Microbiota and Flatography in Pediatric Gastrointestinal Disease

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2017

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

CHAPTER 9

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

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J Pediatr Gastroenterol Nutr. 2016 Dec;63(6):585-91

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.[3,5] 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.[6] 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.[6] 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.[6] 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.[2,15,18] 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.[23,24] 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.[9,13]

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.[13] 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,[25,26] 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*,[27] species that have been observed in higher concentrations in the mucosa of IBD patients.[28,29] 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.[13] 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

					Main outcomes		
Disease	Study (ref.)	Study group cases/controls	Method	Sample medium	Study groups	VOCs used in ROC analysis	*P*; AUC (±95% CI or range)
IBD	de Meij et al (9)	55 (26 UC, 29 CD)/28	eNose	Faeces	UC vs control: - active	Not applicable	$P < 0.001$; AUC 1.00 (±0.00)
					- Remission	Not applicable	$P < 0.001$; AUC 0.94 (±0.05)
					CD vs control: - active	Not applicable	$P < 0.001$; AUC 0.85 (±0.05)
					- Remission		$P < 0.001$; AUC 0.94 (±0.06)
					UC vs CD: - active		$P < 0.001$; AUC 0.96 (±0.03)
					- Remission		$P = 0.002$; AUC 0.81 (±0.08)
	Patel et al (13)	62 (11 UC, 51 CD)/55	SIFT-MS	Breath	IBD vs control	Three different VOCs	$P < 0.001$; AUC 0.96 (0.93–0.99)
					CU vs CD		$P > 0.05$
NEC	Garner et al (12)	6/7	GC-MS	Faeces	Number of VOCs increased with age for non-NEC infants, not for NEC infants		
					Total absence of 4 particular VOCs up to 4 days before NEC		
		34/70	GC-MS	Faeces	NEC vs control	Six different VOCs	
					- Overall; training set		AUC 0.83
					- t(−1) to t(−6); training set		AUC 0.78–0.9
	de Meij et al (10)	13/14	eNose	Faeces	NEC vs control	Not applicable	
					- t(−1), t(0)		$P < 0.001$; AUC 0.99 (±0.04)
					- t(−3), t(−2)		$P = 0.02$; AUC 0.77 (±0.21)

Liver disease							
- NAFLD	Alkhouri et al (8)	37/23	SIFT-MS	Breath	-NAFLD vs control; validation set	Four different VOCs	AUC 0.763
- NASH	Okwu et al (19)	22/74	SIFT-MS	Breath	-NASH vs control; training set	Two different VOCs	AUC 0.73
- CLD	Eng et al (11)	49/55	SIFT-MS	Breath	-CLD vs control; training set	Five different VOCs	AUC 0.97
Acute diarrhoea	Al-Kateb and Cunliffe (20)	27/26	GC-MS	Faeces	Rotavirus infected vs noninfected	Not applicable	''significant''
	Poulton and Tarlow (21)	10/13	Human smell	Faeces	Rotavirus infected vs noninfected	Not applicable	$P < 0.009$
	Probert et al (22)	38/6	GC-MS		Rotavirus infected vs noninfected	Not applicable	$P < 0.0001$
				Faeces	*C. difficile* infection vs noninfected		$P < 0.0001$
IBS	Patel et al (14)	22/55	SIFT-MS	Breath	IBS vs control; training set	Four different VOCs	AUC 0.99

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.[8] 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.[9] Interestingly, these observations correspond with the microbial changes in IBD-subjects with active and inactive disease.[30] 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,[2,28,31-34] 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.[9,13] Supposedly, faecal samples harbour more local and exogenous (derived from the resident microbiome) VOCs than exhaled breath, which predominantly harbours VOCs of systemic origin.[3]

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.[35] Faecal VOC profiling has a reported pooled sensitivity of 86% and specificity of 85% for IBD.[36] 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.[37] 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.[38] The current criterion standard for diagnosis is histological evaluation of a liver biopsy.[39]

Three studies have aimed at linking specific VOCs in exhaled breath to the presence of paediatric liver diseases, including NAFLD,[8] NASH[19] and chronic liver disease.[11] 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).[8,11,19] 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.[11] 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.[40] 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.[40-43]

Table 9.2 Overview of significantly increased exhaled volatile organic compound concentrations in paediatric liver disease linked to the corresponding pathophysiological mechanism

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

CLD = chronic liver disease; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; VOC = volatile organic compounds.
*Pathophysiological source of the specific volatile organic compound; other unknown sources may contribute to VOC increase.
†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.[14] 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%.[14] 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.[19,44] 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.[45] Despite a decline in overall mortality among extremely premature infants, NEC-related mortality in this specific population has risen.[45] 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.[12] 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.[46] 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.[10] 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.[47,48] 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%.[21] 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.[20] 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.[22] 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.[22]

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.[49] 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.[50] For the further development of VOC analysis it is recommendable to work along guidelines with proven efficacy, such as STARD and TRIPOD.[51,52] Ideally, future studies should take effort in standardising and protocolling sampling methods and analysation 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.[3] 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

1. Hippocrates. Aphorisms (translation by F. Adams). Gloucester: Dodo Press, 2009.
2. Broza YY, Haick H. Nanomaterial-based sensors for detection of disease by volatile organic compounds. Nanomedicine (Lond) 2013;8:785-806.
3. van der Schee MP, Paff T, Brinkman P, van Aalderen WM, Haarman EG, Sterk PJ. Breathomics in lung disease. Chest 2015;147:224-31.
4. de Lacy CB, Amann A, Al-Kateb H, Flynn C, Filipiak W, Khalid T, et al. A review of the volatiles from the healthy human body. J Breath Res 2014;8:014001.
5. Sohrabi M, Zhang L, Zhang K, Ahmetagic A, Wei MQ. Volatile organic compounds as novel markers for the detection of bacterial infections. Clin Microbiol 2014;3:2.
6. Arasaradnam RP, Covington JA, Harmston C, Nwokolo CU. Review article: next generation diagnostic modalities in gastroenterology--gas phase volatile compound biomarker detection. Aliment Pharmacol Ther 2014;39:780-9.
7. Buszewski B, Kesy M, Ligor T, Amann A. Human exhaled air analytics: biomarkers of diseases. Biomed Chromatogr 2007;21:553-66.
8. Alkhouri N, Cikach F, Eng K, Moses J, Patel N, Yan C, et al. Analysis of breath volatile organic compounds as a noninvasive tool to diagnose nonalcoholic fatty liver disease in children. Eur J Gastroenterol Hepatol 2014;26:82-7.

9. de Meij TG, de Boer NK, Benninga MA, Lentferink YE, de Groot EF, van de Velde ME, et al. 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. J Crohns Colitis 2014 Sep 22 [Epub ahaed of print].

10. de Meij TG, van der Schee MP, Berkhout DJ, van de Velde ME, Jansen AE, Kramer BW, et al. Early detection of necrotizing enterocolitis by fecal volatile organic compounds analysis. J Pediatr 2015;167:562-7.e1.

11. Eng K, Alkhouri N, Cikach F, Patel N, Yan C, Grove D, et al. Analysis of breath volatile organic compounds in children with chronic liver disease compared to healthy controls. J Breath Res 2015;9:026002.

12. Garner CE, Ewer AK, Elasouad K, Power F, Greenwood R, Ratcliffe NM, et al. Analysis of faecal volatile organic compounds in preterm infants who develop necrotising enterocolitis: a pilot study. J Pediatr Gastroenterol Nutr 2009;49:559-65.

13. Patel N, Alkhouri N, Eng K, Cikach F, Mahajan L, Yan C, et al. Metabolomic analysis of breath volatile organic compounds reveals unique breathprints in children with inflammatory bowel disease: a pilot study. Aliment Pharmacol Ther 2014;40:498-507.

14. Patel SA, Patel N, Okwu V, Matloob A, Grove D, Rome E, et al. Analysis of exhaled volatile organic compounds reveals new biomarkers for irritable bowel syndrome. Program No. 12. ACG Annual Scientific Meeting Abstracts. Philadelphia, PA: American College of Gastroenterology; 2014.

15. Rock F, Barsan N, Weimar U. Electronic nose: current status and future trends. Chem Rev 2008;108:705-25.

16. Langford VS, Graves I, McEwan MJ. Rapid monitoring of volatile organic compounds: a comparison between gas chromatography/mass spectrometry and selected ion flow tube mass spectrometry. Rapid Commun Mass Spectrom 2014;28:10-8.

17. Smith D, Spanel P. Selected ion flow tube mass spectrometry (SIFT-MS) for on-line trace gas analysis. Mass Spectrom Rev 2005;24:661-700.

18. Westhoff M, Litterst P, Freitag L, Urfer W, Bader S, Baumbach JI. Ion mobility spectrometry for the detection of volatile organic compounds in exhaled breath of patients with lung cancer: results of a pilot study. Thorax

19. Okwu V, Matloob A, Grove D, Tian L, Lappe S, Lopez R, et al. Volatile organic compounds in the exhaled breath as biomarkers of nonalcoholic steatohepatitis in obese children. Program No. P871. ACG 2014 Annual Scientific Meeting Abstracts. Philadelphia, PA: American College of Gastroenterology.2009;64:744-8.

20. Al-Kateb H, Cunliffe N, deLacy Costello B, Probert C, Ratcliffe N. Analysis of faecal volatiles from young children infected with and without rotavirus. Gut 2012;61(Suppl 2):A364-A365.

21. Poulton J, Tarlow MJ. Diagnosis of rotavirus gastroenteritis by smell. Arch Dis Child 1987;62:851-2.

22. Probert CS, Jones PR, Ratcliffe NM. A novel method for rapidly diagnosing the causes of diarrhoea. Gut 2004;53:58-61.

23. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 2012;142:46-54.

24. Benchimol EI, Manuel DG, Guttmann A, Nguyen GC, Mojaverian N, Quach P, et al. Changing age demographics of inflammatory bowel disease in Ontario, Canada: a population-based cohort study of epidemiology trends. Inflamm Bowel Dis 2014;20:1761-9.
25. Gevers D, Kugathasan S, Denson LA, Vazquez-Baeza Y, Van TW, Ren B, et al. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe 2014;15:382-92.
26. Morita H, Nakanishi K, Dohi T, Yasugi E, Oshima M. Phospholipid turnover in the inflamed intestinal mucosa: arachidonic acid-rich phosphatidyl/plasmenyl-ethanolamine in the mucosa in inflammatory bowel disease. J Gastroenterol 1999;34:46-53.
27. Bos LD, Sterk PJ, Schultz MJ. Volatile metabolites of pathogens: a systematic review. PLoS Pathog 2013;9:e1003311.
28. Sagar NM, Cree IA, Covington JA, Arasaradnam RP. The interplay of the gut microbiome, bile acids, and volatile organic compounds. Gastroenterol Res Pract 2015;2015:398585.
29. Sartor RB. Genetics and environmental interactions shape the intestinal microbiome to promote inflammatory bowel disease versus mucosal homeostasis. Gastroenterology 2010;139:1816-9.
30. Schwiertz A, Jacobi M, Frick JS, Richter M, Rusch K, Kohler H. Microbiota in pediatric inflammatory bowel disease. J Pediatr 2010;157:240-4.
31. Nyangale EP, Mottram DS, Gibson GR. Gut microbial activity, implications for health and disease: the potential role of metabolite analysis. J Proteome Res 2012;11:5573-85.
32. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505:559-63.
33. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011;334:105-8.
34. Couch RD, Navarro K, Sikaroodi M, Gillevet P, Forsyth CB, Mutlu E, et al. The approach to sample acquisition and its impact on the derived human fecal microbiome and VOC metabolome. PLoS One 2013;8:e81163.
35. Degraeuwe PL, Beld MP, Ashorn M, Canani RB, Day AS, Diamanti A, et al. Faecal calprotectin in suspected paediatric inflammatory bowel disease. J Pediatr Gastroenterol Nutr 2015;60:339-46.
36. Chan DK, Leggett CL, Wang KK. Diagnosing gastrointestinal illnesses using fecal headspace volatile organic compounds. World J Gastroenterol 2016;22:1639-49.
37. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014;384:766-81.
38. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. Pediatrics 2006;118:1388-93.
39. Aggarwal A, Puri K, Thangada S, Zein N, Alkhouri N. Nonalcoholic fatty liver disease in children: recent practice guidelines, where do they take us? Curr Pediatr Rev 2014;10:151-61.
40. Korterink JJ, Diederen K, Benninga MA, Tabbers MM. Epidemiology of pediatric functional abdominal pain disorders: a meta-analysis. PLoS One 2015;10:e0126982.
41. Simren M, Barbara G, Flint HJ, Spiegel BM, Spiller RC, Vanner S, et al. Intestinal microbiota in functional bowel disorders: a Rome foundation report. Gut 2013;62:159-76.

42. Di Lorenzo C, Colletti RB, Lehmann HP, Boyle JT, Gerson WT, Hyams JS, et al. Chronic abdominal pain in children: a clinical report of the American Academy of Pediatrics and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 2005;40:245-8.

43. Chumpitazi BP, Cope JL, Hollister EB, Tsai CM, McMeans AR, Luna RA, et al. Randomised clinical trial: gut microbiome biomarkers are associated with clinical response to a low FODMAP diet in children with the irritable bowel syndrome. Aliment Pharmacol Ther 2015;42:418-27.

44. Alkhouri N, Eng K, Cikach F, Patel N, Yan C, Brindle A, et al. Breathprints of childhood obesity: changes in volatile organic compounds in obese children compared with lean controls. Pediatr Obes 2015;10:23-9.

45. Patel RM, Kandefer S, Walsh MC, Bell EF, Carlo WA, Laptook AR, et al. Causes and timing of death in extremely premature infants from 2000 through 2011. N Engl J Med 2015;372:331-40.

46. Mayor A, Ellaby N, Reade S, Aggio R, Greenwood R, Jackson R, et al. Investigation of faecal volatile organic compounds as biomarkers for the diagnosis of necrotising enterocolitis. Gut 2015;64(Suppl 1):A15-A16.

47. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet 2012;379:2151-61.

48. Konno T, Suzuki H, Imai A, Kutsuzawa T, Ishida N, Katsushima N, et al. A long-term survey of rotavirus infection in Japanese children with acute gastroenteritis. J Infect Dis 1978;138:569-76.

49. Broadhurst DI, Kell DB. Statistical strategies for avoiding false discoveries in metabolomics and related experiments. Metabolomics 2006;2:171-96.

50. Boots AW, Bos LD, van der Schee MP, van Schooten FJ, Sterk PJ. Exhaled molecular fingerprinting in diagnosis and monitoring: validating volatile promises. Trends Mol Med 2015;21:633-44.

51. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Ann Intern Med 2003;138:W1-12.

52. Moons KG, Altman DG, Reitsma JB, Collins GS. New guideline for the reporting of studies developing, validating, or updating a multivariable clinical prediction model: The TRIPOD Statement. Adv Anat Pathol 2015;22:303-5.