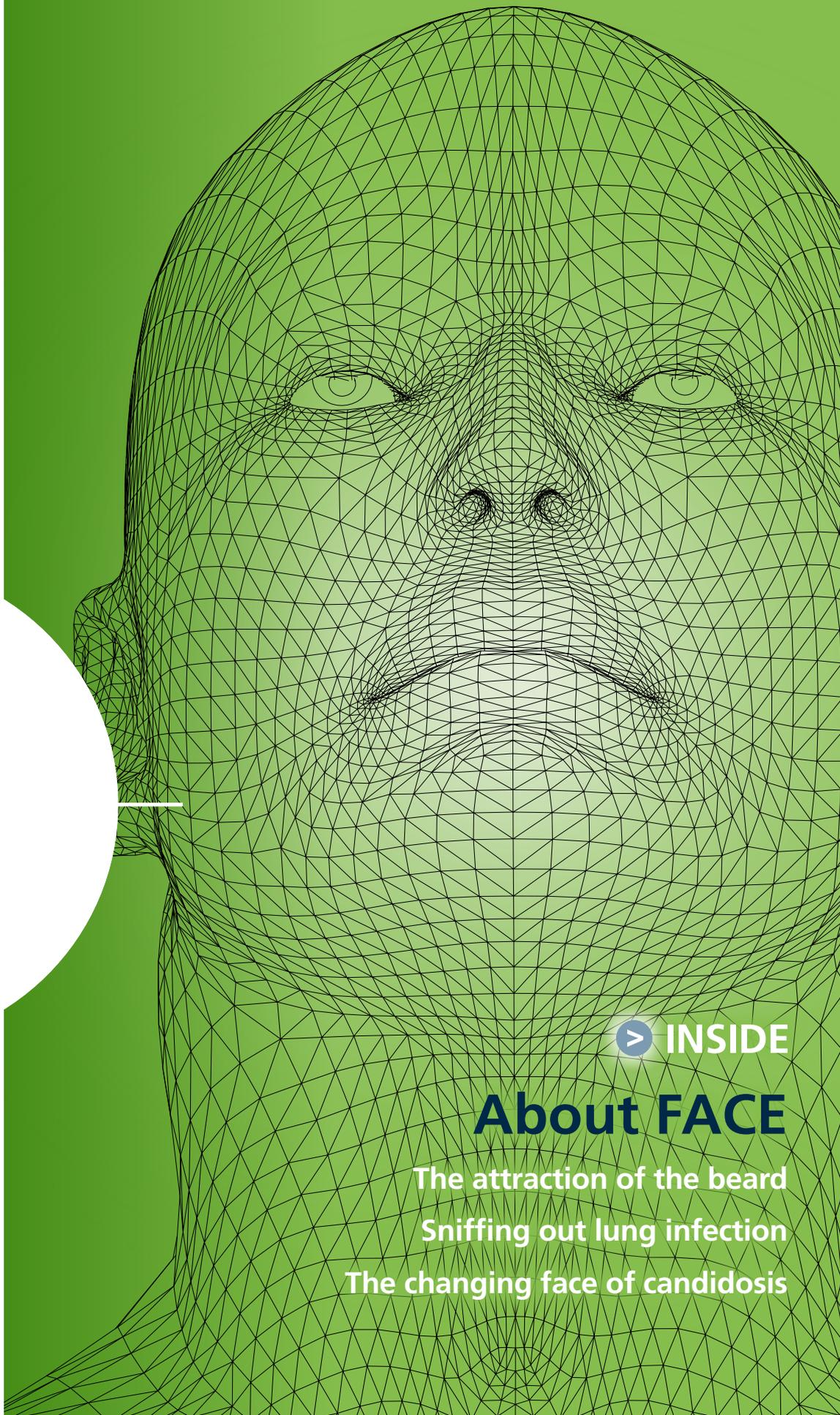


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SNIFFING OUT lung infection

Lung microbiology

Our lungs are continuously exposed to chemical contaminants, as well as microbes, from the air we breathe. Adherence to lung tissue and microbial colony formation consequently occur throughout the respiratory tract and whilst there is rich microbial colonization of the lungs there are also invasive pathogenic organisms as well. The warm and humid environment within the airways provides ideal conditions for growth, including a plentiful supply of oxygen. Microbial exposure is likely to be dependent on the immediate environment, and will differ between, for example, hospitals and the community. Dependent upon these influences, the development of the lung microbiota relies upon two main survival factors – microbial transport in and out of the lungs (and therefore colonization), and the reproductive rates of these resident microbes.

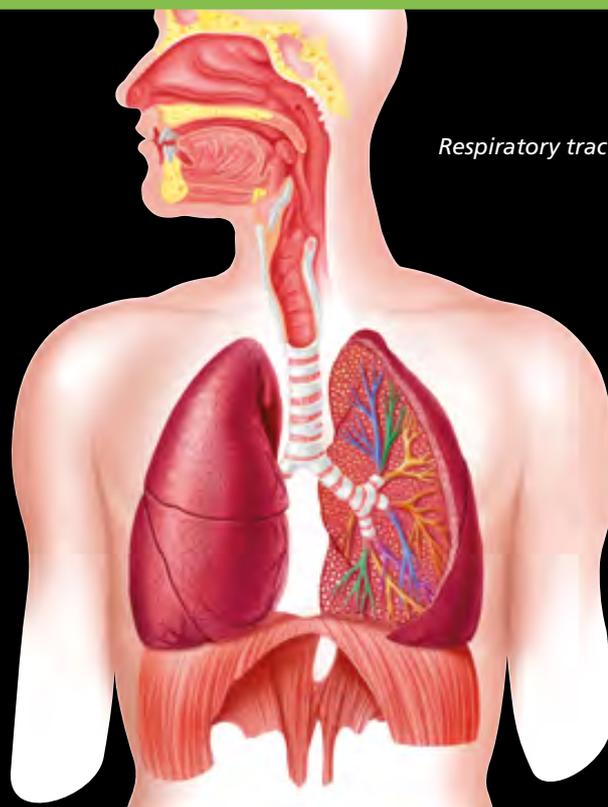
Cells that form the inner lung barrier contain a number of immune signalling and recognition mechanisms which are involved in regulating the balance between the host's adaptive immunity and (what becomes) the commensal microbial flora. If this balance is disrupted by an unrecognized microbial species (triggering an innate immune response), symptoms and signs of a lung infection will occur.

In health, the immune system responds to such exogenous invasion by activating pro-inflammatory signalling pathways and mobilizing associated immune cells. However, a weakened immune system and infection pathogenicity act synergistically leading to severe infection. This is seen in acute infections such as invasive pulmonary aspergillosis (where the main pathogen is *Aspergillus fumigatus*) or ventilator-associated pneumonia, and chronic infections such as *Pseudomonas aeruginosa* in cystic fibrosis patients. These opportunistic pathogens are ubiquitous to the host, and normally would not cause harm with a healthy immune system.

Clinical diagnosis

Initial suspicion of lung infection by a medical professional often involves the identification of progressive symptoms and deteriorating lung health, which may include difficulty in breathing and coughing. Pulmonary pathology tests such as chest scans may show abnormalities. If required, lung-derived samples such as sputum or bronchoalveolar lavage (BAL) are sent for biochemical and genetic analyses. Following this, antibiotic treatment is given to the patient (almost always before the results of the sample culture are available) to reverse the course of the infection.

This diagnostic process can be subject to errors throughout the whole system, from initial intervention to treatment. As the lung can be infected by bacteria, fungi and viruses, appropriate antimicrobial therapy is important as the wrong antibiotic can lead to increased microbial resistance. Differential diagnosis therefore relies upon numerous tests which indicate a specific pathogen, where medical history and chest pathology characteristics play a crucial role. Delayed diagnosis may also occur, with a likely detrimental effect on patient morbidity. These examples of inefficient diagnosis contribute to higher healthcare costs, which includes the time of medical staff, costs per test, drugs



Respiratory tract

and length of stay. Therefore a faster, more accurate and efficient indication of early infection would be beneficial to the patient and improve their overall healthcare.

Breath analysis

Since the advent of modern medicine, physicians have reported that breath scents are associated with disease – the fruity aroma of ketoacidosis (acetone) or the musty smell of hepatic encephalopathy (dimethyl sulfide).

The main advantage of breath analysis for clinical diagnosis is the ability to sample breath gas non-invasively, in contrast to inserting a catheter into the body, such as in blood, urine or BAL sampling, which is pretty uncomfortable for the patient.



Our lungs are continuously exposed to chemical contaminants, as well as microbes, from the air we breathe

FEATURES

The development of powerful analytical and chemometric tools have allowed researchers to look deeper into the chemical cocktail of breath gas. Pioneers such as Antoine Lavoisier and Linus Pauling identified chemicals within breath linked to clinical abnormalities.

It is now understood that breath contains thousands of chemical compounds, within both gas and condensate. The chemical patterns of individual breath samples can distinguish between healthy and diseased subjects. The differences seen may relate to a single or multiple volatile molecules of interest, or the concentration of a known compound may relate to disease phenotype. For example, researchers have been able to identify the chemical responsible for the grape-like aroma of *Pseudomonas aeruginosa* (2-aminoacetophenone) in patients infected by this pathogen.

Current technologies

Breath metabolites are prone to high temporal and diurnal variation, and are inherently unstable. This is because they are highly dependent on their chemical characteristics and the sampling method used. Therefore, for the majority of small molecule studies, enhanced data processing methods and standards are required to ensure robust and unbiased analysis.

The interdisciplinary scientific field of metabolomics aims to define the chemical processes within a biological matrix, and thus corresponds well with the requirements of breath analysis studies. To extract information from large and complex data (as in many metabolomic studies), various chemometric tools are employed, ranging from shotgun unsupervised classification techniques, to complex machine-learning algorithms. The ideal experimental and chemometric method will depend on whether the analysis is targeted (known metabolites) or non-targeted (metabolite discovery followed by pathway exploration).

Advances in metabolite analysis instruments have helped develop tools to sample and analyse breath more efficiently. Electronic sensors (commonly called 'e-noses') and ion-mobility-based methods have been adapted from analysing differences in toxic chemicals in the air, to differentiating between breath samples in healthy and disease states. Both have advantages of portability and low cost; however, some require prior training and are limited to binary classification between sample groups.

Mass-spectrometry-based methods further enhance this breath sample differentiation capability by allowing additional separation and selection of metabolites with high sensitivity and specificity. Of these methods, gas



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Breath analysis

chromatography-mass spectrometry is by far the most popular as the sample is analysed in the gas phase, but also due to its large compound library and requirement for minimal sample preparation. Other mass-spectrometry-based methods, such as proton transfer reaction-mass spectrometry, and selected ion flow tube-mass spectrometry use chemical ionization for even higher sensitivity to known metabolites, with the latter developed specifically for breath analysis. The downside of mass-spectrometry-based analysis are the high maintenance costs and bulky size, and (to date) most are performed in specialized laboratories and not readily used at the patient's bedside.

Development areas

The complexity of searching for a specific chemical marker in breath is significant given the direct influence of the microbiota and surrounding environment. This is especially important when attributing a metabolite to a specific pathogen, when in fact the immune response may produce the same metabolite. Other influences such as pre-infection underlying disease may alter the dynamics of the lung microbiota, and therefore its metabolic footprint. Some of these may be a reduction in a metabolite from the host's response rather than from the invasive microbe.

Another key topic of ongoing research are methods of breath sampling and analysis. Recently, methodology studies have been reviewed to help researchers evaluate the optimal sampling and analysis methods specific to their own needs.

Although not all studies report the same number and name of metabolites, some unique metabolites across multiple studies have been associated with microbial infection. Furthermore, chemical groups such as branched hydrocarbons have been shown to be released (in both infectious and non-infectious diseases) following an immune response. However, the same metabolites can be found in other diseases, and therefore further validation and larger clinical trials are required.

Breath analysis shows potential for delivering early diagnostic markers of lung infection. The intersection of multiple scientific fields makes this a truly interdisciplinary subject area. Although there are some areas that require further validation and development, the future holds much promise for breath analysis to investigate the mechanism behind respiratory infection pathogenesis and support early diagnosis.

FURTHER READING



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