Breath Test

Clinical Application of Breath Analysis in Lower Respiratory Tract Infection Diagnosis

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Lower respiratory tract infections (LRTIs) are responsible for >800,000 deaths per year in children younger than 5 years worldwide.1) Methods to diagnose the etiology of LRTI are limited. Radiological imaging is traditionally used as a gold standard, but lacks precision.2) Obtaining sputum specimens for microbial culture poses a challenge in the pediatric population; sputum induction can be unpleasant and bronchoalveolar lavage is an invasive method. Blood culture lacks sensitivity, and, in general, microbial culture can take several days to yield results.2) Polymerase chain reaction detection of viral pathogens in upper respiratory tract samples does not distinguish symptomatic from asymptomatic viral infections. Metabolomic analysis of volatile organic compounds (VOCs) in exhaled breath is a promising noninvasive alternative to current methods to diagnose LRTI.3) Its principle resembles the principle of breath tests used by the police to detect drunk drivers. In LRTI, different causative pathogens and host–pathogen interactions can change the composition of the VOCs found in exhaled breath, which provides a potential target for diagnosis.4)

This review, aimed at the clinician, summarizes the different breath analysis techniques available, the different respiratory tract infections in which exhaled breath was studied and the challenges that come with breath research in children.

BREATH ANALYSIS TECHNIQUES

VOCs are organic molecules that are gaseous at room temperature, and can originate from host metabolism, pathogen metabolism and host–pathogen interactions. They can be detected using real-time techniques, in which the breath sample is directly introduced into the measuring instrument, or laboratory-based techniques, in which the exhaled breath is collected, stored and preprocessed.5) The former are also known as online techniques, the latter as offline techniques. A targeted approach is used to detect preselected VOCs, whereas an untargeted approach analyses unknown VOCs as well.

MASS SPECTROMETRY TECHNIQUES

Gas chromatography mass spectrometry (GC-MS) is considered the gold standard in breath research and uses preconcentrated breath samples for offline analysis. GC-MS can be used for an untargeted approach, to provide chemical identification of the exhaled VOCs.6,7) Other MS techniques, for example, selected ion flow tube MS, can perform online untargeted or targeted analysis of VOCs, following chemical ionization. They have the potential to be developed into point-of-care tests.4)

ION MOBILITY SPECTROMETRY

Ion mobility spectrometry, frequently coupled to a multicapillary gas chromatography column, allows online analysis of breath samples. In this technique, ions are separated in the gas phase by their mobility through a controlled electrical field.4)

ELECTRONIC NOSE

Electronic nose (E-nose) devices, used for online breath analysis, apply a sensor-based technique which resembles mammalian olfaction. This technique cannot identify

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individual VOCs, but analyzes the whole spectrum of exhaled VOCs. When exposed to a profile of VOCs, the electronic resistance of the E-nose sensors changes, which generates a so-called breathprint. These can be analyzed by pattern recognition algorithms that facilitate discrimination of different VOC profiles.3

SAMPLE COLLECTION
The sampling of exhaled breath is a complex process: a lot of variables have to be taken into account. These include, among others, dietary factors and medication taken, environmental VOCs, the portion of breath sampled, and the collection device used. The European Respiratory Society has formulated recommendations for standardization in exhaled breath research, which is important to allow comparison of different studies.5

FEASIBILITY IN CHILDREN
Sample collection is especially challenging in the pediatric population, because children may be less cooperative and have smaller tidal volumes. Nevertheless, protocols to sample breath from children have been described. Children older than 4 years, may be instructed to exhale breath into a mouthpiece connected to a collection or point-of-care device. Breath from children below the age of 4 years can be collected by letting the child breathe normally through a face-mask placed over the nose and mouth, while making sure it is comfortable and distracted.

RESPIRATORY PATHOGENS
In the field of exhaled breath research, the following LRTIs have been a focus of investigation: pulmonary infection in patients with cystic fibrosis (CF), ventilator-associated pneumonia (VAP), pulmonary tuberculosis (PTB), pulmonary invasive aspergillosis and SARS-CoV-2. Several approaches to breath analysis have been applied: investigating single VOCs, identifying VOC profiles or E-nose breathprints.

BACTERIAL PATHOGENS
Pseudomonas aeruginosa
In CF patients, including children, hydrogen cyanide (HCN), 2-aminoaceto-phenon (2-AA) and methyl thiocyanate are specific VOCs that have been associated with Pseudomonas aeruginosa infection. In a prospective cohort of 233 pediatric CF patients, elevated levels of HCN predicted P aeruginosa infection with a sensitivity and specificity of 33% and 99%, respectively.5 A possible explanation for the low sensitivity found in this study is that some strains of P. aeruginosa produce HCN in concentrations below the cutoff value, thereby generating false-negative results. Furthermore, in adult patients, mouth-exhaled breath can be contaminated with HCN produced in the oral cavity, possibly leading to decreased specificity.2 2-AA had a good sensitivity for detecting colonization with P. aeruginosa in CF patients, but is also found in several foods and beverages. Better results were obtained in studies that used an untargeted approach, reporting profiles of 12–16 VOCs with sensitivities and specificities ranging from 83% to 100% and 71% to 100%, respectively. Finally, the E-nose was only moderately accurate for P. aeruginosa in this population.

BACTERIAL VERSUS VIRAL LRTI
Researchers also investigated whether bacterial and viral etiology of LRTI can be discriminated. Lewis et al14 used GC-IMS in 71 patients with confirmed or probable bacterial or viral respiratory tract infections based on microbiologic, biochemical and radiologic testing. The authors reported that this point-of-care breath test could discriminate between bacterial and viral infection with a sensitivity and specificity of 62% and 80%, respectively, and concluded that these results show promise and warrant further trials.

VIRAL PATHOGENS
SARS-CoV-2
Breath diagnostics were also investigated in the context of the current COVID-19 pandemic. Ruszkiewicz et al11 conducted a feasibility study with 98 participants, using GC-IMS. With a 5-VOC profile, they could discriminate COVID-19 patients from COVID-negative patients presenting with respiratory complaints. The sensitivity and specificity were 82%–90% and 75%–80%, respectively.

In a pilot study performed by Berna et al,12 a 6-VOC profile was discovered to discriminate children with COVID-19 from healthy controls, with sensitivity and specificity of 100% and 67%, respectively. This VOC profile encompasses elevated levels of octanal and heptanal, in line with the findings of Ruszkiewicz et al.11 Finally, Wintjens et al13 assessed the diagnostic performance of an E-nose. In this proof-of-principle case-control study, a total of 219 hospital employees presenting with respiratory complaints, and patients admitted with confirmed COVID-19 were included. Sensitivity and specificity were 86% and 54%, respectively, resulting in a respective negative and positive predictive value of 0.92 and 0.40 in this population with high prevalence.

The high sensitivities and corresponding negative predictive values suggest that exhaled VOC analysis may be a reliable tool to rule out COVID-19 in a population with high prevalence. Since VOC analysis can be performed in a point-of-care fashion, this
tool can play an important role in deciding quickly which patients need further diagnostic work-up.

CHALLENGES AND LIMITATIONS
The challenges in this field of research are diverse. Changes in VOCs are induced by several environmental factors, causing great interindividual and intraindividual variability.5 Also, because the host response to infection influences the exhaled VOCs, the translation of in vitro research to in vivo conditions needs attention.4 Furthermore, the literature regarding VOC-research consists mostly of proof-of-principle studies, aiming to identify volatile biomarkers rather than validating previous findings. Control groups often consist of healthy volunteers, instead of patients presenting with respiratory symptoms. Finally, few researchers included children. Aforementioned limitations preclude wide clinical applicability.

SUMMARY
Breath research shows promise in diagnosing LRTIs with various etiology and is feasible both in children and in adults. In general, combinations of several biomarkers in a VOC profile or E-nose breathprints demonstrate the best sensitivity and specificity. At this moment, breath analysis can be of value as a screening tool. The time is ready for larger scale validation studies, including pediatric patients and using appropriate control groups, to further establish the accuracy of breath analysis in diagnosing etiology of LRTIs, and the potential to replace the current more invasive diagnostic methods.

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