Volatile organic compounds as disease predictors in newborn infants: a systematic review

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TOPICAL REVIEW

Volatile organic compounds as disease predictors in newborn infants: a systematic review

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Abstract

Volatile organic compounds (VOCs) detected in human breath, urine, stool, sweat, saliva, and blood result from metabolic processes in the body during health or disease. Using sophisticated measurement systems, small amounts of these compounds can be detected in the above bodily fluids. Multiple studies in adults and children have shown the potential of these compounds to differentiate between healthy individuals and patients by detecting profiles of compounds in non-invasively collected samples. However, the detection of biomarkers in VOCs from neonates is particularly attractive due to the non-invasive nature of its approach, and its ability to track disease progress by longitudinal sampling. In this work we have reviewed the literature on the use of VOCs in neonates and identified areas for future work. Overview of VOCs and their usefulness as metabolic signatures. Detailed review of studies on VOCs in neonates Learn about potential uses of VOCs as derived from adult and paediatric studies. Examine current limitations and identify future work. Detailed studies on VOCs involving neonatal patients including sick preterm infants and term infants with specific morbidities are needed. These studies should collect longitudinal samples using non-invasive methods for the detection of potential biomarkers. Underlying metabolic processes need to be identified so that any therapeutic options can be clarified.

1. Introduction

Sick newborn infants admitted to a neonatal intensive care unit (NICU) need close continuous monitoring throughout the length of their stay. They are also subjected to multiple phlebotomy episodes and other invasive investigations for diagnostic and monitoring purposes. These investigations are essential but can result in pain and discomfort [1–3], and in iatrogenic blood loss requiring blood transfusions [4, 5]. Long term impacts of neonatal care include mental health problems, including phobias [6], which may be related to their experiences as inpatients. The burden of these problems are significant and the incidence of NICU admissions is increasing in the USA [7] and the UK.

Invasive investigations in NICU for the diagnosis of disease, monitoring disease progress, changing acute management, or determining prognosis remain the gold standard for neonatal care. However, advances in technology have enabled trials of point-of-care tests [8, 9] and non-invasive options. Pulse oximetry is an example of a universally used monitoring device for sick infants [10] which provides a continuous display of the cardio-respiratory status of the patient. Transcutaneous monitoring of carbon dioxide of infants on respiratory support can provide a continuous trend analysis of respiratory status

[11] and consequently reduce phlebotomy episodes for blood gas analysis [12]. Transcutaneous bilirubin measurements for screening neonates has also been in use for several years which also has potential for reducing invasive blood tests [13]. However, another strategy to reduce invasive tests on neonates includes the use of urine, stool and exhaled breath as sources for volatile organic compounds (VOCs) and metabolic end products, which may be useful as biomarkers.

NICU patients experience a range of pathophysiological processes including inflammation, infection, genetic disorders, and other congenital malformations, and often require mechanical or pharmacological support. A combination of the pathophysiology and the external support required can often result in complex morbidities of multiple organ systems like the lungs (bronchopulmonary dysplasia, pneumonia), gut (necrotising enterocolitis), liver (hepatitis, cholestasis), bone (metabolic bone disease), and brain (intraventricular haemorrhage, periventricular leukomalacia) [14]. Currently most of these conditions are diagnosed retrospectively using static and invasive investigation methods, although almost certainly they develop over time and involve multiple metabolic processes. The ability to detect them dynamically and non-invasively well before they present clinically is an attractive proposition. Prime candidates for such detection are the lung and gut morbidities where breath, urine and stool samples can be used to monitor disease-specific changes and offer potentially mitigating therapeutics.

Numerous metabolic processes in the body produce end products which are dissolved in the blood and are excreted by the lungs, kidneys, and bowel. Excretion products from these organs include exhaled breath, urine, and stool, carry signatures of these metabolic end products in the form of VOCs. The ability to recognise specific VOCs by smell as characteristic signs of disease, like the sweet acetonic smell of diabetic ketoacidosis, fishy breath related to liver disease, the urine-like smell in patients of kidney failure, and the mousy/musty odour of patients with untreated phenylketonuria, has been recognised for a long time [15]. While these examples are attributed to specific metabolic products, thousands of other compounds are detectable in exhaled breath and other bodily fluids, leading to the development of the term 'volatilome' to describe the study of complex mixtures of VOCs from breath and body fluids (as well as other areas of science like food processing, plant biology, etc.). Using a combination of highly sensitive techniques including gas chromatography (GC) and mass spectrometry (MS), the volatilome from various body fluids has been used for the detection of biomarkers. The aim of this work is to systematically review of studies of VOC detection in neonatal patients, their potential uses and how the use of VOC diagnostics in adult and paediatric patients may be applicable to the neonatal population. We will also discuss the current limitations of these diagnostic techniques and identify potential future work.

2. Methods of detection and interpretation

A variety of systems and techniques have been employed to analyse gas-phase VOCs from a range of biological samples [15]. Gas chromatography–mass spectroscopy (GC–MS) is considered the gold standard technique, as it provides comprehensive profiles of VOCs and allows quantitation of compounds present within a sample. In combination with sampling methods such as thermal desorption tubes or solid phase microextraction, the method is highly sensitive and can identify single VOCs, and thus can allow identification of underlying metabolic processes involved in its production and excretion. However, although sampling can be carried out *in situ*, the actual analysis needs to be carried out off-site as the equipment required is bulky. GC–MS systems are also relatively expensive and require highly trained operators, and this means that it is challenging to implement GC–MS based methods as a routine diagnostic tool. Sampling is also intermittent and discontinuous; the method is, therefore, not suited to detect compounds in real-time.

In the efforts to create a more practical method of detection for clinical use, 'electronic nose' technology has been developed. These instruments do not detect and identify specific compounds present within a sample, rather attempt to identify patterns of chemicals using an array of non-specific detectors. Electronic noses can have an array of anywhere between 8 and 32 sensors that are designed to detect different groups of compounds, including alcohols, ketones, and low-pressure gases. The 'headspace' (the air above a sample) is injected into the sensor array and each sensor response is unique. A typical feature of this response is then extracted and used to train the instrument with a pattern recognition algorithm, therefore allowing the instrument to be trained to recognise a range of conditions [16]. Although most of the papers reviewed below use this technique, there are obvious limitations with using an eNose for screening purposes. Sensor sensitivity ranges from 1 part per million to 1 part per billion, which is less sensitive than the detection limit of GC–MS and limited data is available on the effect of multiple compounds on detection limits (also a factor which does not affect GC–MS measurements). Sensitivity of sensors can be increased by selective coatings, but this requires prior knowledge of volatile biomarkers [17]. The discriminatory power of sensor arrays depends on the number of sensors and their difference in affinity and is still limited compared to the resolution of GC separation and specificity of GC and MS data combined. Finally, eNoses do not allow

identification of single compounds and cannot point to the underlying metabolic processes.

Electronic nose technology has been further developed since its inception, and now covers techniques including ion mobility spectrometry (IMS), proton transfer reaction mass spectrometry (PTR-MS), GCs employing gas sensors as the detector and optical gas spectrometer instruments, all of which can be configured to give a rapid result within 60 s. IMS and PTR-MS technology generally offers the highest sensitivity of these newer systems and has been further refined as field asymmetric ion mobility spectroscopy (FAIMS). FAIMS shows even greater sensitivity, as it can track the movements of a single ion through an electrical field and can detect minute changes in the composition of VOCs [18]. Potentially, these methods can be utilised in neonates when specific disease predictors are identified.

Selected ion flow tube mass spectrometry (SIFT-MS) is a newer technology that allows for real-time absolute quantification of several trace gases simultaneously, even when an abundance of atmospheric gas is present. VOCs are ionised and reacted with precursor ions and fed into a mass spectrometer which detects precursor and traces gas ions, allowing for identification and quantification of VOCs present in the sample [19]. All direct inlet methods (IMS, PTR-MS, FAIMS and SIFT), however, analyse the entire mixture and rely on sufficient discrimination in mass and the (limited) degree of fragmentation for identification of VOCs. They all use soft ionisation methods, which reduce the number of ions but at the same time do not produce mass spectra that can be compared to existing libraries making identification much more difficult especially in the absence of any retention data.

In summary, powerful chemical analysis techniques are available for the detection of VOCs in human samples. Highly sensitive methods like GC–MS are ideally suited for the identification and quantification of minute quantities of compounds, which are useful for the detection of biomarkers for specific diseases and ideally preferred for neonatal populations where the concentration of VOCs is expected to be low and disease predictors are as yet unknown. Newer technology like SIFT-MS can be used to for the clinical implementation of these identified biomarkers on patients and for real-time measurements.

3. Methods for the literature review

Using a combination of keywords, medical subject headings and Boolean combinations, six databases were searched for papers and abstracts on VOCs in neonates (supplementary information, which is available online at stacks.iop.org/JBR/15/024002/mmedia). Full-text articles were reviewed from an initial shortlist which

was chosen based on the title and abstract. Further manual searches in the bibliography of the chosen papers were undertaken for any further relevant articles which were not included in the earlier searches. For studies in adults and children, the following combination of terms were used to search review papers which were used to identify references to original studies: v*olatile organic compound, VOC, exhaled breath condensate, breath biomarker, chronic obstructive pulmonary disease, COPD, asthma, respiratory infections, pulmonary, gas chromatography mass spectrometry, GCMS, child, children, adult, systematic review.*

4. VOCs in neonates: stool, breath, and urine

The systematic search revealed 13 original papers on VOCs in neonates (10 full-text papers and 3 conference abstracts) and 11 review articles. In addition, four full-text articles were identified which explored the composition of VOCs in the neonatal environment. Details of the filtering of studies at various stages are presented in figure 1. Manual searches in the references of the papers failed to identify any further original studies. A list of the selected papers is presented in table 1 and a summary of the literature is presented below categorised by the sample used in the study. Despite VOCs having been extensively studied in adults, there have been relatively fewer studies in the neonatal population. Most studies have examined the use of VOCs in faecal samples; however, there is some data available examining VOCs detectable in respiratory and urine samples.

4.1. VOCs in neonatal stool

VOCs in neonatal faecal samples have been the most extensively studied for potential diagnostic abilities. Faecal VOCs have been studied as biomarkers for a range of conditions including necrotising enterocolitis (NEC), bronchopulmonary dysplasia (BPD) and late-onset neonatal sepsis (LONS).

Berkhout *et al* examined faecal VOCs in a cohort of infants born at <30 weeks' gestational age, taking daily faecal samples for the first 28 d of life and examining them with a commercially available eNose device. Infants born in three centres in the Netherlands were recruited. They demonstrated that faecal VOC profiles show a significant difference up to 3 d before the onset of clinical symptoms of late-onset sepsis in 127 infants and matched controls without indwelling percutaneous central venous or arterial access [20]. This is particularly true for LONS caused by *Staphylococcus aureus* and *Escherichia coli*, whereas infections caused by coagulase-negative staphylococcus species did not seem to alter faecal VOCs significantly [21], suggesting that LONS may be related to altering intestinal conditions and translocation of
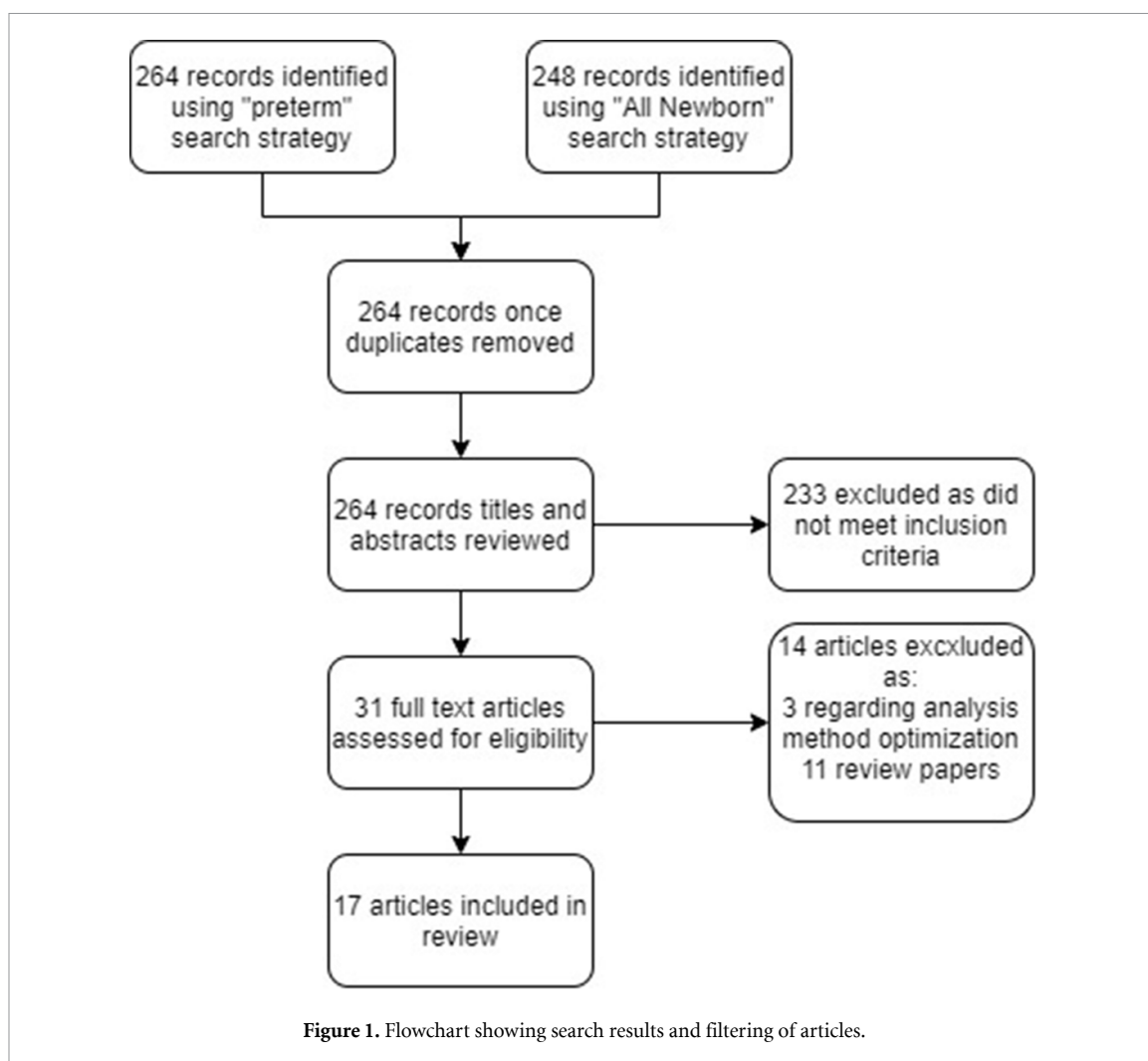
Figure 1. Flowchart showing search results and filtering of articles.

bacterial species to the bloodstream. Berkhout *et al* have also demonstrated that in this same cohort, BPD could be predicted with up to 70% accuracy by the changing faecal VOC profile from day 14 of life in a small group of 15 infants with matched controls [22]. However, the eNose detector is not designed to identify specific compounds thus the metabolic processes involved for these differences could not be identified.

Understandably, most attention has been given to the ability of faecal VOCs to act as biomarkers for developing NEC due to easy access to the sample. de Meij *et al* [23] examined faecal VOCs (also using an eNose) from infants in the Berkhout *et al* cohort described above, to determine whether VOCs altered before the onset of clinical symptoms. VOC profiles from 13 infants with Bell's Stage II or higher NEC were compared with 31 infants who developed sepsis and 14 matched healthy controls. They demonstrated that NEC could be discriminated with increasing accuracy from controls in each of the 5 d leading up to clinical symptoms of the disease, with 65% accuracy five days before and 99% accuracy one day before. Additionally, faecal VOC profiles could be reliably differentiated between those infants developing sepsis and those developing NEC 2 to 3 d prior to onset of clinical symptoms.

A multi-centre study from eight UK neonatal units has examined faecal samples taken from 1326 infants born at 23–34 weeks' gestation using head-space GC–MS. The group identified 224 different VOCs present in their pilot work, with the number of VOCs present increasing as the infants matured, with the possibility of detecting changes several days before symptom-onset [24, 25]. Overall, when the entire cohort was analysed, a broad range of VOCs was found in 32 babies who developed NEC Stage IIa or higher, with three groups of VOCs including aldehydes, butanoic acid and methanedithiones being strongly associated with an increased risk of NEC. All these factors were also associated with increasing postmenstrual age thus may reflect the presence of species in the maturing gut microbiome. Certain VOCs were also found to play a protective role against development of NEC including 3-methyl and 2-methylbutanoic acid. These changes in VOCs could be seen up to 4 d before the onset of clinical symptoms [26].

Table 1. Details of included studies on neonatal patients.

Study ID	**Berkhout *et al* (2017)**
Title	Detection of sepsis in preterm infants by fecal volatile organic compounds analysis: a proof of principle study
Year	2017
Population	Sevnty-six preterm infants (<30 weeks' gestation); 36 with late onset sepsis, 40 matched controls
Sample type	Faeces
Method of detection	eNose (Cyranose 320)
Study ID	**Berkhout *et al* (2018)**
Title	Development of severe broncho-pulmonary dysplasia is associated with alterations in fecal volatile organic compounds.
Year	2018
Population	Thirty preterm infants (<30 weeks' gestation); 15 with BPD, 15 matched controls
Sample type	Faeces
Method of detection	eNose (Cyranose 320)
Study ID	**Berkhout *et al* (2019)**
Title	Late-onset Sepsis in preterm infants can be detected preclinically by fecal volatile organic compound analysis: a prospective, multicenter cohort study.
Year	2019
Population	Two hundred and fifty-four preterm infants (<30 weeks' gestation); 127 with late onset sepsis; 127 matched controls
Sample type	Faeces
Method of detection	Field asymmetric ion mobility spectroscopy
Study ID	**Berkhout *et al* (2020)**
Title	Preclinical detection of non-catheter related late-onset sepsis in preterm infants by fecal volatile compounds analysis.
Year	2020
Population	Forty-nine preterm infants (<30 weeks' gestation); 24 with late onset sepsis, 25 matched controls
Sample type	Faeces
Method of detection	Field asymmetric ion mobility spectroscopy
Study ID	**Cordell *et al* (2013)**
Title	GC-MS analysis of ethanol and other volatile compounds in micro-volume blood samples–quantifying neonatal exposure.
Year	2013
Population	Two hundred and eighty-nine samples from neonatal population
Sample type	Blood
Method of detection	Gas chromatography–mass spectrometry
Study ID	**de Meij TG *et al* (2015)**
Title	Early detection of necrotizing enterocolitis by fecal volatile organic compounds analysis.
Year	2015
Population	Fifty-eight preterm infants (<30 weeks' gestation); 13 NEC, 31 sepsis, 14 matched controls
Sample type	Faeces
Method of detection	eNose (Cyranose 320)
Study ID	**El Manouni *et al* (2018)**
Title	Fecal volatile organic compounds in preterm infants are influenced by enteral feeding composition.
Year	2018
Population	Thirty preterm infants (<30 weeks' gestation)
Sample type	Faeces
Method of detection	eNose (Cyranose 320)
Study ID	**El-Metwally *et al* (2018)**
Title	Urinary metabolites of volatile organic compounds of infants in the neonatal intensive care unit.
Year	2018
Population	Eighty preterm infants (<37 weeks' gestation)
Sample type	Urine
Method of detection	Ultra-high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry

Table 1. (Continued.)

Study ID	**Garner *et al* (2009)**
Title	Analysis of faecal volatile organic compounds in preterm infants who develop necrotising enterocolitis: a pilot study
Year	2009
Population	Thirteen infants <36 weeks' gestation; 6 who developed NEC, 7 matched controls
Sample type	Faeces
Method of detection	Gas chromatography-mass spectrometry
Study ID	**Mayor *et al* (2014)**
Title	Paediatric Faecal VOC analysis: method optimisation.
Year	2009
Population	One hundred and four preterm infants (23–34 weeks' gestation); 34 developed NEC, 70 matched controls
Sample type	Faeces
Method of detection	Gas chromatography–mass spectrometry
Study ID	**Merchant *et al* (2014)**
Title	Analysis of exhaled volatile organic compounds (VOC) in intubated preterm infants.
Year	2014
Population	Five infants; 3 preterm (2 with RDS, 1 with respiratory failure), 2 controls
Sample type	Exhaled breath (sampled from expiratory limb of ventilator circuit)
Method of detection	Gas chromatography–mass spectrometry
Study ID	**Meyer-Monath *et al* (2014)**
Title	Analysis of BTEX and chlorinated solvents in meconium by headspace-solid-phase microextraction gas chromatography coupled with mass spectrometry.
Year	2014
Population	Term infants
Sample type	Meconium
Method of detection	Gas chromatography–mass spectrometry
Study ID	**Prazad *et al* (2008)**
Title	Airborne concentrations of volatile organic compounds in neonatal incubators.
Year	2008
Population	Ten unoccupied incubators
Sample type	Air
Method of detection	Gas chromatography–mass spectrometry
Study ID	**Probert *et al* (2019)**
Title	Faecal volatile organic compounds in preterm babies at risk of necrotising enterocolitis: the DOVE study.
Year	2019
Population	One hundred and two infants <34 weeks' gestation; 32 developed NEC, 70 matched controls
Sample type	Faeces
Method of detection	Gas chromatography–mass spectrometry
Study ID	**Rogosch *et al* (2014)**
Title	Detection of bloodstream infections and prediction of bronchopulmonary dysplasia in preterm neonates with an electronic nose.
Year	2014
Population	Twenty-eight intubated preterm infants
Sample type	Tracheal aspirates
Method of detection	eNose (Cyranose 320)
Study ID	**Steinbach *et al* (2019)**
Title	Bedside measurement of volatile organic compounds in the atmosphere of neonatal incubators using ion mobility spectrometry.
Year	2019
Population	Seventeen incubators occupied by preterm infants; 9 unoccupied incubators
Sample type	Air
Method of detection	Ion Mobility Spectrometry

4.2. VOCs in neonatal respiratory samples

Despite a move towards less invasive respiratory support for premature infants, a significant proportion continue to require intubation and mechanical ventilation during their management. Therefore, exhaled gases, tracheal aspirates and bronchoalveolar lavage samples provide a source of VOCs for potential point-of-care diagnostics. To date, only two studies have examined VOCs in samples from the lungs of neonates.

A pilot study published as a conference abstract of two preterm neonates ventilated for respiratory distress syndrome (RDS) examined VOCs from gas obtained from the expiratory limb of a ventilator circuit, using gas chromatography. These were compared to samples obtained from two term infants electively ventilated for surgery, and one preterm infant ventilated for respiratory failure secondary to congenital hypotonia. They found a total of 79 VOCs present in the samples including carbon monoxide and dioxide, methane, and a wide variety of nonmethane hydrocarbons, halogenated gases, alkyl-nitrates, selected sulphur and oxygen compounds. Seven gases were identified that showed a possible trend towards being higher in those infants with RDS, of particular note was chloroform which was three times more abundant [27].

The single centre study by Rogosch *et al* [28] examined tracheal aspirates obtained by lavage and suction from 28 intubated preterm infants using an eNose system. They found that the eight infants in their cohort with a confirmed bloodstream infection could be differentiated on 'smell print' analysis. They also found a non-significant trend towards being able to differentiate those infants with BPD compared to those without. These studies show the potential of detecting VOCs in neonates to diagnose and guide monitoring of specific diseases.

4.3. VOCs in neonatal urine

There have been several papers examining the detection of peptide compounds in urine to assist with the early diagnosis of NEC [29], but very little attention has been paid to examining VOCs in urine for early diagnostics. A study by El-Metwally [30] compared VOC metabolites in the urine of preterm infants nursed in incubators compared to open cots using ultra-high-performance liquid chromatography coupled with electrospray ionization tandem MS. They demonstrated that detecting these metabolites was possible and that there was a significant difference in the VOC metabolites between incubator- and cot-nursed infants. Their study, although small, suggested that preterm infants nursed in incubators may absorb more VOCs associated with tobacco smoke, plasticizers from medical devices, excipients used in medications, and cleaning/sterilizing agents used in the environment.

4.4. VOCs related to the neonatal environment

In a single-centre study, Steinbach *et al* [31] measured VOCs in 9 occupied and 17 unoccupied neonatal incubators (all preterm infants) using ion-mobility spectrometry. The authors were successful in identifying discriminating VOC profiles from occupied and unoccupied incubators using principal component and decision tree analysis. They concluded that preterm infants produce identifiable VOC profiles in their environment which can potentially be useful for diagnostic purposes.

In variable temperature, humidity and air-circulation conditions, Prazad *et al* [32] used GC–MS to measure VOCs from unoccupied neonatal incubators attempting to identify their exposure to potentially harmful incubator-generated VOCs. Increased concentration of two compounds, 2-heptanone and n-butyl acetate, were found in the incubators compared to ambient air. The concentration of both increased significantly in temperature and humidity conditions typical of the preterm neonatal environment. This study raises important further questions regarding exposure of vulnerable neonates to environmental VOCs and xenobiotics. In addition, their results also suggest that any future work on neonatal samples will need further control samples from their environment to differentiate between metabolic products from the patient or from environmental exposure.

A study by Meyer-Monath *et al* [33] attempted to identify fetal exposure to benzene, toluene, ethylbenzene, *o-m-p*-xylene, trichloroethylene and tetrachloroethylene by analysing the headspace of meconium using GC–MS. Sensitivity was further increased by using headspace-solid-phase microextraction steps prior to actual measurement, and the steps involved were optimised for the purpose.

To determine exposure of neonates to ethanol excipient containing medications, Cordell *et al* [34] successfully demonstrated the identification of several VOCs from small volume blood samples from neonatal patients. The authors plan to use this system to identify the course of ethanol metabolism in neonates receiving ethanol containing medications. While this paper demonstrates the sensitivity of the GC–MS method to detect individual VOCs, using blood-samples makes it an invasive process. However, they established that similar methods could be used on non-invasive samples from patients for identification and quantification of individual VOCs.

5. Limitations of current methods

There are many challenges to implementing current methods of VOC analysis into routine, point-of-care clinical use for diagnostic purposes.

There is evidence that VOC composition is altered by enteral feeding regimens in neonates. A study by El Manouni El Hassani *et al* studied 15 infants of <30 weeks' gestational age who were fed >75% either maternal or donor expressed breast milk comparing their faecal VOC profiles to infants who were fed >75% formula milk, with stable diets over the first 28 d of life. Again, an eNose detector was used to identify VOC profiles. Although the numbers studied were small, they found a significant difference in VOC profiles over the first 21 d of life [35]. This presents a challenge to the use of devices such as the eNose,

which does not identify VOCs present but gives an overview of the VOC mix present in a sample. Preterm infants on neonatal units are often fed a broad mix of diets, dependent on maternal and donor breast milk availability and unit preferences about preterm formula milk use and brand. This study suggests that feeding variables also need to be recorded in studies on VOCs as they can affect the interpretation results.

As demonstrated by El-Metwally [30], the link between VOCs measures and their potential role as a disease biomarker may be confounded by differential environmental exposures or pre-emptive therapeutic interventions. Preterm infants in particular are at high risk of absorbing environmental compounds, and have immature secretory and excretory pathways. It has been demonstrated that the atmosphere within unoccupied incubators has detectable levels of VOCs which are also influenced by the temperature and humidity settings of the incubator [32]. The effect this has on infants is as-yet unclear. Additionally, excipients in medications, typically ethanol, also contain VOCs which are detectable in the bloodstream of those infants receiving them [34]. There is also evidence that trans-placental passage of VOCs occurs in-utero as they can be detected in the meconium of newborn infants [33]. In this work we have not reviewed the causal pathways for the breadth of work identified, and some encouraging results may turn out to be due to xenobiotic exposures (exogenous to the infant) rather than endogenous metabolites.

These observations highlight the potential variations in VOCs between individual infants depending on their antenatal history, previous and current management and NICU environment, and it is unclear whether different forms of biological samples studied may all be affected. It is also unclear if VOC levels are stable or vary in the same individuals over time. Studies examining repeatability of VOC examination in adults with stable COPD have shown variation in profiles on measurements taken on the same day and over a 7 d period [36] and similarly for profiles in healthy and asthmatic children [37]. The repeatability of VOC measurements is unknown in healthy neonates, nor how disease states may impact on the reproducibility of profiles. This may all lead to further complicating the interpretation of VOC patterns and profiles in the early stages of the disease.

Many of the techniques employed in these studies are unable to give a real-time assessment of VOC profiles in a clinical setting, and several methods employed, particularly GC–MS methods and its derivatives, require laboratory processing time. This can limit the clinical applicability of VOCs for early disease detection, as if the VOC profile changes a few days before symptom onset, but sample processing takes longer than this time, there can be no early disease detection and patient benefit. Newer VOC detection technologies as described above can give

rapid real-time results but are largely untested in the neonatal population.

Thus, there are only a few studies of VOCs in neonates, often with a small number of infants and sometimes from the same cohort. However, these studies should be considered as proof of principle showing promise for studying these methodologies in larger longitudinal cohorts of newborn infants with specific conditions such as NEC, sepsis, and BPD. In addition to the issues of prediction and validity, any potential test requires implementation at, or near, the neonatal unit; and a rapid enough result to help guide the clinician. For some measures this lag maybe acceptable in the region of hours or even days (e.g. the development of chronic lung disorders where results may guide long term ventilation of respiratory support strategies), while other diseases processes progress much faster and require rapid turn-around to be of clinical use (e.g. developing infections).

6. Lessons from studies in adults and children

Adult and paediatric studies on the use of VOCs as disease predictors can provide useful direction while setting up similar research programmes for neonatal patients. The literature on adult VOCs is vast and only a few examples are presented below which may potentially guide future research studies in neonates.

6.1. Examples of VOC studies in adults

Boots *et al* [38] describes detection of pulmonary bacterial infections by detection of VOCs in exhaled air. Four different microorganisms (*E. coli, Pseudomonas aeruginosa, S. aureus* and *Klebsiella pneumoniae*) were analysed by GC–MS with 25 VOCs being identified as being differently profiled across the various microorganisms. Additionally, for *S. aureus*, antibiotic sensitive and resistant strains were shown to have different VOC profiles. Kamal *et al* [39] report the results of a study that employed GC–MS to distinguish between viral and bacterial infections in chronic obstructive pulmonary disease (COPD) patients. Preliminary findings from COPD patients identified 14 increased VOCs in patients with positive bacterial sputum cultures. As exhaled breath condensate (EBC) is an easily accessible source of sample in the neonatal population, who are prone to infection and pneumonia, these results could guide research in neonates for early detection of pulmonary infection.

Determination of a set of COPD breath marker molecules has been underway for some years but the results are generally inconsistent. This is typified by Christiansen *et al* [40] who report a systematic review of work done to identify potential COPD breath marker molecules. Twelve papers were included, but no consistent markers were found with only three markers reported in more than one study. Recently, Besa *et al* [41] reported the results of a VOC study

of 45 COPD patients with 51 healthy controls. One hundred and thirty-seven VOCs were found to have a statistically significant difference between the COPD and healthy groups. Six of these VOCs when combined were found to correctly discriminate COPD patients from healthy controls with an accuracy of 70%. Allers *et al* [42] describes a study where two variations on GC–MS devices were employed to investigate correlations between VOCs and COPD with smoking taken into account, and successfully identified four distinct VOCs in total that showed statistically significant differences between healthy controls and patients with COPD. In summary, VOCs from breath samples in adults can be used as biomarkers of disease, an approach which is potentially replicable in the neonatal population who develop inflammatory conditions of the lungs like BPD [43].

Hayton *et al* [44] conducted a systematic review of studies investigating breath predictors of idiopathic pulmonary fibrosis. Of the 14 included studies, a total of 20 VOCs that distinguished pulmonary fibrosis from controls were identified with three identifying alveolar nitric oxide as significantly higher in cases than controls. Lamote *et al* [45] describe a cross-sectional case-control study that identified (and confirmed earlier work) discriminating VOCs as predictors of malignant pleural mesothelioma. Using a GC–MS supplied by Markes (Markes International Ltd, Llantrisant, UK), the following VOCs were identified as discriminatory: nonanal, diethyl ether, limonene, methyl cyclopentane, cyclohexane, and isothiocyanatocyclohexane. These high-sensitivity systems would be useful for the discovery of predictors in the neonatal population before assessing them as disease markers.

6.2. Examples of VOC studies in children

In children, primarily exhaled breath but also faecal VOC profiling are reported, with asthma the frequent reason for profiling of exhaled breath. Exhaled breath of 252 preschool children were analysed using GC–MS in a study that found 17 VOCs within 3256 identified different compounds that discriminated preschool asthmatic children from transient wheezing children [46] with a prediction success rate of 80% in terms of the correct medical diagnosis. Another study analysed the exhaled breath of 64 participants, aged 6–18 years and used the medical diagnosis of asthma within the children to construct models around the measured VOCs that were able to significantly distinguish between those who had and did not have asthma and therefore identify those in need of corticosteroid therapy [47]. Ehrmann *et al* [48] split 82 children into controls, asthmatics, and those with either episodic viral wheeze (EVW) or multiple-trigger wheeze (MTW). Following analysis by GC–MS, they found 20 VOCs to differ between asthmatics and controls, and 13 VOCs to differ between EVW and MTW. Schee

et al investigated the distinction between rhinovirus-induced wheeze and non- rhinovirus-induced wheeze as its presence is associated with increased risk of asthma. The VOC profile they found was able to discriminate wheeze from non-wheeze, but this distinction only persisted after symptomatic recovery for the rhinovirus-induced wheeze [49]. Mastrigt *et al* [50] compared exhaled breath samples from healthy children aged 6–18 years, with those from children with asthma or cystic fibrosis (CF). The study failed to produce a profile that could distinguish asthma from CF but did find a profile that distinguished healthy from asthmatic and CF separately. The above examples suggest that EBC are a ready source of sample for research, which can be used in neonates for monitoring evolving lung infection or inflammation.

7. Future directions

VOCs have been successfully identified and quantified as biomarkers in research studies from all age groups, although significant variation on results and use of different methods limit their clinical implementation. In neonates, only a limited number of studies have attempted to use VOCs as predictors of disease using cross-sectional sampling methods from small cohorts. Due to their potential benefits including non-invasive sampling methods, real-time sampling and detection of an evolving disease process, further studies on VOCs are recommended in the future. These studies should collect longitudinal samples from an appropriate cohort of infants and use highly sensitive methods for the detection and quantification of VOCs to identify potential predictors of disease in neonates. EBCs from ventilated and unventilated neonates remain understudied, partly due to technical difficulties with sample collection from small infants [51]. Integrated systems like the 'Pneumopipe' developed for adults [37, 52] could be applied in neonates and small infants to aid in collection and analysis of EBC from this population group. Careful repeatability and validation of results from further studies will be essential before VOCs can be considered for clinical use in patient populations.

8. Conclusion

VOCs in bodily fluids contain important profiles of metabolic activity and disease-related changes. These profiles can be detected in a variety of samples including breath, urine, stool, saliva, sweat and blood. The biggest attraction of this area of research is that in spite of the mostly non-invasive nature of the sample collection, these metabolic signatures can be detected in minute quantities and have already been shown to be able to differentiate adequately between health and disease.

In neonates, research into VOCs is particularly attractive due to the non-invasive sample collection techniques, non-interference with the neonatal physiology and the potential ability to identify biomarkers for several longitudinal disease processes. So far, only one study has attempted to look at stool in enough detail to identify and quantify metabolic biomarkers for disease. Other studies have used methods which have only attempted to differentiate between patient groups without being able to identify underlying metabolic processes. Clearly, to be successful as a strategy for diagnostic and prognostic purposes, an understanding of the metabolic processes and robust validation as a biomarker would be required for the identification of potential therapeutic pathways. Further research is required in appropriate patient groups using detailed longitudinal sampling of breath, urine and stool for identification and quantification of VOCs as biomarkers of neonatal disease.

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