IV. Exhaled NO in Clinical Situations

4.1. Diagnosis of Asthma

Summary
Exhaled NO measurement has been shown to be superior to the majority of conventional tests recommended in international clinical guidelines for the diagnosis of asthma in symptomatic patients. Correct asthma diagnosis can be ascertained in around 80% of patients, using a cut-off of around 25 ppb in adults and 20 ppb in children (depending on age).

Measurement of exhaled NO provides a useful method of differentiating asthma from other conditions. The diagnostic value of NO measurements and the ability of the method to differentiate between subjects with airway symptoms and patients with true asthma were analysed by Dupont et al. [Dupont LJ et al. 2003] The sensitivity and specificity of the method is dependent on the selection of an appropriate cut-off point. The study involved 240 consecutive, non-smoking, steroid-naïve patients and found that a cut-off point for exhaled NO of 16 ppb (at a flow rate of 200 mL/s), gave a specificity for the diagnosis of asthma of 90% and a positive predictive value of > 90%. [Dupont LJ et al. 2003] With a cut-off of 20 ppb (flow rate 200 mL/s), a specificity of 100% was seen. In a study by Heffler et al. [Heffler E et al. 2006] involving 48 patients with asthma-like symptoms, in which asthma was diagnosed on the basis of 12% improvement in FEV\(_1\) after salbutamol or a methacholine PD\(_{20}\)FEV\(_1\) < 800 µg, none of the asthmatic patients had exhaled NO values < 25 ppb (at 50 mL/s) and all the patients with exhaled NO > 100 ppb (n = 5) were diagnosed with asthma. The sensitivity and specificity of exhaled NO for detecting asthma, using 36 ppb as cut-off point, were 78% and 60% and the positive and negative predictive values were 54% and 82%, respectively.

Henriksen and co-workers investigated the use of exhaled NO levels alone (using a cut-off value of 8 ppb at 250 mL/s) or in combination with airway hyperresponsiveness tests to diagnose asthma in a large population survey (n = 8571). [Henriksen AH et al. 2000] The study showed that 52% of those diagnosed with asthma had NO levels at or above the cut-off value, whereas 80% of those who were thought to be healthy had NO levels below 8 ppb. The authors suggested that combining this NO cut-off level with airway hyperresponsiveness to methacholine (< 2 mg causing a 20% fall in FEV\(_1\) as cut-off value) would allow the diagnosis of asthma with a high specificity. Zietkowski et al. demonstrated that in allergic and non-allergic asthmatics, exhaled NO was significantly correlated with other diagnostic measures such as bronchial hyperresponsiveness to histamine, reversibility of airway obstruction, serum eosinophil cationic protein levels, and blood eosinophilia (but did not correlate with FEV\(_1\)). [Zietkowski Z et al. 2006a] In 31% of non-allergic and 9% of allergic patients, exhaled NO was < 20 ppb (at 50 mL/s).
Chatkin et al. investigated the value of NO in the assessment of chronic cough. [Chatkin JM et al. 1999] Using 30 ppb (at a flow of 45 mL/s) as the cut-off point for exhaled NO gave a sensitivity and specificity of 75% and 87%, respectively, for a diagnosis of asthma. In a non-smoking adult population of asthmatics and healthy controls, an exhaled NO measurement of 30 ppb at a flow rate of 42 mL/s was both sensitive and specific for a diagnosis of asthma. [Deykin A et al. 2002] The positive predictive value was 72%, and the negative predictive value was 71%. If used as a screening test in the general population, the authors calculated that exhaled NO measurements of less than 30 ppb would have a negative predictive value of 98% (Figure IV.1).

In a study of 71 children undergoing elective surgery, Warke and colleagues found that exhaled NO measurements greater than 17 ppb (at 50 mL/s) were both a highly sensitive (81%) and highly specific (80%) means of predicting airway inflammation. [Warke TJ et al. 2002] (See Section 6.5. Bronchoalveolar Lavage.)

Another study by Malmberg et al. [Malmberg LP et al. 2003] showed that airway inflammation was present at the early stages of asthma, even in pre-school children (3.8–7.5 years old). Exhaled NO was superior to baseline lung function measures or indices of bronchodilator responsiveness, as assessed by the oscillometric technique for identifying pre-school children with predominantly atopic probable asthma (Figure IV.2). The optimum cut-off value for exhaled NO was 9.7 ppb (flow rate 50 mL/s) giving a sensitivity of 86% and specificity of 92%. Also, exhaled NO had a high negative predictive value of 95%.
Asthma may be defined either as wheeze within the previous 12 months (current wheeze), doctor-diagnosed asthma, or current wheeze plus confirmed airway hyperresponsiveness. Gender may also affect how asthma is diagnosed. Henriksen et al. found that there is a risk of underestimating the prevalence of asthma, especially amongst girls, when asthma is defined as doctor-diagnosed asthma. [Henriksen AH et al. 2003] A comparison of exhaled NO levels and sputum cell counts with a range of clinical tests normally recommended by international guidelines to confirm the diagnosis of asthma was carried out by Smith and colleagues. In this study, asthma was defined by a positive response to a bronchodilating agent or a positive hyperresponsiveness test, in accordance with ERS/ATS guidelines. Sensitivities for each of the conventional tests (peak flow measurements and spirometry) following a steroid trial were lower (0–47%) than for exhaled NO (88%) and sputum eosinophils (86%). [Smith AD et al. 2004] Both exhaled NO and sputum eosinophils provided significantly higher degrees of diagnostic accuracy than tests based on lung function (Figure IV.3). For exhaled NO, the optimum cut-off point for diagnosing asthma, based on calculating the predictive accuracy for a range of different exhaled NO levels, was 20 ppb (at a flow rate of 50 mL/s). Interestingly the combination of a raised exhaled NO level (> 33 ppb) and abnormal spirometry (FEV\textsubscript{1} < 80% predicted) provides even greater sensitivity (94%) and specificity (93%) for the diagnosis of asthma. [Taylor DR et al. 2006] Although there is strong evidence of the value of exhaled NO in the diagnosis of asthma, the fact that atopy in the absence of asthma is also associated with increased NO levels has led some authors to question the value of exhaled NO as a screening tool. [Prasad A et al. 2006]

4.2. Alternatives to Airway Hyperresponsiveness Test

**Summary**

Exhaled NO is a simple, time-efficient and resource-efficient screening tool for airway hyperresponsiveness and exercise-induced bronchoconstriction. Patients will not demonstrate bronchial hyperresponsiveness using a pre-exercise cut-off of < 12 ppb. In this respect, exhaled NO may replace bronchial provocation tests and outperforms FEV\textsubscript{1} as a predictor for airway hyperresponsiveness.
Airway hyperresponsiveness is the ‘gold standard’ test of airway inflammation and is the hallmark of asthma. However, the test is time consuming and invasive, and impractical for screening. Exhaled NO offers a more practical alternative. Several studies have investigated the link between exercise-induced bronchoconstriction and NO in patients with asthma. [Berkman N et al. 2005; Buchvald F et al. 2005b; Kanazawa H et al. 2000; Kotaru C et al. 2001] In one of these studies, patients who experienced exercise-induced bronchoconstriction had higher concentrations of NO derivatives in their sputum than other asthma patients. [Kanazawa H et al. 2000] Moreover, excess NO production appeared to contribute to the prolonged airway narrowing stimulated by exercise in susceptible patients. These findings are supported in another study, in which inhibition of NO synthesis reduced airway constriction following challenge. [Kotaru C et al. 2001] The authors concluded that NO plays an important role in the pathogenesis of exercise-induced asthma.

De Meer and co-workers assessed the ability of exhaled NO and prechallenge FEV\textsubscript{1} measurements to predict hyperresponsiveness to hypertonic saline in children aged 8–13 years. [de Meer G et al. 2005] Using a cut-off of 43 ppb (sample air collected at 8.3 mL/s), exhaled NO had a positive predictive value of 83% and a negative predictive value of 90%. These values compared favourably with prechallenge FEV\textsubscript{1} (33% and 80%, respectively). Additionally, Lex showed that all children (n = 85) with normal exhaled NO values (< 25 ppb) had normal lung function results after exercise; hence the NPV of elevated exhaled NO for prediction of exercise-induced bronchoconstriction (EIB) was 100%. However, the PPV was only 28%. Considering recent symptom history in addition to elevated exhaled NO improved the PPV to 40%, and resulted in the best combination of sensitivity and specificity. Baseline lung function parameters do not predict EIB. Instead, as proposed by Lex et al., exhaled NO measurements together with a symptom questionnaire can be used as time-saving surrogate tests to exclude EIB in atopic childhood asthma. [Lex C et al. 2007]

EIB is widely used to assess asthma, particularly asthma control. Again, it is an expensive and time-consuming test that is perceived by many patients to be unpleasant. El-Halawani and colleagues examined whether exhaled NO levels before or after exercise (flow rate 50 mL/s) could be used as a surrogate marker of exercise-induced bronchoconstriction in a population referred specifically for evaluation. [El Halawani SM et al. 2003] They showed that patients with very low pre-exercise exhaled NO levels (< 12 ppb) did not demonstrate bronchial hyperresponsiveness to exercise. Hence, exhaled NO measurements may make bronchoprovocation testing unnecessary in patients who complain of exertional dyspnoea (Figure IV.4). Garcia-Rio et al. found that in patients with exercise-induced bronchoconstriction, exhaled NO was reduced at 5, 10 and 15 minutes after exercise and these changes in NO correlated with post-exercise reductions in FEV\textsubscript{1}. [Garcia-Rio F et al. 2006] There was also a significant correlation between baseline NO and the maximal post-exercise decrease in FEV\textsubscript{1}. A correlation between NO levels and exercise-induced bronchoconstriction has also been shown by Carraro et al. [Carraro S et al. 2005]
An exhaled NO < 20 ppb (50 mL/s) in children with asthma not receiving corticosteroids predicts a negative exercise-induced bronchoconstriction test with 90% accuracy. [Buchvald F et al. 2005b] In children who are receiving steroids for their asthma, a cut-off of < 12 ppb provided similar accuracy.

The studies reported above are in agreement with Berkman and colleagues who compared exhaled NO measurements with exercise-induced bronchoconstriction and bronchial provocation tests for the diagnosis of asthma. [Berkman N et al. 2005] The diagnosis of asthma was based upon clinical evidence up to 24 months after testing. Exhaled NO was found to be as good a predictor of asthma as bronchial provocation (Figure IV.5) tests and better than exercise-induced bronchoconstriction.

Figure IV.5. ROC curves for exhaled NO (a), fall in FEV\textsubscript{1} after exercise (b), PC\textsubscript{20} for methacholine (c) and PC\textsubscript{20} for adenosine-5-monophosphate (d) [Berkman N et al. 2005]
4.3. Prediction of Steroid Response

Summary
A high level of exhaled NO (> 47 ppb in adults) serves as a positive indicator that the patient will respond to inhaled corticosteroids. Exhaled NO assessment is important when considering the potential usefulness of inhaled anti-inflammatory therapy.

Exhaled NO levels can be used to identify patients who are likely to respond to anti-inflammatory treatment with corticosteroids. In a paediatric study by Szefler and colleagues, the response to anti-inflammatory treatment was found to be correlated with the exhaled NO level before treatment. A good FEV₁ response (> 15% increase) was associated with high exhaled NO (17.4 ppb), while a poor response (> 5% increase) was associated with lower exhaled NO levels (11.1 ppb). [Szefler SJ et al. 2002] In another study by Szefler et al., children with high NO levels, high levels of other inflammatory markers, and low pulmonary function at baseline were more likely to respond to fluticasone than other children with asthma. [Szefler SJ et al. 2005]

In an adult study, an exhaled NO value > 47 ppb (at 50 mL/s) was a good predictor of steroid response (Figure IV.6). [Smith AD et al. 2005a] Interestingly, the ability of NO to predict responsiveness to steroids was independent of the diagnosis underlying the persistent respiratory symptoms in the participants. Similarly, Zeiger et al. reported that a high NO level at baseline predicts a good response to fluticasone, particularly in terms of the number of asthma control days. [Zeiger RS et al. 2006] In addition, Szefler and co-workers have shown that high baseline NO levels are a predictor of response to inhaled corticosteroid treatment. [Szefler SJ et al. 2005]

On the basis of such evidence, the authors conclude that NO levels can be used to indicate the patient's need for ICS treatment.

Figure IV.6. An exhaled NO level > 47 ppb is significantly better than FEV₁ bronchodilator response in predicting response to inhaled steroids [Smith AD et al. 2005a]
4.4. Response to Anti-inflammatory Treatment

Summary
An impressive number of studies show that anti-inflammatory treatment reduces levels of exhaled NO in asthma. The response to corticosteroids is both rapid (within 1 week, potentially as early as 48 hours) and dose-dependent. The higher the exhaled NO value the higher the dose needed to normalize it. Anti-leukotrienes also reduce exhaled NO levels in asthma, albeit to a lesser extent. Exhaled NO measurement is easily performed and provides objective assessment of the effect of anti-inflammatory therapy.

One of the most useful features of exhaled NO is the response to anti-inflammatory treatment, enabling physicians to monitor the effect of treatment objectively. There are now substantial data showing that corticosteroids reduce exhaled NO in asthma. The response of exhaled NO to anti-inflammatory treatment is rapid [Henriksen AH et al. 1999; Wilson AM et al. 2006] and dose-dependent. [Jones SL et al. 2002; Kharitonov SA et al. 2002; Silkoff PE et al. 2001] Silkoff et al. have demonstrated large reductions in exhaled NO levels in response to anti-inflammatory treatment (95% confidence intervals of –40% to –60%). [Silkoff PE et al. 2004a] Thus, exhaled NO measurements have the potential to be used in monitoring the effects of such treatment.

Although treatment with inhaled corticosteroids reduces exhaled NO, it does not totally suppress it and therefore a correlation with the severity of disease is still evident. [Artlich A et al. 1999; Lanz MJ et al. 1999] (See Section 4.6. Monitoring Asthma Control.) Reid et al. showed that exhaled NO levels were within the normal range in patients with persistent asthma treated with inhaled corticosteroids. However, exhaled NO was still significantly related to airway hyperresponsiveness, blood eosinophils and clinical markers of disease severity. [Reid DW et al. 2003]

Exhaled NO measurement is of great value in monitoring asthma and assessing the response to treatment, i.e. mainly in tracking the degree of inflammation and airway reactivity within individual patients. [Sanders SP 1999]
4.4.1. Corticosteroid Treatment

Inhaled corticosteroids have a marked effect on exhaled NO in keeping with their anti-inflammatory properties. The response to corticosteroids is both rapid (within 1 week, potentially as early as 48 hours) and dose-dependent. The higher the exhaled NO value, the higher the ICS dose needed to normalize it. Exhaled NO measurement is easily performed and provides objective assessment of the effect of anti-inflammatory therapy.

The concomitant effect of inhaled steroid on markers of airway inflammation and exhaled NO has been examined in many studies. Lim and co-workers used a 12-week, double-blind, crossover design to compare the effects of inhaled budesonide with placebo. [Lim S et al. 1999] Each patient underwent 4-week periods of treatment with budesonide and placebo, separated by a 4-week washout period. Treatment with budesonide was associated with a significant drop in exhaled NO in parallel with improvements in other markers of inflammation (PC\textsubscript{20} methacholine, sputum eosinophils, and bronchial biopsy appearances). The improved indices of inflammation were associated with improved FEV\textsubscript{1}, indicating reduced airway obstruction and clinical improvement. However, it should be noted that FEV\textsubscript{1} and exhaled NO respond at different rates; i.e. exhaled NO responding more quickly. In line with these findings, van Rensen et al. reported a fall in exhaled NO with a simultaneous reduction in bronchial hyperresponsiveness (PC\textsubscript{20} histamine) and sputum eosinophils during treatment with inhaled steroids. [van Rensen EL et al. 1999] After the collection of baseline data, patients were treated with twice-daily inhaled steroids, or placebo, for 4 weeks, with further measurements taken at weeks 2 and 4. The final set of measurements was taken two weeks after the end of the treatment period. Markers of airway inflammation, including exhaled NO, improved significantly during the treatment period in patients receiving corticosteroids. Two weeks after the cessation of treatment, there was significant deterioration, compared with the values obtained at week 4. The level of exhaled NO therefore reflected the efficacy of anti-inflammatory therapy (Figure IV.7).

![Figure IV.7. Mean levels of exhaled NO in patients at baseline, 2 weeks, 4 weeks, and after washout in patients treated with steroids and placebo [van Rensen EL et al. 1999]](image-url)
Aziz et al. assessed the effect of seven treatment regimens (placebo, formoterol [12 µg and 24 µg], budesonide [400 µg and 800 µg], and a combination of both [12 µg, 400 µg; 24 µg, 800 µg]), on exhaled NO in 15 patients with atopic asthma. [Aziz I et al. 2000] Significant reductions were observed during treatment with regimens containing budesonide when compared with placebo. For example, mean exhaled NO levels after 2 weeks of budesonide 400 µg and 800 µg treatments were 8.3 ppb and 7.4 ppb at a flow of 83 mL/s. The corresponding value after placebo was 15.9 ppb.

A dose-dependent response of exhaled NO levels to corticosteroid treatment has been reported by Kharitonov et al. [Kharitonov SA et al. 2002] In this study, patients with asthma were treated with placebo, budesonide 100 µg or budesonide 400 µg. NO levels in the active treatment arms were decreased after just 3 days, with the largest decrease occurring in the group receiving the higher dose. [Kharitonov SA et al. 2002] Silkoff et al. also demonstrated an inverse relationship between inhaled BDP dose (0–800 µg/day) and the level of exhaled NO (Figure IV.8). [Silkoff PE et al. 2001] Exhaled NO was more effective for identifying doses of BDP than FEV$_1$ or PC$_{20}$. Furthermore, the fall in exhaled NO levels for a specific dose of BDP was highly reproducible. A study by Jones et al. confirms this dose relationship. [Jones SL et al. 2002] Following withdrawal of inhaled BDP, 65 patients went through a double-blind, parallel group, and placebo-controlled trial of 50, 100, 200 or 500 µg BDP/day for 8 weeks. The relationship between the dose of BDP and change in exhaled NO was linear at 1 week and at the end of the study. The authors concluded that exhaled NO might be useful in guiding dose adjustments of inhaled corticosteroids in patients with persistent asthma. Similarly, in a study by Kelly et al., in which 14 steroid-naive patients with asthma received incremental doses of fluticasone at 50, 100, 200 and 400 µg/day each for 7 days, there was a dose-dependent decrease in exhaled NO for each 100 µg of fluticasone (Figure IV.9). [Kelly MM et al. 2006]

Slats et al. investigated the effect of adding a 2-week course of oral prednisone to maintenance ICS treatment in patients with mild-to-moderate persistent asthma. [Slats AM et al. 2006] Levels of exhaled NO were not significantly changed during prednisone treatment, but the change in exhaled NO was significantly greater with prednisone
compared with placebo ($p = 0.03$). There was no correlation between change in NO and improvement in bronchodilation following deep inspiration, suggesting the presence of residual airway inflammation.

A randomized, double-blind, placebo-controlled, crossover study with 4-week washout periods involving 18 adults with asthma was carried out to investigate changes in exhaled NO levels after inhaled and oral anti-inflammatory therapy. [Lanz MJ et al. 2001] A significant difference in mean exhaled NO levels was found ($p < 0.01$) before and after low-dose inhaled fluticasone propionate (FP; 44 µg): $34 \pm 7$ ppb vs. $13 \pm 3$ ppb, respectively. There was also a significant improvement in FEV$_1$% (from $75 \pm 3$ to $85 \pm 3$; $p < 0.05$). No significant reduction was found in exhaled NO levels with low-dose oral zafirlukast (20 mg) for 4 weeks.

The above studies were performed in adult patients, but corticosteroids, both inhaled and systemically administered, are also effective in reducing the amounts of exhaled NO in children with acute and stable asthma (Figure IV.10). [Baraldi E et al. 1997; Baraldi E et al. 2003; Kroesbergen A et al. 1999; Lanz MJ et al. 1999] For example, Carra et al. showed that exhaled NO levels were reduced by over 40% when children with stable asthma were treated with budesonide (400 or 600 mg/day) for 6 weeks. [Carra S et al. 2001] Covar et al. also showed that budesonide therapy was more effective than nedocromil in reducing exhaled NO levels. [Covar RA et al. 2003] Budesonide-treated children had significantly lower median exhaled NO levels and ECP levels than those receiving placebo (21.5 ppb
[95% CI 13.2, 84.4] vs. 62.5 ppb [95% CI 26.2, 115.0], p < 0.01; 17.4 mg/dL [95% CI 10.1, 24.3] vs. 24.0 mg/dL [95% CI 15.4, 33.9], p = 0.5, respectively).

Exhaled NO in children and adults with asthma has also been shown to respond to a new hydrofluoroalkane-beclomethasone dipropionate aerosol. [Horiguchi T et al. 2006; Petersen R et al. 2004] Exhaled NO was used to investigate the anti-inflammatory effect of changing the regimen of ICS from twice to once daily (keeping the same total daily dose) in children with asthma. [Li AM et al. 2006b]

There was significant improvement in exhaled NO (47.1 ppb vs. 39.9 ppb, p = 0.037), and sputum eosinophils (5.7% vs. 2.5%, p = 0.024) after 8 weeks, with no deterioration in asthma symptom control. Sorkness et al. demonstrated that fluticasone monotherapy produced a significantly greater reduction in exhaled NO (59% reduction at 6 weeks) compared with fluticasone/salmeterol combination therapy or montelukast (both p < 0.001) (Figure IV.11). [Sorkness CA et al. 2007] In this study, fluticasone monotherapy was similarly superior to the other two therapies in terms of asthma control. Another study demonstrated that although exhaled NO in children is within the normal range in most asthmatic children on a moderate dose of inhaled corticosteroids (budesonide), exhaled NO exhibited a heterogeneous response to corticosteroids and levels remained high in a subgroup of clinically well controlled children with asthma. [Buchvald F et al. 2003]

In a study involving both adults and children with uncontrolled asthma, 2 weeks of corticosteroid treatment resulted in a mean percentage reduction of 50.5% (Figure IV.12). [Silkoff PE et al. 2004a] Notably, all patients who did not show a reduction in their NO levels of over 20% did not report a reduction in symptoms. Corticosteroids inhibit the induction of iNOS both in vitro and in vivo, and the fall in exhaled NO appears to be a reflection of this. [Berry MA et al. 2005] A reduction in exhaled NO is clearly demonstrable within 1 week of starting treatment. [Kharitonov SA et al. 1996a]
In acute exacerbations of asthma, the effect of steroid treatment can be seen even faster. Massaro et al. demonstrated a fall in exhaled NO 48 h after starting corticosteroid treatment, [Massaro AF et al. 1995] and Baraldi et al. found a mean reduction in the level of exhaled NO of 46% after 5 days of treatment with oral prednisone, [Baraldi E et al. 1997] which was confirmed in a later study by the same group. [Zanconato S et al. 2002] The use of nebulized budesonide in acute asthma has been shown to significantly reduce NO levels just 6 h after treatment. [Tsai YG et al. 2001] Interestingly, the reduction in NO in this study correlated significantly with changes in peak expiratory flow (PEF).

As may be expected, treatment of asthma with the corticosteroid pro-drug, ciclesonide, is associated with a decrease in exhaled NO. [Kanniess F et al. 2001; Zietkowski Z et al. 2006b] Furthermore, four weeks of treatment with ciclesonide resulted in significantly reduced exhaled NO levels (47 ppb difference compared with placebo [95% CI: 15, 81]), along with improvement in bronchial hyperresponsiveness and reduced levels in sputum eosinophils. [Wilson AM et al. 2006] Ciclesonide in an HFA formulation also significantly reduced exhaled NO levels in a study of patients with moderate persistent asthma by Lee et al. [Lee DK et al. 2005]

4.4.2. Combination Treatment

Long-acting $\beta_2$-agonists (LABA) may mask ongoing underlying inflammation, which can be detected by exhaled NO. When combination treatment is used exhaled NO measurements still reflect the ICS responsiveness.

Asthma management guidelines advocate the addition of a long-acting $\beta_2$-agonist to inhaled corticosteroids as an alternative to increasing the dose of the latter. [British Thoracic Society & Scottish Intercollegiate Guidelines Network 2005] It is thought that long-acting $\beta_2$-agonists may exert a facilitatory effect on inhaled corticosteroids permitting a lower corticosteroid dose. In light of this, studies have investigated the effects of combination therapy on exhaled NO levels. [Buchvald F and Bisgaard H 2003; Currie GP et al. 2003a; Lee DK et al. 2003] Currie et al. evaluated the anti-inflammatory activity of fluticasone plus salmeterol in combination versus a double-dose of fluticasone. [Currie GP et al. 2003a] Fifteen people who had mild-to-moderate asthma that was uncontrolled on inhaled corticosteroids were randomized in a single-blind, 2-period crossover study. In each 2-week study period, patients received fluticasone 250 µg plus salmeterol 50 µg in combination or fluticasone 500 µg. Both fluticasone double-dose and fluticasone plus salmeterol conferred a significant ($p < 0.05$) fall in exhaled NO from baseline. However, between treatments, the reduction in exhaled NO was significantly ($p < 0.05$) greater with fluticasone treatment alone (Figure IV.13). The effect of fluticasone plus salmeterol on exhaled NO has been confirmed in a 3-month study, showing a significant reduction in exhaled NO compared with placebo ($p < 0.001$). [van den Toorn LM et al. 2005]
Buchvald and Bisgaard compared exhaled NO levels after salmeterol or montelukast add-on therapy in 22 asthmatic children receiving regular maintenance treatment with budesonide 400 mg daily. [Buchvald F and Bisgaard H 2003] Exhaled NO levels were significantly higher after salmeterol add-on treatment compared with both placebo (p = 0.003) and montelukast (p = 0.002) add-on treatment. Furthermore, salmeterol also improved lung function (FEV$_1$) significantly compared with placebo and non-significantly compared with montelukast.

4.4.3. Anti-leukotriene Treatment

Anti-leukotrienes also reduce exhaled NO levels in asthma, albeit to a lesser extent than inhaled corticosteroids.

Cysteinyl leukotrienes are produced and released by inflammatory cells in the airways of asthmatic patients and are important mediators of asthma. Leukotriene pathway modifiers have been shown to improve asthma control and they have a more prolonged action than corticosteroids. [Bratton DL et al. 1999] In a study of children with mild-to-moderate stable chronic asthma, not requiring maintenance steroids, Bratton et al. demonstrated that the leukotriene receptor antagonist montelukast sodium reduced exhaled NO by approximately 33%. [Bratton DL et al. 1999] The reduction in exhaled NO was not accompanied by any significant change in tests of pulmonary function, suggesting that exhaled NO is a more sensitive measure of inflammation than tests of lung function. These findings are supported by the results of a double-blind crossover study by Bisgaard et al. [Bisgaard H et al. 1999] who compared a 2-week treatment with montelukast, 5 mg daily, with placebo. Montelukast sodium treatment was associated with a significant (20%) reduction in exhaled NO (Figure IV.14), and most of this effect (15% reduction) was evident within 2 days. Although there was a tendency towards improved lung function with montelukast treatment, this did not reach statistical significance. In another study, 1 week of montelukast treatment resulted in a significant decrease in exhaled NO (baseline vs. Week 1: 33.3 ± 15.5 ppb vs. 14.8 ± 8.6 ppb, p < 0.05), with levels rebounding 2 weeks after treatment was stopped. [Lee MY et al. 2005] Other groups have reported similar responses of exhaled NO to montelukast treatment. [Hung CH et al. 2005; Montuschi P et al. 2006; Straub DA et al. 2005]
One study has described more precisely the time course of changes in exhaled NO with montelukast therapy. Montelukast (10 mg daily), administered in a randomized, double-blind crossover design over 2 weeks, significantly reduced the levels of exhaled NO from the first day of treatment with the maximal effect occurring on the seventh day (median change, 22%; Figure IV.15). [Sandrini A et al. 2003a] Furthermore, the levels of exhaled NO remained lower in comparison to baseline during the washout period.

Ghiro and colleagues studied the effect of adding montelukast to inhaled corticosteroid treatment in children. After 3 weeks there was a significant reduction in exhaled NO values in the group treated with both montelukast and inhaled steroids compared with the group remaining on inhaled corticosteroids only. After withdrawal of the montelukast therapy the NO values rose to baseline levels again. [Ghiro L et al. 2002] This suggests an anti-inflammatory effect of montelukast, additive to that of inhaled corticosteroids. Lee et al. also reported a lowering of NO levels when montelukast was added to an inhaled corticosteroid treatment, in this case after only 1 week of treatment. [Lee DK et al. 2004]

Further support comes from Lipworth and colleagues, who examined the effects of adding the leukotriene antagonist, zafirlukast, or a β2-agonist, to corticosteroid treatment in 24 asthmatic patients. [Lipworth BJ et al. 2000] In this crossover study, addition of zafirlukast for 1 week resulted in a significant decrease in exhaled NO. In contrast, no significant decrease was seen after the addition of the β2-agonist to corticosteroid treatment.

Whelan et al. found montelukast had no significant effect on exhaled NO levels. [Whelan GJ et al. 2003] Concurrent results were reported from a cross-over study by Barnes et al.
Barnes ML et al. 2007] Subjects with asthma and perennial allergic rhinitis were
randomized to montelukast plus cetirizine or extra-fine inhaled beclomethasone plus
intranasal beclomethasone, each taken once daily for 2 months. Exhaled NO and nasal NO
levels were reduced only in the combined topical corticosteroid group, while both
treatments reduced eosinophils and eosinophilic cationic protein. However, when patients
were stratified according to the genotype of the leukotriene C4 (LTC4) synthase A-444C
polymorphism, montelukast significantly reduced the slope of the percentage change in
exhaled NO levels compared with time curve in heterozygotes, suggesting that this
subgroup responded better to montelukast therapy with respect to exhaled NO levels.
Others have also reported no effect of montelukast on exhaled NO. [Barnes ML et al. 2007;
Biernacki WA et al. 2005; Peroni D et al. 2005; Sorkness CA et al. 2007; Spahn JD et al.
2006] Furthermore, in a study by Boushey et al., daily budesonide treatment resulted in a
reduction in exhaled NO levels over 1 year, but daily zafirlukast was associated with an
increase in exhaled NO. [Boushey HA et al. 2005] The lack of an effect of antileukotrienes
on NO levels may be due to differences in the pathology behind increased NO levels and
increased leukotrienes. It has been shown that exhaled leukotriene levels do not respond to
corticosteroids to the extent that NO levels do, possibly indicating weak pathophysiological
relationships. [Mondino C et al. 2004]

Leukotriene antagonist therapy in patients with asthma may also improve exercise
tolerance. Montelukast was shown to reduce exhaled NO levels in response to exercise in
people with mild-to-moderate asthma, but had little effect on bronchial hyperresponsiveness
to methacholine and adenosine challenges. [Berkman N et al. 2003]

4.4.4. Other Anti-inflammatory Treatment

Effective anti-inflammatory treatment reduces clinical and spirometric indicators of asthma
severity, and is thereby in line with reduction of exhaled NO.

In a study of asthmatic patients treated with the soluble interleukin-4 receptor (IL-4R)
improved indices of lung function were accompanied by reductions in exhaled NO. [Borish
LC et al. 1999] Asthma symptoms deteriorated in patients taking placebo or low-dose IL-4R
and they also had increased exhaled NO after steroid withdrawal. However, patients taking
high-dose IL-4R had significantly fewer asthma symptoms and lower exhaled NO
concentrations. Objective tests of lung function, including FEV1 and forced expiratory flow
25–75% (FEF25–75), showed less deterioration with high-dose treatment, and patients in this
group also needed less rescue medication with inhaled β2-agonists. Effective anti-
inflammatory treatment thus reduced clinical and spirometric indicators of asthma severity,
and reduced exhaled NO mirrored this.
4.4.5. IgE Treatment

Omalizumab is a monoclonal antibody to IgE that is used in the treatment of moderate-to-severe atopic asthma. IgE treatment may inhibit airway inflammation measured as a decrease in exhaled NO levels during steroid reduction; however, more clinical studies are required.

One preliminary study suggests that omalizumab reduces exhaled NO levels to a similar extent to inhaled corticosteroids, indicating an anti-inflammatory effect for this agent. [Silkoff PE et al. 2004b]

4.4.6. Immunotherapy Treatment

Immunotherapy produces significant immunomodulatory changes, demonstrated by the rise in exhaled NO.

Dinakar and colleagues investigated changes in exhaled NO during the build-up phase of traditional (slow build up) and rush (rapid build up) immunotherapy regimens. [Dinakar C et al. 2006] In the ‘rush’ group, baseline exhaled NO 12.6 ppb, and this rapidly rose to 17.7 ppb at week 2. The elevated NO levels persisted until week 8, and then dramatically dropped below baseline levels to 8.9 ppb at week 12 (p = 0.038). These changes in exhaled NO were not seen in the traditional immunotherapy group, with difference in exhaled NO levels between the two groups most marked at 4 weeks (p = 0.014). The authors concluded that initiation of immunotherapy produces significant immunomodulatory changes evidenced by the rise in exhaled NO, and that these changes are accelerated by rapid build up of immunotherapy.

In children with seasonal allergic rhinitis, Roberts et al found no difference in exhaled NO between patients treated for two seasons with grass-pollen specific immunotherapy and those receiving placebo. [Roberts G et al. 2006] This may be explained by the finding that patients treated with immunotherapy showed a trend toward less corticosteroid use, suggesting a steroid-sparing effect of immunotherapy.

4.5. Dose Optimization of Steroid Treatment

Summary
A number of studies indicate that monitoring inflammation and titrating the dose of anti-inflammatory medication accordingly has real clinical benefits. As the measurement of exhaled NO is simpler and less time-consuming than measurement of sputum eosinophils, it is better suited for routine clinical practice and allows more frequent assessment of inflammation in the airways.
Evidence is accumulating that exhaled NO monitoring can improve the management of asthma. A paper in the New England Journal of Medicine showed that by using exhaled NO measurements to help guide treatment rather than conventional guidelines, the dose of inhaled corticosteroids can be reduced by more than 40% (Figure IV.16). [Smith AD et al. 2005b] Moreover, despite this reduction in dose, exacerbations were less frequent in the exhaled NO group (by 45%), although this was not statistically significant. These results suggest that the routine use of exhaled NO monitoring could optimize corticosteroid treatment.

An interesting study published in The Lancet shows that steering anti-inflammatory treatment according to the degree of inflammation results in a healthier patient. Green et al. treated two groups of patients, one by normalizing the sputum eosinophil level and the other according to present British guidelines. [Green RH et al. 2002a] Patients in the sputum management group had significantly fewer severe asthma exacerbations than patients managed by standard regimens (35 vs. 109; p = 0.01) (Figure IV.17). They also had fewer emergency room visits (1 vs. 6 of 37 patients, p = 0.047). Another study confirmed the benefit of anti-inflammatory therapy guided by monitoring sputum cell counts. [Jayaram L et al. 2006] This study assessed two treatment strategies over a 2-year period: a clinical strategy based on symptoms and spirometry and a sputum strategy based on sputum cell counts to guide corticosteroid therapy. In the patients treated according to the sputum strategy, the time to the first exacerbation was longer than in the group treated according to the clinical strategy (by 213 days) and the number of exacerbations needing prednisone was reduced (5 vs. 15).

Figure IV.16. Using $\text{FeNO}$ to guide dosing of inhaled corticosteroids results in lower doses being used [Smith AD et al. 2005b]

Figure IV.17. The sputum management group had significantly fewer severe exacerbations compared with the BTS management group (35 vs. 109 total exacerbations, respectively, p = 0.001) [Green RH et al. 2002a]
As the measurement of exhaled NO is simple, more accurate and less time-consuming than the measurement of sputum eosinophils, it is more suitable for routine clinical practice and allows more frequent measurement of inflammation.

In another study, Pijnenburg and colleagues compared using symptoms alone with symptoms plus NO levels to guide treatment in children with asthma over one year. [Pijnenburg MW et al. 2005a] Although the mean dose of steroids was similar in the two groups, hyperresponsiveness improved more in the NO group (Figure IV.18) and there were fewer exacerbations (18 vs. 8).

Similarly, Fritsch et al. compared treatment strategies based on monitoring of symptoms, lung function, and β₂-agonist use with or without monitoring of exhaled NO in children with asthma. [Fritsch M et al. 2006] Patients in the NO group received higher doses of ICS and had significantly higher MEF₅₀% predicted. Significant relationships were observed between exhaled NO and dose of ICS, β₂-agonist use, asthma symptoms, and bronchial hyperresponsiveness. Although the frequency of exacerbations did not differ between groups, the study authors concluded that using NO monitoring as part of the treatment strategy improved small airway function, which may result in improved outcomes in the long term.

4.6. Monitoring Asthma Control

Summary
Although exhaled NO levels are reduced by inhaled corticosteroids they are not totally suppressed and continue to correlate with clinical markers of disease control, such as symptom frequency and with the need for rescue medicine. NO levels are higher in patients who require an increased dose of inhaled corticosteroids.

According to the 2006 Global Initiative for Asthma (GINA) guidelines, released December 12, 2006, the primary aim of asthma management is disease control. [Global Initiative for Asthma 2006] Although exhaled NO is reduced by inhaled corticosteroids, it is not totally suppressed and continues to correlate with disease control. Stirling et al. found lower levels of NO in patients requiring high doses of inhaled or oral steroids compared with
steroid-naive asthmatics. NO levels were still significantly higher than those in normal controls, however. [Stirling RG et al. 1998] Exhaled NO levels correlated closely with clinical markers of disease control (symptom frequency and the need for β₂-agonist medication use). Similarly, Artlich et al. found high levels of exhaled NO in children with recent symptoms of bronchial obstruction, even if steroids were included in the treatment. [Artlich A et al. 1999] Fitzpatrick et al. demonstrated that children with severe asthma had higher levels of exhaled NO compared with children with mild-to-moderate asthma. [Fitzpatrick AM et al. 2006] These high NO levels persisted during long-term follow-up, during which the children were receiving the maximum recommended doses of anti-inflammatory medications. In adult asthma patients, Silvestri et al. found that exhaled NO levels were lower in patients with severe asthma compared with mild-to-moderate asthma. The authors speculate that this was due to the higher doses of ICS used by the former group. [Silvestri M et al. 2006] There was also a positive correlation between exhaled NO and serum levels of the inflammatory marker TNF-α in the patients with severe asthma.

These findings have been confirmed by other groups suggesting that exhaled NO levels are useful to monitor response to changes in medication and to assess compliance with treatment. [Covar RA et al. 2003; Meyts I et al. 2003] Meyts and colleagues compared exhaled NO levels with the clinical assessment of asthma control in 73 children with asthma aged 5–18 years. [Meyts I et al. 2003] They showed that exhaled NO levels were higher in patients in whom asthma control was insufficient (Figure IV.19), whereas levels were not significantly different between children in whom asthma was well controlled and in those whose control was considered to be acceptable. In another study, NO levels correlated significantly to the severity of asthma based on the National Asthma Education and Prevention Program (NAEPP) guidelines (Figure IV.20). [Delgado-Corcoran C et al. 2004] The effect of corticosteroids on exhaled NO is dose related, as reported by Kharitonov et al. in
a study performed on adult asthmatics maintained on twice-daily inhaled budesonide. [Kharitonov SA et al. 2002; Kharitonov SA et al. 1996b] The exhaled NO concentrations increased after a 200 µg reduction in the dose of budesonide. Although there were no significant changes in lung function or daytime asthma symptoms, there was a significant increase in nocturnal asthma symptoms. When the dose of budesonide was increased to 200 µg more than the usual maintenance dose, the level of exhaled NO fell, and this was associated with a reduction in the diurnal variability of PEF and a reduction in nocturnal symptoms. Thus, the level of exhaled NO is a marker of disease activity and may be more sensitive than either lung function tests or clinical symptoms.

Exhaled NO levels have also been assessed in children with stable asthma whose doses of corticosteroids were adjusted according to National Institutes of Health (NIH) guidelines. [Griese M et al. 2000] Notably, NO levels were higher in the children who required an increase in dose, than in similar children who did not need a dose increase (Figure IV.21). Thus, exhaled NO levels correlated (although only weakly) with the decision on treatment, even though they were not used as a guide for that decision. No statistical correlation was found between exhaled NO levels and the disease severity in this study.

In another similar study, exhaled NO levels were also found to be higher in patients whose corticosteroid dose was increased after examination by a paediatric pulmonologist or an allergist, compared with those whose dose was unchanged. [Colon-Semidey AJ et al. 2000] In addition, NO levels correlated with the change in FEV₁. The authors suggested that NO levels could be a clinically useful measure of asthma severity.

In a study involving 100 asthma patients (aged 7–80 years) exhaled NO levels correlated with asthma symptoms during the previous two weeks as tested by spirometry, daily use of rescue medicines, and reversibility of airflow obstruction. [Sippel JM et al. 2000] There was, however, no correlation between exhaled NO levels and history of respiratory failure, healthcare use, fixed airflow obstruction or a validated asthma score. The authors concluded that monitoring of exhaled NO might be useful in outpatient management as a means of determining asthma control. Reid et al. found that exhaled NO levels reflected clinical activity in asthmatic patients treated with inhaled corticosteroid. [Reid DW et al. 2003]
4.7. Monitoring Compliance with Anti-inflammatory Treatment

**Summary**
Exhaled NO measurement is an easily performed, non-invasive tool to monitor adherence to steroid treatment.

Failure to comply with inhaled corticosteroid therapy may be a larger problem than is often recognized. A study by Milgrom *et al.* compared data on inhaler use from electronic inhaler monitors with data collected by asthmatic children using diary cards. [Milgrom *et al.*, 1996] More than 90% of the children exaggerated their use of inhaled steroids. According to the diary cards, median use of inhalers was 95% of the prescribed dosage, whereas the median actual use was 58%. Furthermore, the least compliant patients were the most likely to have acute exacerbations of disease. Compliance is therefore a major issue in the management of childhood asthma.

Beck-Ripp *et al.* studied 34 patients with asthma treated with budesonide for four weeks, followed by a washout period and then randomized to budesonide or no budesonide for 8 weeks. [Beck-Ripp *et al.*, 2002]. NO levels were significantly reduced during the run-in period but increased during the washout period. As might be expected, NO decreased again in those patients who were randomized to budesonide, whereas NO levels were unchanged in patients not receiving budesonide. There was a good correlation between NO levels and compliance ($r_2 = 0.586; p = 0.0003$) (Figure IV.22). A similar correlation was found by Delgado-Corcoran and co-workers ($r_2 = 0.56$), who demonstrated the large difference in NO levels between patients who exhibited poor compliance (< 49% of prescribed regimen) and those who showed good compliance (> 75% of prescribed regimen) (Figure IV.23). [Delgado-Corcoran *et al.*, 2004] However, Katsara *et al.*, using a data logger attached to a pMDI to monitor

![Figure IV.22. Relationship between exhaled NO and compliance with inhaled steroids (Beck-Ripp *et al.*, 2002)](image1)

![Figure IV.23. Exhaled NO levels in patients with asthma who demonstrated poor, moderate or good compliance with corticosteroid treatment (Delgado-Corcoran *et al.*, 2004)](image2)
medication use, found weak and non-significant correlations between exhaled NO and day compliance (number of days the advised dose was taken) \((r = 0.055, p = 0.67)\), and between exhaled NO and dose compliance (number of doses taken over a period vs. number prescribed) \((r = 0.153, p = 0.24)\) in their study of 20 children with asthma. [Katsara M et al. 2006] Almost 80% of exhaled NO values > 12 ppb (at 50 mL/s) were from subjects with a day compliance of < 50% during the preceding month.

4.8. Exhaled NO and Steroid Resistance

**Summary**
Persistently elevated levels of exhaled NO in symptomatic patients on corticosteroid treatment are most likely caused by poor compliance or poor inhaler technique. Possible other reasons include insufficient corticosteroid dosage and continuous high allergen exposure. In rare cases it may indicate true steroid resistance.

Although anti-inflammatory treatment usually reduces NO, some patients continue to have persistently elevated levels of exhaled NO and symptoms, despite corticosteroid treatment. Non-compliance with treatment, poor inhalation technique and, in some cases, inadequate corticosteroid dosage are the most likely reasons for this. Continuous high levels of allergen exposure create an overwhelming inflammatory activity. [Borish LC et al. 1999] There may also be a small number of patients, especially those with severe asthma, who are unresponsive to steroid treatment. [Payne DN et al. 2001b; Stirling RG et al. 1998]

Payne et al. [Payne DN et al. 2001b] assessed NO levels before and after oral prednisolone treatment in children with severe asthma. In some of these patients, NO levels were high at the initial evaluation and remained high following treatment, and this group continued to have persistent symptoms. Notably, some patients had normal levels of NO at baseline that remained normal during treatment. However, several of these patients still had persistent symptoms after treatment. The authors concluded that NO levels may identify different subsets of patients with difficult asthma. Those with high levels of NO, which remain high, may require a higher dose or a different type of anti-inflammatory treatment. In contrast, patients with normal NO levels who respond poorly to anti-inflammatory treatment have little or no eosinophilic inflammation. Thus, continued anti-inflammatory treatment may be inappropriate.

Patients with persistent airway eosinophilia despite steroid treatment have higher NO levels compared with those in whom eosinophil counts responded to treatment. Given that exhaled NO measurements are more convenient than bronchoscopy, exhaled NO may allow the easy identification of patients at risk of severe clinical outcomes. [Silkoff PE et al. 2005]
In one study of atopic children with stable asthma, increasing the steroid dose did not reduce exhaled NO levels [Pijnenburg MW et al. 2005b]. The authors of this study have, therefore, questioned the use of NO measurements in routine practice. The authors however did not report exhaled NO levels before the initiation of treatment and therefore it is possible that NO levels were already optimal in these patients.

### 4.9. Prediction of Loss of Asthma Control

**Summary**

High levels of exhaled NO and sputum eosinophils are markers of loss of asthma control. There is a high positive predictive value (a rise in exhaled NO is very likely to be followed by loss of control), but the negative predictive value is low; i.e. a low exhaled NO value does not exclude the possibility of exacerbation.

Prevention of exacerbations is an important goal in the management of asthma, and prediction of imminent exacerbations before the onset of clinical symptoms and airway obstruction could be of considerable value.

Jatakanon et al. induced mild asthma exacerbations by reducing the dose of steroids in stable asthmatic patients maintained on inhaled corticosteroids. [Jatakanon A et al. 2000] Seven of 15 patients developed mild exacerbations of asthma over the course of the 8-week study. At initial evaluation, the only difference between those who developed an exacerbation was a higher baseline sputum eosinophil count compared with those who did not develop an exacerbation, and exhaled NO increased in those patients who developed exacerbations (Figure IV.24). The increases in sputum eosinophils and exhaled NO were correlated with decreases in airway function (morning PEF and FEV₁). Multiple regression analysis suggested that the change in sputum eosinophils is a potentially useful marker in predicting loss of asthma control as reflected by loss of airway function. In a similar study by Belda et al.,

![Figure IV.24. Exhaled NO levels and symptoms in asthma patients with mild exacerbations after glucocorticoid dose reduction (Jatakanon A et al. 2000)](image-url)
reduction of ICS caused a mild loss of asthma control in approximately half of the patients whose asthma was previously well-controlled. [Belda J et al. 2006] Baseline exhaled NO tended to be higher in the patients who experienced exacerbation compared with those whose asthma remained controlled, but the difference was not statistically significant. Raised exhaled nitric oxide levels tended to predict loss of asthma control (risk ratio of 1.1%, 95% CI 0.94 to 1.2).

Jones and co-workers examined the ability of exhaled NO, sputum eosinophil and hyperresponsiveness to saline measurements to predict loss of asthma control. [Jones SL et al. 2001] Corticosteroid treatment was discontinued for 6 weeks in 78 patients with mild-to-moderate asthma. Exhaled NO > 15 ppb (at a flow of 250 mL/s) at baseline had a positive predictive value of 88% (i.e. exhaled NO values >15 ppb indicate loss of control in 88/100 patients), but the negative predictive value was low (25%) (i.e. a low exhaled NO value does not exclude the possibility of loss of asthma control). (Table IV.1). In addition, NO levels were equally predictive of loss of asthma control. The authors concluded that, because of simplicity of use, exhaled NO was the preferred marker. They also suggest a lower flow rate (i.e. 50 mL/s) may provide greater distinction between those who developed exacerbations and those who did not.

Table IV.1. Exhaled NO levels predict loss of control of asthma. [Jones SL et al. 2001]

<table>
<thead>
<tr>
<th></th>
<th>Positive predictive value (confidence intervals)</th>
<th>Negative predictive value (confidence intervals)</th>
<th>Sensitivity (confidence intervals)</th>
<th>Specificity (confidence intervals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO at visit prior to loss of control &gt; 15 ppb</td>
<td>0.83 (0.67, 0.94)</td>
<td>0.27 (0.14, 0.43)</td>
<td>0.50 (0.37, 0.63)</td>
<td>0.65 (0.38, 0.86)</td>
</tr>
<tr>
<td>Change in NO from baseline to visit prior to loss of control &gt; 60%</td>
<td>0.83 (0.67, 0.94)</td>
<td>0.27 (0.14, 0.43)</td>
<td>0.50 (0.37, 0.63)</td>
<td>0.65 (0.38, 0.86)</td>
</tr>
<tr>
<td>Percentage eosinophils at baseline &gt; 4%</td>
<td>0.80 (0.52, 0.96)</td>
<td>0.21 (0.14, 0.43)</td>
<td>0.21 (0.12, 0.34)</td>
<td>0.80 (0.52, 0.96)</td>
</tr>
<tr>
<td>Saline PD_{15} at baseline &lt; 12 mL</td>
<td>0.77 (0.60, 0.90)</td>
<td>0.25 (0.11, 0.43)</td>
<td>0.53 (0.38, 0.67)</td>
<td>0.50 (0.25, 0.75)</td>
</tr>
</tbody>
</table>

The predictive value of exhaled NO was confirmed in a study of 37 well-controlled asthmatic patients, where the determination of AMP responsiveness and exhaled NO was used to identify those patients whose conditions deteriorated when the ICS dose was reduced. [Prieto L et al. 2003] In another study, exhaled NO was significantly higher in patients who subsequently had an exacerbation within 2 weeks of the measurement.
(29.7 ± 14.5 vs. 12.9 ± 5.2). [Harkins MS et al. 2004] Logistic regression showed that exhaled NO was the only significant predictor of exacerbation (Table IV.2).

Table IV.2. Most significant assessments predicting asthma exacerbations. [Harkins MS et al. 2004]

<table>
<thead>
<tr>
<th>Term</th>
<th>Standard error</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁</td>
<td>0.793</td>
<td>0.56</td>
</tr>
<tr>
<td>Exhaled NO</td>
<td>0.107</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Gelb et al., examined the roles of traditional spirometry and exhaled NO in predicting asthma exacerbations in 44 patients with stable asthma. [Gelb AF et al. 2006] During 18 months of follow-up, 22 patients experienced exacerbations. Among patients with baseline FEV₁ ≤ 76% predicted, 65% had exacerbations, while in those with baseline FEV₁ > 76% predicted, only 15% had exacerbations. Using a cut-off FEV₁ of 76% predicted to predict exacerbation gave a sensitivity of 0.91 and specificity of 0.50. When baseline exhaled NO was > 28 ppb (mean of three measurements at 100, 150 and 200 mL/s), exacerbations occurred in 76% of patients, while if baseline exhaled NO was < 28 ppb, exacerbations occurred in 33%. Using a cut-off point of 28 ppb gave a sensitivity of 0.59 and specificity of 0.82. Combined baseline exhaled NO > 28 ppb and FEV₁ < 76% predicted identified 13 stable asthmatics with 85% probability for future exacerbation, whereas 9 asthmatics with exhaled NO < 28 ppb and FEV₁ > 76% predicted had a 0% probability of exacerbation (Figure IV.25). The study concluded that combining exhaled NO and spirometry can be used to stratify risk for asthma exacerbation.

4.10. Emergency Care

Summary

Further research is required to determine the value of exhaled NO measurement in emergency care.

Two studies suggest that exhaled NO is a sensitive marker of acute asthma exacerbations in children. In a study of 10 children with acute asthmatic exacerbation presenting at the emergency room, the group had a mean exhaled NO level of 48 ± 8 ppb prior to
glucocorticoid treatment and 17 ± 1 ppb after treatment (p < 0.002). [Lanz MJ et al. 1999]
Both FEV\textsubscript{1}% and PEF improved after treatment. Similar results have been reported following
treatment with nebulized terbutaline and i.v. betamethasone. [Lee MY et al. 2006] However,
in one study NO levels were not a useful marker for assessing severity, response to treatment
in patients with acute exacerbation. [Gill M et al. 2005]

4.11. Safe Withdrawal of Inhaled Corticosteroids

**Summary**
Elevated exhaled NO predicts a relapse of symptoms after withdrawal from inhaled
corticosteroids. An exhaled NO level of > 49 ppb at 2-4 weeks after steroid withdrawal was
highly indicative of asthma relapse.

Many children with asthma do not develop airway hyperresponsiveness after withdrawal of
inhaled corticosteroids. One study investigated whether differences between children with
and without airway hyperresponsiveness after withdrawal of inhaled corticosteroids were
compatible with differences between transient and persistent wheezers found in other
studies. The investigators found that hyperresponsive children had more atopic features
(positive RAST, high IgE, eczema), lower FEV\textsubscript{1} values, and lower levels of soluble
intercellular adhesion molecule-1 (ICAM\textsubscript{1}) than children who were not hyperresponsive.
[Visser MJ et al. 2002] Hyperresponsive children also had elevated exhaled NO levels and
poorer lung function. After withdrawal of inhaled corticosteroids, children with airway
hyperresponsiveness had similar features to children in epidemiological studies with
persistent wheeze. Children without airway hyperresponsiveness after withdrawal of
inhaled corticosteroids may have had transient wheeze.

Exhaled NO levels may help physicians
decide whether it is safe to avoid
Pijnenburg and co-workers showed that
an exhaled NO level of > 49 ppb 4 weeks
after steroids were stopped in symptom
free asthmatic children was a good
predictor of asthma relapse (Figure IV.26).
[Pijnenburg MW et al. 2005c] It has also
been shown that steroid treatment in
asymptomatic adolescents with a history
of atopic asthma may reduce airway
remodelling. [van den Toorn LM et al.
2005] Exhaled NO levels are reduced in

![Figure IV.26. F\textsubscript{NO} \textgreater 49 ppb is a good predictor of asthma relapse in asymptomatic children not receiving treatment [Pijnenburg MW et al. 2005c]](image)
such individuals in response to treatment. Thus, the response of exhaled NO to treatment in asymptomatic individuals with a history of asthma may indicate whether treatment should be continued or not. Zacharasiewicz et al. showed that exhaled NO levels predict which patients will have worsening of symptoms if steroid doses are reduced. [Zacharasiewicz A et al. 2005] In this study, children had their steroid doses reduced by 50% every 8 weeks (if clinically indicated). An NO level increasing to at least 22 ppb (at 50 mL/s) was associated with an odds ratio of 10.4 for an exacerbation. In the study by Belda et al., there was a non-significant trend towards higher baseline NO levels among patients who experienced asthma exacerbation after reduction in ICS dose. [Belda J et al. 2006] Prieto et al. found in a study of 37 asthmatic patients that having both bronchoconstriction in response to AMP challenge and exhaled NO levels of \( \geq 15 \) ppb at baseline was a predictor for exacerbation after ICS dose reduction. [Prieto L et al. 2003]

4.12. Other Clinical Indications

4.12.1. Chronic Cough

Summary

An increased level of exhaled NO may assist in the differential diagnosis of chronic cough. Patients with asthma and chronic cough, and patients with a high cough frequency have higher exhaled NO levels than controls.

Accurate diagnosis of patients complaining of chronic cough is essential for the underlying disease to be treated correctly. In more than 90% of cases, symptoms are a result of smoking, postnasal drip, gastro-oesophageal reflux, asthma or COPD. Chatkin et al. [Chatkin JM et al. 1999] investigated the value of exhaled NO in the assessment of chronic cough. Exhaled NO was measured in adults with chronic cough, known asthmatics and healthy controls. Patients with chronic cough and asthma had significantly higher exhaled NO values than non-asthmatics with chronic cough or healthy controls (Figure IV.27). Using 30 ppb (at a flow of 45 mL/s) as the cut-off point for exhaled NO gave a sensitivity and specificity of 75% and 87%, respectively, for a diagnosis of asthma. Nogami et al. also described a significant negative correlation between exhaled NO levels and bronchial hyperresponsiveness in patients with chronic cough. [Nogami H et al. 2003]

![Figure IV.27. A cut-off point of 30 ppb at an exhalation flow of 45 mL/s provides a good specificity and sensitivity for the detection of asthma [Chatkin JM et al. 1999]](image)
These findings are supported by other studies, [Avital A et al. 2001; De Diego A et al. 2005; Kanazawa H et al. 2004] one of which involved children aged 2–7 years. [Avital A et al. 2001] In this study, children with mild intermittent asthma had exhaled NO levels of 5.6 ± 0.4 ppb (flow rate not specified) compared with 3.2 ± 0.3 ppb in those with chronic cough and 2.2 ± 0.2 in healthy individuals.

Similar to asthma, inhaled corticosteroids reduce exhaled NO levels in patients with chronic cough. In one study involving 88 patients, the reduction in NO levels was accompanied by a modest improvement in the severity of cough. [Chaudhuri R et al. 2004]

The relationship between coughing frequency and exhaled NO levels has also been investigated in children with asthma. Li et al. showed that cough frequency in children with stable asthma was increased compared with normal controls and that it correlated with exhaled NO levels ($r = 0.781, p < 0.001$; Figure IV.28) but not with FEV$_1$ or sputum eosinophil counts ($r = -0.270, p = 0.157; r = 0.173, p = 0.508$, respectively). [Li AM et al. 2003] In another study of children with mild intermittent asthma, however, Li et al. found a positive correlation between cough frequency and neutrophil levels but not with exhaled NO, [Li AM et al. 2006a] and NO levels do not appear to correlate with cough frequency in children with PCD. [Zihlif N et al. 2005] In the clinical setting, these findings may make more invasive tests for asthma unnecessary, such as bronchial challenge. High values of NO are not specific for asthma, but make the diagnosis more likely and may help to indicate which patients should be studied further.

4.12.2. Rhinitis

**Summary**

Elevated exhaled NO in patients with allergic rhinitis suggests the presence of inflammation in the lungs even in the absence of asthma symptoms. Since allergic rhinitis is a major risk factor for asthma, regular assessment of lower airway inflammation is recommended for patients with rhinitis.

Hyperreactivity occurs in patients with rhinitis, even in those without clinical asthma symptoms. Exhaled NO levels are elevated not only in asthma but also in rhinitis, reflecting the unification of the upper and lower airways.
Studies have demonstrated elevated levels of exhaled NO in patients with allergic rhinitis but no symptoms of asthma. [Cardinale F et al. 2005; Downie SR et al. 2004; Jouaville LF et al. 2003] Cardinale et al. demonstrated significantly higher exhaled NO levels in children with allergic rhinitis (15 ppb) or asthma (23 ppb) compared with healthy controls (6 ppb, all at a flow rate of 50 mL/s). [Cardinale F et al. 2005] There was a significant positive correlation between exhaled NO and total serum IgE in the children with asthma (p < 0.0001) and allergic rhinitis (p < 0.01), and between exhaled NO and the number of positive skin prick tests (asthma, p < 0.0001; allergic rhinitis, p < 0.01). Lopuhaa et al. discovered that while exhaled NO at baseline was lower in non-asthmatic patients with rhinitis compared with asthmatic patients (p < 0.006), after bronchial allergen challenge a greater increase in NO levels in the patients with rhinitis resulted in similar post-challenge NO levels in the two groups of patients. [Lopuhaa CE et al. 2003]

Högman et al. elucidated the source of NO in the airways of subjects with asthma or allergic rhinitis by measuring exhaled NO at three different flow rates (5, 100 and 510 mL/s) and using a new algorithm to partition NO. [Högman M et al. 2002b] Compared with healthy controls, NO was increased in asthma (8 vs. 18 ppb) but not allergic rhinitis (13 ppb). The rate of transfer of NO across the airway wall was increased in allergic asthma and allergic rhinitis compared with controls, despite the fact that the rhinitis patients had no pulmonary symptoms.

Elevated exhaled NO in patients with allergic rhinitis suggests the presence of inflammation in the lungs even in the absence of asthma symptoms. Since allergic rhinitis is a major risk factor for asthma, regular assessment of lower airway inflammation is recommended for patients with rhinitis. Heffler et al. assessed the validity of exhaled NO measurement to identify asthma in 48 patients with allergic rhinitis. [Heffler E et al. 2006] Exhaled NO was significantly higher in the 18 patients who were diagnosed with asthma based on lung function (60 ppb) compared with patients who did not have asthma (30 ppb, p = 0.001). None of the patients with asthma had exhaled NO < 25 ppb, and all of the patients with exhaled NO > 100 ppb were diagnosed with asthma. Other studies have also found higher levels of exhaled NO in patients with allergic rhinitis and asthma compared with allergic rhinitis alone. [Aronsson D et al. 2005; Cardinale F et al. 2005; Sandrini A et al. 2003b] Sandrini et al. showed that exhaled NO levels in patients with allergic rhinitis and asthma were reduced after treatment with nasal triamcinolone. [Sandrini A et al. 2003b]
4.12.3. COPD

**Summary**
Exhaled NO may be increased in unstable COPD. It is likely that the pathogenesis of COPD is variable. It may be associated with eosinophilic inflammation and increased exhaled NO levels. This form of COPD responds to anti-inflammatory treatment.

To date, assessments of exhaled NO in patients with COPD have provided seemingly conflicting results. Maziak and co-workers showed that patients with COPD, particularly those with unstable disease, had higher levels of exhaled NO than smokers with chronic bronchitis. [Maziak W et al. 1998] These results were supported by Kanazawa et al. who found that exhaled NO levels were higher in patients with COPD than in healthy controls (12.1 ± 1.9 ppb vs. 5.2 ± 1.4 ppb). [Kanazawa H et al. 1998] Others have reported similar results. [Agusti AG et al. 1999; Corradi M et al. 1999] Ansarin and co-workers reported that NO levels were higher in patients with COPD than in controls, but were lower than in patients with asthma. [Ansarin K et al. 2001] NO levels correlated with lung function tests in this study. In a study by Clini and colleagues, however, patients with severe but stable COPD have been shown to have abnormally low levels of exhaled NO. [Clini E et al. 1998] Other studies by the same group have shown that NO levels were significantly lower in COPD patients with cor pulmonale than in those patients who did not have this complication (5.7 ± 1.9 ppb vs. 8.9 ± 4.7 ppb) [Clini E et al. 2000] and pulmonary rehabilitation in patients with mild-to-moderate COPD was associated with an increase in exhaled NO. [Clini E et al. 2002] Zeitkowski et al. have also reported that NO levels are high in COPD and vary with smoking status (Figure IV.29). [Zietkowski Z et al. 2005] Furthermore, Rutgers et al. [Rutgers SR et al. 1999] and Delen et al. [Delen FM et al. 2000] both reported no differences in NO levels between patients with stable COPD and healthy controls. Papi et al. discovered that exhaled NO was higher during COPD exacerbation compared with during stable convalescence. [Papi A et al. 2006] Others have shown that exhaled NO is not associated with severity of COPD. [Olin AC et al. 2006a]
A possible explanation for these conflicting results may be differing inflammation pathologies in patients with COPD. This condition is recognized as an inflammatory disorder associated with sputum neutrophilia and, in some cases, eosinophilia. In patients with fixed airflow obstruction, those with a history of asthma have significantly more eosinophils in blood, sputum, BAL and airway mucosa than patients with a history of COPD. Exhaled NO was also significantly higher in the patients with a history of asthma, 38 ppb compared with 11 ppb (flow rate not stated) in the patient group with a history of COPD (p < 0.01; Figure IV.30).

[Fabbri LM et al. 2003] Another study by Clini et al. showed that exhaled NO and peak work rate increased in patients with COPD of differing severity after a pulmonary rehabilitation programme. [Clini E et al. 2002] Machado et al. have shown exhaled NO to be lower in patients with \( \beta_1 \)-antitrypsin deficiency than in COPD patients without this deficiency. [Machado RF et al. 2002]

Some patients with COPD respond to corticosteroids whereas others do not. One study suggests that patients who respond to these anti-inflammatory agents tend to be those with higher counts of eosinophils. [Brightling CE et al. 2000] In another study, patients with COPD who experienced partial reversibility of airflow limitation after salbutamol treatment had higher levels of sputum eosinophils than those who showed no response to this bronchodilator. [Papi A et al. 2000]

Furthermore, those who showed a partial response had higher levels of exhaled NO compared with healthy controls and those who showed no response (Figure IV.31). NO levels have been shown to decrease in response to corticosteroids in COPD patients. [Ferreira IM et al. 2001; Zacharasiewicz A et al. 2005]
Other factors that may affect results in COPD patients are seasonal changes. Bhowmik et al. showed that NO levels in patients with COPD increased in autumn and winter, and in association with viral infection. [Bhowmik A et al. 2005] Patients with exacerbations also had higher NO levels in this study.

Interestingly, one study has shown that various factors, including exhaled NO, airflow limitation and sputum eosinophils and neutrophils are separate and largely independent components of COPD pathophysiology. [Lapperre TS et al. 2004] It is possible that exhaled NO has the potential to distinguish COPD patients who will respond to anti-inflammatory treatment.

4.13. Quality of Life

Summary
Elevated exhaled NO levels correlate inversely with quality of life in patients with asthma (i.e. a decreased exhaled NO value is associated with better quality of life). However, in patients without asthma symptoms, there is no correlation between exhaled NO and quality of life.

It is increasingly evident that exhaled NO measurements reflect the underlying airway inflammation in asthma. The general well-being of patients correlates with the degree of inflammation status in their airways. In a longitudinal study, Grönke et al. studied patients with severe asthma who had NO levels, sputum eosinophils, lung function and quality of life (Juniper scale) assessed over an 18-month period. [Grönke L et al. 2002b] Lower NO levels correlated with higher quality of life measures ($r = -0.61$ at 18 months; $p < 0.05$), whereas there was no correlation between lung function and quality of life. Sputum eosinophils also correlated with quality of life, but less strongly than NO. Roberts and co-workers confirmed a similar correlation between exhaled NO and quality of life. [Roberts G et al. 2005] Assessing children with allergic disease, they found a significant negative correlation between NO levels and scores on the Paediatric Allergic Disease Quality of Life Questionnaire ($r = 0.41$). Ehrs et al. found no correlation between exhaled NO and quality of life in a sample of patients who had been diagnosed with asthma but were currently free from symptoms and not using corticosteroid treatment. [Ehrs PO et al. 2006] However, Miedinger et al. found no correlation between exhaled NO or other clinical measures and score on the University of Sydney Asthma Quality of Life Questionnaire in a sample of patients with a range of asthma severity. [Miedinger D et al. 2006]

Summary

Measurement of exhaled NO has the potential to ensure better clinical outcomes and improve the cost-effectiveness of asthma diagnosis and treatment.

The Gaining Optimal Asthma Control (GOAL) study showed that a strategy aiming for total control of asthma leads to better clinical outcomes and that cost-effectiveness of this approach compares favourably with other uses of scarce healthcare resources. [Briggs AH et al. 2006] Exhaled NO can be used to assess the effects of anti-inflammatory treatment. This brings the potential to guide dosing, ensure better clinical outcomes and improve the cost-effectiveness of asthma diagnosis and treatment. Zeiger et al. have shown that exhaled NO levels may predict which patients with mild-to-moderate asthma will benefit from addition of an inhaled corticosteroid rather than from addition of a leukotriene receptor antagonist to their standard bronchodilator treatment. [Zeiger RS et al. 2006] NO levels have been shown to correlate to symptom scores by Spergel and co-workers. [Spergel JM et al. 2005] The authors concluded that using NO levels to monitor asthma severity and control would aid asthma management. Indeed, this seems to have been confirmed by Smith et al. who have shown that monitoring NO levels can lead to reduced steroid doses without loss of asthma control (see Section 4.5. Dose Optimization of Steroid Treatment). [Smith AD et al. 2005b] NO levels also correlate with quality of life measures. [Roberts G et al. 2005] Thus, monitoring exhaled NO could be a surrogate marker of the effects of treatment on well-being, and therefore be used to assess the value of treatment. However, in considering potential economic benefits of NO measurements, it should be noted that one study has shown that NO levels do not correlate with healthcare use. [Sippel JM et al. 2000]

Berg et al. used a decision-tree model to assess the cost-effectiveness of NO monitoring in the diagnosis and management of asthma from a German healthcare perspective. [Berg J et al. 2006] They found that asthma diagnosis based on exhaled NO measurements alone cost more per patient than standard diagnostic methods, but offered improved accuracy. The use of NO monitoring in treatment decisions was less costly than management based on standard guidelines, while providing the same health benefits. The authors concluded that the cost of using NO monitoring in the diagnosis stage would be offset by cost savings during asthma treatment in the form of reduced ICS doses and fewer exacerbations.
References


SCIENTIFIC BACKGROUNDER


