

Non-invasive monitoring of airway inflammation

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NON-INVASIVE CLINICAL MEASUREMENTS are needed to assess the type and extent of airway inflammation in individuals in order to diagnose, manage and monitor their asthma. Furthermore, non-invasive tests can be used to investigate the genesis and pathophysiological mechanisms of asthma.

General principles

In considering non-invasive tests to assess inflammation in asthma, three issues are particularly important:

- the methods used to collect samples;
- the nature of the samples; and
- the markers analysed as indicators of inflammation.

These factors limit the age range of subjects tested, the setting and physical handling of specimens, and the validity of any assessment. For a test that might have an impact on clinical management, cost is also an issue, but, to date, there have been no published cost-benefit studies on the various non-invasive tests of inflammation.

An ideal non-invasive test should be easy to perform, reliable, accurate and informative. To be useful in the early stages of asthma, results should be valid from early infancy, and the same methodology should be applicable throughout childhood. Currently, no non-invasive tests of pulmonary inflammation for use in children meet these criteria.

Types of tests and applicability

The commonly used non-invasive tests employ one of three methods to obtain samples from the lower (intrathoracic) airways:

- induced sputum;
- exhaled gas collection or real-time analysis; and
- condensation of exhaled gas.

Because of the methods used to obtain sputum, the induced-sputum test is only suitable for school-age children and therefore cannot be used to investigate the early, initiation phase of asthma. Although standardised methods for the analysis of exhaled gases, in particular nitric oxide (NO), have been recommended for children, there are few data assessing the validity or the effects of age on the various methods. The latter issue relates specifically to the effect of expiratory flow on exhaled NO, and the fact that expiratory flow increases with age. Even though the effect of expiratory flow can be eliminated, little is known about NO production, diffusion and metabolism during infancy. As to breath

ABSTRACT

What we know

- Various techniques are available that purport to measure aspects of airway inflammation non-invasively, including analysis of volatile molecules in exhaled breath and components of breath condensates.
- Adapting and validating these methods for use in young children and infants poses significant methodological problems, but progress has been made, particularly with regard to measurements of exhaled nitric oxide.
- Future studies to validate such tests are likely to require access to airway tissue for examination as a "gold standard".

What we need to know

- How can we obtain airway tissue from infants and young children to better characterise the early airway changes in asthma?
- How do non-invasive tests of airway inflammation compare with a validated gold standard?
- How sensitive and specific are non-invasive tests of inflammation for predicting outcomes in asthma, including response to therapy?

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condensates, there are no reliable reported methods for such tests in infants, although it is possible that changes in expiratory flow will not significantly affect the composition of the condensate, particularly with regard to metabolites of NO, such as nitrites and nitrates.

Recommendations for the standardised performance of induced-sputum procedures and the measurement of exhaled NO have been published. However, there is little information on the effect of age on the validity of the various methods.

Validity of non-invasive tests

The validity of the various methods has usually been determined by assessing intrasubject variability, by comparing with results obtained by more invasive methods, and by monitoring responses to anti-asthma medications, in particular glucocorticoids.

In adult studies, it has been possible to compare markers of inflammation examined by non-invasive methods with the same markers examined using more invasive methods, such as bronchoalveolar lavage and bronchial biopsy. However, there are limited data in children and virtually no useful data in preschool children. Furthermore, studies in children are almost entirely limited to those with severe disease.¹

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Induced sputum

The utility of induced-sputum analyses for the study of asthma in children has been reviewed recently.² In essence, standardised techniques have been developed to induce sputum, isolate cell pellets and measure cytokine and other inflammatory markers in sputum. Asthma appears to be characterised by eosinophilic inflammation, and therefore much attention has been paid to the analysis of eosinophils obtained from induced-sputum specimens. Eosinophil numbers in sputum reflect the proportion of eosinophils in the total cell count obtained from bronchoalveolar lavage, but there is controversy about whether sputum eosinophil numbers reflect tissue eosinophilia. However, changes in sputum eosinophil numbers do reflect changes in eosinophil numbers in bronchoalveolar lavage and biopsy after treatment with anti-inflammatory drugs. The interpretation of data on eosinophils in the lungs is complex, and, at present, important information such as the state of activation and movement of cells cannot be deduced easily.

Exhaled gases

The measurement of volatile components in exhaled gas has elicited much interest since the earliest reports that levels of NO were raised in the breath of patients with asthma compared with those of healthy individuals.³ Endogenous NO is synthesised from L-arginine by isoenzymes of NO synthetase (NOS). At least three isoforms exist: neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). The activities of nNOS and eNOS are calcium dependent, whereas the iNOS isoenzyme can be induced by inflammatory cytokines. NO is produced by several classes of pulmonary cells, including inflammatory, endothelial and airway epithelial cells, and is easily detected in exhaled air.³

Data on NO in asthma have recently been reviewed.⁴ In asthmatics, NO in exhaled breath is thought to reflect the activity of iNOS, as levels are lower after treatment with either a specific iNOS antagonist or anti-inflammatory agents such as glucocorticoids.⁵ Although there are recommendations regarding the measurement of the fractional concentration of NO in exhaled breath (FE_{NO}) in children,⁶ there are few data either assessing the validity of the various measurements in infants or comparing the performance of the various techniques. The most frequently reported technique measures mixed expired FE_{NO} during tidal breathing, either on-line in real time or off-line after collection into a small-volume, inert balloon. However, since FE_{NO} is flow-dependent,⁷ one can predict that factors that change expiratory flow (age, respiratory illness) are likely to have an impact on the measured FE_{NO}. A further potential limitation to the use of FE_{NO} as a simple marker of inflammation relates to observations that atopy alone causes elevation in FE_{NO}.⁸ Although subclinical inflammation in healthy subjects with atopy might explain this phenomenon, an effect on NO biology of atopy has not been excluded and is supported by observations in asthmatics that atopy has an independent effect on FE_{NO}.⁹

Breath condensates

Breath-condensate analysis might be one solution to some of the methodological problems with exhaled-gas analysis. Preliminary data suggest that measurements of nitrites and hydrogen peroxide are not flow-dependent and also better reflect general airway inflammation. For example, in patients with cystic fibrosis, levels of nitrite and hydrogen peroxide are raised,¹⁰ but FE_{NO} is not. Furthermore, non-volatile components of the airway lining fluid, such as cytokines and other inflammatory markers, can also be detected in breath condensates. Further work is required to establish reliable methods in infants, and to compare the results obtained in breath condensates with those using bronchoalveolar lavage.

Future research

The development of tools that provide insight into the early airway manifestations of asthma is important if we are to learn more about asthma genesis. One important barrier to this is the difficulty of obtaining airway specimens from young children. The definitive method of validating these indirect methods of assessing airway inflammation is the "gold standard" — biopsy specimens — which has been used to examine the relationship between airway eosinophilia and FE_{NO}.¹ Non-bronchoscopic lavage techniques in unselected populations of children undergoing routine surgery might also provide an acceptable means of acquiring data for comparison with non-invasive tests. Another approach is to incorporate a number of different, standardised techniques into longitudinal cohort studies to determine the sensitivity and specificity of individual tests in relation to outcomes relevant to asthma.¹¹

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