

## VI. Exhaled NO: Correlation with Inflammatory Markers

It is likely that changes in the levels of different inflammatory markers after corticosteroid treatment reflect the influence of steroids on different mediators of the inflammatory response. [van Rensen EL *et al.* 1999] Exhaled NO reflects airway inflammation, supported by studies correlating NO levels with conventional markers of airway inflammation. [Covar RA *et al.* 2003; Currie GP *et al.* 2003b; Delclaux C *et al.* 2005; Silvestri M *et al.* 2003; van Amsterdam JG *et al.* 2003a; Zietkowski Z *et al.* 2006a] The correlations reported are often only moderate or weak, but this is to be expected as the different markers reflect different aspects of inflammation. More importantly, evidence has shown that exhaled NO levels correlate with the results from examinations of bronchial biopsies and bronchoalveolar lavage (BAL) – the ‘gold standard’ assessment of airway inflammation. [Payne DN *et al.* 2001a; van den Toorn LM *et al.* 2001; Warke TJ *et al.* 2002] Compared with procedures such as BAL and airway biopsy, measurement of NO is non-invasive, safe, provides rapid results and causes no inconvenience to the patient.

### 6.1. Airway Hyperresponsiveness

Airway hyperresponsiveness is measured as the concentration of inhaled bronchoconstrictor agent required to produce a 20% drop in forced expiratory volume in 1 second ( $FEV_1$  [ $PC_{20}$ ]). Conflicting evidence regarding the correlation between NO levels and hyperresponsiveness has been reported. However, a good correlation between exhaled NO and bronchial hyperreactivity has been shown by many investigators. Al-Ali and Howarth demonstrated a relationship between exhaled NO levels and the  $FEV_1$  ( $PC_{20}$ ) dose of inhaled

histamine (compared with post-saline  $FEV_1$ ) in 26 non-smoking, atopic asthmatic patients with a mean age of 27 years (Figure VI.1). [Al-Ali MK and Howarth PH 2001] Salome *et al.* also reported a significant correlation between exhaled NO and hyperresponsiveness to histamine in young adults. [Salome CM *et al.* 1999] Similar relationships between exhaled NO and airway hyperresponsiveness have been demonstrated by other groups [Dupont LJ *et al.* 1998; Fabbri LM *et al.* 2003; Jatakanon A *et al.* 1998; Katz SD *et al.* 2005; Langley SJ *et al.* 2003b; Strunk RC *et al.* 2003] who used methacholine rather than histamine as the

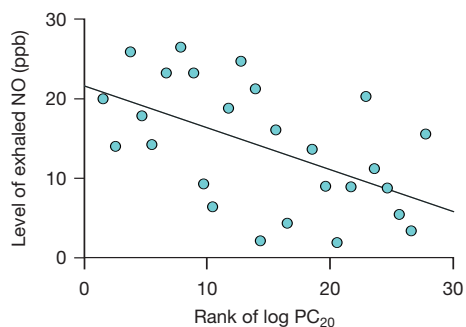


Figure VI.1. Relationship between exhaled NO levels and  $PC_{20}$  histamine daily ( $r = -0.51$ ,  $p = 0.008$ ) [Al-Ali MK and Howarth PH 2001]

bronchoconstrictor. In addition, a correlation has been reported between NO and hyperresponsiveness to saline. [Jones SL *et al.* 2001; Steerenberg PA *et al.* 2003] Lúdvíksdóttir *et al.* measured exhaled NO in a group of atopic and non-atopic asthmatic patients, and healthy controls. [Ludvíksdóttir D *et al.* 1999] Exhaled NO was elevated only in atopic patients, and in this group, it correlated well with the airway hyperresponsiveness to methacholine. In non-atopic patients and controls, there was no relationship between airway hyperresponsiveness and exhaled NO. Similar results have been reported by Rouhos and co-workers. [Rouhos A *et al.* 2005] Nishio *et al.* demonstrated a correlation between exhaled NO and airway hyperresponsiveness to acetylcholine in steroid-naïve asthmatic children, but not in children receiving ICS treatment. [Nishio K *et al.* 2006]

Many studies show no correlation between exhaled NO levels and airway hyperresponsiveness. In a study of children with mild intermittent asthma, Silvestri *et al.* were unable to find a correlation between exhaled NO and hyperresponsiveness. [Silvestri M *et al.* 2000] Olin and co-workers also reported no correlation with methacholine hyperresponsiveness in pulp mill workers exposed to ozone. [Olin AC *et al.* 1999] In addition, adults with mild asthma have been reported to show no correlation between NO and hyperresponsiveness. [Olin AC *et al.* 1999; van Rensen EL *et al.* 1999]

A study by van den Toorn and colleagues failed to show a significant correlation between hyperresponsiveness to methacholine and exhaled NO in patients with ongoing asthma and those in clinical remission. [van den Toorn LM *et al.* 2000] There was, however, a significant relationship between exhaled NO and hyperresponsiveness when adenosine-5-monophosphate (AMP) was used. It has been shown by de Meer *et al.* that hyperresponsiveness to AMP tends to correlate with serum eosinophilia, whereas responsiveness to methacholine correlates with FEV<sub>1</sub>, indicating that the former is a better marker of inflammation. [de Meer G *et al.* 2002] Several studies support the theory that the bronchial response to AMP is more closely associated with airway inflammation than the response to direct bronchoconstrictors such as histamine or methacholine. [Alving K *et al.* 1993; van den Berge M *et al.* 2001] A study in asthmatic patients in whom disease was already stabilized and well controlled by use of inhaled corticosteroids, showed that both bronchoconstriction in response to AMP and increased exhaled NO levels were significant predictors for failure in inhaled corticosteroid dose reduction. [Prieto L *et al.* 2003] Therefore, determination of AMP responsiveness and exhaled NO levels may be useful in identifying which patients might deteriorate when their dose of inhaled corticosteroid is reduced. Grönke *et al.* found that there was a significant correlation between NO levels and hyperresponsiveness to methacholine in patients who have had asthma for 16 or fewer years, but that this correlation was not present in patients who have had asthma for longer duration. [Grönke L *et al.* 2002a] Importantly both Grönke and de Meer's groups showed a significant correlation between NO levels and sputum eosinophils, suggesting that

methacholine hyperresponsiveness in patients with a long duration of asthma does not reflect active eosinophilic inflammation.

## 6.2. Sputum Eosinophils

Eosinophilic inflammation is a hallmark of bronchial asthma. [Bousquet J *et al.* 1990] Two studies by Jatakanon *et al.* [Jatakanon A *et al.* 2000; Jatakanon A *et al.* 1998] have demonstrated a relationship between exhaled NO and the fraction of eosinophils in induced sputum. The first study measured sputum eosinophils and exhaled NO in a group of stable asthmatics maintained on  $\beta_2$ -agonists alone, and found a significant correlation between the two parameters. [Jatakanon A *et al.* 1998] The second study examined a group of patients in whom mild exacerbations of asthma were induced by reducing the dose of inhaled maintenance steroids. [Jatakanon A *et al.* 2000] In those patients who developed an exacerbation of asthma, increases in both sputum eosinophils and exhaled NO were significantly correlated with deterioration in airway function. In addition, changes from baseline in exhaled NO levels and the concentration of sputum eosinophils were greater in patients after they had experienced an exacerbation compared with the changes seen in those who did not experience an exacerbation (Figure VI.2). There was also a significant correlation between the change in exhaled NO and the change in FEV<sub>1</sub> and the amount of rescue bronchodilator required.

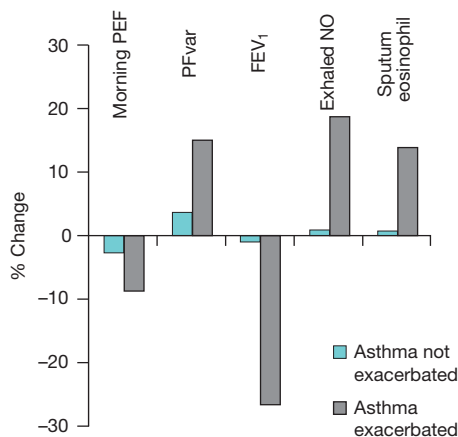


Figure VI.2. Effect of asthma exacerbation on changes in markers of airway inflammation. Peak expiratory flow = PEF; change in peak-flow variability = PF<sub>var</sub>; forced expiratory volume in one second = FEV<sub>1</sub> [Jatakanon A *et al.* 2000]

Other studies have drawn similar conclusions. Piacentini *et al.* [Piacentini GL *et al.* 1999a] found that exhaled NO correlated with sputum eosinophils, particularly in steroid-naïve patients, and Mattes *et al.* reported a correlation between markers of eosinophilic airway inflammation in children with corticosteroid-dependent asthma. [Mattes J *et al.* 1999] A small study of steroid-naïve non-atopic asthmatic patients showed that levels of sputum eosinophils were positively correlated with dose of inhaled beclomethasone dipropionate (BDP), indicating that the monitoring of eosinophilic airway inflammation may also be useful in the assessment of the effects of inhaled steroids in patients without history of allergy. [Dal Negro R *et al.* 2003] Berry and colleagues found a significant but non-linear correlation between exhaled NO and sputum eosinophils in 566 adult patients with asthma (Figure III.2). [Berry MA *et al.* 2005] There were no clinically important confounding factors

to this model in non-smokers. A good correlation between sputum eosinophils and exhaled NO levels was also found in children by Malmberg *et al.* [Malmberg LP *et al.* 2005]

It is worth noting that not all studies have found correlations between these inflammatory markers. For example, Lex and colleagues found that only 6 of 23 children with increased levels of NO had sputum eosinophilia. [Lex C *et al.* 2005] Furthermore, Deykin *et al.* reported that sputum eosinophils predicted loss of asthma control, whereas NO levels did not. [Deykin A *et al.* 2005]

In summary,  $FE_{NO}$  and sputum eosinophilia are two methods for assessing airway inflammation. Most studies show a good correlation between the methods. Sputum eosinophilia in many cases is more informative and sensitive than  $FE_{NO}$ , but is less patient friendly a method without immediate results like  $FE_{NO}$ .

### 6.3. Blood Eosinophils

Measurement of sputum eosinophilia requires induction of sputum and is an inconvenient and perhaps hazardous procedure for both adults and children. It may be more convenient to measure the eosinophil count in peripheral blood, which correlates well with the degree of allergic sensitization in children. [Frangova YV *et al.* 1996] A statistically significant correlation between both exhaled NO and total number of blood eosinophils and percentage of blood eosinophils in children with atopic asthma has been demonstrated by Silvestri *et al.* (Figure VI.3). [Silvestri M *et al.* 1999] In a study of children with exacerbations of asthma, Lanz *et al.* found that exhaled NO was a more sensitive marker of disease activity than either serum eosinophilic cationic protein (ECP) or soluble interleukin-2 receptor (SIL-2R).

[Lanz M] *et al.* 1997] Furthermore, repeated measurements after treatment suggested that exhaled NO was a more useful indicator of response to corticosteroid therapy than either serum ECP or SIL-2R. Crater *et al.* found a highly significant correlation between exhaled NO

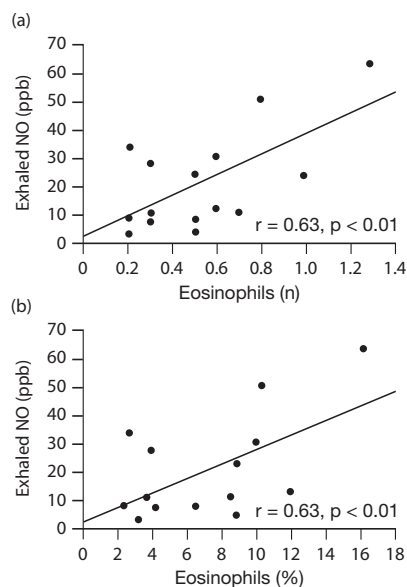


Figure VI.3. Correlation between NO levels in orally exhaled air and blood eosinophilia in asthmatic patients treated with inhaled  $\beta_2$ -agonists on an as-necessary basis. (a) Number of eosinophils; (b) percentage of eosinophils [Silvestri M *et al.* 1999]

and peripheral blood eosinophilia in adult patients with acute and stable asthma. [Crater SE *et al.* 1999] A combination of exhaled NO > 10 ppb and eosinophilia > 200 cells/ $\mu$ L had a sensitivity of 90% in predicting acute airway obstruction (flow rate not measured in mixed-air technique). Pedroletti and co-workers have also found correlations between NO levels and serum eosinophils in asthma patients. [Pedroletti C *et al.* 2005] In another study, serum eosinophilia was associated with higher NO levels, particularly in atopic individuals (Figure VI.4). [Barreto M *et al.* 2005]

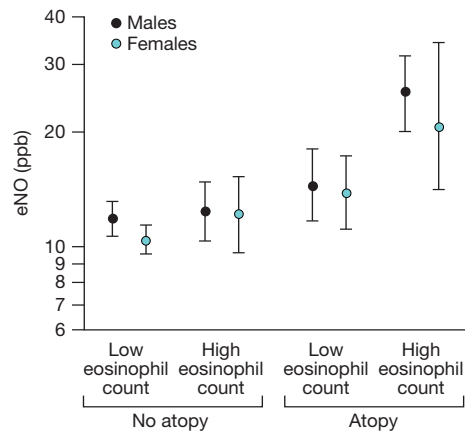


Figure VI.4. High NO levels correlate with eosinophilia particularly in atopic patients [Barreto M *et al.* 2005]

## 6.4. Biopsy

In an early study by Lim *et al.* steroid treatment was associated with a significant reduction in epithelial and submucosal immunoreactivity scores for eosinophils in bronchial biopsy specimens ( $p < 0.001$  and  $p < 0.005$ , respectively), and significant reductions in the exhaled NO concentration ( $p < 0.001$ ). [Lim S *et al.* 1999] A further study by the same group found a significant correlation between bronchial mucosal eosinophils and lung function ( $r = 0.43$ ,  $p < 0.05$ ), and significantly lower levels of exhaled NO in patients treated with inhaled steroids ( $p < 0.05$ ). [Lim S *et al.* 2000] There was no direct correlation between mucosal eosinophils and exhaled NO in this study. Two more studies have provided strong evidence that exhaled NO reflects airway inflammation. van den Toorn and co-workers assessed the quantity of major basic protein in bronchial biopsies of patients with asthma and individuals in clinical remission. [van den Toorn LM *et al.* 2001] Significant correlations between major basic protein density and exhaled NO levels occurred in both groups (Figure VI.5). Evidence of airway remodelling was also found in patients in remission, even though the median duration of remission was 5 years.

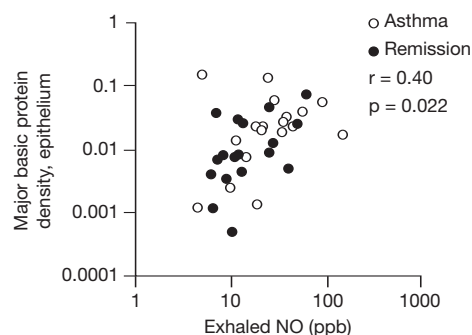


Figure VI.5. Exhaled NO levels correlate with major basic protein density in bronchial biopsies from asthma patients and individuals in remission from asthma [van den Toorn LM *et al.* 2001]

Payne and colleagues examined children with difficult asthma and showed a significant correlation between exhaled NO levels and eosinophil scores in biopsies ( $r = 0.54$ ,  $p = 0.03$ ). [Payne DN *et al.* 2001a] In those patients with evidence of adherence to prednisolone, a NO level of less than 7 ppb (at a flow rate of 200–280 mL/s) was associated with an eosinophilic score in the non-asthmatic range, whereas all patients with persistent symptoms and eosinophil counts above the non-asthma range had NO levels above 7 ppb (Figure VI.6). However, in another study the same group did not find any correlation between NO levels and inflammatory cells in biopsies from children with difficult asthma. [Payne DN *et al.* 2004] Clearly, further studies in this special group are required.

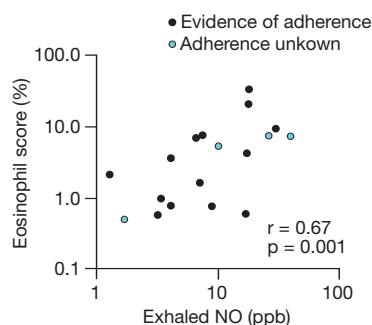


Figure VI.6. Exhaled NO levels correlate with biopsy examinations in children with difficult asthma [Payne DN *et al.* 2004]

There is evidence that invasive procedures such as bronchoscopy can invoke a systemic inflammatory response in some patients. Krenke *et al.* assessed the effect of bronchoscopy on exhaled NO levels to investigate whether this procedure caused local inflammation in the airways. [Krenke R *et al.* 2006] In a sample of 55 patients with a range of respiratory conditions, mean exhaled NO before bronchoscopy was 21.0 ppb. Levels decreased to 14.8 ppb one hour after bronchoscopy, reached a nadir at two hours (14.4 ppb,  $p < 0.05$ ), and returned to baseline levels 24 hours after bronchoscopy (22.8 ppb).

## 6.5. Bronchoalveolar Lavage

A study by Lim *et al.* confirms a correlation between exhaled NO and inflammatory markers in BAL fluid. [Lim S *et al.* 2000] Both exhaled NO and the percentage of BAL eosinophils were reduced by budesonide treatment, although only the decrease in NO was statistically significant. However, the reductions in exhaled NO and percentage of BAL eosinophils showed a statistically significant correlation ( $p < 0.05$ ). In a study by Warke *et al.* including 71 children with atopic asthma, atopic non-asthmatics and non-atopic normal controls, there was a significant correlation between the percentage of eosinophils in BAL fluid and exhaled NO. [Warke TJ *et al.* 2002] A BAL eosinophilic value of 0.86% represents the 95% confidence interval (CI) for the 95th percentile in normal children. Using this as a cut-off value for airway inflammation, exhaled NO levels greater than 17 ppb (at 50 mL/s) predict airway inflammation with a sensitivity of 81% and a specificity of 80% (Figure VI.7). The authors concluded that exhaled NO measurement is a useful method of indirectly assessing

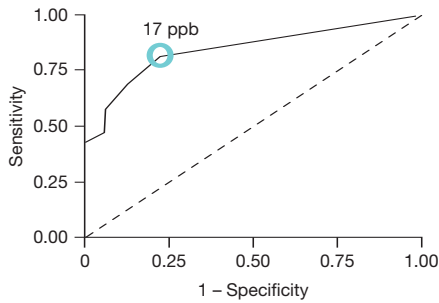


Figure VI.7. Receiver-operated characteristic (ROC) curve for the presence of airway inflammation; exhaled NO > 17 ppb predicts inflammation with a sensitivity of 81% [Warke TJ et al. 2002]

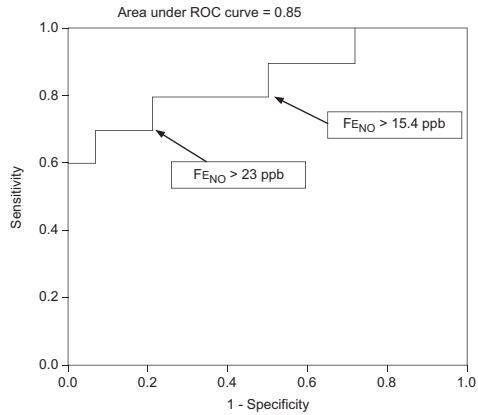


Figure VI.8. ROC curve for  $FE_{NO}$  for predicting BAL eosinophilia in children with asthma [Lex C et al. 2006]

eosinophilic airway inflammation in asthmatic children. Similarly, in a study of 27 children with moderate-to-severe asthma by Lex *et al.*, there was a significant correlation between exhaled NO and BAL eosinophils ( $r = 0.54$ ,  $p = 0.006$ ) (Figure VI.8). [Lex C *et al.* 2006] All patients who had both high levels of induced sputum eosinophils and elevated exhaled NO (> 23 ppb) had elevated eosinophils in BAL (positive predictive value of 100% and negative predictive value of 65%).

## 6.6. IgE

Exhaled NO levels and eosinophil counts have shown a positive correlation with IgEs specific to house dust mites (Figure VI.9) [Sacco O *et al.* 2003] Atopic children with mild intermittent asthma, sensitized to house dust mite species, *Dermatophagoides pteronyssinus* (Dp) or *D. farinae* (Df), showed positive correlations between serum levels of total, Dp-specific or Df-specific IgE with eosinophil counts and exhaled NO levels.

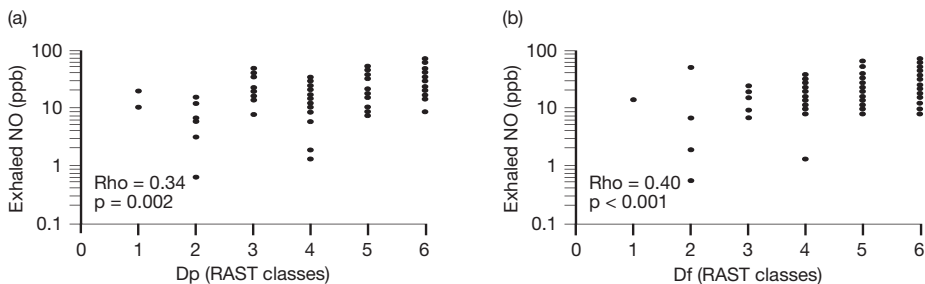


Figure VI.9. There are significant correlations between exhaled NO levels and (a) house dust mite Dp-specific IgE levels; (b) Df-specific IgE levels ( $p < 0.01$ ). *Dermatophagoides pteronyssinus* = Dp, *D. farinae* = Df [Sacco O et al. 2003]

## SCIENTIFIC BACKGROUNDER

The Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid (CLIC) trial showed that exhaled NO significantly correlated with peripheral blood eosinophils, IgE and plasma ECP but not urinary leukotriene E4 (uLTE4) in 144 children with mild-to-moderate asthma who were between 6 and 17 years of age (Figure VI.10). [Strunk RC *et al.* 2003]

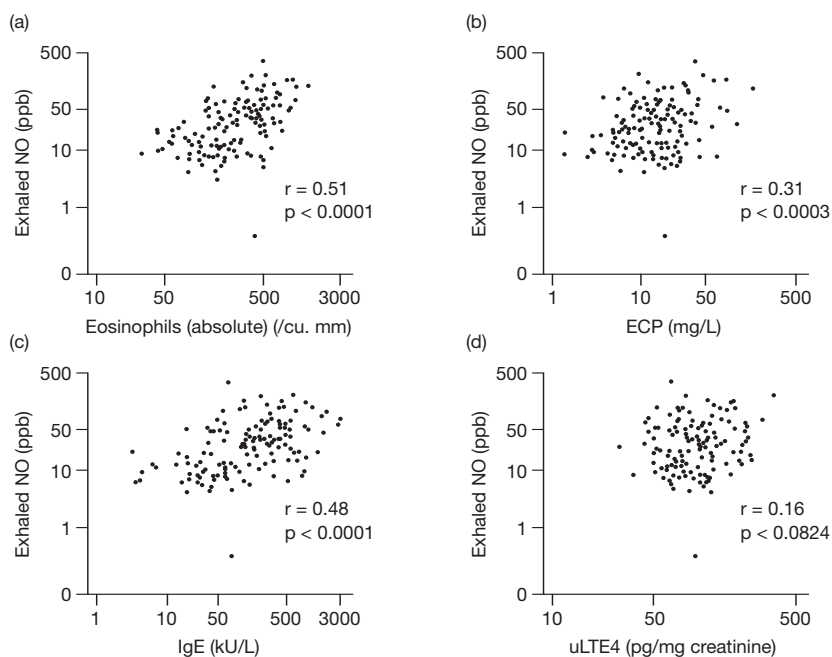


Figure VI.10. Scattergrams showing that exhaled NO significantly correlates with (a) peripheral blood eosinophils; (b) plasma eosinophilic cationic protein (ECP); and (c) IgE; but not with (d) urinary leukotriene E4 (uLTE4) measurement on log scale [Strunk RC *et al.* 2003]

## 6.7. Breath Condensate

Numerous chemical entities in condensed breath can be analysed and some of these may be related to inflammation. However, studies assessing such parameters and comparing them with exhaled NO levels have produced mixed results. Baraldi *et al.* reported that exhaled breath condensate levels of 3-nitrotyrosine were over five times higher in children with asthma than in controls. [Baraldi E *et al.* 2006] As expected, NO levels were also higher in the asthmatic group (44.6 ppb vs. 7.5 ppb, at 50 mL/s). However, no correlation was seen between NO levels and the condensate measurement. The same group assessed cysteinyl leukotrienes concentrations in breath condensate of asthmatic children with and without EIB and found that this parameter correlated with the post-exercise decrease in FEV<sub>1</sub>. [Carraro S *et al.* 2005] Exhaled NO also correlated with the change in FEV<sub>1</sub>. In another study from the same group, concentrations of cysteinyl leukotrienes and 8-isoprostane were increased in patients with asthma compared with controls. [Zanconato S *et al.* 2004] Only the leukotriene parameter distinguished severity, in patients with unstable asthma. They had higher levels than those who were steroid-naïve or those treated with steroids for mild-to-moderate asthma. Exhaled NO levels were also higher in patients with unstable asthma, but in addition NO levels were higher in the patients not receiving steroids compared with those who had treated mild-to-moderate disease. Breath condensate pH has also been studied and shown to correlate inversely with disease severity, but not with NO levels. [Leung TF *et al.* 2006] However, others have found that breath condensate pH is not different between patients with asthma and controls. [Ojoo JC *et al.* 2005] Jackson *et al.* observed no correlation between exhaled NO and total nitrogen oxide levels in breath condensate. [Jackson AS *et al.* 2007] This study also found no correlation between breath condensate and BAL for any of the biomarkers assessed.

One reason for the apparent poor correlation between exhaled NO and markers in breath condensate may be the poor reproducibility of breath condensate measurements. For example, Chladkova and co-workers reported that the % coefficient of variation of nitrite and nitrate measurements in breath condensate of asthma patients were 21% and 88%, respectively. [Chladkova J *et al.* 2006] In another paper, Franklin *et al.* reported that the coefficient of repeatability for nitric oxide metabolites in breath condensate was 103.4% for interday variability and 118.6% for intraday variability. [Franklin P *et al.* 2006] Likewise, Chladkova *et al.* demonstrated that breath condensate levels of nitrite and nitrate did not differ between patients with asthma and healthy controls, and that the short-term reproducibility of nitrite measurements in breath condensate was not as good as that reported for exhaled NO, likely due to unidentified factors influencing the breath condensate sampling technique. [Chladkova J *et al.* 2006] Notably, dietary intake has been shown to affect nitrite levels in breath condensate. [Martens H *et al.* 2005] Another possible reason for the lack of correlation between NO and breath condensate measurements may

be that breath condensate parameters reflect different aspects of disease other than inflammation. Mondino *et al.* have shown that, whereas exhaled NO levels in patients with asthma respond to inhaled steroids, various breath condensate parameters (leukotriene B<sub>4</sub>, 8-isoprostane and prostaglandin E<sub>2</sub>) do not. [Mondino C *et al.* 2004] Therefore, these parameters may reflect other elements of airway disease.

## 6.8. Neutrophils

Prominent neutrophilia has been documented in certain asthma cases. [Basha MA *et al.* 1994; Cox G 1998; Djukanovic R 1994] Results suggest the presence of a distinct subgroup of patients with the clinical signs of asthma but who have predominantly neutrophilic airway inflammation; their airway secretions do not contain eosinophils. They also do not respond to steroid therapy, unlike patients with typical eosinophilic airway inflammation. [Cox G 1998; Green RH *et al.* 2002b; Keatings VM *et al.* 1997] There is increasing evidence that neutrophils may play a role in acute severe asthma. High levels of neutrophils have been demonstrated in fatal asthma of sudden onset. [Sur S *et al.* 1993] Neutrophil numbers and activation are also increased during exacerbations of asthma. [Fahy JV *et al.* 1995] Jatakanon and colleagues showed that neutrophils were increased in patients with severe asthma compared with healthy volunteers ( $p < 0.05$ ) and patients with mild asthma ( $p < 0.05$ ). [Jatakanon A *et al.* 1999] Low levels of NO are observed in neutrophilic asthma.

Ramesh and colleagues showed that production of nitrite and L-citrulline by neutrophils increased significantly as the severity of asthma increased from mild to severe ( $p < 0.001$ ). [Ramesh G *et al.* 2001] Among all asthmatics, there was an inverse relationship between peak expiratory flow and nitrite and L-citrulline, while NO production by neutrophils was increased in bronchial asthma, suggesting an association between NO production and progressive airway narrowing.

## 6.9. Phenotype

Findings from BAL and airway biopsy studies have shown that asthma appears to have different phenotypes with different types of airway inflammation. Exhaled NO may offer a non-invasive method for classifying these different phenotypes. For example, asthma can be divided pathologically into two inflammatory types based on the presence or absence of bronchial eosinophils, which appear to be associated with different structural changes in the airways. As a marker of inflammation, variations in exhaled NO are increasingly recognized as an important component of the asthma phenotype.

In subjects with severe refractory asthma, Silkoff *et al.* demonstrated that exhaled NO could differentiate between those with eosinophilic and non-eosinophilic inflammation. [Silkoff PE *et al.* 2005] Subjects with eosinophilia (as identified by BAL) had significantly higher

exhaled NO levels compared with those without eosinophilic infiltration ( $p=0.0084$ ). Exhaled NO levels were correlated with levels of tissue eosinophils ( $p = 0.007$ ), lymphocytes ( $p = 0.003$ ), and mast cells ( $p = 0.05$ ). Exhaled NO levels  $> 72.9$  ppb (at a flow rate of 50 mL/s) were associated with a sensitivity of 0.56 and specificity of 1.0 for identification of eosinophil status. Using exhaled NO as a marker for asthma phenotype can be problematical, however, in patients with more severe asthma being treated with high doses of ICS. In a study by Lemiere *et al.* involving 32 patients with severe asthma, eosinophilic and non-eosinophilic phenotypes were identified using both bronchial biopsy and induced sputum cell counts. [Lemiere C *et al.* 2006] Exhaled NO was increased in the eosinophilic phenotype identified from bronchial biopsy, but not on the basis of induced sputum. Subjects with high sputum eosinophil counts had more asthma exacerbations compared with those with lower sputum eosinophil counts. In contrast, there were no differences in clinical characteristics between subjects with eosinophilic and non-eosinophilic phenotypes according to bronchial biopsies or exhaled NO.

As well as local airway inflammation, CD4+ T cells are involved in the systemic pathogenesis of asthma. CD4+ Th2 lymphocytes are responsible for allergen-induced airway inflammation via production of interleukins, while Th1-derived interferon is mainly released on stimulation of the immune system. The relative contribution of Th2 and Th1 cells is controlled by T-regulatory cells, which cells mediate peripheral tolerance towards allergens. Karagiannidis *et al.* investigated the influence of high-altitude climate therapy on airway inflammation in patients with moderate and severe asthma. [Karagiannidis C *et al.* 2006] The frequency of CRTh2-expressing T cells decreased, while regulatory T cells remained stable. Overall, inflammatory cells switched towards a tolerogenic phenotype under high-altitude climate therapy. Exhaled NO significantly decreased within 3 weeks of therapy in patients with allergic and intrinsic, moderate and severe asthma.

Asthma can also be categorized into atopic and non-atopic phenotypes. Franklin *et al.* demonstrated that in adult subjects, elevated exhaled NO measurements were associated with a phenotype characterized by atopy and increased airway responsiveness regardless of the presence of asthma or asthma-like symptoms. [Franklin PJ *et al.* 2004a]

$\alpha_1$ -anti-trypsin (ATT) deficiency is an autosomal recessive disorder caused by mutations of the protease inhibitor system, and is associated with a range of lung disease severities. In ATT deficiency, chronic obstructive pulmonary disease is associated with the PiZ homozygous phenotype (PiZZ), while airway hyperresponsiveness tends to be associated with the PiM heterozygous phenotype (PiMS/MZ). In a study of 40 patients with ATT deficiency, exhaled NO was significantly lower in those with the PiZZ phenotype (4.5 ppb) than in matched subjects with airway obstruction but no ATT deficiency (8.2 ppb), healthy controls (9.3 ppb, all at 50 mL/s), or patients with other phenotypes. [Malerba M *et al.* 2001] Exhaled NO may be useful as an early marker of lung involvement in ATT deficiency.

## SCIENTIFIC BACKGROUNDER

Twin and family studies have shown genetic and environmental influences on the development of asthma. Lund *et al.* performed a population-based study to estimate the relative contribution of genes, family environment, and non-shared environmental influences to variation in exhaled NO and airway responsiveness in 377 adult twins. [Lund MB *et al.* 2006] Genetic effects accounted for 60% of the variation in exhaled NO, while family environment explained 30% of the variation in airway responsiveness, and non-shared environment explained the remaining variation for both measures. The association between exhaled NO and responsiveness ( $r = 0.14$ ,  $p = 0.006$ ) was primarily explained by common genetic factors.

Welschler *et al.* found an association between exhaled NO and variations in the neuronal NO synthase gene. [Wechsler ME *et al.* 2000] In patients with asthma, both mean exhaled NO ( $p = 0.00008$ ) and variability around the mean ( $p = 0.000002$ ) were significantly lower in individuals with a high number ( $> 12$ ) of AAT repeats at intron 20 than in those with fewer repeats. In contrast, Leung *et al.* found that single-nucleotide polymorphisms in neuronal and endothelial NO synthase enzymes were not associated with asthma or exhaled NO levels in Chinese children. [Leung TF *et al.* 2005] These findings may be explained by the fact that variability in exhaled NO appears to be determined by expression of inducible epithelial NO synthase. Lane *et al.* demonstrated that expression of this isoform of NO synthase was associated with exhaled NO levels in children ( $p < 0.001$ ). [Lane C *et al.* 2004] This relationship was stronger in asthmatic children ( $r = 0.828$ ,  $p = 0.006$ ) than in asymptomatic atopic ( $r = 0.752$ ,  $p = 0.02$ ) or healthy children ( $r = 0.525$ ,  $p = 0.008$ ). In this study, there was no significant correlation between exhaled NO and neuronal or endothelial NO synthase expression. Further studies are clearly required to elucidate the associations between exhaled NO and the genetic basis of asthma phenotypes.

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