Breathomics in pulmonary disease
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Summary

Background
After technological validation of a new diagnostic test, an extensive evidence-based evaluation is required. Studies to assess the diagnostic accuracy have a common basic structure. This thesis describes the necessary steps for the introduction of exhaled breath metabolomics, in short breathomics, into medical diagnostics of pulmonary diseases. Using electronic noses (eNoses) and gas chromatography mass spectrometry (GC-MS) the overall molecular pattern of breath can be assessed in a single measurement, providing a specific breath fingerprint, in short breathprint. We mainly focused on the validation of breathomics in the obstructive airway diseases asthma and COPD. In addition, we assessed what comprises the exhaled signal in asthma and COPD, and the value of breathomics in subphenotyping the heterogeneous disorder of COPD. Methodological issues concerning breath testing were investigated to allow large-scale application of this technique. Finally, a sidestep to vascular pulmonary medicine was undertaken to assess the diagnostic potential in acute pulmonary embolism.

Breath analysis in perspective
In chapter 2, exhaled breath testing is put in a medical perspective as an alternative, empirical approach for the commonly used pathophysiological reasoning. Omics techniques allow analysis of the overall molecular pattern of complex samples in a single measurement. Chapter 2 describes the recent advances, current status and future developments of breathomics using GC-MS and electronic noses both from a medical and technical point of view. Examples of medical advantages of breathomics are the noninvasiveness of the test, the possibility to store breath samples, the availability of portable devices and the applicability of the technique in many diseases. Disadvantages are the inability to distinguish between endogenous and exogenous compounds and the influence of many factors, such as medication and diet, on the composition and quality of air samples. Technical advantages of eNose measurements are the limited costs, reversibility and short response time of sensors, and the use of pattern recognition algorithms. The latter enables a single device to be used in different diseases by simply changing the diagnostic algorithm. Drawbacks are the necessity of training the device before application, incompatibility between devices and limited stability of sensors. This perspective focuses on advances made in the field of pulmonology, but recommendations for application can be easily translated to other fields of medicine. Steps towards introduction of metabolic breath tests into daily clinical practice are currently undertaken and require joined forces between medical and technical task forces.
Diagnostic validation

The diagnostic validation of metabolomic exhaled breath testing using an electronic nose is studied in chapters 3 and 4. These chapters describe the standard diagnostic evaluation of a test following the STARD guidelines. The first step of internal validation for the discrimination between asthma and COPD is described in chapter 3. For the discrimination between ‘classic’ (reversible) asthma and COPD a 96% accuracy was reached. Asthma could also be distinguished from healthy non-smoking controls (95% accuracy) and smoking controls (93%). COPD patients could only be partly discriminated from asymptomatic smoking controls (66% accuracy). An obvious explanation for the low accuracy is the presence of future COPD patients in the group of asymptomatic smokers. Follow-up studies should clarify this finding. Importantly, the discrimination between groups was reproducible in duplicate measurements, and repeatable when using a different electronic nose device.

As the second validation step, the same diagnostic model was tested in a newly recruited group of patients with ‘classic’ asthma and COPD in chapter 4. This external validity study showed an accuracy of 83% for the correct identification of asthma and COPD. Additionally, a difficult-to-diagnose group of patients with asthma due to fixed airways obstruction similar to COPD was tested. Breathprints were accurately discriminated from COPD breathprints with 88% accuracy. We concluded that the obstructive airway diseases asthma and COPD are characterized by specific breathprints, enabling discrimination between the disorders by non-invasive breath testing. This may reduce misdiagnosis in obstructive airway diseases leading to appropriate treatment. The final step for validation of the diagnostic algorithm is currently undertaken, in which new patients with suspected disease (i.e. an intention to diagnose) are recruited and classified. The result is then compared with the result of the current gold standard test, lung function.

The exhaled signal

In chapter 5, we investigated what comprises the signal in the exhaled breath in asthma. The hallmark feature of asthma is (variability of) airway obstruction and airway caliber. In order to assess the influence of the change in airway caliber on exhaled breathprints as measured by electronic nose, ten asthma patients were exposed to methacholine challenge to induce bronchoconstriction. In addition, we tested whether inhalation of saline also induced changes in breathprints. We showed that the variability of airway caliber and obstruction as a result of methacholine challenge altered the breathprints. However, this change was similar after saline challenge, indicating a change in breathprint induced by nebulized aerosols rather than by bronchoconstriction. We concluded that breathprints in asthma measured by eNose are not confounded by the level of airway obstruction and acute changes in airway caliber.

In chapter 6, we assessed whether the exhaled signal in mild to moderately severe COPD (GOLD stages I-II) is related to airway inflammation. Associations between eNose and GC-MS
profiles, individual VOCs and inflammatory markers in sputum were investigated. We showed that in COPD both eNose and GC-MS breathprints were highly associated with inflammatory markers in sputum. In addition, a number of individual compounds, most likely derived from oxidative stress pathways, were associated with inflammatory parameters in sputum. Our results suggest that exhaled breath molecular profiling may be used as a non-invasive and fast way to assess and monitor airway inflammation in COPD. Whether this is also the case in asthma is currently investigated.

Subphenotyping COPD

COPD is a very heterogeneous disorder and many methods have been proposed to subphenotype this disease into meaningful clusters of similar cases that help to improve patient care and provide directions for future research. The classification of COPD is currently based on the severity of airflow limitation, but this may not adequately reflect the phenotypic heterogeneity of COPD in the (ex-) smoking community and in first line care. In chapter 7, we investigated whether exhaled breathprints contribute to the formation of clusters of COPD subphenotypes in a community-based population of 300 heavy (ex-) smokers. We performed a cluster analysis on parameters from different domains, including lung function, chest CT scanning, questionnaires and breath molecular profiling by electronic nose. Three clusters were identified: (1) severe airway obstruction with low quality of life, (2) mild COPD with cardiovascular comorbidity, with and without emphysema and (3) chronic bronchitis. We found that there are indeed differences in breathprints between the COPD clusters that provide additional information besides clinical, pathological, functional and patient-related outcomes.

Methodological issues

Methodological and technical considerations of exhaled breath metabolomics in medicine are highlighted in chapter 8. Large-scale studies in multi-center trials are hampered by incompatibility between devices, making centralized measurements necessary. We investigated whether a known discriminatory exhaled breath signal (asthma vs. healthy controls) remains viable after adsorption, storage and desorption of breath samples on sorbent tubes (Tenax GR®). We showed that the electronic nose and GC-MS signals of exhaled breath from asthmatics and controls were stable for at least a storage period of 2 weeks, mimicking transportation time in multi-center trials. This indicates that centralized analysis of breath samples is feasible.

Acute pulmonary embolism

Exhaled breath testing is not only useful in detecting airway diseases such as asthma and COPD, but also in the case-finding of other pulmonary and non-pulmonary diseases as
described in literature. In chapter 9 we investigated in a proof-of-principle study whether acute pulmonary embolism (PE) can also be detected in breath. We showed that when electronic nose breath testing is combined with a commonly used clinical decision rule (Wells score), the percentage of patients suspected of PE that will have to undergo additional CT-imaging may be decreased. This holds especially for patients without comorbidities, reaching an accuracy of 85% for the discrimination between patients with confirmed PE and patients with suspected PE in whom PE was ruled out by chest CT scanning. In patients with comorbidities associated with distinctive breath profiles (asthma, lung cancer etc.) the eNose was unsuccessful to discriminate between PE and non-PE (accuracy 65%). A solid and large training set of ‘pure’ i.e. non-comorbid PE patients should overcome this limitation and provide a gold standard PE breathprint.

Conclusions

This thesis describes the first and necessary steps towards introduction of exhaled breath molecular profiling into clinical diagnostic practice in respiratory medicine.

• Great advances have been made with respect to discrimination of diseases by comparing overall breathprints provided by electronic noses since the discovery of this technology (chapter 2).
• Step 1 in the validation of electronic noses in the diagnosis of classical asthma and COPD, following the STARD guidelines, showed excellent internal validity of 96% accuracy (chapter 3).
• Step 2 showed good external validity of 83% accuracy in a newly recruited group of patients (chapter 4).
• Step 3 showed good external validity of 88% accuracy in a difficult-to-diagnose group of asthma patients with fixed airflow limitation and COPD patients (chapter 4).
• Variability in airways obstruction and caliber in asthmatics showed no influence on the exhaled breathprint as measured by electronic nose (chapter 5).
• In contrast, inflammatory markers in sputum were highly associated with electronic nose breathprints and individual VOCs in COPD (chapter 6).
• Exhaled breathprints have additional value in subphenotyping COPD into clusters that may help to improve patient care and provide directions for future research (chapter 7).
• Electronic nose and GC-MS signal stability for asthma over a 2-week transportation period on Tenax® tubes, indicating that centralized analysis of exhaled breath samples in multicenter studies is feasible (chapter 8).
• Exhaled breath testing using an electronic nose may increase the exclusion of patients at high risk for pulmonary embolism (PE) without additional imaging (CT) testing, especially when combined with a commonly used clinical decision rule (chapter 9).