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# Exhaled volatile organic compounds in adult asthma: a systematic review

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**The clinical utility of exhaled breath sampling contrasts the complexity required for VOC analysis. Modern studies are now starting to apply robust methodological standards demonstrating breathomics to be sensitive to underlying inflammation.** http://bit.ly/2WS1Sfx

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ABSTRACT The search for biomarkers that can guide precision medicine in asthma, particularly those that can be translated to the clinic, has seen recent interest in exhaled volatile organic compounds (VOCs). Given the number of studies reporting "breathomics" findings and its growing integration in clinical trials, we performed a systematic review of the literature to summarise current evidence and understanding of breathomics technology in asthma.

A PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)-oriented systematic search was performed (CRD42017084145) of MEDLINE, Embase and the Cochrane databases to search for any reports that assessed exhaled VOCs in adult asthma patients, using the following terms (asthma AND (volatile organic compounds AND exhaled) OR breathomics).

Two authors independently determined the eligibility of 2957 unique records, of which 66 underwent full-text review. Data extraction and risk of bias assessment was performed on the 22 studies deemed to fulfil the search criteria. The studies are described in terms of methodology and the evidence narratively summarised under the following clinical headings: diagnostics, phenotyping, treatment stratification, treatment monitoring and exacerbation prediction/assessment.

Our review found that most studies were designed to assess diagnostic potential rather than focus on underlying biology or treatable traits. Results are generally limited by a lack of methodological standardisation and external validation and by insufficiently powered studies, but there is consistency across the literature that exhaled VOCs are sensitive to underlying inflammation. Modern studies are applying robust breath analysis workflows to large multi-centre study designs, which should unlock the full potential of measurement of exhaled volatile organic compounds in airways diseases such as asthma.

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## Introduction

In observing the phenotypic heterogeneity of the asthma population [1, 2] and its varied response to currently available treatments [3–5], it is clear that asthma describes a spectrum of biological complexity. Rather than diagnostics, the challenge for clinicians and researchers is the accurate stratification of patients based upon their underlying physiology/pathophysiology and the identification of treatable traits [6, 7]. Critical to this ambition of precision medicine is the identification of biomarkers. Those biomarkers that can be easily sampled at the point of care have the highest translational potential [8]. In this regard, the clinical adoption of carbon-13/14 urea for *Helicobacter pylori* [9] and exhaled nitric oxide fraction ($F_{e}NO$) for airway inflammation [10] demonstrates the utility of exhaled breath: this medium can be sampled safely, non-invasively and repeatedly, almost without exhaustion (*e.g.* capnography [11]). Moreover, through contact with the airways, exhaled breath measurements can potentially offer a direct insight into the organ of interest [12].

Although primarily composed of water vapour and inert gases, exhaled breath also contains thousands of volatile organic compounds (VOCs) [13]. VOCs are the main molecular substrate triggering our sense of smell, and characteristic breath odours have been used to identify illnesses since Hippocrates [14]. The medical applications of this strategy were transformed in 1971 by the demonstration that exhaled breath contained >250 VOCs [15]. We now recognise that changes in exhaled VOC concentrations can reflect different disease states [16, 17], suggesting a role for exhaled VOC analysis in non-invasive and early diagnostics [14].

Modern VOC analysis can be considered under one of two broad methodological headings: pattern recognition based sensors or chemical analytical techniques [18]. Pattern recognition based sensors, synonymous with electronic noses, are modelled on the mammalian nose. These e-noses contain an array of cross-reactive sensors, which react promiscuously and non-selectively to VOCs. The final "breathprint" reflects the differential signalling of multiple sensors to partially overlapping VOCs [19, 20]. These breathprints then require analysis by pattern recognition algorithms [21, 22], similar to how our brain would interpret smells. Chemical analytical techniques typically refer to mass spectrometry (MS) [23] or MS hybrid techniques [24–26], in which ions created by VOCs can be measured based upon their mass/charge (m/z) ratio.

In asthma, exhaled VOC analysis has demonstrated excellent accuracy for discriminating patients from healthy controls and other respiratory conditions [27]. The purpose of this systematic review is to describe the evidence for exhaled VOCs as a biomarker not only for diagnostics, but also other major clinical dilemmas faced by adult asthma clinicians: phenotyping, treatment stratification, treatment monitoring and exacerbation prediction/assessment [28]. We discuss these findings as well as methodologies surrounding breath sampling and VOC analysis; the challenges faced by this technology and future directions for research.

## Methods

### *Search strategy and information sources*

A PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)-oriented systematic search (CRD42017084145) (the full protocol can be accessed at www.crd.york.ac.uk/PROSPERO/) was performed until December 5, 2018 in Embase, MEDLINE and Cochrane Libraries. The search was conducted using keywords for "asthma", "volatile organic compounds", "exhaled" and "breathomics" and full Boolean operator terms (described in detail in the supplementary material). English-language peer-reviewed full-text articles were assessed for eligibility.

### *Eligibility*

The ideal study for our review would prospectively recruit patients with asthma, diagnosed according to internationally recognised guidelines [29] and measure exhaled VOCs consistent with recently defined recommendations [30] including well-defined cut-off values against adequate reference standards. However, this would be difficult to define due to clinical and technological evolution over the timespan of the review. Similarly, the ideal study design for the detection and validation of exhaled VOC markers would be different for each of the clinical dilemmas that we wished to explore (diagnostics, phenotyping, treatment stratification, treatment monitoring and exacerbation prediction/assessment); thus, we used liberal inclusion criteria.

Studies were therefore included if 1) they measured exhaled breath VOC(s) in 2) a distinct group or patients with asthma defined by a trained physician or according to Global Initiative for Asthma or the American Thoracic Society/European Respiratory Society guidelines. Studies were excluded if they looked at non-adult (*i.e.* age <18 years) asthma patients; due to differences in diagnostic criteria between children and adults, as well as an attempt not to overlap with a recent review in paediatric populations [31].

### Study selection
The titles and abstracts of all the records returned by the literature search were independently reviewed by two reviewers, to identify potentially relevant studies. The reviewers used the web-based software platform Rayyan (https://rayyan.qcri.org/) for screening and reviewing. Searches of study bibliographies were conducted to identify additional studies. Using the prespecified inclusion/exclusion criteria, these two reviewers then independently reviewed the full texts of potentially relevant studies to select trials for inclusion in the review. Disagreement was resolved by discussion, but in the event of non-consensus, it was planned that a third reviewer would be involved.

### Data extraction
We extracted information from each study regarding 1) study design, 2) population, 3) breath collection methodology, 4) statistical analysis and 5) results.

### Risk of bias
Risk of bias for each included study was assessed using the quality assessment of diagnostic accuracy studies (QUADAS)-2 tool [32]. The tool assesses the risk of bias across four domains: 1) patient selection, 2) index test, 3) reference standard and 4) flow/timing. Applicability is assessed across the first three domains.

### Method of analysis
Studies were qualitatively assessed according to their methodology and results.

## Results
### Search results
Our search strategy yielded 2955 unique titles from Embase, MEDLINE and the Cochrane Library and two further titles through correspondence with authors in the field. Of these, 2891 were excluded after title and abstract review, leaving 66 full-text articles for screening (full-text exclusions are listed in the supplementary material). Reference list searching yielded no new studies. 22 articles were deemed to fulfil the inclusion/exclusion criteria (figure 1). All articles were assessed for risk of bias using the QUADAS-2 scoring system.

### Study characteristics
Across these 22 studies, 1409 patients with asthma were investigated. The majority were recruited from European centres with just four studies recruiting from the USA [33], Australia [34, 35] and New Zealand [36].

Almost two-thirds (61.0%) came from three studies [21, 37, 38]: the median sample size of studies was 25. As per the inclusion criteria, five studies only required a physician diagnosis of asthma while the other 18 specifically met recognised international diagnostic guidelines. Six studies used oral corticosteroid use as an exclusion criterion and 10 used smoking history.

The broad inclusion criteria of our search meant that several study designs were observed. Most studies employed a cross-sectional design with six including a longitudinal component. One study was purely correlational [39], but the remaining offered comparisons between groups (table 1). Many of these studies address more than one of the aforementioned clinical dilemmas (*e.g.* diagnosis and phenotyping) (table 1).

### Diagnosis
In this review, we identified 14 studies which used VOC signals to discriminate asthma from other disease states; most often healthy controls, although four studies compared to a chronic obstructive pulmonary disease (COPD) group and one to lung cancer. One further study specifically compared asthma with allergic rhinitis to allergic rhinitis alone [44]. Notably, a study of 27 mild asthma patients found e-nose to outperform $F_{e}NO$ and simple spirometry in distinguishing asthma from healthy controls [42].

### Phenotype
Six studies investigated the relationship between exhaled VOCs and airway inflammation in patients with asthma. Exhaled VOCs, identified using gas chromatography-mass spectrometry (GCMS), that were discriminatory between baseline and loss of control (following steroid withdrawal) [50] and which contributed to classification (of sputum cellular phenotype) models were found to correlate with sputum eosinophils [45]. Two studies used e-nose VOC profiles to correlate to bronchoalveolar lavage (BAL) and sputum eosinophil percentages finding an R of 0.76 [39] and 0.60 [36], respectively. As both studies of mild asthma patients used similar breath collection methodologies, these results might suggest that the e-nose profile better relates to small airway events, as represented by BAL sampling, but could be

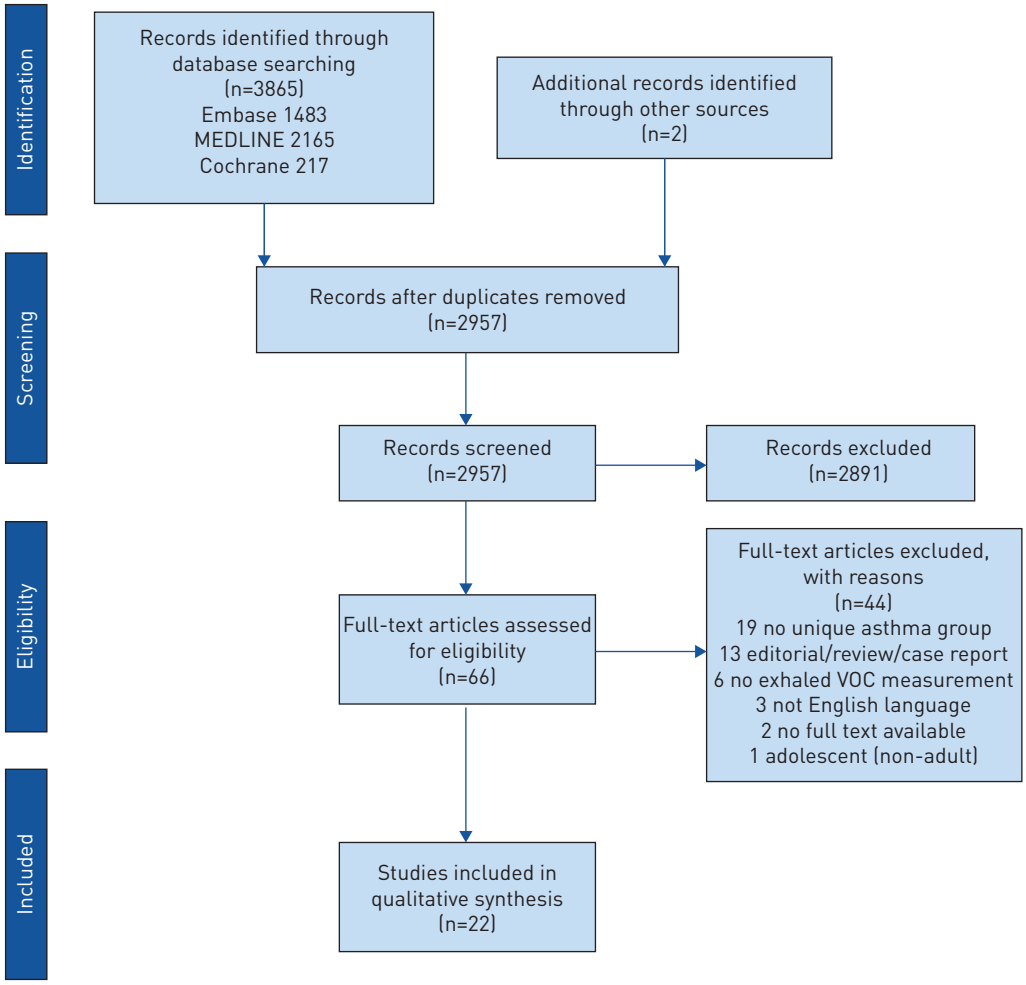


FIGURE 1 Flow diagram of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)-oriented systematic search. VOC: volatile organic compound.

confounded by the fact that patients withheld steroid therapy before sputum, but not bronchoscopy sampling.

Three further studies assessed the ability of exhaled VOCs to discriminate inflammatory phenotype, defined by sputum granulocyte percentages. The e-nose discriminated sputum eosinophilia (3% cut-off) from paucigranular disease, with an area under the receiver operating characteristic curve (AUC) of 0.79 [46]. GCMS analysis discriminated eosinophilic (2% cut-off) from non-eosinophilic sputum with an AUC of 0.98 [45]. A more extensive GCMS study was able to discriminate eosinophilic (3% cut-off) from paucigranulocytic and neutrophilic (76% cut-off) with an AUC of 0.99 and 0.92, respectively [38]. Hoverer, it is not possible to reliably compare e-nose with GCMS approaches from these studies, due to separate breath-collection strategies and statistical analytical approaches. Critically, the Belgian study was able to test its model in a replication cohort demonstrating AUCs of 0.68 and 0.71 for eosinophilic *versus* paucigranulocytic and neutrophilic, respectively [38].

The ability of exhaled VOCs to discriminate patients by their inflammatory profile is further supported by unbiased cluster analysis of e-nose profiles of the U-BIOPRED cohort [47]. These clusters differed by systemic inflammatory profiles and anti-inflammatory medication profile use. They did not differ by sputum eosinophils or neutrophil percentages, but a change in sputum eosinophil percentages was seen in those patients that migrated clusters at 18 months. Using a different set of e-nose derived principal components, this study was also able to discriminate sputum transcriptomic associated clusters [54] in a small pilot analysis (n=28) [47].

### *Treatment stratification*

In patients who had been withdrawn from their regular inhaled corticosteroid therapy, the e-nose breathprint was found to be accurate in predicting who would respond to an oral corticosteroid course

TABLE 1 Summary of titles included, organised by clinical dilemma

| First author (year) [reference] | Design | Methodology | Brief summary of main findings |
|---|---|---|---|
| **Diagnosis** | | | |
| Dragonieri (2007) [40] | Cross-sectional | e-nose (Cyranose 320)<br>GCMS | e-nose breathprints accurately discriminate asthma from age-matched controls<br>Less successful at discriminating mild and severe asthma<br>Explorative GCMS analysis identified compounds in asthma |
| Fens (2009) [41] | Cross-sectional | e-nose (Cyranose 320) | e-nose breathprints accurately discriminate asthma from COPD (smoking and nonsmoking as well as ICS-treated and non-ICS-treated)<br>e-nose breathprints accurately discriminate asthma from nonsmoking controls |
| Montuschi (2010) [42] | Cross-sectional | e-nose (Tor Vergata)<br>GCMS | e-nose breathprints accurately discriminate asthma from healthy controls<br>e-nose superior to *F*eNO and spirometry for diagnostic accuracy<br>Late expiratory phase breath sampling (described as "alveolar air") gives better separation than mixed expiratory for the e-nose<br>No correlation between e-nose and *F*eNO or spirometry |
| Fens (2011) [43] | Cross-sectional# | e-nose (Cyranose 320) | e-nose breathprints accurately discriminate asthma with fixed airways obstruction and reversible airway obstruction from COPD<br>Less successful at discriminating fixed airways obstruction from reversible airways obstruction |
| Timms (2012) [34] | Cross-sectional | e-nose (Cyranose 320) | e-nose breathprint could discriminate asthma with GORD from healthy controls and asthma without GORD from healthy controls<br>Could also discriminate asthma from COPD |
| de Vries (2015) [21] | Cross-sectional | e-nose (Spironose) | e-nose breathprints accurately discriminate asthma from COPD and healthy controls<br>Less successful at discriminating asthma from lung cancer<br>No significant difference in breathprints from asthma patients at different sites |
| Dragonieri (2019) [44] | Cross-sectional# | e-nose (Cyranose 320) | e-nose breathprints accurately discriminate asthma with allergic rhinitis from allergic rhinitis (no asthma) and healthy controls |
| **Phenotyping** | | | |
| Ibrahim (2011) [45] | Cross-sectional | GCMS | VOC model accurately discriminates asthma from healthy controls<br>VOC model accurately discriminates (using sputum granulocyte percentages) eosinophilic from non-eosinophilic asthma (superior to *F*eNO) and neutrophilic from non-neutrophilic asthma phenotypes<br>VOC model accurately discriminates controlled from uncontrolled asthma (using ACQ score) |
| Meyer (2014) [37] | Cross-sectional | GC ToF MS | VOC model accurately discriminates asthma from healthy controls<br>Identified 7 clusters (using 16 discriminatory VOCs and clinical parameters) with different clinical phenotypes |
| Plaza (2015) [46] | Cross-sectional | e-nose (Cyranose 320) | e-nose breathprints accurately discriminates (using sputum granulocyte percentages) eosinophilic from neutrophilic asthma, eosinophilic from paucigranular asthma and neutrophilic from paucigranular asthma |
| Fens (2015) [39] | Cross-sectional | e-nose (Cyranose 320) | e-nose breathprints associated with bronchoscopy lavage fluid eosinophil percentages (no relationship found between *F*eNO and BAL eosinophils) |
| Brinkman (2019) [47] | Longitudinal and cross-sectional# | e-nose (composite platform) | Identified 3 clusters (using e-nose breathprints) with significant differences in chronic OCS usage and blood eosinophil and blood neutrophil percentages<br>The majority of patients had migrated clusters at follow-up; patients that migrated clusters had changes in their sputum eosinophils |

Continued

TABLE 1 Continued

| First author (year) [reference] | Design | Methodology | Brief summary of main findings |
|---|---|---|---|
| Schleich (2019) [38] | Cross-sectional# | GC ToF MS | VOC model accurately discriminates (using sputum granulocyte percentages) eosinophilic from paucigranular asthma, eosinophilic from neutrophilic asthma and neutrophilic from paucigranular asthma<br>VOC model accurately discriminates eosinophilic from non-eosinophilic asthma (with similar accuracy to *F*eNO and blood eosinophils) and neutrophilic from non-neutrophilic asthma<br>VOC model unable to discriminate smokers, ex-smokers and nonsmokers<br>VOC model unable to discriminate ICS-treated and ICS-naïve patients |
| **Treatment stratification** | | | |
| van der Schee (2013) [36] | Longitudinal and cross-sectional | e-nose (Cyranose 320) | e-nose breathprints accurately discriminate asthma from healthy controls (maintained after asthma patients treated with oral prednisolone)<br>Accurately discriminates (at the point of full treatment withdrawal) patients who had lost control from ICS withdrawal from patients who had not<br>Accurately discriminates (at the point of full treatment withdrawal) patients who were OCS-responsive from OCS-unresponsive patients<br>e-nose breathprint correlates with sputum eosinophils |
| **Treatment monitoring** | | | |
| Paredi (2000) [48] | Cross-sectional | GC FID | Exhaled ethane levels increased in asthma patients not receiving steroid therapy compared to steroid treated asthma patients and controls<br>Ethane concentrations increased in patients with more severe bronchoconstriction and gas trapping |
| Bruce (2009) [35] | Cross-sectional | Breath ethanol device (Alcometer SD-400TM) | Exhaled ethanol levels are transiently elevated following the use of metred dose inhalers using hydrofluoroalkane propellants |
| Brinkman (2018) [49] | Longitudinal and cross-sectional# | GC ToF MS | Exhaled VOCs associated with urinary detection of salbutamol and oral corticosteroids |
| **Exacerbation assessment/ prediction** | | | |
| Olopade (1997) [33] | Longitudinal and cross-sectional | GC FID | Exhaled pentane concentrations increased in acute exacerbation compared to healthy controls (higher in those requiring admission from the emergency department)<br>Pentane concentration significantly decreased following treatment to concentrations similar to concentrations found in healthy controls and stable (outpatient) asthma |
| Brinkman (2017) [50] | Longitudinal | e-nose (composite platform) and GCMS | VOC model discriminates baseline from loss of control and loss of control from recovery<br>e-nose breathprint accurately discriminates baseline from loss of control and loss of control from recovery<br>GCMS identified compounds correlated with sputum eosinophil but not sputum neutrophils; e-nose breathprints did not correlate with either |
| **Main findings not applicable to clinical categories** | | | |
| Lazar (2010) [51] | Longitudinal and cross-sectional | e-nose (Cyranose 320) | e-nose breathprint discriminates post-methacholine from baseline and post-salbutamol (also post-methacholine) from baseline, but unable to discriminate post-methacholine and post-salbutamol (also post methacholine)<br>e-nose breathprint discriminates post-saline from baseline and post-saline from post-salbutamol (also post-saline) |

Continued

TABLE 1 Continued

| First author (year) [reference] | Design | Methodology | Brief summary of main findings |
|---|---|---|---|
| van der Schee (2013) [52] | Cross-sectional | e-nose (Cyranose 320) and GCMS | Peak intensities of GCMS identified compounds did not show changes related to storage time (of up to 14 days)<br>VOC model and e-nose breathprints discriminate asthma from healthy controls with similar accuracies following different storage times |
| Lärstad (2007) [53] | Cross-sectional | GC FID | Isoprene and pentane concentrations increase with breath-holding<br>Ethane concentrations decreased at higher flow rates; pentane increased |

Studies are categorised according to their main aim. The main aims of the studies are briefly summarised and are by no means exhaustive. GCMS: gas chromatography-mass spectrometry; COPD: chronic obstructive pulmonary disease; ICS: inhaled corticosteroids; *F*eNO: exhaled nitric oxide fraction; GORD: gastro-oesophageal reflux disease; VOC: volatile organic compound; ACQ: Asthma Control Questionnaire; GC ToF MS: gas chromatography–time-of-flight mass spectrometry; BAL: bronchoalveolar lavage; OCS: oral corticosteroids; GC-FID: gas chromatography with flame-ionisation detection. #: external validation of results.

(AUC=0.88), defined as a ⩾12% increase in forced expiratory volume in 1 s ($FEV_1$) and/or two or more doubling doses of inhaled AMP to cause a 20% decline in $FEV_1$ [36]. While the steroid-responsive group (n=11) had higher *F*eNO and sputum eosinophil measurements at baseline, as compared to the steroid-unresponsive group (n=9), the respective predictive AUCs of 0.55 and 0.61, respectively, for these measures were not as good as that for e-nose [36]. Other than in the aforementioned steroid withdrawal studies, we found no studies designed to look at treatment responders *versus* nonresponders.

### *Treatment monitoring*

E-nose-derived clusters differ by oral corticosteroid use [47], and exhaled pentane levels are reduced in patients receiving inhaled corticosteroid treatments [48], consistent with the premise that VOCs are sensitive to ongoing inflammation. However, while oral corticosteroid use weakens the diagnostic (asthma *versus* healthy controls) accuracy of *F*eNO, this is not the case for the e-nose breathprint [36]. Following oral corticosteroid treatment of asthma patients, the e-nose continued to be able to diagnose asthma, suggesting discrimination independent of ongoing inflammation, possibly related to the medication itself. Similarly, the discrepancy between e-nose VOC profiles and BAL and sputum eosinophil percentages [36, 39], may be explained by treatment effect: if the VOC profile were sensitive to eosinophilic inflammation alone, a better relationship would be expected without the suppressive and noise effect of steroid therapy. Consistent with this, the U-BIOPRED group have reported that exhaled VOCs correlate with urinary levels of salbutamol and oral corticosteroid (by liquid chromatography-high resolution mass spectrometry) [49], confirming that some exhaled VOCs are related to asthma medications.

### *Exacerbation prediction*

Markers of lipid peroxidation are elevated in acute exacerbations before returning to normal levels following treatment [33]. In addition, they are increased in patients with more severe disease (as defined by more reduced lung function) [48]. Principal components derived from e-nose breathprints can discriminate episodes of stability (baseline or at recovery with oral corticosteroids) from episodes of loss of control, due to treatment withdrawal. While exhaled VOCs have been measured prospectively in children to predict exacerbation events [55], no such studies have been performed in adults. In the two steroid withdrawal studies captured in this systematic review, one did not measure exhaled VOCs at baseline [36], and in the other, all but one patient suffered a loss of control on treatment withdrawal [50]. Therefore, these studies were unable to compare exacerbators against non-exacerbators.

### *Breath sampling*

Concerning sampling, storage and analysis, few studies use the same strategies. Although a degree of consistency is seen from the studies originating from the same group [21, 40, 41, 43, 50–52], wide methodological variation is seen at every step in exhaled breath methodology. This is illustrated in table 2 and expanded upon in the supplementary material.

Most studies instruct patients to perform a single vital capacity manoeuvre: from maximal inspiration to maximal expiration. The majority of these studies collect VOCs from this total expiratory phase, as it is

TABLE 2 Methodological variation across studies split by breath fraction from which volatile organic compounds were captured

| First author (year) [reference] | Breathing manoeuvre for breath collection | Breath fraction | Flow rate | Breath collection container |
|---|---|---|---|---|
| **Mixed expiratory** | | | | |
| Olopade (1997) [33] | Single vital capacity from maximal deep inspiration | Mixed expiratory | Not specified | Tedlar bag |
| Larstad (2007) [53] | Various manoeuvres tested including breath-holding | Mixed expiratory | Various tested (computerised biofeedback) | Tedlar bag |
| Dragonieri (2007) [40] | Single vital capacity from maximal deep inspiration | Mixed expiratory | 0.1–0.2 $L \cdot s^{-1}$ | Tedlar bag |
| Fens (2009) [41] | Single vital capacity from maximal deep inspiration | Mixed expiratory | 0.1–0.2 $L \cdot s^{-1}$ | Tedlar bag |
| Lazar (2010) [51] | Single vital capacity from maximal deep inspiration | Mixed expiratory | 0.1–0.2 $L \cdot s^{-1}$ | Tedlar bag |
| Fens (2011) [43] | Single vital capacity from maximal deep inspiration | Mixed expiratory | 0.1–0.2 $L \cdot s^{-1}$ | Tedlar bag |
| Timms (2012) [34] | Single vital capacity from maximal deep inspiration | Mixed expiratory | Not specified | Tedlar bag |
| van der Schee (2013) [52] | Single vital capacity from maximal deep inspiration | Mixed expiratory | 0.1–0.2 $L \cdot s^{-1}$ | Nalophan bag |
| van der Schee (2013) [36] | Single vital capacity from maximal deep inspiration | Mixed expiratory | 0.1–0.2 $L \cdot s^{-1}$ | "Inert bag" |
| Meyer (2014) [37] | No special provision | Not specified (assume mixed expiratory) | Not specified | Tedlar bag |
| de Vries (2015) [21] | Single vital capacity from maximal deep inspiration following 5-s breath-hold | Mixed expiratory | <0.4 $L \cdot s^{-1}$ | Not applicable (online data collection) |
| Plaza (2015) [46] | Single vital capacity from maximal deep inspiration | Mixed expiratory | 0.1–0.2 $L \cdot s^{-1}$ | Tedlar bag |
| Fens (2015) [39] | Single vital capacity from maximal deep inspiration | Mixed expiratory | 0.1–0.2 $L \cdot s^{-1}$ | Tedlar bag |
| Brinkman (2017) [50] | Single vital capacity from maximal deep inspiration | Mixed expiratory | 0.1–0.2 $L \cdot s^{-1}$ | Tedlar bag |
| Brinkman (2019) [47] | Single vital capacity from maximal deep inspiration | Mixed expiratory | 0.1–0.2 $L \cdot s^{-1}$ | Tedlar bag |
| Dragonieri (2019) [44] | Single vital capacity from maximal deep inspiration | Mixed expiratory | 0.1–0.2 $L \cdot s^{-1}$ | Tedlar bag |
| Schleich (2019) [38] | Single vital capacity from maximal deep inspiration following 5-s breath-hold | Mixed expiratory | Not specified | Tedlar bag |
| Brinkman (2018) [49] | Single vital capacity from maximal deep inspiration | Mixed expiratory | 0.1–0.2 $L \cdot s^{-1}$ | Tedlar bag |
| **Other** | | | | |
| Paredi (2000) [48] | Single vital capacity from maximal deep inspiration | Late expiratory (calculated time required to washout dead space) | 10–11 $L \cdot min^{-1}$ (equivalent to 0.1–0.2 $L \cdot s^{-1}$) | "Collapsible reservoir" |
| Bruce (2009) [35] | Not specified (single vital capacity from maximal deep inspiration described by Lion Laboratories) | Not specified (late expiratory described by Lion Laboratories manual) | Not specified (minimum required flow described by Lion Laboratories manual) | Not applicable (online analysis) |
| Montuschi (2010) [42] | Single vital capacity from maximal deep inspiration | Compared mixed expiratory to late expiratory (using 150 mL as dead space) | Not specified | Tedlar bag |
| Ibrahim (2011) [45] | Tidal breathing | Late expiratory (respiratory pattern monitored by a pressure transducer) | Not specified | Directly adsorbed |

logistically most simple to achieve, but is contaminated by air within the oropharynx. Several studies have excluded dead space using strategies ranging from the crude (valves and estimated volumes [56, 57]), to highly engineered (pressure sensors [58]).

Just 13 studies measured exhaled flow rate. 12 of these regulated exhaled breath flow between 0.1 and 0.2 $L \cdot s^{-1}$, which minimises nasal contamination of the sample by ensuring closure of the soft palate [59, 60], rather than due to a determination of optimal VOC capture. Most studies collect breath samples in an inert polymer, usually Tedlar, bag followed by storage onto thermal desorption tubes containing some adsorbent material, usually Tenax. Once the exhaled breath is collected, it can be either analysed "online" or "offline". Online refers to real-time analysis, but other than the Alcometer, a blood alcohol "breathalyser" [35], no studies have used such a platform.

### *VOC analysis*

Since its first description in 2007 [40], the majority of modern studies utilise e-nose technology. Preprocessing procedures on raw data is described in few studies, and even then only briefly [38, 47]. Variation in statistical methodology, ranging from univariate to machine-learning techniques (table 3) broadly illustrates evolution over time: statistical analysis now involves some form of dimension-reduction strategy coupled to some form of machine-learning classifier. Several studies used "leave one out cross-validation techniques" but, until recently, external validation was notable by its rarity.

### *Risk of bias*

In this systematic review, the QUADAS-2 scoring system highlights concerns in the published literature across several domains [32] (figure 2). Many studies applied smoking history, use of oral corticosteroids (due to the severity of illness) and common comorbidities as exclusion criteria to patient recruitment. By removing diagnostically challenging patients, studies are likely to provide over-optimistic discriminatory estimates. In addition, poor reference standards might undermine published results. Examples include diagnostic studies using asthma diagnosis based on physician assessment only [61] and inflammatory phenotyping studies based on sputum granulocyte percentages [62] or gastro-oesophageal reflux disease diagnosis without objective evidence. However, the most significant source of bias related to the analysis of exhaled VOC data (both GCMS or e-nose). In the absence of a clear consensus on the best statistical approach to multivariate data and an over-reliance on internal validation, the statistical findings described by authors are likely to be over-optimistic [63].

The flow and timing between index test and reference standards did not clearly introduce any bias, and the applicability of the identified studies to our review question was generally very good. The applicability of some study designs, such as cluster analysis, to our review question, which relates to clinical applications, was not clear. Similarly, studies relying on GCMS-based analysis techniques for acute assessment were found to be of limited translation potential.

## Discussion

### *Technological evolution*

Reviewing the past few decades of asthma–VOC literature demonstrates the rapid technological evolution of this field. Early breath research was limited to a "bottom-up" approach: VOCs were targeted *a priori* and analysed using expensive and laborious chemical techniques. Consequently, these studies were limited in numbers and focused on markers of oxidative stress [33] (inflammation not specific to asthma [48, 64]). The modern parallel developments of improved separation techniques, improved lower limits of detection, e-nose technology and high-throughput omics analysis platforms allow the full spectrum of exhaled VOCs to be analysed "top-down" as highly dimensional composite profiles: "breathomics".

Modern breathomics can refer to several VOC analysis platforms [18, 65], but mass spectrometry continues to be the gold standard [66], due to its ability to identify composition and concentrations of individual VOCs. However, it is too expensive in terms of expertise and equipment. Although the e-nose sensor array is cheaper, quicker and easier to use [40, 57], it sacrifices that ability to reliably trace back to analytes of interest [67]. For clinicians, the focus on "treatable traits" [7, 68] requires a probabilistic approach: stratification of a patient based upon a clinically relevant quality (diagnosis, prognosis, treatment response) [69]. Understanding the mechanisms behind that biomarker/biomarker profile is not necessary [70], so sensor-based systems are ideally suited. In contrast, if the biomarker is being interrogated for mechanistic purposes [71] (*e.g.* drug target discovery), then the costs of chemical analytic techniques are likely justifiable. That is not to say that the two technologies are mutually exclusive: both can be successfully integrated into the same study [31], and improved understanding of the mechanisms and factors contributing to VOC profiles could inform future sensor-based technologies.

### *Breath collection heterogeneity*

Of course, the strength of both platforms is intimately linked with the quality of its reference library. Efforts to build these libraries are already underway [72, 73] (www.breathomix.com/breathbase-data) and will only improve as more studies engage with such processes. Critically, such libraries can only be

TABLE 3 Statistical approaches to exhaled volatile organic compound (VOC) data, organised by type of data

| First author (year) [reference] | Signal | Statistical technique | External validation |
|---|---|---|---|
| **Univariate statistical analysis** | | | |
| Olopade (1997) [33] | Pentane | Wilcoxon signed rank test | No |
| Paredi (2000) [48] | Ethane | ANOVA | No |
| Lärstad (2007) [53] | Ethane, pentane, isoprene | Wilcoxon signed rank test | No |
| Bruce (2009) [35] | Blood alcohol | Mann–Whitney | No |
| **Multivariate statistical analysis** | | | |
| Dragonieri (2007) [40] | Pre-processed sensor data | PCA<br>Classification by linear discriminant analysis on all PC factors | No |
| Fens (2009) [41] | Raw sensor data | PCA<br>Classification by linear discriminant analysis on PC factors that were discriminant between groups | No |
| Montuschi (2010) [42] | Raw sensor data | PCA<br>Classification by feed-forward neural network | No |
| Lazar (2010) [51] | Pre-processed sensor data | PCA<br>Mixed model analysis on PC factors that were discriminant between groups | No |
| Fens (2011) [43] | Raw sensor data | PCA<br>Classification by linear discriminant analysis on PC factors that were discriminant between groups | Yes |
| Ibrahim (2011) [45] | VOCs | Logistic regression for each VOC<br>PCA for VOCs that were discriminant between groups.<br>Classification by multivariate logistic regression on all PCs | No |
| Timms (2012) [34] | Raw sensor data | PCA<br>Classification by canonical model on all PC factors | No |
| van der Schee (2013) [52] | Select VOCs and raw sensor data (respectively) | PCA<br>Classification by linear discriminant analysis on PC factors that were discriminant between groups and classification by linear canonical discriminant analysis on compound intensities (respectively) | No |
| van der Schee (2013) [36] | Raw sensor data | PCA<br>Classification by linear discriminant analysis on PC factors that were discriminant between groups | No |
| Meyer (2014) [37] | Clinical features, asthma medications, VOCs | Hierarchical SPSS two-step cluster analysis | No |
| de Vries (2015) [21] | Pre-processed sensor data | PCA<br>Classification by linear discriminant analysis on PC factors that were discriminant between groups | No |
| Plaza (2015) [46] | Raw sensor data | PCA<br>Classification by linear discriminant analysis on PC factors that were discriminant between groups | No |
| Fens (2015) [39] | Raw sensor data | PCA<br>Multivariate regression analysis on all PCs and BALF eosinophil counts | No |
| Brinkman (2017) [50] | Pre-processed data | PCA on variables of interest determined by ANCOVA and Pearson's correlation to ACQ. Paired t-test on PCs with eigenvalue >1 | No |
| Brinkman (2019) [47] | Pre-processed data | Non-hierarchical K-means clustering | Yes |
| Dragonieri (2019) [44] | Pre-processed sensor data | PCA<br>Classification by linear discriminant analysis on PC factors that were discriminant between groups | Yes |
| Schelich (2019) [38] | VOCs | Conditional inference forest algorithm and variable importance measure for each VOC. Selection of identifiable and discriminatory VOCs. Classification by random forest algorithm | Yes |
| Brinkman (2018) [49] | Pre-processed data | Multivariate modelling of GCMS fragments compared to LCMS drug outcomes by AUC | Yes |

PCA: principal component analysis; PC: principal component; BALF: bronchoalveolar lavage fluid; ACQ: Asthma Control Questionnaire; GCMS: gas chromatography-mass spectrometry; LCMS: liquid chromatography-mass spectrometry; AUC: area under the receiver operating characteristic curve.

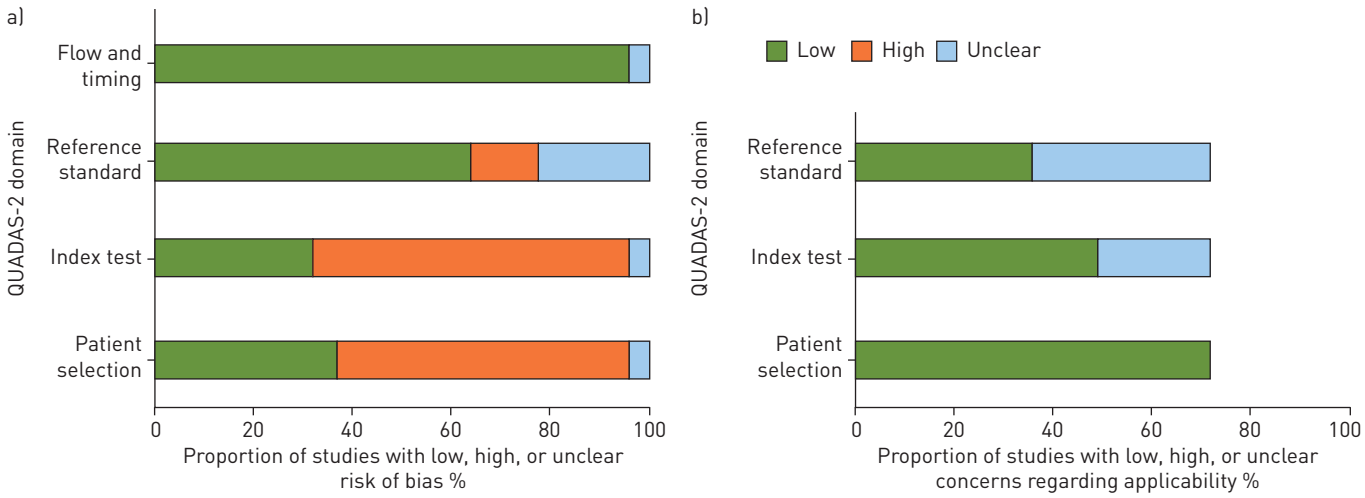


FIGURE 2 Quality assessment of diagnostic accuracy studies (QUADAS)-2 graphical display. a) Risk of bias; b) applicability concerns.

referenced against when using the same methodological parameters, so sharing meta-data relating to sample handling, processing and analysis is of paramount importance. The methodological heterogeneity of the breath analysis literature is well documented [30, 74]. While exhaled breath sampling is simple for patients, by definition, the analyte of interest is volatile, and each stage of exhaled breath analysis introduces additional sources of variation [74].

Like exhaled nitric oxide concentrations [60, 75], individual VOC concentrations [53] and e-nose sensor deflections [21] can be influenced by flow rate and breath-holding [21]. Similarly, most studies collect VOCs from the total expiratory phase, as it is logistically most simple to achieve. E-nose breathprints using this breath sample show more inferior diagnostic potential (asthma *versus* healthy controls) than when excluding air within the oropharynx [42], possibly reflecting the dilution of discriminatory VOCs by this contaminant air. There has been a range of strategies used to exclude air from this dead space, including valves and estimated volumes, which are inconsistent and unreliable [56, 57], to a highly engineered system of pressure sensors [45, 58, 76], which is expensive and bulky. The optimal solution needs to balance practicality and precision, so as not to negate the clinical utility of breath sampling. The breath sampler developed by a broad consortium of breath researchers and engineers (www.breathe-free.org), represents one such solution. A similar pragmatism is likely necessary for the exclusion of exogenous VOCs. Subtracting ambient VOC concentrations from exhaled VOC concentrations (alveolar gradient) [77] risks the loss of salient signals and ignores VOC interactions within the airways [78, 79]. Although unable to eliminate all exogenous VOCs, filters can at least reduce background contamination [40].

Once collected, unless performing online analysis, the storage medium bears consideration. Most early studies stored breath samples in an inert polymer (usually Tedlar) bag, but the concentrations of compounds stored in Tedlar bags show compound-specific decay rates and the bags themselves can introduce contaminants [80, 81]. Van der Schee *et al.* [52] found no variation when breath samples were stored for up to 2 weeks, and many studies try to minimise storage time [40, 42]. Alternative or subsequent storage solutions: thermal desorption tubes containing some sort of adsorbent material (porous organic polymers, activated charcoal, carbon molecular sieves or graphitised carbon blacks) do not guarantee against this decay [81]. No adsorptive material can completely capture all the VOCs in the breath without some degree of loss [82].

Moreover, different materials are vulnerable to breakthrough (non-quantitative adsorption of analytes) and memory effect (incomplete desorption resulting in interference with subsequent measures) [83]. Therefore, ideally, the choice of adsorbent materials and the duration of storage [52] should be determined by compounds of interest [82, 84]. Most studies now adsorb onto Tenax TA (2,6-diphenyl-*p*-phenylene oxide) [85], due to its hydrophobicity, thermal stability and its ability to absorb a wide range of VOCs [86].

### *Data handling heterogeneity*

Even then, a robust and reliable sample collection is relatively straightforward when compared to data management, analysis, and interpretation [87]. There are many thousands of VOCs in exhaled breath [13], and a proportion of these will relate to non-disease-related factors: age [88], sex [89], diet [90], exercise

[91] and smoking [92], as well as therapies [51, 93], the resident microbiome [18] and environmental exposures [70, 81, 94–96]. Furthermore, it is also appreciated that not all VOCs originate from the airways. Non-asthma–VOC research proposes a model of blood/gas coefficients [97] and quantification of regional lung ventilation and perfusion [98] to describe the delivery and migration of systemically generated VOCs to exhaled breath.

Almost all the identified discriminatory VOCs identified in asthma studies are straight-chain, branched or aromatic hydrocarbons [81], and while there are proposed endogenous origins to such compounds [79], they frequently occur exogenously [99] and may simply reflect differential uptake of environmental VOCs [100]. Consequently, those VOCs that occur commonly and in high concentrations or those that are common to many inflammatory states [64, 101], are over-represented in the literature. For airways diseases, it is likely that actually, only a fraction of the VOCs identified in exhaled breath actually relate to airway events.

The majority of studies in this review explore their data using principal component analysis (PCA). Dimension reduction in this manner usefully allows focus on the variation of interest [28]. Classification by only using discriminatory principal components results in better diagnostic classification (asthma *versus* healthy controls) than when using all principal components unselectively [42]. Different principal components are used to discriminate diseases and inflammatory phenotype [39, 45], just as e-nose discrimination of transcriptomic-associated clusters [54] in the U-BIOPRED study relies on different principal components to those used to cluster patients according to inflammation [47]. As such, the VOCs used to discriminate across sputum granulocyte phenotypes were unable to discriminate between inhaled corticosteroid (ICS)-treated or ICS-naïve patients [38]. The disadvantage of PCA is its sensitivity to outliers, the handling of which is critical but rarely commented upon [102]. Moreover, data restructuring in this manner abstracts the relationship between trait/mechanism and biomarker.

For supervised statistical analyses, we commonly see linear discriminant analysis and partial least squares discriminant analysis, both of which investigate linear relationships. Complex biological systems are characterised by non-linear relationships, and although non-linear statistical techniques deliver the highest accuracy, their application may be limited by difficulty in their interpretation [103]. In the absence of a clear consensus on the optimal statistical approach, studies are likely to publish highly internally valid results which, in the absence of external validation, probably overestimate real-world findings [63]. This influence of data handling on biomarker identification means that transparency is more important than ever. The TRIPOD (Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis) recommendations on reporting multivariable prediction models [104] and STARD (Standards for Reporting of Diagnostic Accuracy Studies) guidelines on reporting of diagnostics accuracy studies [105] provide useful frameworks for future publications.

### *Limitations of systematic review*

We specifically sought to describe biomarkers for precision medicine in asthma [7]; however, few studies specifically measure underlying biology or treatable traits, focusing instead on historical diagnostic labels [106]. In addition, we found that studies focused on milder cohorts, poorly representative of the heterogeneity of asthma, which is greatest in those with severe disease [1, 2, 107, 108]. Although we deliberately excluded paediatric studies, they have been summarised elsewhere [31]. Our broad and inclusive search strategy successfully captures the full spectrum of study designs, populations and outcome measures seen in exhaled VOC research. While this undermines some of the QUADAS risks-of-bias scores, we believe that our search allows for a comprehensive review of this emerging field.

### *Future considerations*

It is evident that the field of breathomics is addressing the limitations mentioned herein. The breath research community has developed a framework for methodological standardisation [30, 109] and the latest breathomics findings are coming from large, multicentre studies with defined validation cohorts [22, 38, 47]. Large-scale studies, which recruit over a protracted period and over multiple sites [81] introduce new challenges. Such studies demand a multibatch experimental design (also unavoidable due to limitations of VOC storage solutions). Regular instrument proficiency testing and the use of quality control samples [110] is critical for demonstrating consistent quality assurance throughout such a study. Metabolomics studies demand standard operating protocols for both the analytical and computational workflows [81], including strategies to monitor within- and between-batch measurement variations [111]. This extends to data preprocessing of MS data (denoising and baseline correction, alignment, peak picking and merging of the peaks) as well as normalisation, scaling and transformation of the data, essential to constructing a reliable data matrix [103]. Consistent with previous reviews, we found that details of this were rarely reported [63], although recent publications are reversing this trend [38, 47]. The ultimate intention for breathomics

research would be interlaboratory and equipment comparison [112]. Only then will true external validation (where findings are replicated in a new study) be possible [113].

Robust biomarkers for T2-low asthma remain elusive [71, 114–116] despite these patient groups being most treatment-resistant and having the worst clinical outcomes [2, 117, 118]. The airway microbiome and host–microbial interactions have been proposed as a way to understand these poorly characterised asthma endotypes [119, 120]. Where bacteria are abundant producers of VOCs [121–123], they are likely to contribute to the spectra of exhaled VOCs. E-nose breathprints have been able to specifically discriminate patients colonised with or without potentially pathogenic bacteria in COPD [124], and the same is likely to be the case for asthma. For example, nonanal, identified to discriminate neutrophilic from eosinophilic asthma [38], is produced by bacterial species and has antimicrobial properties of its own [125], which may, in turn, contribute to the reduction in microbial diversity seen in neutrophilic asthma [126].

As airways diseases move closer to precision medicine, study designs will also have to consider the best way to assess VOCs. Biomarker studies are only as good as the gold standard to which they are compared. Existing gold standard biomarkers, such as *F*eNO and blood eosinophils, may not accurately predict T2-high biology [62]. Failings of a new biomarker (such as VOCs) may, in fact, reflect imperfect gold standard tests [127]: e-nose, for example, can discriminate (sputum) transcriptomic associated clusters (TAC clusters) [47] more effectively than existing biomarkers. Therefore, studies that aim to assess the biomarker potential of breathomics should consider moving away from solely comparing to these existing biomarkers and towards the direct assessment of biology, possibly alongside other omics platforms [128]. GCMS studies might also consider parallel *in vitro* studies as a means to validate *in vivo* findings [129, 130]. Alternatively, the focus should be on crucial disease traits such as treatment response [69], exacerbation prediction [28] and treatment adherence [131, 132]. Early evidence shows e-nose to demonstrate superior accuracy at predicting clinical response to steroids (compared to *F*eNO or sputum eosinophils) [36, 42], as well as accurate GCMS detection of VOCs, linked to salbutamol and OCS use [49].

### Conclusions

Breathomics is still a relatively new field, and the guarded optimism with which findings are treated is justified (figure 2). As with other omics technologies, breathomics generally suffers from a lack of external validation, compounded by limited patient numbers and the risk of false discoveries due to model overfitting [12, 103, 113]. Nevertheless, the sensitivity of exhaled VOCs to underlying inflammation is corroborated across several studies using both sensor- and chemical-based platforms. Encouragingly, we see successful discrimination of asthma patients based upon their inflammatory phenotypes in a large replication cohort [38].

Strictly, true external validation has still not yet been met: the discriminatory VOCs in this study were not the same as those in a similar study [45] nor relevant *in vitro* work [130]. These might be explained away by differences in study design/methodology and the failings of headspace samples to accurately reflect complex interactions within the airways [133], but equally demonstrate the scale of what is still unknown in breathomics. Immediate priorities for breathomics include standardisation of reporting, a better understanding of the best statistical approaches and access to data-sharing platforms. Armed with these tools and study designs that maximise biomarker discovery, the next generation of breathomics evidence should show the full potential of exhaled VOCs.

Conflict of interest: A. Azim has nothing to disclose. C. Barber has nothing to disclose. P. Dennison has nothing to disclose. J. Riley is employed by and has shares in GSK. P. Howarth works for GSK in an educational role and has shares in GSK.

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# Supplementary Material A Search Strategy

A PRISMA-oriented systematic search (CRD42017084145) (the full protocol can be accessed at https://www.crd.york.ac.uk/PROSPERO/) was performed until 05/12/2018 in EMBASE, MEDLINE and Cochrane Libraries.

## Search Strategy Structure

1 AND (2 AND 3) OR 4

1= Asthma Terms

2= Volatile Organic Compound Terms

3= Exhaled Terms

4= Breathomic Terms

## Embase Search

1. exp asthma/

2. asthma.mp.

3. (bronch* adj3 hyper*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

4. exp bronchus hyperreactivity/

5. respiratory tract inflammation/ or exp allergic airway inflammation/

6. exp airway obstruction/

7. (revers* adj3 air*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

8. 1 or 2 or 3 or 4 or 5 or 6 or 7

9. exp volatile organic compound/

10. VOC.mp.

11. (volatile adj3 organic).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

12. exp gas chromatography/

13. (gas adj3 chromatograph*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

14. 9 or 10 or 11 or 12 or 13

15. exp exhalation/

16. exhal*.mp.

17. expir*.mp.

18. exp breathing/

19. breath*.mp.

20. 15 or 16 or 17 or 18 or 19

21. 14 and 20

22. exp breath analysis/

23. (breath* adj2 test).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

24. exp electronic nose/

25. (elect* adj2 nos*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

26. (breath* adj2 metab*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

27. breathomic*.mp.

28. e$nose.mp.

29. 22 or 23 or 24 or 25 or 26 or 27 or 28

30. 21 or 29

31. 8 and 30

## MEDLINE Search

1. exp Asthma/

2. asthma*.mp.

3. (bronch* adj3 hyper*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

4. (airway adj3 inflamm*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

5. (revers* adj3 air*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

6. 1 or 2 or 3 or 4 or 5

7. VOC.mp.

8. exp Volatile Organic Compounds/

9. (volatile adj3 organic).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

10. exp Chromatography, Gas/

11. (gas adj3 chromatograph*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

12. 7 or 8 or 9 or 10 or 11

13. exp Exhalation/

14. exhal*.mp.

15. expir*.mp.

16. breath*.mp.

17. 13 or 14 or 15 or 16

18. 12 and 17

19. exp Breath Tests/

20. (breath adj2 test).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

21. (elect* adj2 nos*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

22. (breath* adj2 metab*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

23. breathomic*.mp.

24. e$nose.mp.

25. exp Electronic Nose/

26. 19 or 20 or 21 or 22 or 23 or 24 or 25

27. 18 or 26

28. 6 and 27

## Cochrane Search

#1 MeSH descriptor: [Asthma] explode all trees

#2 asthma:ti,ab,kw

#3 bronch* near/3 hyper*:ti,ab,kw

#4 MeSH descriptor: [Bronchial Hyperreactivity] explode all trees

#5 MeSH descriptor: [Airway Obstruction] explode all trees

#6 revers* near/3 air*:ti,ab,kw

#7 #1 or #2 or #3 or #4 or #5 or #6

#8 MeSH descriptor: [Volatile Organic Compounds] explode all trees

#9 "Voc":ti,ab,kw

#10 volatile near/3 organic:ti,ab,kw

#11 MeSH descriptor: [Chromatography, Gas] explode all trees

#12 gas near/3 chromatog*:ti,ab,kw

#13 #8 or #9 or #10 or #11 or #12

#14 exhal*:ti,ab,kw

#15 expir*:ti,ab,kw

#16 breath*:ti,ab,kw

#17 #14 or #15 or #16

#18 #13 and #17

#19 MeSH descriptor: [Breath Tests] explode all trees

#20 breath* near/2 test:ti,ab,kw

#21 MeSH descriptor: [Electronic Nose] explode all trees

#22 elect* near/2 nose:ti,ab,kw

#23 breath* near/2 metab*:ti,ab,kw

#24 breathomic*:ti,ab,kw

#25 e?nose:ti,ab,kw

#26 #19 or #20 or #21 or #22 or #23 or #24 or #25

#27 #18 or #26

#28 #7 and #27

## Appendix B Breath Sampling

| Author | Ambient VOC Exclusion | Breathing Manoeuvre for Breath Collection | Breath Fraction | Flow Rate | Breath/VOC Storage | VOC Extraction |
|---|---|---|---|---|---|---|
| **Olopade (1997)[1]** | Ambient Air Collected in Parallel | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | not specified | Tedlar bag | n/a |
| **Paredi (2000)[2]** | Ambient Air Collected in Parallel | Single Vital Capacity from Maximal Deep Inspiration | Late Expiratory (calculated time required to washout deadspace) | 10-11 L/min (equivalent to 0.1-0.2 L/s) | "collapsible reservoir" | n/a |
| **Larstad (2007)[3]** | Purified Air through non-rebreathe valve | Various Manoevres Tested | Mixed Expiratory and Late Expiratory | various tested (computerised biofeedback) | Tedlar bag to tubes containing Tenax, Carbopack, Carboxen | Thermal Desorption |
| **Dragonieri (2007)[4]** | Inspired VOC filtered air through non-rebreathe valve and collected ambient air in parallel | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | 0.1 -0.2L/s | Tedlar bag | n/a |
| **Fens (2009)[5]** | Inspired VOC filtered air through non-rebreathe valve and collected ambient air in parallel | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | 0.1 -0.2L/s | Tedlar bag | n/a |
| **Bruce (2009)[6]** | not specified | not specified (Single Vital Capacity from Maximal Deep Inspiration described by lion laboratories) | not specified (late expiratory described by lionlaboratories manual) | not specified (minimum required flow described by lionlaboratories manual) | n/a (online analysis) | n/a |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Montuschi (2010)[7]** | Ambient Air Collected in Parallel | Single Vital Capacity from Maximal Deep Inspiration | compared Mixed Expiratory to Late Expiratory (using 150mls as deadspace) | not specified | Tedlar bag | Solid Phase Micro Extraction |
| **Lazar (2010)[8]** | Inspired VOC filtered air through non-rebreathe valve and collected ambient air in parallel | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | 0.1 -0.2L/s | Tedlar bag | n/a |
| **Fens (2011)[9]** | Inspired VOC filtered air through non-rebreathe valve and collected ambient air in parallel | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | 0.1 -0.2L/s | Tedlar bag | n/a |
| **Ibrahim (2011)[10]** | Inspired VOC filtered air through non-rebreathe valve | Tidal Breathing | Late Expiratory (respiratory pattern monitored by pressure transducer) | not specified | Directly adsorbed onto tubes containing Tenax, Carbotrap | Thermal Desorption |
| **Timms (2012)[11]** | not specified | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | not specified | Tedlar bag | n/a |
| **van der Schee (2013)[12]** | Inspired VOC filtered air through non-rebreathe valve and collected ambient air in parallel | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | 0.1 -0.2L/s | Nalophan bag | n/a |
| **van der Schee (2013)[13]** | Inspired VOC filtered air through non-rebreathe valve and collected ambient air in parallel | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | 0.1 -0.2L/s | "inert bag" | n/a |
| **Meyer (2014)[14]** | not specified | No special provision | not specified (assume Mixed Expiratory) | not specified | Tedlar bag to tubes containing Carbopack | Thermal Desorption |

| | | | | | | |
|---|---|---|---|---|---|---|
| **de Vries (2015)[15]** | Ambient Air Collected in Parallel | Single Vital Capacity from Maximal Deep Inspiration following 5second breath hold | Mixed Expiratory | <0.4L/s | n/a (online data collection) | n/a |
| **Plaza (2015)[16]** | Inspired VOC filtered air through non-rebreathe valve and collected ambient air in parallel | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | 0.1 -0.2L/s | Tedlar bag | n/a |
| **Fens (2015)[17]** | Inspired VOC filtered air through non-rebreathe valve and collected ambient air in parallel | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | 0.1 -0.2L/s | Tedlar bag | n/a |
| **Brinkman (2017)[18]** | Inspired VOC filtered air through non-rebreathe valve and collected ambient air in parallel | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | 0.1 -0.2L/s | Tedlar bag to tubes containing Tenax | Thermal Desorption |
| **Brinkman (2018)[19]** | Inspired VOC filtered air through non-rebreathe valve and collected ambient air in parallel | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | 0.1 -0.2L/s | Tedlar bag to tubes containing Tenax | Thermal Desorption |
| **Dragonieri (2018)[20]** | Inspired VOC filtered air through non-rebreathe valve and collected ambient air in parallel | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | 0.1 -0.2L/s | Tedlar bag | n/a |
| **Schleich (2019) [21]** | Ambient Air Collected in Parallel | Single Vital Capacity from Maximal Deep Inspiration following 5 second breath hold | Mixed Expiratory | not specified | Tedlar bag to tubes containing Tenax | Thermal Desorption |
| **Brinkman (unpublished) [22]** | Inspired VOC filtered air through non-rebreathe valve and collected ambient air in parallel | Single Vital Capacity from Maximal Deep Inspiration | Mixed Expiratory | 0.1 -0.2L/s | Tedlar bag to tubes containing Tenax | Thermal Desorption |

Table 1 Breath Sampling and Storage Methodology. Abbreviations: VOC = Volatile Organic Compounds

## Appendix C Risk of Bias Tables (QUADAS 2)

| | Risk of Bias | | | | | Concerns regarding Applicability | | |
|---|---|---|---|---|---|---|---|---|
| **AUTHOR** | **PATIENT SELECTION** | **INDEX TEST** | **REFERENCE STANDARD** | **FLOW AND TIMING** | | **PATIENT SELECTION** | **INDEX TEST** | **REFERENCE STANDARD** |
| Olopade (1997)[1] | high | low | low | low | | low | low | low |
| Paredi (2000)[2] | high | low | unclear | low | | low | unclear | low |
| Larstad (2007)[3] | unclear | low | high | low | | low | low | unclear |
| Dragonieri (2007)[4] | high | high | low | low | | low | low | low |
| Fens (2009)[5] | high | high | low | low | | low | low | low |
| Bruce (2009)[6] | low | unclear | low | low | | low | unclear | unclear |
| Montuschi (2010)[7] | low | high | low | low | | low | low | low |
| Lazar (2010)[8] | high | high | low | low | | low | low | unclear |
| Fens (2011)[9] | low | high | low | low | | low | low | low |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ibrahim (2011)[10] | high | high | high | low | | low | unclear | low |
| Timms (2012)[11] | low | high | high | low | | low | low | low |
| van der Schee (2013)[12] | high | high | low | low | | low | low | unclear |
| van der Schee (2013)[13] | high | high | low | low | | low | low | low |
| Meyer (2014)[14] | high | high | unclear | low | | low | unclear | unclear |
| de Vries (2015)[15] | high | high | low | low | | low | low | low |
| Plaza (2015)[16] | high | high | unclear | low | | low | low | low |
| Fens (2015)[17] | high | high | low | unclear | | low | low | low |
| Brinkman (2017)[18] | low | high | low | low | | low | low | low |
| Brinkman (2018)[19] | low | low | unclear | low | | low | low | unclear |
| Dragonieri (2018)[20] | low | low | unclear | low | | low | low | low |
| Shleich (2019) [21] | high | low | low | low | | low | low | unclear |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Brinkman (unpublished) [22] | low | low | low | low | | low | unclear | unclear |

# Appendix D Reasons for Full Text Exclusion

| Author (Year) | Title | Reason for Exclusion |
|---|---|---|
| Baraldi (2004) | Exhaled gas analysis and airway inflammation | review |
| Basanta (2012) | Methodology validation, intra-subject reproducibility and stability of exhaled volatile organic compounds | asthma in mixed group |
| Bjerg and Lotvall (2013) | Sniffing out steroid responsiveness in asthma using an electronic nose | editorial |
| Bogozi (2015) | Fiber-enhanced Raman multi-gas spectroscopy: What is the potential of its application to breath analysis? | review |
| Boys (2012) | An electronic nose can aid diagnosis of gastro-oesophageal reflux disease in COPD | no full text available |
| Brinkman (2019) | Breathomics and treatable traits for chronic airway diseases | no full text available |
| Bukreeva (2017) | [Photoacoustic spectroscopy evaluation of the impact of smoking on the composition of exhaled air in patients with bronchopulmonary diseases] | not English language |
| Couto (2017) | Oxidative stress in asthmatic and non-asthmatic adolescent swimmers-A breathomics approach | adolescent |
| De Vries (2018) | Clinical and inflammatory phenotyping by breathomics in chronic airway diseases irrespective of the diagnostic label | asthma in mixed group |
| Dragonieri (2017) | Electronic Nose Technology in Respiratory Diseases | review |
| Gaugg (2017) | Metabolic effects of inhaled salbutamol determined by exhaled breath analysis | no exclusive asthma group |
| Gomm (1991) | The effect of salbutamol on breath alcohol testing in asthmatics | no exclusive asthma group |
| Gorham (2009) | Ethane and n-pentane in exhaled breath are biomarkers of exposure not effect | no exclusive asthma group |
| Greulich (2011) | An electronic nose can differentiate between patients with obstructive sleep apnoea syndrome and healthy controls | no exclusive asthma group |
| Guatura (2000) | Increased exhalation of hydrogen peroxide in healthy subjects following cigarette consumption | no VOC |
| Guo (2010) | A novel breath analysis system based on electronic olfaction | review |
| Ignacio Garcia (2002) | Influence of asthma inhalers on a breath alcohol test | not English language |
| Jachau (2006) | Influence of ethanol-containing medical drugs on breath alcohol concentration as measured by using the Alcotest 7110 Evidential MK III | no exclusive asthma group |
| Kharitonov (2002) | Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma | no VOC |
| Kikowatz (2009) | Differential ion mobility spectroscopy: non-invasive real-time diagnostics and therapy control in metabolic diseases | no exclusive asthma group |

| | | |
|---|---|---|
| Machado (2009) | Identifying chronic obstructive pulmonary disease and asthma by exhaled breath analysis: Does the (e)Nose know? | editorial |
| Maniscalco (2017) | Coexistence of obesity and asthma determines a distinct respiratory metabolic phenotype | no VOC |
| O'Connell (2006) | Asthmatics: Too drunk to drive? The time curve of exhaled ethanol levels after use of Salamol in normal subjects | no exclusive asthma group |
| Paredi (2002) | Analysis of expired air for oxidation products | review |
| Prabhu (1991) | Airflow obstruction and roadside breath alcohol testing | asthma in mixed group |
| Reti (2005) | Salamol asthma inhaler fails roadside alcohol breath testing | case report |
| Scaglione (2009) | Salbutamol and false-positive breath-alcohol test | not English language |
| Schivo (2013) | A mobile instrumentation platform to distinguish airway disorders | no VOC |
| Self (2017) | False-positive breathalyser tests and use of metered-dose inhalers: Does inhalation technique affect test results? | case report |
| Shehada (2016) | Silicon Nanowire Sensors Enable Diagnosis of Patients via Exhaled Breath | no exclusive asthma group |
| Shin (2015) | Quantification of Aerosol Hydrofluoroalkane HFA-134a Elimination in the Exhaled Human Breath Following Inhaled Corticosteroids Administration | no exclusive asthma group |
| Siblia (2014) | Identification of airway bacterial colonization by an electronic nose in Chronic Obstructive Pulmonary Disease | no exclusive asthma group |
| Smith and Spanel (2017) | On the importance of accurate quantification of individual volatile metabolites in exhaled breath | review |
| Suzuki (2000) | Difference in urinary LTE4 and 11-dehydro-TXB2 excretion in asthmatic patients | no VOC |
| Tan (2015) | To validate standard measurements of lung function in a user-friendly novel spirometer and investigate the accuracy of an exhaled breath gas sensor in asthmatics and non-asthmatics | no VOC |
| Timms (2011) | Diagnosing GORD in respiratory medicine | review |
| Tirzite (2017) | Detection of lung cancer in exhaled breath with an electronic nose using support vector machine analysis | no exclusive asthma group |
| Westenbrink (1991) | The effect of asthma inhalers on the A.L.E.R.T. J3A, Breathalyzer 900A and Mark IV G.C. Intoximeter | no exclusive asthma group |
| Xi (2013) | Diagnosing obstructive respiratory diseases using exhaled aerosol fingerprints: A feasibility study | no exclusive asthma group |
| Xi (2015) | Detecting obstructive lung diseases with aerosol breath test: A non-invasive diagnostic method using fractal analysis and SVM classification | no exclusive asthma group |
| Xi (2014) | Exhaled aerosol pattern discloses lung structural abnormality: a sensitivity study using computational modeling and fractal analysis | no exclusive asthma group |
| Xi (2015) | Detecting Lung Diseases from Exhaled Aerosols: Non-Invasive Lung Diagnosis Using Fractal Analysis and SVM Classification | no exclusive asthma group |

| | | |
|---|---|---|
| Yoon (2017) | Toward breath analysis on a chip for disease diagnosis using semiconductor-based chemiresistors: recent progress and future perspectives | review |

## Appendix E PRISMA Checklist

| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review, meta-analysis, or both. | 1 |
| **ABSTRACT** | | | |
| Structured summary | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | 2 |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of what is already known. | 3-4 |
| Objectives | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). | 5 |
| **METHODS** | | | |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | 5 |
| Eligibility criteria | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. | 5 |
| Information sources | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. | 5 |
| Search | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. | Supplementary Material |
| Study selection | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). | 5 |

| | | | |
|---|---|---|---|
| Data collection process | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. | 5 |
| Data items | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. | 5 |
| Risk of bias in individual studies | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | 6 |
| Summary measures | 13 | State the principal summary measures (e.g., risk ratio, difference in means). | n/a |
| Synthesis of results | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$) for each meta-analysis. | n/a |
| Risk of bias across studies | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). | n/a |
| Additional analyses | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | n/a |
| **RESULTS** | | | |
| Study selection | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | 8 |
| Study characteristics | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | 9,10,13,14,15,16,17, Supplementary Material |
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | 18, Supplementary Material |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | n/a |
| Synthesis of results | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | n/a |
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | 18 |
| Additional analysis | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | n/a |

| | | | |
|---|---|---|---|
| **DISCUSSION** | | | |
| Summary of evidence | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | 19-23 |
| Limitations | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | 22 |
| Conclusions | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | 23 |
| **FUNDING** | | | |
| Funding | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | Declarations |

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# Exhaled volatile organic compounds in adult asthma: a systematic review

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**The clinical utility of exhaled breath sampling contrasts the complexity required for VOC analysis. Modern studies are now starting to apply robust methodological standards demonstrating breathomics to be sensitive to underlying inflammation.** http://bit.ly/2WS1Sfx

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ABSTRACT The search for biomarkers that can guide precision medicine in asthma, particularly those that can be translated to the clinic, has seen recent interest in exhaled volatile organic compounds (VOCs). Given the number of studies reporting "breathomics" findings and its growing integration in clinical trials, we performed a systematic review of the literature to summarise current evidence and understanding of breathomics technology in asthma.

A PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)-oriented systematic search was performed (CRD42017084145) of MEDLINE, Embase and the Cochrane databases to search for any reports that assessed exhaled VOCs in adult asthma patients, using the following terms (asthma AND (volatile organic compounds AND exhaled) OR breathomics).

Two authors independently determined the eligibility of 2957 unique records, of which 66 underwent full-text review. Data extraction and risk of bias assessment was performed on the 22 studies deemed to fulfil the search criteria. The studies are described in terms of methodology and the evidence narratively summarised under the following clinical headings: diagnostics, phenotyping, treatment stratification, treatment monitoring and exacerbation prediction/assessment.

Our review found that most studies were designed to assess diagnostic potential rather than focus on underlying biology or treatable traits. Results are generally limited by a lack of methodological standardisation and external validation and by insufficiently powered studies, but there is consistency across the literature that exhaled VOCs are sensitive to underlying inflammation. Modern studies are applying robust breath analysis workflows to large multi-centre study designs, which should unlock the full potential of measurement of exhaled volatile organic compounds in airways diseases such as asthma.

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