Eur Respir J 2000; 16: 1008–1015
Printed in UK – all rights reserved

European Respiratory Journal
ISSN 0903-1936

SERIES "NONINVASIVE MONITORING OF AIRWAY INFLAMMATION"
Edited by H. Magnussen and F.E. Hargreave
Number 5 in this series

Noninvasive assessment of airway inflammation in children: induced sputum, exhaled nitric oxide, and breath condensate

P.G. Gibson*, R.L. Henry**, P. Thomas***

Noninvasive assessment of airway inflammation in children: induced sputum, exhaled nitric oxide, and breath condensate. P.G. Gibson, R.L. Henry, P. Thomas. ©ERS Journals Ltd 2000.

ABSTRACT: Noninvasive markers of airway inflammation are needed for use in research and clinical practice in childhood asthma. Induced sputum and exhaled nitric oxide are well established as direct markers of inflammation for use in asthma research.

Sputum can be induced from children of >6 yrs using inhalation of hypertonic saline, and, if appropriate, can be combined with an assessment of airway responsiveness to hypertonic saline. The success rate of sputum induction in children is 68–100%. Most studies have processed sputum using the plug selection method, and show that the dominant cell in sputum from normal children is the macrophage, and that the upper normal limit for sputum eosinophils in children is 2.5%.

The inflammatory response in childhood asthma is characterized by elevated numbers of sputum eosinophils, and eosinophil cationic protein concentration, as well as increased nitric oxide and hydrogen peroxide levels in exhaled breath. Sputum eosinophils correlate with objective markers of disease severity in steroid-naive children with asthma, and in severe asthma. Inflammatory marker levels are lower in children using glucocorticosteroids.

Induced sputum and exhaled gases are important markers of inflammation in childhood asthma. The clinical utility of these markers warrants further study.
Eur Respir J 2000; 16: 1008–1015.

*Dept of Respiratory and Sleep Medicine, John Hunter Hospital, Newcastle, **School of Paediatrics, University of New South Wales, Sydney and ***School of Medicine, University of New South Wales and Dept of Respiratory Medicine, Prince of Wales Hospital, Randwick, Sydney, New South Wales, Australia.

Correspondence: P.G. Gibson, Dept of Respiratory and Sleep Medicine, John Hunter Hospital, Locked Bag 1, Hunter Region Mail Centre, Newcastle, New South Wales, 2310 Australia. Fax: 61 249213469

Keywords: Airway inflammation
childhood asthma
exhaled breath analysis
sputum eosinophilia

Received: July 14 2000
Accepted after revision July 25 2000

Airway inflammation is a major characteristic of childhood asthma. Investigation of the mechanisms and features of airway inflammation has helped to define the pathogenesis of asthma in adults and there is a need to extend this research into childhood asthma. Noninvasive markers of airway inflammation are needed for use in research and clinical practice. Induced sputum and exhaled nitric oxide are well-established direct markers of inflammation, and, in this review, the technique and application of induced sputum and measurement of exhaled gases to the assessment of airway inflammation in childhood asthma is detailed.

Induced sputum

Since the initial report of induced sputum analysis in children with asthma in 1992 [1], there have been >20 reports in the literature demonstrating sputum induction to be safe and successful in children. Sputum can be induced in children using inhalation of hypertonic saline [1], which is delivered *via* an ultrasonic nebulizer, either as a 4.5% solution or as increasing concentrations of saline (3, 4 and 5%). Children aged 6–18 yrs have been studied using sputum induction. Successful sputum induction at <6 yrs may be limited by spirometric technique and a low tidal volume which limits the dose of saline that can be delivered [2]. Over 500 children have been studied, with the range of diagnoses including stable asthma (n=308), acute asthma (n=38), healthy controls (n=185) and cystic fibrosis (n=31).

Inhalation of hypertonic saline may cause airway narrowing that can be reduced by pretreatment with a β_2-agonist [3]. β_2-Agonist pretreatment does not alter the cellular differential of induced sputum in adults [4]. Most studies of sputum induction in children have used β_2-agonist pretreatment. An alternative technique is to combine sputum induction and bronchial provocation challenge using hypertonic saline [5]. This combined challenge provides a measure of both airway inflammation and airway responsiveness in a single test. Whichever technique is used, it is necessary to monitor lung function during sputum induction in order to detect and treat airway obstruction.

Previous articles in this series: No 1: L. Jayaram, K. Parameswaran, M.R. Sears, F.E. Hargreave. Induced sputum cells counts: their usefulness in clinical practice. *Eur Respir J* 2000; 16: 150–159. **No. 2:** O. Holz, J. Kips, H. Magnussen. Update on sputum methodology. *Eur Respir J* 2000; 16: 335–359. **No. 3:** R.A. Jörres. Modelling the production of nitric oxide within the human airways. *Eur Respir J* 2000; 16: 555–560. **No. 4:** S.A. Kharitonov, P.J. Barnes. Clinical aspects of exhaled nitric oxide. *Eur Respir J* 2000; 16: 781–792.

Table 1. – Sputum induction in children with asthma and controls

First author [Ref.]	Success rate %	Subjects n		Sputum processing method	Sputum eosinophils %		Sputum ECP $\mu g \cdot L^{-1}$	
		Asthma	Control		Asthma	Control	Asthma	Control
Pin [1]	76	13	26	Selected	1.7	0.15	-	-
Twaddell [6]	92	8	0	Selected	33.0	-	-	-
Piacentini [7]	100	16	0	Selected	14.0	-	-	-
Sorva [8]	76	14	15	Selected	-	-	591	140
Gibson [5]	92	61	109	Selected	8.0	0.3	-	-
Jones [9]	100	1	0	Selected	2.3	-	-	-
Cai [10]	84	50	72	Selected	4.3	0.3	-	-
Piacentini [11]	100	9	0	Selected	2.2	-	-	-
Mattes [12]	96	25	9	Selected	2.2	0.4	453	234
Piacentini [13]	81	25	0	Selected	13.0	-	69	-
Lonnkvist [14]	100	10	0	Selected	-	-	28	-
Oh [15]	100	30	14	Whole	5.9	0.8	141	36
Grootendorst [16]	95	20	0	Whole	0.4	-	193	-
Gibson [17]	88	42	0	Selected	16.0	-	1077	-

ECP: eosinophil cationic protein.

Determinants of success

The reported success rates of sputum induction in children are 68–100% (table 1). Factors relating to the children, the methods used and the operator collecting sputum all contribute to the success rate of sputum induction. Two major factors of technique have been identified which are potentially important contributors to the success rate. Hypertonic saline results in greater success, more sputum and a shorter collection time in comparison to normal saline. Combining a hypertonic saline challenge test with sputum induction may result in worse success rates than induction after pretreatment with a β_2-agonist. It has been found that experienced enthusiastic operators are more likely to obtain sputum [10]. It appears that there is a learning curve for each individual and that initial success rates may be poor.

During an acute exacerbation of asthma [6, 18], the time taken to collect sputum was shorter and the sputum was more likely to be expectorated spontaneously than after the exacerbation had resolved. In addition, the use of normal saline rather than hypertonic saline was sufficient to induce sputum in acute asthma but not during stable asthma. An unpublished analysis of data from the Airway Research Centre (Newcastle, New South Wales, Australia) suggests that induced sputum collection is more likely to be successful if the background asthma control is worse compared with well-controlled asthma or normal children (P. Jones, School of Paediatrics, University of Newcastle, Australia, personal communication).

Mechanisms

The mechanism of sputum production after hypertonic saline challenge is not established. Potential mechanisms include a reduction in the viscosity of tracheobronchial mucus, increased mucociliary clearance, increased mucus production or an increase in the volume of airway secretions [19]. Inhalation of hypertonic saline also causes bronchoconstriction and cough which are mediated by mast cell degranulation and stimulation of afferent nerves in the airway [19–21]. These factors could also enhance sputum production.

Sample collection and analysis

Sputum is a variable mixture of tracheobronchial secretions, saliva and hypertonic saline. Saliva is a contaminant that can confound the interpretation of sputum results. The two greatest problems to overcome when using sputum for analysis are, first, how to deal with contaminating saliva and, secondly, how to quantify results. Reliable results can be obtained when these sources of variability are controlled [22].

Salivary contamination can be minimized by microscopic selection of viscid mucocellular portions of sputum for analysis (selected method) [1, 23]. An alternative method of dealing with salivary contamination is to collect and analyse sputum and saliva together (whole sputum method) [24]. The salivary results are subtracted from the sputum results to give a "corrected cell-count". Both methods have been shown to be useful in assessing airway inflammation in adults; however, the selected sputum method may provide more viable cells, more eosinophils and a higher concentration of eosinophil cationic protein (ECP) [25]. In children, the majority of studies have used the selected portion method and only two studies have used the whole expectorate method of processing induced sputum (table 1) [15, 16]. There has been no formal comparison of these two processing techniques in children. Sputum cells need to be dispersed using dithiothreitol (DTT) prior to cytocentrifugation. Dispersed cell preparations are useful for cytochemistry and immunocytochemistry. The sputum samples may also be suitable for molecular biological techniques and cell culture, although these applications have yet to be described in children.

Quality

The quality of induced sputum samples in children is reported as good to satisfactory in most studies; however, data have seldom been provided to validate this. In particular, the extent of salivary contamination has been reported as part of a component quality score in only some studies [1, 5, 9, 10, 16, 26]. Most studies have not reported the extent of salivary contamination. A

Table 2. – Measurement properties of sampling techniques in children

	Sputum	Exhaled NO	Breath condensate
Reproducibility			
Within sample time	Good	Good	?
Over time	Good	Good	?
Responsiveness to condition			
Allergen exacerbation	?	Yes	?
Asthma exacerbation	Yes	Yes	?
Corticosteroid therapy	?	Yes	Yes
Validity in relation to objective measures of lung function	Satisfactory	Satisfactory	?
Adverse events			
Fall in FEV1	Yes	No	No
Fall in S_{a,O_2}	?	No	No
Subject discomfort	Minor	No	No
Costs (major equipment)			
Sample collection	Ultrasonic nebulizer; spirometer	Mouthpieces and Teflon tubing	Mouthpiece; nonrebreathing glass tube system
Subject discomfort	Haemocytometer; cytocentrifuge; microscope	Chemiluminescence analyser	Fluorimeter

FEV1: forced expiratory volume in one second; S_{a,O_2}: arterial oxygen saturation.

percentage of squamous cells of >50% constitutes excessive squamous contamination, and the sample should be recollected [27].

Measurement issues

Sputum differential cell counts have been reported to give reproducible results [1]. The reproducibility of fluid phase marker levels in children have not been reported. Sputum eosinophil counts are responsive to change. Elevated levels are seen in acute asthma [6, 18] and cell counts fall after corticosteroid therapy [15]. There is a satisfactory relationship between sputum eosinophil counts and measures of lung function. Table 2 summarizes the measurement properties of sputum cell counts. The induced sputum technique requires an ultrasonic nebulizer for sample collection and access to a centrifuge, cytocentrifuge and microscope for sample processing and analysis.

Studies comparing bronchial wash and bronchoalveolar lavage (BAL) to the induced sputum technique in adults show that there is good agreement between the type of cells recovered by sputum and bronchoscopic samples [28, 29]. Selected sputum is more concentrated than BAL fluid, having a higher density of recovered cells and higher levels of fluid-phase markers [29]. This issue has yet to be examined in children. Serum ECP levels have been compared to induced sputum in adults, and sputum eosinophil number has been found to be a more sensitive and accurate means of detecting airway inflammation than serum ECP levels. Sputum eosinophilia has a sensitivity of 70% and specificity of 90% compared to serum ECP level, which has a sensitivity of 50% and specificity of 50% [30]. In children, sputum ECP level was not correlated with serum ECP level [13] and the improvement in clinical asthma with inhaled glucocorticosteroids (GCSs) was associated with a fall in sputum ECP level but no change in serum ECP level [8]. These data suggest that direct measures of airway inflammation using airway samples such as sputum may be more useful than indirect measures in childhood asthma.

Normal values

The normal range for sputum cell counts in children is now well established (table 3) [10]. The dominant cell in sputum from normal children is the macrophage, and the upper normal limit for sputum eosinophils in children is 2.5%.

Sputum eosinophils

The eosinophil plays an important role in the pathogenesis of asthma [31]. Levels of eosinophils [1, 5, 10, 12, 15] and the biochemical marker ECP [8, 12, 15] are higher in asthmatic sputum than in sputum from normal

Table 3. – Cell counts in sputum from normal children

	Normal	Atopic normal	Nonatopic normal
TCC 10^6 cells·mL^{-1}	5.14 (1.2–9.08); 1.5 (0.8–3.9)	1.75 (0.89–2.6); 1.0 (0.55–2.15)	8.04 (0.63–15.5); 1.8 (1.05–6)
Eosinophils %	1.57 (0.62–2.52); 0.3 (0–1.05)	2.16 (0.83–3.48); 0.5 (0–2.8)	1.13 (0–2.54); 0 (0–0.6)
Mast cells %	0.024 (0–0.05); 0 (0–0)	0.03 (0–0.07); 0 (0–0)	0.02 (0–0.06); 0 (0–0)

Data are presented as mean (95% confidence interval); median (interquartile range). TCC: total cell count. (From [10].)

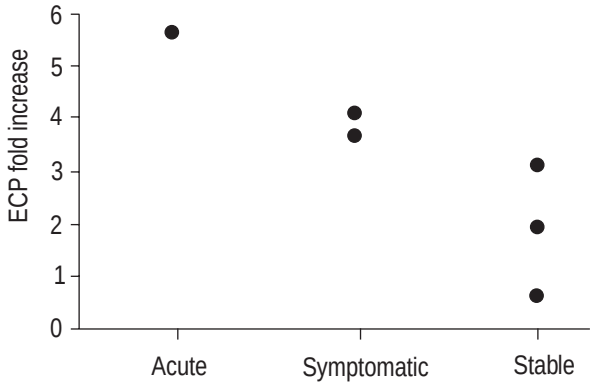

Fig. 1. – Increase in sputum eosinophil cationic protein (ECP) levels compared to controls in acute asthma, symptomatic asthma and stable asthma in children. (Data from studies in table 1.)

subjects (table 1, fig. 1) and may not be completely suppressed by inhaled GCS therapy [10]. Children with methacholine airway hyperresponsiveness but no symptoms of current or past asthma do not show increased numbers of sputum eosinophils [26]. These studies indicate that eosinophils and their secretory products form the characteristic profile of airway inflammation in symptomatic childhood asthma.

Sputum eosinophilia may reflect disease activity in childhood asthma (fig. 2). Sputum eosinophilia increases several-fold during exacerbations of asthma [6, 10, 15, 17, 18]. In addition, sputum albumin, ECP and interleukin (IL)-5 levels were higher in current symptomatic episodes and during exacerbations [15, 18]. Sputum eosinophil levels correlate with objective markers of disease severity in childhood asthma. Higher levels of induced sputum eosinophils and ECP are associated with greater airflow obstruction (reflected in the forced expiratory volume in one second (FEV_1)) [6, 8, 16, 27]. The same association holds in severe asthma, in which sputum eosinophil counts correlated with the degree of airflow obstruction in children attending the emergency department with severe asthma [11]. Degree of sputum eosinophilia was also associated with the degree of airway responsiveness (fig. 3) [5, 11, 26], and eosinophil numbers are reduced with allergen avoidance [7, 14]. These studies indicate a potential role for the measurement of levels of eosinophils and their degranulation products in monitoring disease severity.

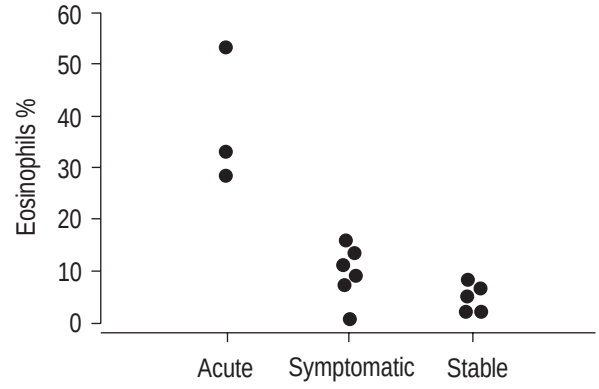

Fig. 2. – Induced sputum eosinophil levels in acute asthma, symptomatic asthma and stable asthma in children. (Data from studies in table 1.)

Sputum eosinophil cationic protein in asthma

Release of eosinophil granule proteins such as ECP can occur with eosinophil activation and/or lysis [14]. Sputum supernatant ECP level is used as an index of eosinophil degranulation. Reported levels of sputum ECP vary greatly. The reasons for this are not clear, but may relate to measurement procedures and subject selection. In normal children, mean sputum ECP levels can be detected at levels higher than those reported in blood. The origin of ECP in these noneosinophilic sputum samples is not clear. In stable asthma, there is a two- to three-fold increase in sputum ECP levels (fig. 1). Extremely high levels of >1,000 $\mu g \cdot L^{-1}$ have been reported in children with acute severe asthma [18], suggesting that there is intense eosinophil degranulation in acute exacerbations of asthma. Inhaled GCSs reduce sputum ECP levels, and the change in sputum ECP concentration is associated with the reduction in asthma symptoms [8].

Sputum mast cells

Mast cells are seldom seen in the sputum of healthy children [1, 5, 10]. They may be infrequently seen in sputum from children with asthma, where their levels are correlated with airway responsiveness to 4.5% saline (fig. 3) [5].

Epithelial cells

Shed epithelial cells can be detected in sputum in increased numbers in unstable asthma [10]. When asthma control improves with allergen avoidance, epithelial desquamation is reduced [11].

Sputum in cystic fibrosis

Attempts to extrapolate induced sputum techniques to cystic fibrosis (CF) have led to the recognition that different processing techniques may be required. Sputum induction tends to be easier than in asthma, given that so many children with CF exhibit mucus hypersecretion from bronchiectasis. The resultant viscid sputum is difficult to process using standard methods with DTT but an alternative three-enzyme method has been described [32]. Total cell and neutrophil counts were significantly increased when using the enzyme mixture for sputum cell dispersal compared with DTT. This effect probably represents incomplete cell dispersal with DTT. Unfortunately, elastase immunoreactivity was reduced by enzyme treatment, which was due to the galactosidase in the enzyme mixture.

Airway inflammation, as measured by total cell counts in sputum, is a prominent feature of CF, with cell counts approximately five to ten times higher than those reported in normal children [32–33]. Neutrophils are the dominant inflammatory cells. Sputum has been used as an outcome factor to measure response to therapy, such as recombinant human deoxyribonuclease (rhDNase). HENRY *et al.* [33] found evidence of decreases in neither sputum total

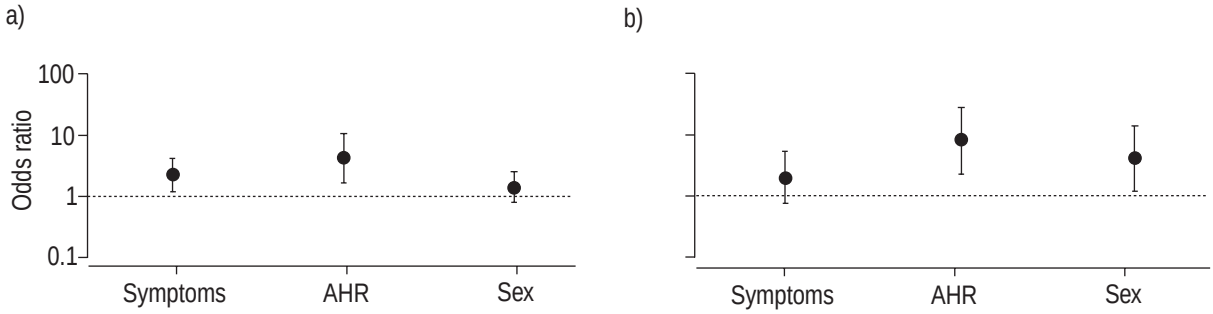

Fig. 3. – Odds ratios (with 95% confidence intervals) for the association between levels of: a) sputum eosinophils; and b) sputum mast cells and asthma symptoms ($p<0.05$), airway hyperresponsiveness (AHR) ($p<0.05$) and sex ($p<0.05$ for mast cells and male sex). (From [5].)

cell counts nor neutrophil counts after rhDNase therapy, even though there were improvements in lung function. These observations were consistent with the adult data of SHAH *et al.* [34], who reported that rhDNase therapy was associated with an initial rise in neutrophil elastase activity with a significant fall by 1 month but no changes in IL-8 level. The overall reduction in proteolytic activity was small and the residual neutrophil elastase and IL-8 levels remained very high. The latter study prompted the comment that "concern arises as to whether treatment with rhDNase only affected superficial removal of secretions, whereas deeper down on the mucosal surface, tissue damage continued as before" [35].

The need to develop better outcome measures for clinical trials in CF has been well recognized [36]. One of the possible outcomes was "a simple, inexpensive test of airway inflammation". At this stage it is not known whether induced sputum will be a useful tool for monitoring changes in airway inflammation in response to interventions in CF.

Exhaled gases: nitric oxide

Since the description of nitric oxide as a mediator in 1987 [37], observations in animals and humans have indicated that NO is detectable in exhaled breath [38]. PERSSON *et al.* [39] and KHARITONOV *et al.* [40], indicated that elevated levels of this gas were found in the breath of those adults with asthma who did not use GCSs. Many subsequent publications have confirmed these observations in adults, and others have extended them to children [41–44]. Variations in the methods of collection of samples have also been described, and guidelines with suggestions for the collection and analysis of exhaled NO (eNO) have been published in the European [45] and, more recently, American literature [46].

Studies of eNO in adults have largely indicated that, in asthma, elevation of eNO levels is seen in those who do not use inhaled GCSs, in those who have an exacerbation of their disease and after allergen challenge in susceptible allergic asthmatic subjects, in association with inflammatory cell influx. eNO is also elevated in other conditions such as upper respiratory tract infection, whereas it is decreased in Kartagener's syndrome and after alcohol ingestion and cigarette smoking. Elevated levels in asthma decrease towards those seen in normal subjects after administration of GCSs, either by oral or inhaled routes [43, 47]. In adults these elevated levels have been associated with other measures of inflammation, and eNO is now established as a recognized marker of inflammation. eNO appears to be related to bronchial responsiveness, and, not surprisingly, in both adults and children, it shows little correlation with isolated spirometric measurements or acute changes in forced expiratory volume [43, 48], although correlation has been observed in some studies [49]. Few published studies have assessed the clinical relevance of eNO, and such studies are awaited. Thus the literature regarding findings in children have so far reflected those in the adult world.

Techniques

These have been described in the reviews and guidelines published [45, 46]. In summary, eNO may be measured on-line *via* chemiluminescence, using a manoeuvre to close the soft palate and avoid nasal gas contamination of the sample (table 2). If ambient NO concentrations are high, then breathing NO-free air is advised. An alternative method, particularly useful for large epidemiological studies, is to use a gas-impermeable bag, and the same caveats apply. eNO samples collected by this latter method have been shown to correlate well with those measured directly on-line [50]. The collection of eNO samples from infants and young children has required other techniques. In this situation, some investigators have used a face mask, which would be contaminated with nasal NO [51], whereas others have used a technique adapted from one used for measuring pulmonary function [48].

Population studies of eNO in normal and asthmatic children have been published [44, 52–54], and even measurements in preterm infants have been reported [51]. These studies suggest that the concentration of eNO measured may be lower than that in adults, but, when corrected for body mass, these differences would appear to be insignificant. Few studies, if any, have compared children with adults using the same techniques and equipment.

Relationship of exhaled nitric oxide to asthmatic airway inflammation

Indices of inflammatory cells and their markers. eNO is generated by isoforms of nitric oxide synthase (NOS; types I–III) in the airway epithelium and from pulmonary macrophages. Constitutive NOS (type III) is probably responsible for the basal levels of eNO seen in normal subjects. If the airways are inflamed, then inducible NOS

(iNOS; type II) is expressed in the epithelium, and this causes a rise in eNO levels. In addition, inflammatory cells are known to express iNOS, and this may contribute to these elevated levels. In adults, it has been shown that inhaled budesonide causes a decrease in eNO which correlates with the associated decrease in bronchoalveolar lavage eosinophil number [55]. In children, eNO levels were also positively correlated with the absolute levels of peripheral blood eosinophils [56], sputum ECP and sputum eosinophils [28]. In adults, eNO has been shown to correlate with both sputum eosinophil number and methacholine responsiveness [57]. Thus eNO levels in allergic asthma appear to be related to the degree of eosinophilic inflammation.

The relationship of eNO with neutrophils in the airway or lung is less clear. It might be expected that, in the presence of neutrophil inflammation, eNO would be elevated. The majority of studies in CF in which there is an intense neutrophil infiltrate have reported that the eNO level is either the same or only slightly elevated compared with normal children. Some studies have reported that, despite this, breath nitrite (a NO metabolite) levels were elevated [58]. One explanation for this apparently contradictory finding is that NO is converted to peroxynitrite and nitrate by superoxide in activated neutrophils. Thus it would appear unlikely that eNO levels would correlate well with pulmonary neutrophil numbers, but no formal studies have addressed this issue. Reports of eNO levels in CF have varied, but, unlike bronchiectasis in adults, there is little evidence that the levels are significantly raised [41]. This may be for the reasons mentioned above, and because NO is rapidly converted to other oxides of nitrogen by superoxide.

Viral upper respiratory tract infections and influenza vaccination have been associated with elevation of eNO levels in adults, perhaps reflecting lymphocytosis in the airway [59, 60], but, again, there have been few studies, and none in children.

Indictors of atopy. FRANKLIN *et al.* [54] demonstrated that, in children, levels of eNO increased with severity of atopy as measured by a number of positive skin-prick tests, perhaps implying subclinical airway inflammation in these individuals. Corroborating this concept is the observation that elevated eNO is associated with the pollen season in those asthmatic children sensitive to grass pollen, despite there being little change in spirometric results [61].

Relationship of exhaled nitric oxide to airway responsiveness. Although studies in adults have suggested a correlation between eNO levels and tests of bronchial reactivity, there are few data published in this area in the paediatric literature [57].

Clinical studies

Few studies have addressed the utility of using eNO as a surrogate marker of long-term asthma control in either adults or children. Some studies have looked at acute asthma and related eNO levels to clinical improvement [47], and others have evaluated it as a screening tool for the diagnosis of asthma [61], finding that those with atopic asthma appeared to be identified, whereas those with nonatopic asthma were not. A study involving a small number of asthmatic adults demonstrated a fall in eNO levels on the lowest dose of inhaled GCS, whereas eosinophil numbers in sputum only fell when a larger dose of GCS was administered, but how this relates to asthma control is not clear. Larger trials in children and adults are needed before any conclusions can be drawn.

Nasal nitric oxide

The nasal sinuses are a rich source of NO and speculation has centred upon the reason for this phenomenon. It is possible that NO has an antibacterial effect in the upper airway and that this aids in reducing inhalation of pathogens, or that inhalation of NO into the lower airway might induce beneficial broncho- and vasodilatation. Children and adults have high levels of NO in the nose compared with the lower airway, and allergic rhinitis is associated with particularly elevated levels in children, as in adults [62]. Topical GCSs reduce the levels of nasal NO associated with a reduction in symptoms and this implies a reduction in iNOS activity. There have been no studies in children comparing results of either rhinomanometry or differential nasal cytology with nasal NO levels. Levels of nasal NO in children with CF appear to be below those seen in normal children [41].

Other exhaled gases

Exhaled carbon monoxide, which is probably derived from the degradation of haem to bilirubin in the lung during oxidative stress, and exhaled 8-isoprostane are also present in exhaled breath and are thought to represent oxidative stress. Data on these exhaled gases have not been reported for children, and the less-well-described exhaled gases ethane and pentane, which may represent lipid peroxidation activity, also deserve further clarification.

Breath condensate

Subglottic gas is saturated with water and can be collected as exhaled water vapour condensate. This provides an opportunity to measure inflammatory markers in breath condensate (table 2). The principle has been applied to adults with asthma, in whom high concentrations of nitrogen oxides, such as nitrite and nitrate, as well as hydrogen peroxide, have been reported [63]. Breath condensate assays are reproducible, and normal data are available which show little variation with age [64]. Children with asthma have higher values than normal subjects [64, 65] and those with asthma who are treated with anti-inflammatory medications exhibit lower H_2O_2 levels than those with asthma who are not on GCSs.

Conclusions

Noninvasive markers such as induced sputum and exhaled gases are important measures of airway inflammation in childhood asthma. Induced sputum is readily obtained in children of >6 yrs. Sputum eosinophil numbers are increased in children with asthma, and the degree of eosinophilia relates to clinical disease activity. Exhaled

nitric oxide level has been developed over the last decade as a potent research tool indicating asthmatic inflammation, which is easily performed, once the initial expense of the equipment has been overcome. Gas analysis is ideal for the paediatric population given the ease of collection and noninvasive nature of the technique. It will continue for many years to be used as a nonspecific marker of inflammation in conjunction with more detailed cellular profiles and markers for these cells. The interest in other exhaled gases is to be encouraged and perhaps a profile of exhaled gases might indicate which individual inflammatory cells have been recruited into the airway or lung. Whether measurement of inflammatory markers might contribute to the clinical management of childhood asthma remains to be evaluated in large studies.

References

1. Pin I, Gibson PG, Kolendowicz R, *et al*. Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 1992; 47: 25–29.
2. Riedler J, Robertson CF. Effect of tidal volume on the output and particle size distribution of hypertonic saline from an ultrasonic nebulizer. *Eur Respir J* 1994; 7: 998–1002.
3. Smith SM, Anderson SD. Inhalation provocation tests using non-isotonic aerosols. *J Allergy Clin Immunol* 1989; 84: 781–790.
4. Cianchetti S, Bacci E, Ruocco L, *et al*. Salbutamol pretreatment does not change eosinophil percentage and eosinophilic cationic protein concentration in hypertonic saline-induced sputum in asthmatic subjects. *Clin Exp Allergy* 1999; 29: 712–718.
5. Gibson PG, Wlodarczyk JW, Hensley MJ, *et al*. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. *Am J Respir Crit Care Med* 1998; 158: 36–41.
6. Twaddell SH, Gibson PG, Carty K, Woolley KL, Henry RL. Assessment of airways inflammation in children with acute asthma using induced sputum. *Eur Respir J* 1996; 9: 2140–2148.
7. Piacentini GL, Martinati L, Mingoni S, Boner AL. Influence of allergen avoidance on the eosinophilic phase of airway inflammation in children with allergic asthma. *J Allergy Clin Immunol* 1996; 97: 1079–1084.
8. Sorva R, Metso T, Turpeinen M, Juntunen-Backman K, Bjorksten F, Haahtela T. Eosinophil cationic protein in induced sputum as a marker of inflammation in asthmatic children. *Pediatr Allergy Immunol* 1997; 8: 45–50.
9. Jones PD, Henry RL, Gibson PG, Hankin R, Carty K. Chemotherapy for malignancy induces a remission in asthma symptoms and airway inflammation but not airway hyperresponsiveness. *Pediatr Pulmonol* 1998; 26: 74–77.
10. Cai Y, Carty K, Henry RL, Gibson PG. Persistence of sputum eosinophilia in children with controlled asthma when compared with healthy children. *Eur Respir J* 1998; 11: 848–853.
11. Piacentini GL, Vincentini L, Mazzi P, Chilosi M, Mastinoti L, Boneo AL. Mite-allergen avoidance can reduce bronchial epithelial shedding in allergic asthmatic children. *Clin Exp Allergy* 1998; 28: 561–567.
12. Mattes J, Storm-van′s K, Reining U, *et al*. NO in exhaled air is correlated with markers of eosinophilic airway inflammation in corticosteroid-dependent childhood asthma. *Eur Respir J* 1999; 13: 1391–1395.
13. Piacentini GL, Bodini A, Costella S, *et al*. Exhaled nitric oxide and sputum eosinophil markers of inflammation in asthmatic children. *Eur Respir J* 1999; 13: 1386–1390.
14. Lonnkvist K, Hallden G, Dahlen SE, *et al*. Markers of inflammation and bronchial reactivity in children with asthma, exposed to animal dander in school dust. *Pediatr Allergy Immunol* 1999; 10: 45–52.
15. Oh JW, Lee HB, Kim CR, *et al*. Analysis of induced sputum to examine the effects of inhaled corticosteroid on airway inflammation in children with asthma. *Ann Allergy Asthma Immunol* 1999; 82: 491–496.
16. Grootendorst DC, van den Bos J-W, Romeijn JJ, *et al*. Induced sputum in adolescents with severe stable asthma. Safety and the relationship of cell counts and eosinophilic cationic protein to clinical severity. *Eur Respir J* 1999; 13: 647–653.
17. Gibson PG, Norzila M, Fakes K, Simpson J, Henry RL. Pattern of airway inflammation and its determinants in children with severe asthma. *Pediatr Pulmonol* 1999; 28: 261–270.
18. Norzila M, Fakes K, Henry RL, Simpson J, Gibson PG. IL-8 secretion and neutrophil recruitment accompanies induced sputum eosinophil activation in children with acute asthma. *Am J Respir Crit Care Med* 2000; 161: 769–774.
19. Umeno E, McDonald DM, Nadel JA. Hypertonic saline increases vascular permeability in the rat trachea by producing neurogenic inflammation. *J Clin Invest* 1990; 85: 1905–1908.
20. Finney MJB, Anderson SD, Black JL. Terfenadine modifies airway narrowing induced by the inhalation of nonisotonic aerosols in subjects with asthma. *Am Rev Respir Dis* 1990; 141: 1151–1157.
21. Eschenbacher WL, Boushey HA, Sheppard D. Alteration in osmolarity of inhaled aerosols cause bronchoconstriction and cough, but absence of a permanent anion causes cough alone. *Am Rev Respir Dis* 1984; 129: 211–215.
22. Kips JC, Peleman RA, Pauwels RA. Methods of examining induced sputum: do differences matter? *Eur Respir J* 1998; 11: 529–533.
23. Gibson PG, Girgis-Gabardo A, Morris MM, *et al*. Cellular characteristics of sputum from patients with asthma and chronic bronchitis. *Thorax* 1989; 44: 693–699.
24. Fahy JV, Liu J, Wong H, Boushey HA. Cellular and biochemical analysis of induced sputum from asthmatic and from healthy subjects. *Am Rev Respir Dis* 1993; 147: 1126–1131.
25. Spanevello A, Beghe B, Bianchi A, *et al*. Comparison of two methods of processing induced sputum: selected *versus* entire sputum. *Am J Respir Crit Care Med* 1998; 157: 665–668.
26. Pin I, Radford S, Kolendowicz R, *et al*. Airway inflammation in symptomatic and asymptomatic children with methacholine hyperresponsiveness. *Eur Respir J* 1993; 6: 1249–1256.
27. Timmins N, Simpson JL, Fakes K, Gibson PG. The effects of salivary contamination on sputum cell counts: establishing quality criteria. *Respirology* 2000; 5: A35.
28. Grootendorst DC, Sont JK, Willems LN, *et al*. Comparison of inflammatory cell counts in asthma: induced sputum *vs* bronchoalveolar lavage and bronchial biopsies. *Clin Exp Allergy* 1997; 27: 769–779.
29. Pizzichini E, Pizzichini MM, Kidney JC, *et al*. Induced sputum, bronchoalveolar lavage and blood from mild asthmatics: inflammatory cells, lymphocyte subsets and

soluble markers compared. *Eur Respir J* 1998; 11: 828–834.
30. Pizzichini E, Pizzichini MM, Efthiamidis A, Dolovich J, Hargreave FE. Measuring airway inflammation in asthma: eosinophils and eosinophilic cationic protein in induced sputum compared with peripheral blood. *J Allergy Clin Immunol* 1997; 99: 539–544.
31. Frigas E, Gleich GJ. The eosinophil and the pathophysiology of asthma. *J Allergy Clin Immunol* 1986; 77: 527–532.
32. Cai Y, Carty K, Gibson PG, Henry RL. Comparison of sputum processing techniques in cystic fibrosis. *Pediatr Pulmonol* 1996; 22: 402–407.
33. Henry RL, Gibson PG, Carty K, Cai Y, Francis JL. Airway inflammation after treatment with aerosolized deoxyribonuclease in cystic fibrosis. *Pediatr Pulmonol* 1998; 26: 97–100.
34. Shah PL, Scott SF, Knight RA, Hodson ME. The effects of recombinant human DNase on neutrophil elastase activity and interleukin-8 levels in the sputum of patients with cystic fibrosis. *Eur Respir J* 1996; 9: 531–534.
35. Zach M. The role of recombinant human DNase in the treatment of patients with cystic fibrosis: many promises, more problems. *Thorax* 1996; 51: 750–755.
36. Ramsey BW, Boat TF. Outcome measures for clinical trials in cystic fibrosis. Summary of a Cystic Fibrosis Foundation consensus conference. *J Pediatr* 1994; 124: 177–192.
37. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; 327: 524–526.
38. Gustafsson LE, Leone AM, Persson MG, Wiklund NP, Moncada S. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochem Biophys Res Commun* 1991; 181: 852–856.
39. Persson MG, Zetterstrom O, Agrenius V, Ihre E, Gustafsson LE. Single-breath nitric oxide measurements in asthmatic patients and smokers. *Lancet* 1994; 343: 146–147.
40. Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994; 343: 133–135.
41. Lundberg JO, Nordvall SL, Weitzberg H, Kollberg H, Alving K. Exhaled nitric oxide in paediatric asthma and cystic fibrosis. *Arch Dis Child* 1996; 75: 323–326.
42. Nelson BV, Sears S, Woods J, *et al*. Expired nitric oxide as a marker for childhood asthma. *J Pediatr* 1997; 130: 423–427.
43. Baraldi E, Azzolin NM, Zanconato S, Dario C, Zacchello F. Corticosteroids decrease exhaled nitric oxide in children with acute asthma. *J Pediatrics* 1997; 131: 381–395.
44. Byrnes CA, Dinarevic S, Shinebourne EA, Barnes PJ, Bush A. Exhaled nitric oxide levels in normal and asthmatic children. *Pediatr Pulmonol* 1997; 24: 312–318.
45. Kharitonov S, Alving K, Barnes PJ. Exhaled and nasal nitric oxide measurements: recommendations. The European Respiratory Society Task Force. *Eur Respir J* 1997; 10: 1683–1693.
46. Silkoff PE. ATS Statement. Recommendations for standardised procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children 1999. *Am J Respir Crit Care Med* 1999; 160: 2104–2117.
47. Lanz MJ, Leung DY, White CW. Comparison of exhaled nitric oxide to spirometry during emergency treatment of asthma exacerbations with glucocorticosteroids in children. *Ann Allergy Asthma Immunol* 1999; 82: 161–164.
48. Wildhaber JH, Hall GL, Stick SM. Measurements of exhaled nitric oxide with the single breath technique and positive expiratory pressure in infants. *Am J Respir Crit Care Med* 1999; 159: 74–78.
49. Artlich A, Hagenah JU, Jonas S, Shrens P, Gortner ?. Exhaled nitric oxide in childhood asthma. *Eur J Pediatr* 1996; 155: 698–701.
50. Paredi P, Loukides S, Ward S, *et al*. Exhalation flow and pressure-controlled reservoir collection of exhaled nitric oxide for remote and delayed analysis. *Thorax* 1998; 53: 775–779.
51. Artlich A, Busch T, Lewandowski K, Schaible T, Flake KJ, Gortner L. Exhaled nitric oxide in preterm infants. *Respir Physiol* 1998; 114: 195–200.
52. Dinarevic S, Byrnes CA, Bush A, Shinebourne EA. Measurement of expired nitric oxide levels in children. *Pediatr Pulmonol* 1996; 22: 396–401.
53. Baraldi E, Azzolin NM, Craco A, Zacchello F. Reference values of exhaled nitric oxide in healthy children 6–15 years old. *Pediatr Pulmonol* 1999; 27: 54–58.
54. Franklin PJ, Taplin R, Stick SM. A community study of exhaled nitric oxide in healthy children. *Am J Respir Crit Care Med* 1999; 159: 69–73.
55. Kharitonov SA, Chung KF, Evans D, O′Connor BJ, Barnes PJ. The elevated level of exhaled nitric oxide is mainly derived from the lower respiratory tract. *Am J Respir Crit Care Med* 1996; 153: 1773–1780.
56. Silvestri M, Spallaross D, Franova Yourukova V, Battistini E, Fregonese B, Rossi GA. Orally exhaled nitric oxide levels are related to the degree of blood eosinophilia in atopic children with mild-intermittent asthma. *Eur Respir J* 1999; 13: 321–326.
57. Jakakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998; 53: 91–95.
58. Ho LP, Innes JA, Greening AP. Nitrite levels in breath condensate of patients with cystic fibrosis is elevated in contrast to exhaled nitric oxide. *Thorax* 1998; 53: 680–684.
59. Kharitonov SA, Yates D, Barnes PJ. Increased nitric oxide in exhaled air of normal subjects with upper respiratory tract infections. *Eur Respir J* 1995; 8: 295–297.
60. Thomas PS, Ng C, Elsing M, Yates DH. Influenza vaccination: changes in exhaled nitric oxide and sputum cytology. *Respirology* 1999; 4: 355–358.
61. Frank TL, Adisesh A, Pickering AC, *et al*. Relationship between exhaled nitric oxide and childhood asthma. *Am J Respir Crit Care Med* 1998; 158: 1032–1036.
62. Baraldi E, Carra S, Dario C, *et al*. Effect of natural grass pollen exposure on exhaled nitric oxide in asthmatic children. *Am J Respir Crit Care Med* 1999; 159: 262–266.
63. Antczak A, Nowak D, Shariati B, Krol M, Piasecka G, Kurmanowska Z. Increased hydrogen peroxide and thiobarbituric acid-reactive products in expired breath condensate of asthmatic patients. *Eur Respir J* 1997; 10: 1235–1241.
64. Jobsis Q, Raatgeep HC, Schellekens SL, Hop WC, Hermans PW, de Jongste JC. Hydrogen peroxide in exhaled air of healthy children: reference values. *Eur Respir J* 1998; 12: 483–485.
65. Jobsis Q, Raatgeep HC, Hermans PW, de Jongste JC. Hydrogen peroxide in exhaled air is increased in stable asthmatic children. *Eur Respir J* 1997; 10: 519–521.