Wake-up call by breathomics in sleep apnoea

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A first step on the evidence-based route towards establishing diagnostic accuracy of eNose assessments in OSAS http://ow.ly/kskNW

Respiratory diseases are amongst the most complex pathophysiological entities in medicine. There is no such thing as a one-item disease in our field, which brings the appeal of respiratory diseases for basic and clinical scientists. The most prevalent respiratory diseases are chronic, exhibiting multiple mechanistic pathways that can vary during the course of the disease. This has not only hampered pathogenetic research, but has also impeded adequate disease phenotyping [1]. Apparently, it takes more than a few clinical and serum markers to establish the true biomedical entity of complex diseases. The good news is that, as we speak, medicine is making a step-change in achieving exactly that [2].

It is now increasingly recognised that the clinical, physiological, cellular and molecular mechanisms of disease cannot be addressed separately. Instead, it is more likely that an integrative approach that links these various domains of disease will get closer to a true understanding of disease [1, 2]. The problem in chronic respiratory medicine has been that each of the disease domains by itself is characterised by multiple perturbed mechanisms in parallel or in series, so that adequately describing various disease states requires capturing this seeming ''chaos'' by: 1) high-throughput measurements in each of these disease domains; 2) sophisticated multi-scale mathematical integration of these data [3] to come to; 3) unbiased discovery of (changes in) mechanistic pathways. ''Omics'' techniques follow this approach [4–6].

Based on developments in molecular medicine and bioinformatics during the past decade [7], a ''systems medicine'' approach has now become a very powerful option for capturing the complexity of diseases in general [1] and respiratory diseases in particular [3]. The required high-throughput ''omics'' tools are now available for use in blood, secretions and tissue [4], and these have already shown their value in discriminating various chronic respiratory diseases and different phenotypes within a disease [5, 6]. The advantage of respiratory medicine is that our organ of interest is continuously exchanging molecular constituents with the environment. Hence, metabolomics in exhaled air (breathomics) can put respiratory research in an advanced position. Indeed, it appears that volatile or non-volatile molecular fingerprints of exhaled air (breathprints) are associated with clinical, inflammatory and metabolic profiles in various chronic respiratory diseases [8–10] and systemic diseases, such as diabetes mellitus [11]. The technical development of hand-held, real-time equipment for pattern recognition of exhaled volatile organic compounds (VOCs) by an electronic nose (eNose) has particularly pushed this field forward [12, 13].

In the current issue of the journal GREULICH *et al.* [14] present data on the detection of obstructive sleep apnoea syndrome (OSAS) by volatile markers in exhaled air using a particular brand of eNose. Why did they do this study? OSAS does not appear to represent merely a mechanical problem of the extrapulmonary

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airways, but is strongly associated with metabolic syndrome, including glucose intolerance, dyslipidaemia and hypertension [15, 16]. Hence, there is a good rationale for examining exhaled molecular profiles as a representation of systemic metabolic changes in OSAS. The authors postulated that breathprints by an eNose can discriminate OSAS patients from healthy controls and that those breathprints change after continuous positive airway pressure (CPAP) intervention. The study combined such an unbiased breathomics approach with hypothesis-driven outcomes in exhaled breath condensate (EBC: pH, conductivity) and pharyngeal washings (proteases, protease inhibitors).

The results of the study show that eNose breathprints allow distinction of OSAS patients and controls with a sensitivity of 0.93 and a specificity of 0.7 [14]. This level of discrimination was confirmed when using ''split-half'' internal validation of the dataset. Even though the selected biomarkers in EBC and pharyngeal washings were not individually different between OSAS and controls (except pharyngeal α1-antitrypsin), adding these markers to the eNose breathprints improved the area under the curve of the receiver operating characteristic curve from 85 to 100%. Further plausibility of the relationship between the eNose signals and OSAS was provided by demonstrating changes in breathprints after 3 months of CPAP therapy. The authors conclude that eNose assessments can distinguish OSAS from controls with high accuracy.

The novelty of this study is the metabolomics approach of exhaled air in OSAS patients. This extends previous studies in search of exhaled biomarkers in OSAS using *a priori* selected volatile and non-volatile compounds [16]. Even though eNose measurements cannot identify individual volatile compounds in exhaled air, the composite molecular patterns are apparently providing an exhaled signature of OSAS. For diagnostic purposes such a probabilistic approach can be highly effective. However, when aiming to unravel the pathophysiological pathways in OSAS, individual molecular compounds should be identified, *e.g.* by gas chromatography–mass spectrometry [8, 9]. Other high-throughput techniques for analysing (non-volatile) compounds in EBC, such as liquid chromatography–mass spectrometry [10] or high-resolution nuclear magnetic resonance spectroscopy [17], are also very promising, but to the best of our knowledge have not been applied yet to OSAS patients.

The present study has several limitations. First, selection of subjects for such a proof of concept should be based on including the extremes: a gold standard group of patients and completely unaffected controls. The patients were selected by apnoea/hypopnoea index and clinical symptoms. Nevertheless, they represented a heterogeneous group, almost inevitably including comorbidities, such as obesity, diabetes, coronary artery disease and arterial hypertension. Even though the authors argued that these comorbidities were equally distributed along the breathprint signals in OSAS, it cannot be excluded that this had a confounding influence similar to the eNose outcomes in pulmonary embolism [18]. Hence, an increased sample size with statistical correction for potential confounders, such as body mass index, is required to analyse the specificity of the present results for OSAS.

Secondly, the intervention of the study should be considered with caution. The CPAP was uncontrolled, so that any other influences during the 3 months follow-up cannot be excluded. And finally, the study used a mixed approach of unbiased and biased biomarker assessments. Obviously, the selection of hypothesis-driven outcomes determines the study results. At this stage it seems to be mandatory to apply high-throughput technologies first [4], in view of biomarker discovery and hypothesis-generation, rather than arbitrarily selecting particular markers.

An exhaled metabolomic signature of OSAS may not be unexpected [16, 17, 19]. Indeed, several exhaled inflammatory and oxidative stress markers have already been described in OSAS as being the possible result of intermittent hypoxia and reperfusion during repetitive episodes of nocturnal apnoea. The first contribution came in 1997 when OLOPADE *et al.* [20] reported high levels of exhaled nitric oxide and pentane in OSAS. Subsequently, several soluble inflammatory markers have been investigated in EBC, such as leukotriene B4, cysteinyl leukotrienes (C4, D4 and E4), interleukins (IL-6, IL-8 and IL-10) and tumour necrosis factor-α [21]. All these markers appear to be increased in OSAS, but most of them are also elevated in obese non-OSAS subjects, often enrolled as controls. This shows the need for studies on the specificity of (airways) inflammation in OSAS.

The redox balance appears to be changed in the EBC of OSAS patients, as derived from the levels of 8-isoprostane, hydrogen peroxide, nitrite and nitrate, and recently urates [21, 22]. EBC has also been used to examine these biomarkers, these include: erythropoietin that is supposed to be increased locally by intermittent hypoxia; leptin which may act in chemotaxis and induction of inflammatory cytokine secretion; and intercellular adhesion molecule (ICAM)-1 that is predictive of future cardiovascular risk. Leptin and ICAM-1 appear to be increased in EBC of OSAS patients, whereas erythropoietin was unchanged when compared to healthy controls [21, 23–25]. These data provide insight into which molecular pathways may be driving the observed changes in breathprints in OSAS.

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What will be the diagnostic potential of eNose assessments in OSAS? The study by GREULICH *et al.* [14] should be considered as a first step on the evidence-based route towards establishing diagnostic accuracy [26, 27]. Subsequently, the data require external validation in an independent population, as has been done for asthma and chronic obstructive pulmonary disease [6]. But will it be applicable in daily practice? The specificity and positive predictive value of the eNose for OSAS is not high enough to establish the diagnosis. One could argue that the preferred usage of this cheap, noninvasive technology may rather be the exclusion of OSAS by high sensitivity and high negative predictive value. The eNose certainly did better in this regard, particularly when supported by additional biomarkers in EBC and pharyngeal washings [14]. The current data suggest that eNose measurements should be developed towards a first-line diagnostic tool to exclude OSAS prior to more advanced diagnostic procedures, which may also lead to cost savings.

Taken together, composite metabolomics analysis of exhaled breath can become an aid in the diagnostic work-up and monitoring of OSAS, similar to inflammatory airways diseases [28]. The critical point in validating the diagnostic accuracy of this fingerprint approach is to avoid false-positive and false-negative results. Therefore, it is mandatory to adopt well-established statistical approaches that allow reliable interpretation of metabolomics datasets [29]. Only then will we move from seeming ''chaos'' to diagnostic consensus, which is within reach in this new biomedical era [30].

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