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 distinct 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.[4] 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.[3,4] 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 trough 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 electronical 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-aminoacetophenon (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.[6] 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.[4] 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.[4] Finally, the E-nose was only moderately accurate for *P. aeruginosa* in this population.

VENTILATOR-ASSOCIATED PNEUMONIA

In patients with VAP, an untargeted approach yielded profiles of 8–12 VOCs with sensitivities and specificities ranging from 76% to 98% and 73% to 97%, respectively.[4] Notably, *Acinetobacter baumannii* VAP could be discriminated from its colonization. Although the E-nose was shown to correlate well with chest-computed tomography and a clinical pulmonary infection score, it currently lacks the accuracy to diagnose VAP.

PULMONARY TUBERCULOSIS

For pulmonary tuberculosis (PTB), the untargeted gas chromatography approach yielded profiles of 7–23 VOCs with sensitivities ranging from 62% to 100% and specificities ranging from 60% to 84%. The reported profiles consisted mostly of alkane derivates as markers of oxidative stress, and cyclohexane, naphthalene and benzene derivates as volatile metabolites of *Mycobacterium tuberculosis*. Methyl nicotinate is a proposed specific VOC for TB.[4,7]

The E-nose seems the most interesting tool for VOC analysis in PTB because it shows moderate to good accuracy with sensitivities and specificities ranging from 77% to 99% and 42% to 99%, respectively.[7] Being a handheld point-of-care device, it could be a suitable tool for PTB screening in rural areas of developing countries. Interestingly, HIV-status does not appear to be a confounder.

BACTERIAL VERSUS VIRAL LRTI

Researchers also investigated whether bacterial and viral etiology of LRTI can be distinguished. Lewis et al[8] 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.

Other studies investigated the accuracy of an E-nose in COPD patients, determining whether exacerbation with bacterial etiology could be distinguished from viral or noninfected etiology.[4,9] They reported sensitivities and specificities in the range of 73%–93% and 60%–72%, respectively.

FUNGAL PATHOGENS

Aspergillus fumigatus

In pulmonary invasive aspergillosis, 2-pentylfuran was investigated as a volatile biomarker, demonstrating moderate accuracy. Koo et al[10] reported a profile of 4 VOCs (sesquiterpenes) that could discriminate patients with invasive *Aspergillus fumigatus* infection from patients with pneumonia caused by other fungi or by bacteria with a sensitivity of 94% and a specificity of 93%. Additionally, the E-nose displayed moderate to good sensitivities and specificities.[4]

VIRAL PATHOGENS

SARS-CoV-2

Breath diagnostics were also investigated in the context of the current COVID-19 pandemic. Ruszkiewicz et al[11] 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 al[13] 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

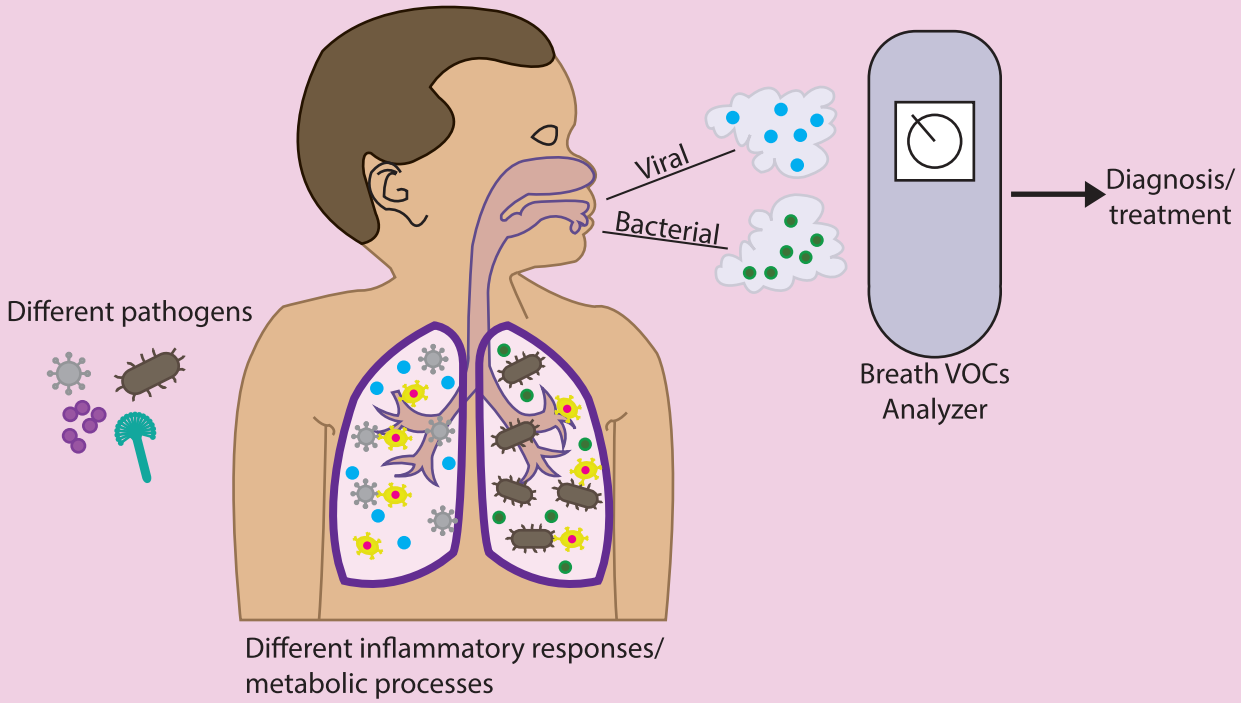

FIGURE 1. Schematic overview of the principle of VOC analysis in the context of lower respiratory tract infection.

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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