Identification of Putative Candidate Biomarkers for Asthma Using the Breath Biopsy VOC Atlas®

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Aim

To classify asthma-associated VOCs as Pathophysiology-Associated or -Specific Biomarkers by comparing literature-reported compounds with a representative baseline from the Breath Biopsy VOC Atlas®, supporting biological interpretation and informing breath-based diagnostic development.

1. Introduction

Breath contains a complex mixture of volatile organic compounds (VOCs) originating from local biological processes or arising systemically. In asthma, inflammatory and immunological mechanisms alter VOC profiles, highlighting the potential of breath analysis for non-invasive biomarker discovery.

A major challenge in breathomics is linking VOCs to the biological pathways they reflect[1]. Many VOCs report on processes less well characterized than metabolites from established matrices such as plasma or tissue, and without robust reference data, it is difficult to distinguish VOCs reflecting pathophysiological significance from those arising through normal biological or behavioural variability.

The Breath Biopsy VOC Atlas® is a curated reference dataset of VOCs detected across a representative population, enabling contextualization against baseline biological variability. This supports biological interpretation and the provisional classification of candidate biomarkers:

- **Pathophysiology-Associated Biomarkers (PABs):** VOCs present in the Atlas cohort but altered under pathophysiological conditions, potentially reflecting broadly responsive processes such as inflammation or metabolic stress.
- **Pathophysiology-Specific Biomarkers (PSBs):** VOCs absent from the Atlas cohort, provisionally linked to distinct pathophysiological mechanisms.

2. Methods

Literature Review

A systematic search identified 39 studies reporting asthma-associated breath VOCs using chemically-resolved methods. Studies varied in collection approaches (Tedlar® bags, ReCIVA® Breath Sampler, direct sampling), analytical platforms (GC-MS, GC×GC-MS, SIFT-MS, SESI-MS), and patient demographics (children, adults, elderly; mild to severe asthma). Approximately 200 unique candidate VOCs were extracted for comparison (see Figure 2a).

VOC Atlas Comparison

The Breath Biopsy VOC Atlas®[2] served as a reference dataset comprising breath VOCs detected across a representative population (demographics in Table 1), including healthy and disease-affected individuals without specific disease enrichment. Samples were collected using the ReCIVA® Breath Sampler (Figure 1) and analyzed by TD-GC-MS.

Defining VOC Presence ("On-Breath")

A VOC was considered "on-breath" if:

- Significantly elevated above paired system background ($p < 0.05$, paired t-test),
- Fold difference >2,
- Detected in >10 samples across the Atlas cohort.

Biomarker Classification

Candidate VOCs were classified as:

- Pathophysiology-Associated Biomarkers (PABs): Detected in the Atlas cohort but reported altered in asthma.
- Pathophysiology-Specific Biomarkers (PSBs): Not detected in the Atlas cohort, provisionally interpreted as specific to an aspect of asthma-related pathophysiology.

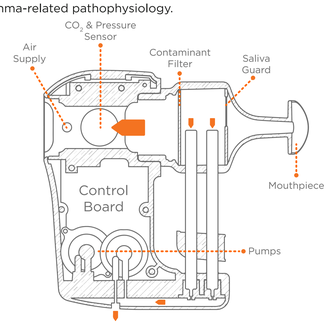

Figure 1 – Schematic of the ReCIVA® Breath Sampler. The device controls the inhalation of clean air and targeted collection of exhaled breath onto sorbent tubes, using CO_2 and pressure sensors to monitor the breath cycle and ensure sample integrity.

Study Cohort

Age Group (Yr)	Male (n)	Female (n)
18-30	10	14
31-50	19	18
51-70	12	15
71+	4	2

Table 1 – Age and sex distribution of participants in the Breath Biopsy VOC Atlas® reference dataset (n = 94), used to define representative baseline breath VOC profiles for biomarker classification.

3. Results

Asthma VOCs from literature

A systematic PRISMA-format search (Figure 2a) identified 315 papers related to asthma and breath VOC analysis. After abstract screening, 39 publications were selected for detailed review, with a final 21 studies included for compound identification. Studies using eNose technology, exhaled breath condensate, or those lacking healthy comparator groups were excluded.

Included studies drew on data from large-scale clinical cohorts such as ALLIANCE, EMBER, ADEM, U-BIOPRED, TASMA, and MICROMAP, and featured diverse patient populations and methodologies:

- **Patient focus:** 10 studies examined asthmatic children, 1 examined elderly patients, and 13 focused on adults.
- **Disease severity:** 5 addressed severe asthma exclusively; 2 focused on mild-to-moderate cases.
- **Collection/analysis:** 6 used filtered air, 16 used Tedlar® bags, and 4 included internal reference standards for GC-MS analysis.

In total, 225 unique VOCs were identified as potentially discriminatory between asthmatic and non-asthmatic individuals. Of these, 47 VOCs appeared in multiple publications, strengthening confidence in their relevance to asthma. Chemical classification of the VOCs is shown in Figure 2b, with alkanes, alcohols, alkenes, aldehydes, cyclic hydrocarbons, carboxylic acids, and ketones representing the dominant chemical classes.

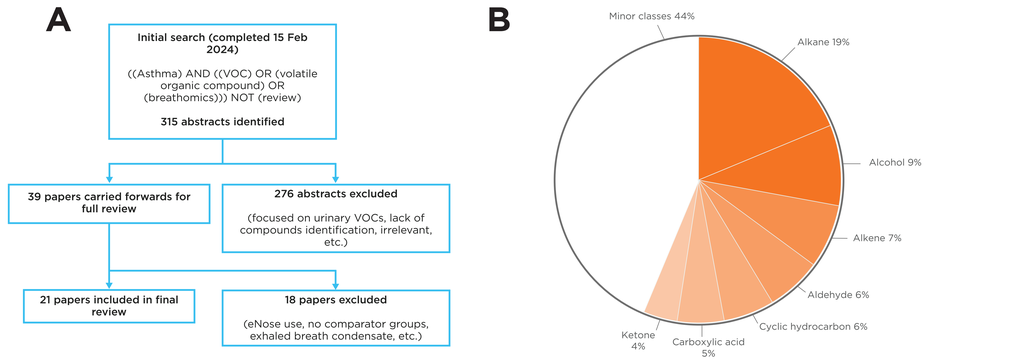

Figure 2 – a) PRISMA-style flow diagram summarising the literature screening process for asthma-related breath VOC studies. An initial search identified 315 abstracts; after abstract screening and full-text review, 21 studies were included for compound identification. b) Distribution of chemical classes among volatile organic compounds (VOCs) identified from asthma-related breath studies. Major classes included alkanes, alcohols, alkene, cyclic hydrocarbons, carboxylic acid and ketones. Minor chemical classes (38 classes in total) accounting for less than 4% individually were grouped as 'minor classes'.

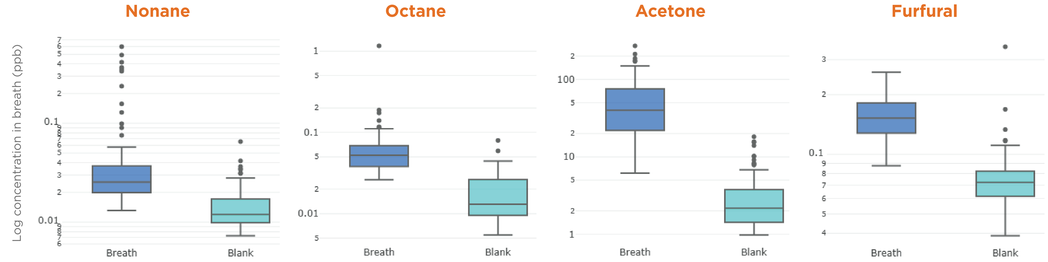

Figure 3 – Representative boxplots showing breath and blank concentrations for candidate VOCs. Nonane and octane are potential disease-specific biomarkers, while acetone and furfural are potential disease-associated biomarkers. Concentrations are plotted as log concentration in breath (ppb). Boxes represent the interquartile range (IQR), whiskers extend to 1.5× IQR, and outliers are shown individually. (n = 94 healthy volunteers).

Asthma VOCs from literature

Of the 225 VOCs identified from the literature, 46 were also found in the Breath Biopsy VOC Atlas®.

- 36 VOCs were present in the representative Atlas cohort and were classified as Pathophysiology-Associated Biomarkers (PABs).
- 10 VOCs were not detected in the Atlas and were provisionally classified as Pathophysiology-Specific Biomarkers (PSBs).

Representative examples are shown in Figure 3:

- Potential pathophysiology-specific biomarkers such as nonane and octane, which have been previously linked with lipid peroxidation[3].
- Potential pathophysiology-associated biomarkers such as acetone and furfural, which have been associated with fatty acid metabolism[4] and dietary intake[5].

In addition to presence/absence classification, the VOC Atlas provides quantitative concentration data (in ppb), enabling interpretation of observed changes in the context of baseline biological variability.

4. Conclusions

The Breath Biopsy VOC Atlas® provides a representative reference dataset that enables contextualization of breath VOCs relative to baseline biological variability. By comparing literature-reported asthma biomarkers with Atlas data, 46 VOCs were provisionally classified as either Pathophysiology-Associated Biomarkers (PABs) (n = 36) or Pathophysiology-Specific Biomarkers (PSBs) (n = 10).

This classification framework supports the interpretation of VOCs in terms of their potential biological origin:

- PSBs, such as nonane and octane, may reflect processes like lipid peroxidation associated with asthma pathophysiology.
- PABs, including acetone and furfural, likely reflect modifiable or systemic factors such as metabolism and diet.

However, important methodological challenges remain. The Atlas was generated using standardized breath collection (ReCIVA® device) and TD-GC-MS analysis, whereas the literature studies reviewed here often use varied approaches such as Tedlar® bag sampling. Therefore, it is important to recognise that variability in "on-breath" VOC detection may partly reflect differences in sampling and analytical methods, rather than true biological absence or presence.

Ongoing efforts to harmonize breathomics methodologies are expected to reduce these discrepancies over time. As the Breath Biopsy VOC Atlas® continues to expand, both in participant numbers and across different disease settings, the ability to interpret VOC changes within their biological context will strengthen further.

5. References

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Scan the QR code to access the Breath Biopsy VOC Atlas®