Exhaled biomarkers in asthma

The exhaled biomarker puzzle: bacteria play their card in the exhaled nitric oxide–exhaled breath condensate nitrite game

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Exhaled NO and nitrite as potential biomarkers in asthma

The measurement of exhaled biomarkers has gained increasing interest in recent years, mainly driven by the unmet clinical need to monitor airway inflammation and the response to anti-inflammatory treatment. The current issue of *Thorax* contains two important publications in this rapidly growing field. The study by Pijnenburg *et al* shows how exhaled nitric oxide (NO) measurement can serve clinical practice,[1] while the investigation by Marteus *et al* draws attention to the potential pitfalls of measuring nitrite in exhaled breath condensate (EBC).[2]

It was hardly more than a decade between the discovery by Gustaffson *et al* in 1991 that the exhaled breath contains NO and the approval of such a measurement for clinical practice to monitor the effect of anti-inflammatory treatment in asthma.[3 4] The road has been paved by approximately 2000 publications on the measurement of the fractional concentration of exhaled nitric oxide (FE_{NO}) in health and disease, including three guidelines which provide methodological recommendations by internationally known experts in the field and endorsed by the European Respiratory Society (ERS) and/or the American Thoracic Society (ATS).[5–7] By using these recommendations, exhaled NO can be measured reproducibly and data from different laboratories can be compared.

Exhaled NO has been extensively studied as a marker of airway inflammation in asthma and it serves as a prototype for the application of biomarkers to the management of the inflammatory component of asthma. Can monitoring FE_{NO} in addition to symptoms and spirometry contribute to asthma control? The paper by Pijnenburg *et al* in this issue of *Thorax* provides a positive answer to this question.[1] In a longitudinal study the authors determined whether FE_{NO} predicted asthma relapse in 40 children with asymptomatic asthma followed for 24 weeks after discontinuation of treatment with inhaled corticosteroids (ICS). The children were enrolled in the study at the moment when discontinuation of ICS was considered because of lack of symptoms for more than 6 months at a stable dose of ICS. This ensured that the study was undertaken in a real clinical context (and the withdrawal of treatment did not occur solely for the purpose of the study). The main finding was that an increase in FE_{NO} predicted loss of asthma control in patients with no symptoms or changes in spirometric parameters. The authors found that increased FE_{NO} predicted asthma relapse with a sensitivity of 71% and a specificity of 93% using a cut-off FE_{NO} value of 49 ppb. This finding has important clinical implications because an increase in FE_{NO} warns the clinician of worsening airway inflammation, indicating the need to start treatment before symptoms appear. In another longitudinal study Jones *et al*[8] studied FE_{NO} as a predictor of loss of asthma control in relation to withdrawal of steroids in adults. Exhaled NO levels were measured weekly for 11 weeks in 78 subjects with asthma who abruptly stopped treatment with ICS. The authors found that, in subjects who eventually experienced loss of control, exhaled NO levels increased more rapidly and to significantly higher levels than in those remaining clinically stable. Similar to the results of Pijnenburg *et al* in children, they found that exhaled NO levels measured at the visit before loss of control occurred predicted the upcoming exacerbation (positive predictive value of 80–90%) at a time when symptoms were stable.

Although as yet we do not know whether using exhaled NO measurements to guide anti-inflammatory treatment in asthma in addition to traditional means of monitoring would improve asthma control, both studies indicate that exhaled NO can serve as a marker of loss of asthma control and may be useful in clinical decision making.

While a decade was enough for exhaled NO measurement to enter clinical practice, the same decade was only good enough to give a boom to research for measurement of biomarkers in EBC. It is easy to collect, requiring only the non-invasive collection of exhaled breath for 10–20 minutes in a cold trap. The fluid obtained is a complex diluted solution of diverse biomarkers with various chemical stabilities including a number of constituents.[9 10] Because of the complexity of EBC and the fact that it is a much diluted sample, there are still a number of uncertainties surrounding it. Although there is an expectation that this sampling method will be clinically useful, there are still several unresolved issues, many of which are highlighted in the report by the ERS/ATS Task Force entitled ''Exhaled Breath Condensate'' which is awaiting ATS approval for publication.

In this issue of *Thorax* an important study by Marteus *et al*[2] addresses the relation between exhaled NO and EBC nitrite/nitrate concentration. The authors performed a carefully designed study which assessed the source of nitrite in orally collected EBC, compared nitrite levels between oral and tracheal EBC samples, and investigated the influence of nitrate intake and antibacterial mouthwash on the nitrite concentration in oral EBC samples, nasal air condensates, and on FE_{NO}. Their findings can be summarised as follows: (1) nitrate levels in EBC are influenced by dietary intake; (2) nitrate is reduced to nitrite primarily by bacterial activity which takes place mainly in the oropharyngeal tract in healthy subjects; and (3) there is a substantial contribution of nitrite from the oropharyngeal tract during oral EBC collection. The findings of this study draw attention to the acknowledged pitfall of oral EBC sampling—namely, the potential naso-oropharyngeal influence on mediator levels. They also question, to some extent, the reliability of oral EBC nitrite in reflecting lower airway NO production and its ability to serve as a marker of airway inflammation.[11–15] The study also highlights the importance of potential external contamination (the authors covered the condensing surface with a specific plasma layer to minimise nitrite contamination) and emphasises the need for great care when measuring mediators which occur in such a low concentration in the EBC.

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Interventional bronchoscopy

Brave new world for interventional bronchoscopy

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New applications for interventional bronchoscopy

Until recently, interventional bronchoscopy was limited to foreign body removal, debulking endobronchial tumours, or insertion of stents for the palliation of lung cancer. Most of these procedures are performed with a rigid bronchoscope under general anaesthesia by thoracic surgeons. As a result, only a few respiratory physicians developed an interest in interventional bronchoscopy. The small range of interventions has meant that, up to now, interventional bronchoscopy has been less glamorous than, for example, interventional cardiology.

Will this situation change? Firstly, there is an increased interest in transbronchial fine needle aspiration (TBNA) for staging lung cancer and in endobronchial ultrasound guided TBNA.[1] The latter technique samples suspicious lymph nodes as small as 5 mm and has the potential for replacing mediastinoscopy. Secondly, tumours can be debulked with electrocautery, photodynamic therapy or lasers, and stents can be inserted under local anaesthesia with flexible bronchoscopes.

Recent research has driven an expansion of interventional bronchoscopy for some of the more common non-malignant respiratory diseases. Bronchial thermoplasty for the treatment of asthma is close to receiving FDA approval. This procedure, performed under local anaesthesia, involves the obliteration of smooth muscle in airways larger than 3 mm by applying radiofrequency energy. An endobronchial probe is passed through the working channel of the bronchoscope and applied to the airway wall. A controlled amount of energy is delivered which heats and destroys the muscle. Smooth muscle ablation causes a reduction in bronchial hyperreactivity and early studies suggest an improvement in asthma control. Pilot human trials have shown that the method is safe and can decrease airway hyperreactivity in patients with moderate asthma.[2 3] Studies on patients with more severe or steroid dependent asthma are currently underway.

A number of procedures are being developed to improve breathlessness in severe emphysema. These aim to achieve volume reduction by bronchoscopy rather than surgery. The basic idea is to induce collapse of the worst affected lobe or segments by blocking the relevant airways with one-way valves. A number of such valves are available and have been designed to block inspiration while allowing drainage of expired air and secretions. This results in controlled deflation of the target segment or lobe. The valves are inserted directly through the working channel of the fibrescope or over a guide wire using the Seldinger technique. More than 100 patients have been treated in this way and the safety record to date is encouraging.[4–6] The valves remain in place and have only seldom been implicated in cough or distal infection. Worthwhile improvements in lung function and quality of life have been reported in up to a third of patients and failure is probably due to collateral ventilation from surrounding lung units. This is not surprising since only patients with very severe emphysema have so far been treated and more work needs to be done to define the most suitable patients. A pivotal randomised trial is now underway.

While bronchoscopic valve placement has been proposed for patchy (heterogeneous) emphysema, an alternative intervention has been suggested for diffuse (homogeneous) emphysema. This involves the creation of extra-anatomical airways to bypass the flow limiting segment airways in expiration. A needle catheter is used to make fenestrations connecting emphysematous lung to nearby cartilaginous airways, and these holes are held open by ''spiracles'' (similar to small vascular stents). Pilot studies have shown that these fenestrations can be created safely and have a beneficial effect on lung function.[7 8] There is, however, a tendency for the fenestrations to become blocked by granulation tissue.

Bronchoscopic instillation of delivery systems for slow release of drugs may open up new perspectives in the localised treatment of lung conditions. Some polymers have thermotropic properties and so behave as liquids at room temperature but form gels at body temperature. These can be injected into a part of the lung where they are cleared slowly and act as a drug efflux reservoir. These polymers also have a number of influences on cells ranging from effects on drug efflux channels to energy depletion in mitochondria. One such