Breath analysis for label-free characterisation of airways disease

Stephen J. Fowler

Affiliation: Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, The University of Manchester and Manchester University NHS Foundation Trust, Manchester, UK.

Correspondence: Education and Research Centre (2nd Floor), Wythenshawe Hospital, Southmoor Road, Manchester M23 9LT, UK. E-mail: Stephen.fowler@manchester.ac.uk

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Potential breath volatile signatures emerge for key clinical characteristics and phenotypes in airways disease http://ow.ly/7HzJ30hoCIJ

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The appeal of exhaled breath gas as a potential source of novel respiratory biomarkers is clear. It provides an inexhaustible source of a medium that can be sampled noninvasively, and one that has been in direct contact with the organ of interest. Targeting volatile organic compounds (VOCs) allows us to see even deeper: these small molecules diffuse across barriers and hence breath sampling also captures VOCs from the pulmonary interstitium, the circulation and beyond. Whilst analytical chemists, engineers, and a few forward-thinking clinical researchers have been exploring the breath volatilome since the early 1970s, it is only in the last decade or so that clinical interest has really grown [1–6]. There are probably two main reasons for the slow speed of uptake. First, the medium is extremely complex, and the source of VOCs difficult to define. The air we breathe out contains VOCs from three main sources: the external environment (either inhaled or absorbed through the skin or *via* ingestion) [7, 8], and metabolism both human [9, 10] and non-human (the microbiome) [11, 12]. To further complicate matters these volatiles may interact, and may be themselves utilised in metabolic processes. The second challenge is common to all 'omics research: the lack of external validation needed to give confidence in findings and support clinical effectiveness studies.

The potential for false-positive results from 'omics studies means that rigorous validation is vital for enhancing the chances of making genuine biomarker discoveries that can be replicated and, most importantly, that merit investigation for clinical use [13]. There is a hierarchy of validation: internal cross-validation, where data come from the same cohort, but a portion are left out of the modelling, before the model is tested in that "unseen" data; external cross-validation within the same study, having a pre-planned two-part study whereby the model is built using a "training set" as above, then tested in a newly collected set of samples; and third (the most rigorous) is true external validation, where findings are replicated in a new study, preferably performed by a different group and in a geographically separate area [14].

To date no breath studies have been published (it would be useful to know if any have been completed but remain unpublished) that meet the most stringent test of validation, and would lead to the necessary clinical effectiveness studies that could test clinical utility. The paper of de Vries *et al.* [15] published in this issue of the *European Respiratory Journal*, is one of the few to have provided at least the second level. Their study addresses another of the current hot topics in airways disease, and one that lends itself well to an unbiased 'omics approach: can breath profiles predict the airway cellular phenotype, regardless of the

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diagnostic label (asthma or COPD)? There is gathering support in the airways community for the concept that we should not be treating people with these "diseases" by rigid protocols, but aiming to personalise treatment based on the characteristics present in any given patient. This has been addressed in different ways, by focusing on endotypes (mechanistically defined) [16], phenotypes (variously defined, but based on the expression of disease in an individual) [17–19] or treatable traits (a holistic approach that allows us to address the person in front of us, especially useful in severe disease; What makes this person breathless? Why do they cough? Why are they tired?) [20]. One of these traits/phenotypes (the two terms may sometimes be synonymous) is eosinophilic airway inflammation; targeting this is beneficial to patients regardless of their disease label [21–24]. We have some surrogates that are relatively easy to measure, such as blood eosinophils and exhaled nitric oxide, and these are sufficient for the current phase of stratified medicine, but not very accurate. With a sufficiently sensitive and specific biomarker, we could improve on this further and deliver the ambition of truly personalised medicine.

de Vries *et al.* [15] have set up a very large prospective programme to collect "breathprints" of adults and children with airways disease, and have used it to investigate whether specific breath profiles are related to clinical characteristics. Some of these are of clear clinical relevance and, if validated, could provide clinically useful biomarkers that would improve care in the very near future. Others have potential to be hypothesis-forming or lead to novel mechanistic insights, for example those related to atopy, systemic neutrophilia and exacerbation rate.

In this study the breath volatile profile was measured using a bespoke, integrated sampling and analytical instrument called the "Spironose" [25]. The version used in the present study is somewhat different to that published previously, comprising seven metal oxide semiconductor sensors across four breath sensor arrays (and another identical four which detect environmental VOCs), and the precise structural arrangement is unclear. Although there is no specific reason to suspect these seven sensors perform differently to the four already published, full validation data are critical to assure confidence in the performance of this arrangement, as volatile sensors are prone to instability and signal drift [26]. It is important that the authors publish these data in order to support future findings of the impressive body of work being undertaken in the Breathcloud programme. If validated, the platform potentially already provides a point-of-care test that could be implemented widely for breath detection in the clinic.

The underlying concept for such eNoses is that each sensor reacts predictably to VOC exposure by changing resistance, and produces a signal output that in this case effectively provides a seven-valent volatile fingerprint for that breath sample. It may be that this version of the Spironose can be further simplified, as the response of some of these sensors correlate closely and do not provide useful additional data to the analysis. A disadvantage of this analytical method, but one that would not impact its clinical utility, is that the sensors do not allow identification of the volatiles used in classification. Hence, for mechanistic insights, such measurements need to be run in parallel by gas chromatography mass spectrometry [27].

For the assessment of the end-points studied, including for the most clinically appealing, systemic eosinophilia, the authors provide a relatively strong degree of validation, which gives real confidence in their findings. They generated the classification model(s) using a "training" dataset of 321 participants (for a breath study this is very large indeed) and then validated it in a separate "validation set" of data from a further 114 participants. Although the authors state that the validation set was "independent", it is not evident that the data were collected from a cohort separated in time and location from the test set. Further support for the relationship between breath and inflammatory profiles comes from the regression analysis that confirmed that the model could predict not only the presence/absence of the dominant inflammatory type, but also that there was a dose-response effect. Our group have previously reported a similar effect (for sputum inflammatory cells), albeit with a much smaller sample size, and this strongly supports a link (direct or indirect) between airway inflammation and breath volatiles [28].

The authors also report, for the first time, the results of an unbiased breath-based cluster analysis. This gives a really fascinating glimpse into what potential new paradigm studying the breath could reveal. We know that rats can "sniff" tuberculosis and dogs can "sniff" prostate cancer, but can an electronic nose detect our "phenotype" just by sniffing our breath? Again the authors have provided validation, and it appears that not only can some phenotypes be reliably associated with a "smell", but they also match clusters of asthma that have been derived using entirely different sets of clinical variables [29, 30]. In both the test and validation sets a breathprint was found relating to a cluster that was (mostly) overweight, female, poorly controlled but with low markers of inflammation; another is strongly associated with type-II inflammation. Where these clusters are currently clinically actionable (as in the second case) an eNose may have clinical application in the near future; for the others then identification of compounds is required to explore potential novel mechanisms.

This admirably large study contributes significantly to the growing breath literature and encourages us to explore further the links between volatiles and disease characteristics in airways disease. It also supports the current move away from rigid disease-based classifications, and promotes the drive for biomarkers that can predict clinically relevant (and treatable) traits.

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