20% of children and adolescents in developed countries are overweight or obese. Due to this epidemiology of childhood overweight and obesity, an increasing number of children are at early age confronted with liver problems. Nonalcoholic fatty liver disease (NAFLD), affecting approximately ten percent of obese children and with nonalcoholic steatohepatitis (NASH) as the most aggressive phenotype, is the most frequently encountered liver complication in obesity. The current criterion standard for diagnosis is histological evaluation of a liver biopsy.

Three studies have aimed at linking specific VOCs in exhaled breath to the presence of paediatric liver diseases, including NAFLD, NASH and chronic liver disease. All children (both cases and controls) participating in the NAFLD and NASH studies were either overweight or obese, whilst the majority of the children in the chronic liver disease study had normal weights. The three studies together identified ten different VOCs with significantly higher concentrations in the cases as compared to controls, all but two of them, pentane and 3-methylhexane, identified in only one study (Table 9.2). In addition, only the study in children with CLD reported significantly lower concentrations of some VOCs – 1-nonene, (E)-2-nonene and dimethyl sulphide – compared to healthy controls. This illustrates the limitation of the VOC studies performed so far, with a lack of methodological standardisation and a high rate of type I errors thwarting the possibility to draw firm conclusions.

In summary, VOC analysis in paediatric liver disorders shows promising results, which however are not yet translatable into a practical approach. Standardisation of methodology is necessary before the clinical potential of VOCs in paediatric liver diseases can be valued.

IRRITABLE BOWEL SYNDROME

Functional abdominal pain disorders are the most prevalent gastrointestinal disorders in the paediatric population with a worldwide prevalence of 8.8%, irritable bowel syndrome (IBS) being the most prevalent phenotype. The exact pathogenesis of IBS remains to be elucidated, but it presumably involves a complex interplay between altered gut motility, visceral hyperalgesia, diet-induced microbial dysbiosis, altered immune responses, and psychosocial disturbance.
### Table 9.2: Overview of significantly increased exhaled volatile organic compound concentrations in pediatric liver disease linked to the corresponding pathophysiological mechanism

<table>
<thead>
<tr>
<th>Increased VOC concentrations</th>
<th>Condition</th>
<th>P vs controls</th>
<th>Pathophysiological mechanism&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsaturated hydrocarbons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Decene</td>
<td>CLD&lt;sup&gt;y&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>Oxidative stress involved in the pathogenesis of liver disease (11)</td>
</tr>
<tr>
<td>1-Octene</td>
<td>CLD&lt;sup&gt;y&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>1-Heptene</td>
<td>CLD&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>3-Methylhexane</td>
<td>CLD&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Pentane</td>
<td>NAFLD</td>
<td>0.002</td>
<td>Lipid peroxidation mediated by oxidative stress (11,38)</td>
</tr>
<tr>
<td>Pentane</td>
<td>NASH</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>Saturated hydrocarbons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoprene</td>
<td>NAFLD</td>
<td>0.022</td>
<td>Byproduct of cholesterol biosynthesis (38)</td>
</tr>
<tr>
<td>Oxygen containing compounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>NAFLD</td>
<td>0.008</td>
<td>Produced by hepatocytes during lipolysis or lipid peroxidation (8,39)</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>NAFLD</td>
<td>0.034</td>
<td>Intermediate breakdown product of ethanol metabolism (8)</td>
</tr>
<tr>
<td>Nitrogen-containing compounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethylamine</td>
<td>NAFLD</td>
<td>0.003</td>
<td>Produced by intestinal microbiota and subsequently metabolized in liver (8)</td>
</tr>
<tr>
<td>Alcohols</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>NASH</td>
<td>0.021</td>
<td>Metabolized in liver (39)</td>
</tr>
</tbody>
</table>

CLD = chronic liver disease; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; VOC = volatile organic compounds.

<sup>a</sup>Pathophysiological source of the specific volatile organic compound; other unknown sources may contribute to VOC increase.

<sup>y</sup>Includes children with autoimmune hepatitis, primary sclerosing cholangitis, biliary atresia, NAFLD, viral hepatitis, alpha-1 antitrypsin deficiency, congenital hepatic fibrosis, progressive familial intrahepatic cholestasis, Alagille syndrome, vascular disorders, idiopathic hepatitis, parenteral nutrition-related cholestasis, and idiopathic cholestatic liver disease.
Only a single study has been performed aiming at the identification of IBS-specific VOCs in a paediatric population. VOC analysis of exhaled breath by means of SIFT-MS showed significant higher concentrations of benzene, dimethyl sulphide, 1-octene and 3-methylhexane in IBS patients compared to healthy controls. Based on these specific VOCs, IBS patients could be discriminated from controls with an accuracy of 96%. Limitation of this study was the presence of a significantly higher BMI percentile in the IBS group. However, several studies have demonstrated higher concentrations of 1-octene and 3-methylhexane in overweight and obese children compared to lean controls. As the discriminate analysis model was not externally validated, it impossible to state whether the observed differences were really IBS-specific or the consequence of the increased BMI.

While the exact pathogenesis of IBS remains unclear, there is no doubt that it involves both somatic as psychological factors. Although this complex interplay may thwart the detection of IBS-specific VOCs, it is tempting to speculate that the identification of IBS-specific VOCs might increase our insight into the pathophysiological processes underlying IBS.

NECROTISING ENTEROCOLITIS

NEC is the most common severe gastro-intestinal disease in premature infants with very low birthweights. Despite a decline in overall mortality among extremely premature infants, NEC-related mortality in this specific population has risen. At present, three studies have looked into the potential of faecal VOCs as (early) biomarkers of NEC.

Garner and colleagues analysed faecal VOCs in six infants with NEC and seven matched controls by means of GC-MS. While in the control group the abundance of VOCs increased significantly with age, this was not seen in the NEC group. Moreover, four particular esters were consistently absent in all faecal samples up to four days prior to the clinical onset of NEC: 2-ethylhexyl acetic ester and decanoic, dodecanoic and hexadecanoic acid ethyl ester. All of these esters were found in at least one of the faecal samples of the non-NEC infants; however, none of the esters was present in every sample of each control. The authors suggest that this finding is a reflection of changing physiology or changing gut microbiome; they speculate that the absent esters might have been oxidised or digested by esterases or are lacking due to downregulation of their synthesis.

In a recently published abstract, the same group confirmed their previous findings in a much larger cohort. Using the same technique, they analysed faecal samples from up to
six days prior to the clinical diagnosis of NEC from 34 premature infants with NEC and 70 matched healthy premature controls. In total, six VOCs differed significantly between NEC and control samples, three being positively and three negatively associated with NEC. Using these six particular VOCs, NEC cases could be discriminated from controls with an area under the receiver operating characteristic curve (AUROC) of 0.83. Between one and six days prior to the clinical onset of NEC, the AUROC ranged from 0.78 to 0.9. Information on sensitivity and specificity is lacking.

Recently, the eNose was used to follow the course of VOC profiles of 13 infants developing NEC compared with that of 14 matched preterm controls. This study also showed that NEC is preceded by changes in VOC production. From three days prior to the clinical onset of NEC onward, VOC profiles differed significantly between the two study groups, with increasing accuracy towards the onset of NEC.

In conclusion, VOC-analysis appears to be a promising method for the detection of NEC in a preclinical stage. Timely recognition of this preclinical stage could steer treatment and prevent the development of (more serious stages of) NEC. Future, prospective multicentre studies including large numbers of subjects are needed to determine which molecular compounds emerge preceding and during NEC, to assess the influence of different variables (e.g., gestational age, feeding pattern and antibiotic therapy) on VOC composition, and to discriminate NEC from other causes of inflammation, before VOC-based decisions can be made in daily practice. Obviously, assessment of optimal therapeutic interventions upon early detection of NEC, to decrease the related high morbidity and mortality rates, will become the next challenge to be met.

**INFECTIOUS DIARRHOEA**

Diarrhoeal diseases remain a leading cause of death in infants worldwide. In 2010, over 800,000 (10.5% of total) deaths among children under five years of age were attributable to diarrhoea, rotavirus being the leading infectious cause. Experienced nurses working at paediatric wards often report that stool and flatus of infants with rotavirus gastroenteritis produce a characteristic smell. A study dating from 1987 demonstrated that based on their olfactory skills, nurses were able to differentiate between rotavirus and nonrotavirus diarrhoea with an accuracy of 69%. Presently, only two studies have been published that tried to objectify this finding.
Al-Kateb and colleagues used GC-MS to compare faecal VOCs from 27 Malawian children with rotavirus gastroenteritis with those from 26 controls with unspecified gastrointestinal problems. They found VOCs, in particular aldehydes and 2,3-butandione, to be more abundant in the rotavirus group. Probert et al. published a study comparing faecal samples from 38 patients, adults and children with infectious diarrhoea, with faecal samples from six healthy adults by means of GC-MS. Depending on the VOC pattern they could differentiate rotavirus, Campylobacter jejuni and Clostridium difficile diarrhoea (5 to 6 patients each, adults and children) from each other with positive and negative predictive values of between 40 and 100% and 77 and 100%, respectively. C. difficile diarrhoea was associated with increased levels of 5-methyl-2-furancarboxaldehyde and/or 2-furancarboxaldehyde and total absence of 3-methylindole; rotavirus diarrhoea with the absence of ethyl decanoate combined with the presence of ammonia; and C. jejuni diarrhoea with the absence of terpenes and hydrocarbons.

Given the small groups in both studies, it is impossible to draw firm conclusions. It should be noted that the study by Probert et al. was not confined to children, which makes comparison with the Malawi study difficult.

**FUTURE PERSPECTIVES AND CONCLUSION**

In this review we have focused on the use of VOCs as biomarkers in paediatric gastrointestinal conditions. Although most studies are limited in sample size, the present evidence suggests that infants and children with both functional and organic conditions can be discriminated from controls based on their VOC profiles, enforcing the potential of VOC analysis in diagnosis and follow-up. Whilst this field is gaining momentum, research is in early stages and several barriers are still on the road towards successful application of this novel approach in daily clinical practice. One caveat with every study using VOC analysis, however, is that the number of measured variables by far exceeds the number of analysed samples. As a result, one can expect an excess in false positive associations. This is underscored by the fact that there is little correlation between the VOC profiles reported for the different conditions. At present, therefore, the clinical implications of the findings remain largely uncertain. Additionally, most studies did not control for environmental factors, which makes it difficult to determine if the observed differences were caused by the variable (condition) of interest or also or even largely by the presence of confounding variables (e.g. diet, age, exogenous VOC exposure). Unfortunately, none of the results so far have been validated in external cohorts.
Another challenging aspect to confront is standardisation of sampling methods and assessment. Unpublished results from our laboratory suggest that differences in collecting, storing and preparing the samples may have major consequences for the test results. Differences in methodology between studies, therefore, might yield incomparable outcomes.\(^5\) For the further development of VOC analysis it is recommendable to work along guidelines with proven efficacy, such as STARD and TRIPOD.\(^5\) Ideally, future studies should take effort in standardising and protocolling sampling methods and analyses techniques. Thus the conditions can be set up to enable multicentre trials and pooling of data, which is necessary for the confirmation of the causal relationship between certain conditions and the associated VOC changes.

To implement VOC analysis as a diagnostic tool, therefore, several analytical and clinical aspects need to be attacked. First of all, selection of the optimal analytical technique is needed. Obviously, the choice of technique also depends on the purpose of VOC assessment. Chemical analysis provides detailed (molecular) insight into the VOC composition in a given situation, but at high costs in terms of purchase and maintenance. Pattern-recognition (eNose) devices are relatively cheap and highly user friendly, but presently there is uncertainty regarding the robustness and reproducibility of the test results. We strongly believe, therefore, that technical validation is an essential step in the development of any VOC-based application. So chemical analytical and pattern-recognition techniques have their own places as complementary approaches. Understanding of the compounds relevant to a specific condition can drive the selection and development of optimal sensors for that condition. Alternatively, in research situations, when there is no need for a point-of-care application, chemical analytical techniques may be preferable.

Another issue is the choice of the optimal substrate. Since VOC profiles originate from a combination of exogenous, local and systemic sources, the choice of substrate will influence VOC outcome.\(^2\) This choice will also be influenced by the organ or condition under consideration. For instance, faeces may be superior to breath in case of gastrointestinal conditions, as is suggested by the IBD studies discussed earlier, where fecal VOCs, and not breath VOCs, enabled discrimination between CD and UC. For liver disease, on the other hand, it could be argued that breath analysis is preferable, although at present there are no comparative studies.

Lastly, the clinical applicability of VOCs as a diagnostic tool needs to be validated. Most studies so far have compared VOC profiles of patient groups with those of healthy controls.
In clinical practice, however, tests are performed to confirm or exclude a certain condition in a given patient. This requires biomarkers with optimal accuracy. Any VOC-based test used in clinical practice needs to be tailored to that extent and evaluated under nonstandardized conditions.

In conclusion, a considerable volume of proof-of-principle studies have shown the potential of volatile biomarkers in diagnosing, monitoring and predicting outcomes in paediatric gastrointestinal conditions. The study groups are, however, generally small and there is lack of standardised methodology, while very few study results are reproduced. We are confident that, when these issues are adequately addressed, there is high potential in clinical practice for this novel approach. VOC analysis harbours promises as a future clinical screening and monitoring tool in paediatric gastrointestinal condition. Strong points are its noninvasive character and the possibility to deliver fast results. Next to standardisation of methodology, priority should be given to the identification of key volatiles, enabling the development of disease-specific eNose sensors.

REFERENCES


