TOWARDS BETTER TREATMENT OF CHILDHOOD ASTHMA: INFLAMMOMETRY WITH EXHALED NITRIC OXIDE

Naar een betere behandeling van astma bij kinderen:
inflammometrie met stikstofmonoxide in uitademingslucht

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voor ons pap en mam

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Chapter 1

Introduction

1.1 ASTHMA

Asthma is the most common chronic disease in school age children, with a prevalence of 9.1% for recent wheeze in Dutch children.[1,2] Symptoms due to asthma are a significant cause of morbidity.[2-4] Compared with asymptomatic children, those with asthma missed more days of school – ranging from 2 to 15 days, depending on asthma severity.[5] In the United Kingdom diagnosed asthma accounts for 5% of primary care doctor consultations and 14% of all hospital admissions in children [6]. There has been a worldwide increase in the prevalence of asthma over the last decades, which tends to level off over the last 10 years.[1,7-10]

Asthma presents as episodic wheezing, dyspnoea and/ or cough and reversible airflow obstruction as shown by pulmonary function tests. A key feature in the pathogenesis of asthma is chronic airway inflammation, characterized by the presence of inflammatory cells and the release of many inflammatory mediators in the airways.[11-13] This may result in structural, irreversible changes of the airway wall, a complex process called remodelling of the airways that includes increases in airway smooth muscle mass and subepithelial fibrosis.[11,14]

Pathophysiology

Many cell types play important roles in the inflammatory process of asthma, in particular resident cells – including mast cells and macrophages – and immune cells, that are recruited to the site of inflammation, such as eosinophils, T-cells and neutrophils.[11,15] Other local, structural types such as epithelial cells, fibroblasts and airway smooth muscle cells are important in the pathogenesis of asthma, and also actively contribute to the inflammatory process.[16] These cells, their chemokines, cytokines and other mediators all take part in the pathophysiological processes that are associated with asthma.[14,16]

Eosinophils play an essential role in allergic inflammation (*Figure 1.1*) and infiltration of airway mucosal tissue with eosinophils is characteristic for allergic asthma.[11] Eosinophils secrete three classes of mediators: lipid derived mediators, which are involved in the acute inflammatory responses, cytotoxic granule components and proinflammatory cytokines. Platelet activating factor (PAF), an eosinophil chemoattractant and activator, belongs to the first class; PAF increases vascular permeability and induces smooth muscle contraction.[17] The granules in eosinophils contain high concentrations of toxic proteins – such as eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), which is also called eosinophil protein X (EPX), and eosinophil peroxidase (EPO). Major basic protein (MBP) forms the core of eosinophilic granules and is able to lyse alveolar epithelial cells and to perturb epithelial barrier function.[18,19] The secretion of ECP is initiated by Fc epsilon specific IgE binding upon and cross linking with antigens and is potentiated by IL-5;[20] ECP is toxic to epithelial cells and has the capacity to induce histamine release by mast cells. EPO may contribute to oxidative damage. Eosinophils are capable of producing a range of cytokines, including tumour

necrosis factor α (TNF-α), transmembrane growth factor (TGF) -α and β, interleukin 1(IL-1) and granulocyte macrophages colony stimulating factor (GM-CSF).[21-23] In turn, these cytokines enhance eosinophil recruitment, survival and activation, which may contribute to the chronicity of allergic inflammation.[17]

Another key cell type in the asthmatic inflammatory process is the *mast cell*, which is present in the bronchus, secreting inflammatory mediators like histamine, prostaglandin D2 and leukotriene C4 in response to antigen-IgE complex binding. Amongst others, these cells synthesize TNFα, GM-CSF, IL-3, IL-4, IL-5 and IL-6.[17,24]

Macrophages may be activated by antigen or allergen challenge and may produce a large variety of cytokines. Although they may act as antigen presenting cells (APC), dendritic cells present in the airways are much more effective in this respect.[25,26]

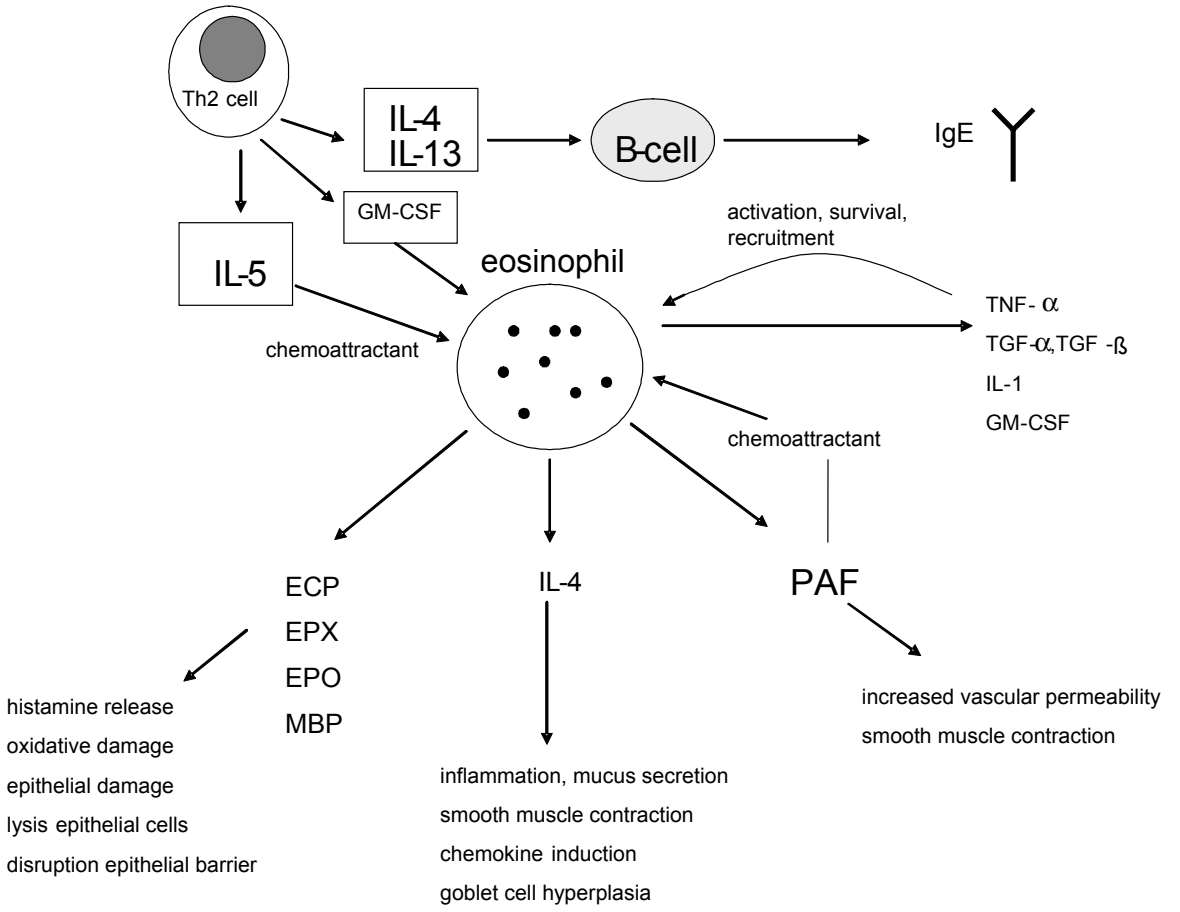

Figure 1.1 *Schematic representation of the central role of the eosinophil in asthmatic inflammation*

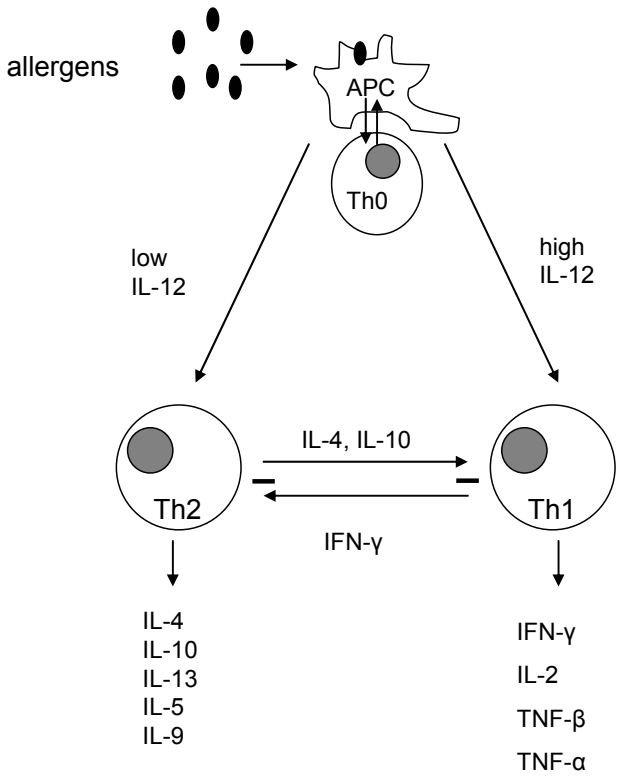

Figure 1.2 *Schematic presentation of the antigen to a naive T-cell (Th0 cell).*
IL-12, produced by APC during antigen presentation, may skew the responding T cell into a Th1 phenotype.
Th1 cells produce IFN-γ, which may inhibit Th2 cells
IL-4 and IL-10 inhibit Th1 cells.
APC antigen presenting cell
- suppression

T-cells, and especially the balance between T-helper 1 (Th1) and T-helper 2 (Th2) cells, have a central role in asthma (*Figure 1.2*). After stimulation with an allergen Th1 cells produce interferon-γ (IFN-γ), TNFα and β, and IL-2, whereas Th2 cells mainly produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13.[27,28] The cytokines produced by Th1 and Th2 cells may antagonize each other.[28] IFN-γ, for example, may inhibit the production of typical Th2 cytokines; reciprocally, IL-4 and IL-10 are implicated in the down regulation of Th1 cytokines. As it could be produced by APC during antigen presentation, IL-12 is a dominant factor that may skew the responding T-cell into a Th1 phenotype.[25] Although both Th1 and Th2 responses are crucial in host defence, Th2 is mainly associated with the pathogenic T-cell responses in asthma. As such, IL-4 is necessary for the production of IgE.

Adhesion molecules are essential in the recruitment and migration of inflammatory cells from the blood vessel to the airway. Increased contact between leukocytes and the endothelium is mediated by vascular selectins. These selectins allow for interaction of leukocytes with adhesion molecules like intercellular adhesion molecule (ICAM) 1 and 2 and vascular cell adhesion molecule (VCAM) 1 on the endothelium.[17,25] This interaction enables migration through the endothelium and

within the extravascular compartment. VCAM-1 on the endothelium allows for selective binding of cells, which enhances recruitment of eosinophils. VCAMs can be induced by IL- 4 or by TNF-α which induces also ICAM 1.[17] Migration to the airway surface is stimulated by chemokines. Frequently produced by epithelial cells, chemokines regulate leukocyte recruitment and are potent histamine-releasing factors.[25]

Pathology

Microscopic features of asthma consist of accumulation of debris and mucus in the airways, disruption and shedding of epithelium, goblet cell hyperplasia, submucosal gland hypertrophy, thickening of the epithelial reticular basement membrane (RBM), submucosal and airway wall oedema, vascular leakage and increase in airway smooth muscle mass.[11] The airway inflammatory infiltrate in asthma is characterized by activated eosinophils, mast cells, macrophages and Th2 cells.[11,12,16,29] In some cases there is an influx of neutrophils, in particular in acute exacerbations, and in severe steroid resistant asthma or acute sudden onset fatal asthma.[30-33] The epithelial cells in asthma are shed and activated, look fragile and are less viable.[11,34,35] Shedding may be a consequence of the release of toxic proteins, inflammatory mediators, free radicals and proteases from inflammatory cells. Activated epithelial cells release mediators and cytokines that can induce bronchoconstriction, inflammation and remodelling.[16]

The clinical features of asthma are at least partly due to bronchoconstriction, inflammation and remodelling.

Treatment

All patients with symptomatic asthma should be provided with a short acting β_2-agonist on demand.[36,37] Anti-inflammatory agents constitute the mainstay of maintenance therapy, with inhaled corticosteroids (ICS) as first choice.[37,38]

1.2 Symptoms and Inflammation

The goal of asthma treatment is to achieve control of the disease, defined as minimal symptoms and need for rescue medication, no exacerbations, no limitation of physical activity, and normal lung function, without treatment-induced side effects.[36,38] The treatment of asthmatic children in most cases relies on symptoms reported by the child and/ or the parents.[36-38] Yet, relationships between inflammation and symptoms, asthma control and asthma severity are complex and still unclear. Lung function tests such as forced expiratory volume in 1 second (FEV_1) reflect airway patency and correlate poorly with airway inflammation.

Communication and perception

In daily practice, patients and their physicians only roughly estimate asthma control and make therapeutic decisions on this subjective assessment. However, a

substantial proportion of asthmatic patients with moderate or severe symptoms believe their asthma is well or completely controlled, even if they miss school or work because of asthma symptoms.[39,40] This discrepancy between perceived asthma control and reported symptom severity suggests that patients accept their symptoms and adapt their lifestyle accordingly.

An extra challenge in the treatment of asthmatic children is symptom communication. It is usually the parents that provide paediatricians with information about children's asthma. Treatment decisions nevertheless should also be based on the child's own perception of symptoms, next to accurate interpretation and evaluation of signs by the parents, and be established through effective communication with the physician and appropriate action by the physician.[41,42] However, up to 78 percent of parents underestimate the severity of their child's asthma and report good control.[41,43,44] Low socioeconomic status and parental smoking were shown to be risk factors for parental underestimation.[45]

Professionals and families may describe asthma in very different terms, leading to a communication gap. Vague, global questions on asthma control are more likely to lead to incomplete information and misinterpretation than specific questions on day time and night time symptoms.[45]

Then, perception of asthma symptoms and bronchoconstriction may be impaired, especially in patients who suffer frequent exacerbations and those with severe asthma.[46] In these patients, ongoing inflammation as measured with eosinophil counts in induced sputum is associated with poorer perception of airway obstruction.[46] Poor perception might be explained by adaptation to more severe airway narrowing, resulting in a higher threshold for experiencing symptoms. In adult patients with near-fatal asthma, reduced chemosensitivity to hypoxia and blunted perception of dyspnoea predispose to fatal attacks.[47]

Correlation with inflammation

Several studies show poor or absent correlation between current or recent symptoms and markers of airway inflammation, including airway hyperresponsiveness (AHR) to methacholine, fractional concentration of nitric oxide in exhaled air (F_{ENO}), ECP in serum and sputum, induced sputum eosinophils and inflammatory cells in bronchial biopsies.[29,39,42,48-68] On the one hand, patients without any evidence of eosinophilic inflammation, either in sputum or bronchial biopsies, may be highly symptomatic.[29,58,69] On the other hand, asymptomatic adolescents in clinical remission of asthma and asymptomatic individuals may show increased AHR, ongoing inflammation and airway remodelling in bronchial biopsies.[50,53,70,71] Similarly, repeated low-dose allergen exposure in asthma can lead to airway inflammation without worsening of symptoms.[60] In the same way, airway inflammation is often not effectively suppressed during regular treatment in asthma, even when clinical symptoms are adequately controlled.[51,62] The relationship between symptoms and inflammation may differ between patients

taking corticosteroids and steroid-naive patients. The correlation may be strongest in steroid-naive or difficult asthma at the other end of the spectrum, where inflammatory markers were found to be related to symptom frequency and rescue beta-agonist use.[29,46,48,65,72,73] During asthma attacks an increase in airway mucosal eosinophil count was found associated with increased symptoms and AHR.[74] A study in adults demonstrated a correlation between clinical asthma severity, as assessed with the use of symptom scores and peak flow measurements, and inflammation in bronchial brushes.[73] All in all, the relationship between symptoms and airway inflammation in asthma remains unclear.[66]

The discrepancy between asthmatic symptoms and airway inflammation implies that decisions on anti-inflammatory treatment based on symptoms in the clinical setting can be inappropriate. Therefore, there is a need for new tools to assess the severity of asthma, guide treatment decisions and reduce asthma morbidity. We are on a quest for the ideal approach that not only is safe, simple, noninvasive, cheap, easy to perform, reproducible and accurate, but also reflects control of inflammation and enables to monitor the changes induced by therapeutic interventions in individual patients.

1.3 WHY SHOULD WE TREAT INFLAMMATION?

Chronic airway inflammation is the key target for treatment with inhaled steroids.

Remodelling

Inflammation in asthma is accompanied by structural changes of the airway wall, referred to as remodelling (see also section 1.1).[75-80] Mediators and growth factors released by inflammatory cells have the potential to induce acute damage and structural changes, which may be the main cause of irreversible airway obstruction and progressive loss of lung function.[81] Remodelling involves interactions between inflammatory cells and their products, intracellular and extracellular signals, and the balance between matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases.[77] Repeated mechanical changes of the airways during bronchospasm may contribute to remodelling.[79] Airway inflammation and airway remodelling are linked; yet the precise relationship between the two remains unclear. Accumulating evidence suggests remodelling to occur parallel to chronic inflammation.[80] There is some evidence that airway remodelling already may take place at a young age. Thickened RBM was demonstrated in school-age children with mild to moderate asthma, and, to a lesser extent, in atopic children without asthma.[82,83] In children aged from 6 to 16 years suffering from difficult asthma, RBM thickening was seen to an extent similar to that in adults with mild or acute life-threatening asthma.[84] No relations were found between age, duration of asthma, FEV_1, mucosal eosinophilic inflammation and RBM thickening.[84] Bronchial biopsies in six children with difficult, severe asthma showed changes consistent with airway remodelling, in the absence of significant airway inflammation.[85] Then,

in a study by Pohunek *et al*, the early presence of eosinophilic inflammation and remodelling was shown in children aged from 1 to 12 years with respiratory symptoms, even before the diagnosis of asthma was made.[86] However, infants with recurrent wheeze or cough and airflow limitation with or without reversibility, showed neither thickening of the RBM, nor airway eosinophilia.[87] The finding of airway eosinophilia and RBM thickening in very young children with mild asthma suggests that remodelling begins early in the course of the disease (although perhaps not in infancy) and probably parallels, or even precedes, the development of chronic inflammation.[79,85]

Asthma is associated with reduced lung function development, and lung function at a young age is a determinant of lung function in adult life.[88,89] Persistent, and even subclinical airway inflammation may lead to altered airway development, remodelling of the airway wall and eventually to permanently impaired airway function.[53,79,90] Early detection and treatment of inflammation might therefore be important in strategies aiming at improving asthma control and long-term prognosis. We might even hypothesize that evaluating the presence of inflammation in asymptomatic patients may identify individuals who could do with closer follow up and, possibly, anti-inflammatory medication.

Improving asthma outcome by treating inflammation

Two studies in adults support the concept that ICS dose titration on airway inflammation can improve asthma treatment. Green *et al*. conducted a trial in 74 asthmatic patients, randomized to management on the guidance of induced sputum eosinophil counts or a standard clinical approach based on British Thoracic Society (BTS) guidelines.[91] Treatment decisions in the BTS group were based on assessments of symptoms, peak expiratory flow (PEF), and β_2-agonist use; in the other group they were based on maintaining a normal sputum eosinophil count (< 3%) with a minimum dose of anti-inflammatory treatment. Patients treated on the guidance of eosinophil counts had fewer asthma exacerbations and hospital admissions compared with the BTS group, with similar average daily doses of ICS. Sont *et al*. demonstrated that better asthma control could be achieved in adult patients who received ICS based on their AHR levels, though at the cost of higher steroid doses.[92] The same study found a reduction in RBM thickness only with the AHR strategy. This poses the question whether the optimal therapy should aim at reversing inflammation, so as to prevent irreversible airflow obstruction.[92]

These observations have led to the concept that controlling airway inflammation is an important therapeutic goal. Moreover, treating symptoms might even be dangerous if the underlying inflammation should not be tackled.[93]

Although it is clear that monitoring airway inflammation is critical to achieve effective control, the current guidelines still adhere to monitoring of symptoms.[36,37]

1.4 How to Measure Inflammation?

Markers of airway inflammation can be studied by direct or indirect sampling techniques. Direct techniques make use of material obtained from the bronchi, including mucosal biopsies, brushes and bronchoalveolar lavage fluid (BALF). Indirect methods include examination of (induced) sputum, blood, urine or exhaled gases or breath condensate.

Bronchial biopsies

The standard by which to determine airway inflammation remains the bronchial biopsy. Mucosal biopsies show changes occurring in the mucosa directly, the state of activation of cells and their products can be observed and localization of cells within the submucosa and epithelium can be visualized. Bronchoscopy is indispensable to obtain biopsies. In experienced hands, this procedure is safe, even in severe asthma.[16,94] It has several limitations, however. First, it is invasive, unsuited for repeated use and ethical in children only if there are specific indications.[94] Second, data are lacking on the reproducibility of several parameters obtained from bronchial biopsies, especially in children.[95] Third, specimens from the large airways only can be obtained, and specimens are small and vulnerable to damage by the biopsy procedure.[16,96] Transbronchial biopsies have the advantage that also smaller airways may be sampled, but the procedure carries a much higher risk of pneumothorax or bleeding.[97] Transbronchial biopsies are mainly indicated in lung transplant patients. Viable epithelial cells can be obtained with bronchial brushings, which provides for studying these cells as well as inflammatory cells infiltrating the epithelium or presenting at the surface of the epithelium.[98]

Bronchoalveolar lavage fluid (BALF)

During bronchoscopy BALF can be obtained for cytology studies. A disadvantage of this procedure is that it cannot be stated with certainty from which part of the lungs the fluid is derived, be it the small airways, large airways or alveoli. Furthermore, variable dilution, sampling errors and variable return of fluid makes the interpretation difficult.[16]

Induced sputum

Children are seldom able to produce sputum spontaneously. Inducing sputum production by inhalation of normal or hypertonic saline may provide sputum samples and supernatant in which cells and soluble constituents like ECP can be determined.[29] Potential mechanisms by which sputum is then produced are reduction in mucus viscosity, increased mucociliary clearance, increased mucus production and increase in the volume of airway secretions. Inhalation of hypertonic saline stimulates cough by mast cell degranulation and stimulation of afferent nerves in the airway. The procedure of sputum induction is relatively noninvasive, gives reproducible and valid results, which are responsive to clinical changes.[99-103] The procedure is feasible in 60 - 100% of children aged 7 years or

older with variable repeatability and interobserver agreement.[58,104,105] Normal values of sputum cell counts are available for children and adults, and the upper limit of normal values for eosinophils in sputum in children is 2.5%.[106] However, the procedure carries a risk of serious bronchoconstriction and oxygen desaturation, which makes it necessary to monitor lung function during sputum induction. In spite of this risk, sputum induction may be performed in children with difficult asthma after pre-treatment with a bronchodilator.[105] Skilled investigators are required and immediate, expert processing is critical for valid and reproducible results. The procedure takes about 1 hour, sputum processing about 90 minutes. The sputum induction procedure may be combined with bronchoprovocation with hypertonic saline.

Inflammatory indices in induced sputum broadly correlate with those obtained by bronchoscopy and with AHR to methacholine.[67,107-109] Sputum eosinophilia separates steroid-naive asthmatics from normals.[58] The sensitivity and specificity of sputum eosinophilia > 5% for the diagnosis of asthma in children is respectively 85 and 93%.[102] Eosinophil counts normalize after treatment with steroids and are higher during exacerbations.[102,110,111] During asthma exacerbations also high neutrophil levels may be found.[99,100,106] Up to 80% of corticosteroid-naive patients and > 50% of steroid-treated subjects with asthma symptoms have sputum eosinophilia. At best, there is a weak relationship between the severity of asthma as defined by lung function, AHR or symptoms and sputum eosinophil count.[51,106,112,113] Eosinophil counts in induced sputum can be used to monitor effects and to guide titration of the minimal effective dose of anti-inflammatory treatment.[114]

Eosinophils and eosinophilic products in blood

The eosinophil is the predominant cell in the inflammatory process in asthma. Circulating levels of eosinophils correlate weakly with symptoms and AHR.[115] Serum ECP levels also correlate poorly with symptoms, asthma severity and AHR, are influenced by atopy and eczema and decrease after institution of treatment with ICS.[58,110,113,116-123] While correlations between ECP in serum and ECP and eosinophils in BALF, eosinophils in sputum and in bronchial biopsies have been evidenced, there are conflicting data on the correlation of ECP in serum with sputum ECP.[113,121,122,124,125] In summary, although blood eosinophil counts and serum ECP correlate with several other inflammatory markers in sputum and BALF, they lack specificity and merely reflect the degree of activation of the circulating pool of eosinophils rather than the local process in the airways.[119,121,126-129] Sputum eosinophil counts and ECP are more accurate markers for asthmatic airway inflammation.[130]

Bronchoprovocation tests

Airway hyperresponsiveness (AHR) is a characteristic feature of asthma. Lung function laboratory testing of AHR makes use of different challenges. Direct challenges involve agonists such as methacholine or histamine that cause airflow

limitation predominantly via a direct effect on airway smooth muscle. This in contrast to indirect challenges – exercise, hyperventilation, inhalation of hypertonic saline or adenosine 5'-monophosphate (AMP)– which act by releasing endogenous mediators that cause the airway smooth muscle to contract. Indirect bronchial stimuli may more directly reflect the ongoing airway inflammation and mimic the natural situation, and are therefore more specific to identify active asthma.[131] Bronchoprovocation with AMP has been shown to be useful when evaluating the effectiveness of different treatment regimens with ICS.[132]

There are conflicting data on possible correlations between AHR and the numbers of inflammatory cells in sputum, BALF or bronchial biopsy.[62,68,133] While some studies showed a correlation between both eosinophils and mast cells within the bronchial mucosa and the degree of AHR in steroid-treated and steroid-naive patients, other studies could not confirm these findings.[62,73,134] It may be possible that with a shorter duration of asthma, AHR is more related to inflammation, whereas in long standing asthma AHR is also determined by baseline lung function or remodelling.[133]

Although bronchial provocation tests might be used as a surrogate marker of AHR, these tests are time consuming, costly, and carry the risk of inducing severe bronchospasm.

Exhaled breath condensate (EBC)
EBC is collected by cooling or freezing exhaled air. Various non-volatile substances such as hydrogen peroxide, leukotrienes, nitrite and nitrate have been detected in breath condensate. Standardized sampling techniques are being developed and normal values of hydrogen peroxide for children are available.[135-137] Collection of EBC is noninvasive and requires minimal cooperation of the child. Although the determination of different substances is promising, correlations with other indices of inflammation are still lacking and not all technical problems have been dealt with yet.[138,139]

None of the discussed methods to measure inflammation seems perfect. Still, there is a need for clinically feasible ways to diagnose and guide treatment of patients with asthmatic inflammation. If a new measurement is to be used to improve asthma management, it should not only be noninvasive or relatively noninvasive, easy to perform and safe, cheap, standardized, reproducible and accurate, but also be able to reliably discriminate different airway diseases and their severity, and to monitor changes after interventions.

1.5 NITRIC OXIDE

1.5.1 Biology of nitric oxide in the airways

Nitric oxide (NO) is a reactive, free radical gas with one unpaired electron, and forms in the airways when L-arginine is oxidized to L-citrulline (*figure 1.3*).[140-142]

This reaction is catalyzed by the NO-synthases (NOS), of which at least three distinct isoforms exist.[143] The constitutive NOS (cNOS) are calcium and calmodulin dependent and activated by small rises in intracellular calcium. There are two forms of cNOS: neuronal NOS (nNOS or NOS1) and endothelial NOS (eNOS or NOS3).[143] The unmasking of endothelium derived relaxing factor as NO won Ignarro and co-workers a Nobel Prize in 1998.[144] Mediators like bradykinine, acetylcholine, histamine, leukotrienes and platelet activating factor can stimulate cNOS.[143] The expression of inducible NOS (iNOS or NOS2) is increased in inflammatory states and is induced by IFN-γ, endotoxin, TNF-α, TNF-ß, IL-1 and several other cytokines.[145]

Different isoforms of cNOS and iNOS are found in different cell types. Endothelial NOS occur in endothelial cells of pulmonary blood vessels, bronchial epithelium, type II alveolar epithelial cells, nasal mucosa and the membrane of ciliary microtubules. Neuronal NOS are located in nerves in the airways, which supply vessels and smooth muscle. INOS have been detected in macrophages, type II alveolar epithelial cells, fibroblasts, airway and vascular smooth muscle cells, respiratory epithelial cells, mast cells, endothelial cells, neutrophils, hepatocytes and chondrocytes.[140-142,146,147] Some cells may have two or more cNOS and others, including neutrophils, will express cNOS under physiological conditions and iNOS when primed by inflammatory stimuli.

NO in the respiratory tract has a half-life of only 1 - 5 seconds and will be quickly oxidized, reduced or complexed with other biomolecules like haemoglobin. In the presence of oxygen, NO in an aqueous environment will be mostly converted to the end-products nitrate (NO_2-) and nitrite (NO_3-). In both gas phase and aqueous solution NO reacts quickly with super oxide (O_2-) to form peroxynitrite (OONO-).[140]

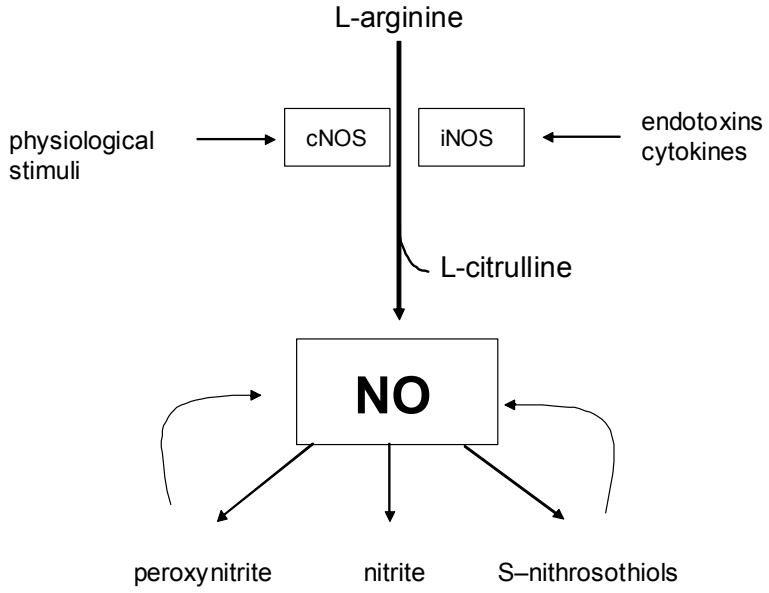

Figure 1.3 *Synthesis of nitric oxide from L-arginine.*
cNOS constitutive NOS
iNOS inducible NOS

NO reacts with thiol-containing molecules such as cysteine and glutathione to form S-nitrosoproteins and S-nitrosothiols, which may again release NO and act as carrier or storage for NO.[140] S-nitrosothiols are potent bronchodilators. Reactive oxygen species (ROS) can interact with NO to form reactive nitrogen species (RNS). ROS comprise a large variety of free oxygen radicals and oxygen derivatives (super oxide anion and hydroxyl radicals, hydrogen peroxide, peroxynitrite and ozone). ROS and RNS are important in the killing of microorganisms but at high concentrations they may exert deleterious effects within the airways, leading to apoptosis and necrosis.

NO and nitrogen oxides have very diverse functions in the airways and blood vessels. They may cause smooth muscle relaxation and vasodilatation, thereby matching regional airflow and bloodflow.[148,149] NO modulates the pulmonary artery response to catecholamines, reverses hypoxic pulmonary vasoconstriction and mediates drops in vascular resistance[150] (*Table 1.1*). NO is involved in platelet inhibition, ciliary function, stimulation of hormonal release and is able to kill tumour cells, inhibit viral replication and eliminate other pathogens.[150,151] NO functions as a neurotransmitter of inhibitory nonadrenergic noncholinergic (iNANC) nerves.[150] NO plays an important role in pulmonary disease – e.g. asthma, pulmonary hypertension, immune-complex mediated lung injury and acute respiratory distress syndrome (ARDS).[141] In asthma (see section 1.5.3) NO has a dual part. On the one hand, involvement in modulating basal airway tone and airway reactivity and counterbalancing constrictor stimuli; on the other hand, promoting inflammation and oedema when present at high levels, partly by its reaction products such as ONOO-.[150] Controlled generation of ONOO- may play a role in defending the host against bacteria, whereas excess generation is likely to lead to lung damage.

In summary, there is a delicate, complex balance between the beneficial and harmful effects of NO in the airways, partly dependent on its concentration in the airway.

Table 1.1 Possible effects of nitric oxide [140,142,150-152]

Bronchodilatation
Vasodilatation
iNANC neurotransmission
attenuation of AHR
reversal of hypoxic pulmonary vasoconstriction
stimulation of submucosal gland secretion
stimulation of platelet reactivity
stimulation of ciliary function
killing tumour cells
inhibition of viral replication
elimination of pathogens

1.5.2 Measurement of exhaled nitric oxide

Several techniques are in use to measure NO. The most common is the chemiluminescence assay, based on detection of light when NO reacts with ozone (O_3) to form its unstable product NO_2• and O_2. NO_2• disintegrates to NO_2 while emitting a photon. The photons released by the reaction can be detected with a photomultiplier tube. Electrochemical methods are based on the electrochemical oxidation of NO on solid electrodes. Other techniques include tuneable diode laser absorption spectroscopy and laser magnetic resonance spectroscopy.[153]

NO can be detected in the exhaled air of humans and several factors influence the fraction of NO in exhaled air (F_{ENO}).[153-155]
F_{ENO} is highly dependent on a person's exhalation flow rate, in the sense that F_{ENO} levels drop with higher flow.[156,157] At increased flow, exhaled gas has less 'residence time' in the conducting airways, and thus there is less time for the airway epithelium to load the passing expirate with NO, resulting in lower F_{ENO} values.
Much higher concentrations of NO relative to the lower airways are found in the nose and paranasal sinuses.[158-160] Contamination of exhaled air with nasal air should therefore be avoided in testing. This may be achieved through closing the velum by exhaling against a resistance with a positive mouth pressure.[160] Also, seeing that environmental NO can reach high values, especially during peak traffic hours, contamination with ambient air should be avoided.[161,162] Inhalation of NO-free air prior to exhalation will avoid contamination with ambient air.

Several non-disease related factors have been found to influence F_{ENO} as well (*table 1.2*). Children, in contrast to adults, show an age-dependent increase in F_{ENO}.[163,164] Adult men seem to have higher F_{ENO} levels than women, but in Caucasian children there is no effect of sex.[165-167] However, a recent study revealed higher F_{ENO} levels in Chinese boys than in girls; moreover F_{ENO} was higher in Chinese compared with Caucasian children.[168] In adults, spirometric manoeuvres transiently reduce F_{ENO}, as do bronchoprovocation tests, exercise and induction of sputum with hypertonic saline.[169-176] It has been speculated that changes in bronchial size and exhalation flow may be responsible for reduced F_{ENO} levels after exercise and forced respiratory manoeuvres. F_{ENO} levels may be lower with more severe airway obstruction, possibly due to smaller ventilated airway surface.[169,174,175,177-181] Alcohol intake shortly before F_{ENO} measurements is associated with slight decreases in F_{ENO} levels, probably due to inhibition of iNOS expression by ethanol.[182-185] Findings on the effects of caffeine, which alters cyclic AMP regulation of NOS by inhibiting phosphodiesterase, are conflicting.[186-188] Active smoking decreases F_{ENO} as does exposure to environmental tobacco smoke.[189-192] Cigarette smoke extract decreases iNOS expression from lung epithelial cells by decreasing iNOS mRNA transcription.[193] It is not clear if F_{ENO} exhibits a circadian rhythm.[194-196] Respiratory tract infections may give rise to an increase in F_{ENO} by virus-induced cytokine release.[197-199]

Table 1.2 Non-disease related confounders that may influence FE_{NO}

Confounder	**Effect on FE_{NO}**
age	children: age-dependent increase [163,164]
gender	adults: M > F, no effect in children? [165-167]
	Chinese boys > girls [168]
race	Caucasians lower compared to Chinese [168]
spirometry	transient reduction [169,172-174]
exercise	transient reduction [170,171]
bronchoprovocation tests	transient reduction [173,175]
sputum induction	transient reduction [176]
airway obstruction	reduction [169,174,175,177-181]
alcohol	reduction [182-185]
caffeine	conflicting results [186-188]
Smoking active	reduction [189]
passive	reduction, effect in daily life? [190-192,218]
time of the day	probably no circadian rhythm [194-196]

Several methods to collect exhaled air and measure FE_{NO} are available (*figures 1.4, 1.5 and 1.6*). Online methods use an NO analyzer designed to directly sample exhaled air and offline methods analyze exhaled gas first collected in a reservoir. Either a single breath technique or tidal breathing can be used. The recommended technique is the single breath online (SBOL) measurement, which however is not feasible in young, preschool children as they cannot comply with the required respiratory manoeuvres (*figure 1.4 and 1.5*).[153,155,200] Several alternative techniques have been developed to measure FE_{NO} in young children, varying from modifications of the standard online technique to offline tidal breathing methods without flow control (*figure 1.6*).[155,200-209] Updated recommendations for the measurement of FE_{NO} have recently been published.[153,155]

The reproducibility of FE_{NO} measurements is excellent with a very high intra-class correlation (> 0.9) for repeated within-sitting measurements.[210] This translates into a within-subject standard deviation for repeated measurements of 2.1 ppb for adults and 1.6 ppb for children (both at a 50 ml/s flow rate).[210,211] Similarly high degrees of reproducibility have been reported by other authors.[212-214]

Several recent studies have attempted to provide reference ranges for adults and children. Most studies were done in small numbers of adults, but in general reported the upper limit of normal values to be around 33 ppb.[210,215] A few studies in children were performed, using different methodology however, which makes it difficult to compare data.[209,210,212,216,217] A large reference values study with standardized techniques is lacking.

Figure 1.4 *Almost 4 year old boy performing a single breath online manoeuvre for $F_{E_{NO}}$ measurement. The child inhales NO-free air through the mouthpiece and exhales directly into the analyzer. Dynamic flow restriction and a visual feedback aid help to maintain the expiratory flow constant at 50 ml/s.*

Figure 1.5 *Six year old girl performing a single breath online manoeuvre with the NIOX MINO™, a portable NO-analyzer, that uses an electrochemical sensor. Deep inhalation through the mouthpiece provides NO-free air via an NO-scrubber. Next, exhalation for 10 sec with a constant flow of 50 ml/s is required. A visual and audible feedback system helps to maintain pressure within pre-set limits to ensure constant flow.*

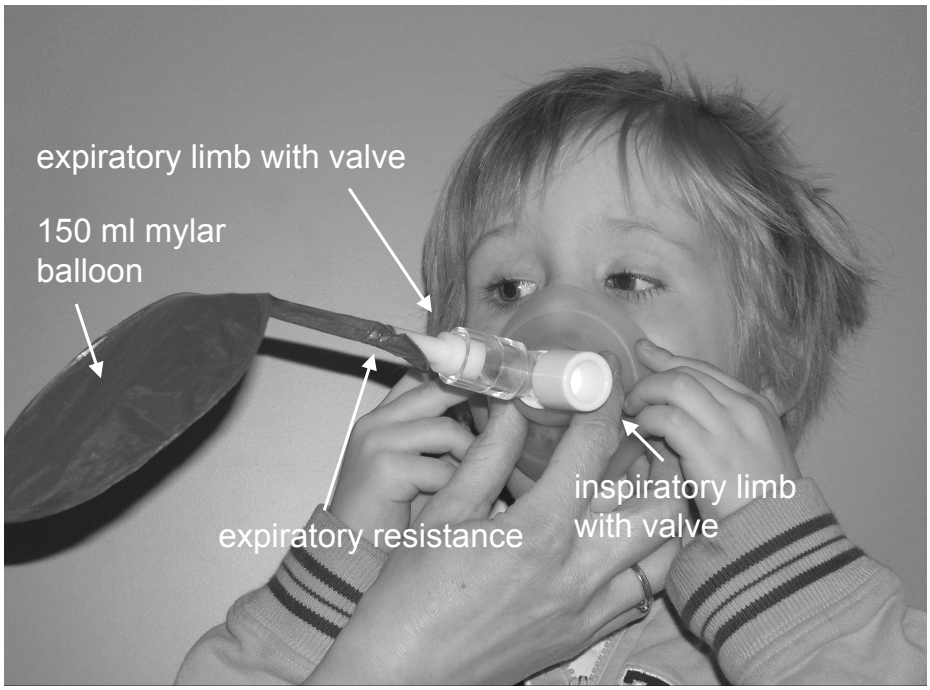

Figure 1.6 *This boy demonstrates the collection of exhaled air for FE_{NO} analysis with the tidal breathing offline method. The child inhales ambient air via the inspiratory limb with an inspiratory valve on the right. Then he exhales into the expiratory limb with valve, through a resistance, into a mylar balloon.*

1.5.3 Asthma, allergy and FE_{NO}

The considerable interest in FE_{NO} since the 1990s has resulted in the publication of more than 1200 research papers on this topic. Nitric oxide can be detected in the exhaled air of humans and several other mammalians, like rabbits, guinea pigs and horses.[154,219] The first report documenting increased FE_{NO} levels in asthma appeared in 1993.[220] Since then, many authors confirmed this finding – both in adults and in children – and proved that this increase mainly results from induction of iNOS by proinflammatory cytokines.[146,221-230]

Correlation with inflammatory markers

In atopic asthmatic adults and children, FE_{NO} levels correlate with eosinophil counts in induced sputum or BALF and with eosinophil infiltration of the airway wall.[50,53,107,133,231-237] The only biopsy study in children concerned patients with difficult asthma after treatment with oral prednisone, and showed a correlation between MBP density in biopsies as a marker of airway eosinophilia and FE_{NO}.[52] Two studies in adult asthmatics failed to show a correlation between FE_{NO} and eosinophilia in bronchial biopsies.[238,239] One used an expiratory flow of 5 – 6 l/min to measure FE_{NO}, which might explain the lack of correlation, higher flows being less sensitive.[238] The second study included no more than 9 patients, and showed a weak correlation between total inflammatory cells in biopsies and FE_{NO}.[239] Mattes *et al*. demonstrated a positive correlation between FE_{NO} and sputum ECP, but not serum ECP,[231] whereas Piacentini *et al*. found a relation between serum ECP and FE_{NO}.[234]

FE_{NO} correlates with total IgE and specific IgE to house dust mite and there is a moderate correlation with the number of positive skin prick tests.[54,240-244]
Data on the correlation between FE_{NO} and blood eosinophilia are conflicting.[54,166,235,239,241,245-249]

Correlation with asthma severity, asthma control and lung function
Several studies have addressed correlations between FE_{NO} and asthma symptoms, lung function and AHR. At best, these correlations are weak or moderate. Some studies showed a correlation between symptoms (recent symptoms, symptom frequency, symptom scores, symptom control) or rescue β_2-agonist use[48,61,235,241,250-253] whereas others did not.[54,235,254,255] Similarly conflicting findings were demonstrated for correlations between FE_{NO} and pulmonary function tests (like FEV_1, PEF or PEF variability); while most studies failed to find a correlation[48,54,225,239-241,245,248,249,253,255-257] some nevertheless did.[233,234,251,254,255,258]

Most bronchoprovocation studies, but not all,[54,248,258] found a negative correlation between bronchoprovocation tests and FE_{NO}.[107,233,239,249,255]

These discrepancies may be explained by the different methodologies used to measure FE_{NO} as well as the heterogeneity of studied populations, in particular regarding atopy and use of steroids. The various parameters studied – symptoms, lung function, AHR and inflammatory markers – most likely represent different aspects of the inflammatory and remodelling process that characterizes asthma.

The influence of atopy on FE_{NO}
Several studies have confirmed that FE_{NO} levels in atopic subjects are elevated, no matter whether they have significant lower respiratory tract symptoms or not.[240,241,243,246,259-264] It has even been suggested that FE_{NO} is merely reflecting atopy rather than airway inflammation. Clearly, FE_{NO} levels of atopic asthmatics are higher than those of non-atopic asthmatics.[215,243,244,248,260,261,264,265] Lower NO production by non-atopic asthmatics might be explained by a different pathophysiological mechanism for these two types of asthma.[266] Alternatively, we may hypothesize that differences in NOS gene polymorphisms are partly responsible for these differences in FE_{NO}.

Then, there is controversy about whether atopic, healthy subjects have higher FE_{NO} in comparison with non-atopic healthy subjects. Where several studies find similar FE_{NO} values,[215,244,260,264,265] others show higher FE_{NO} for the atopic subjects,[49,163,243] partly dependent on the allergen to which sensitization was developed. Differences in expiratory flow rate, in the definition of atopy, in the presence of allergic rhinitis or recent exposure to allergen, they all may be responsible for these discrepancies.

Some studies addressed the question if atopy rather than asthma would explain elevated FE_{NO}. With multinomial logistic regression analysis, Malmberg *et al*. showed that symptoms suggestive of asthma and sputum eosinophilia are more

important determinants of high levels of FE_{NO} than atopy, suggesting that FE_{NO} is primarily a marker of eosinophilic airway inflammation.[267] When asthma and airway eosinophilia were adjusted for, atopy did not have an independent effect on FE_{NO}.[267] Also, factor analysis conducted by Leung *et al*. suggested atopy-related indices and airway inflammation to be separate dimensions in the assessment of childhood asthma.[268] This suggestion was supported by several authors [215,269]. In contrast, Franklin and co-workers found that raised FE_{NO} in children and adults seemed to be associated with atopy and level of AHR, but not with physician diagnosed asthma.[166,241] FE_{NO} was raised in subjects with both atopy and increased AHR, irrespective of symptoms or an asthma diagnosis.[241] Atopy appears to be at least a co-factor of FE_{NO} variation – both in asthmatic children and in healthy subjects.

Elevated FE_{NO} values in atopic, asymptomatic subjects may be explained by subclinical airway inflammation. Indeed, airways eosinophilia has been shown in asymptomatic atopics, and in asymptomatic subjects in clinical remission of atopic asthma.[50,270] Also, sensitized patients exposed to low doses of allergens developed airway inflammation as measured with sputum eosinophils and increased FE_{NO} while remaining asymptomatic with stable lung function.[60] An increase in FE_{NO} is seen in sensitized subjects during exposure to allergens, irrespective of being asthmatic or not.[60,61,262,271,272]

Role of FE_{NO} in diagnosing asthma

FE_{NO} measurements are helpful in discriminating asthma from non-asthma, at least when the underlying inflammation is eosinophilic.[273-278] Dupont *et al*, in a study among 240 non-smoking steroid-naive individuals of whom 160 (67%) fulfilled criteria for the diagnosis of asthma, found FE_{NO} levels to be highly predictive of asthma – with sensitivity and specificity 85% and 90%, respectively.[276] Comparable sensitivities and specificities were obtained in similar studies.[275,277] Predictive values were almost identical to those obtained using induced sputum cell counts and better than those from conventional diagnostic tests.[275,277] Regrettably, data on the predictive value of FE_{NO} for the diagnosis of asthma in preschool children are still lacking. FE_{NO} measurements may also be helpful in the differential diagnosis of patients with chronic respiratory symptoms, as normal values make eosinophilic inflammation unlikely.[273-276]

In conclusion, FE_{NO} is elevated in steroid-naive asthmatics and correlates with several inflammatory markers including eosinophils in airway mucosal biopsies. Hence, FE_{NO} is a valid marker of asthmatic airway inflammation.

1.5.4 Effects of asthma treatment on FE_{NO}

Treatment with inhaled or oral steroids causes FE_{NO} levels to drop in patients with asthma.[110,222,233,279-286] Steroids reduce FE_{NO} by inhibiting the induction of iNOS in two ways. There is a direct effect on nuclear factor-kappa B (NF-κβ), which is involved in the transcription of iNOS, and corticosteroids inhibit the production of proinflammatory cytokines, which can induce iNOS expression.[146,280,287-290] Both

the magnitude of FE_{NO} reduction and the time interval over which reduction occurs are dose-dependent, and the response is reproducible.[281,283,285] During the first week of regular treatment with ICS, FE_{NO} will gradually decrease to reach peak reduction at 2 to 4 weeks.[110,233,279] At higher doses of ICS FE_{NO} levels tend to plateau, with sputum eosinophilia still uncontrolled.[110] However, Jones *et al*. found highly significant correlations between the *changes* in FE_{NO} and *changes* in induced sputum eosinophils with ICS therapy.[291] This suggests that FE_{NO} is a sensitive marker to monitor eosinophilic airway inflammation.

Blocking the effects of cysteinyl leukotrienes, the leukotriene receptor antagonists (LTRA) form another class of anti-inflammatory drugs. Cysteinyl leukotrienes are mediators released in asthma with both direct bronchoconstricting and proinflammatory action.[292] One of the LTRA, montelukast, reduces FE_{NO} rapidly, although not always to normal values and to a lesser extent than do low dose ICS.[293-295] Reduction was independent of the concurrent use of ICS.[295]

Several studies found short acting β_2-agonists to increase FE_{NO} levels by their effect on airway calibre and hence ventilated airway surface,[169,174,178] but other studies did not find significant changes in FE_{NO} before and after bronchodilation.[177,179,181] Long acting β_2-agonists (LABA) have a bronchodilator effect, but anti-inflammatory properties for these drugs have been claimed as well. In two studies no effect of LABA on FE_{NO} was found, neither as monotherapy or added to ICS.[179,296]

One study explored how omalizumab, a monoclonal anti-IgE antibody, in combination with ICS would effect FE_{NO}.[297] Patients treated with ICS and placebo showed an increase of FE_{NO} while ICS were tapered off, whereas FE_{NO} remained stable in patients on ICS and omalizumab.[297] There are no studies exploring effects of omalizumab monotherapy on FE_{NO}.

In summary, FE_{NO} is a valid marker of eosinophilic airway inflammation that has the potential to serve as an indicator of the adequacy of anti-inflammatory treatment. Thus, FE_{NO} may help to rationalize and improve steroid therapy in asthmatic children.

1.5.5 FE_{NO} and other respiratory and non-respiratory diseases

FE_{NO} measurements could be helpful in several other respiratory and non-respiratory diseases as well (*Table 1.3*). For example, viral infections, which are extremely common in children, may increase FE_{NO} values. These and three rare disorders in which there may be a role for FE_{NO} will be discussed here.

Viral infections

FE_{NO} values are elevated during upper and lower respiratory tract infections, both in healthy subjects and in asthmatics.[197-199,298] Viral infections may induce iNOS;[298] the concurrent rise in NO is probably beneficial to the host as NO inhibits viral replication and may mediate the anti-viral effects of IFN-γ.[298,299] Increased

generation of NO during experimental rhinovirus infection is associated with fewer symptoms and more rapid viral clearance.[298]

Cystic fibrosis

FE_{NO} measurements have not proven to be clinically helpful in patients with cystic fibrosis (CF). Values are normal or low, despite the massive airway inflammation involved.[224,300,301] There are several possible explanations for low FE_{NO} values in CF. First, iNOS has been shown to be deficient or show reduced expression.[302-304] Also, as CF-patients showed elevated nitrite levels in breath condensate, trapping and metabolisation of NO in secretions and mucus might occur in CF airways.[305-307]

Primary ciliary dyskinesia

In primary ciliary dyskinesia (PCD), FE_{NO} levels are significantly lower than in healthy persons, although with some overlap.[308,309] Moreover, *nasal* NO (nNO) is *extremely* low in patients with PCD of all ages, and fully discriminates between affected and unaffected individuals.[308,310-313] The diagnostic sensitivities and specificities of nNO for PCD range from 89 to 100%, and from 97 to 100%, respectively.[308,311,312] Low FE_{NO} and nNO levels may also be found in non-PCD bronchiectasis and sinus disease, respectively.[308,312,313] Again, several explanations present themselves. Firstly, lower or altered NOS activity may account for low nNO in PCD. Administration of L-arginine as a substrate for NO was found to increase nasal and exhaled NO formation in PCD patients, yet normal values were not reached.[314,315] This finding favours the hypothesis that decreased NOS activity is the mechanism involved; the more so as even young infants with PCD showed low nNO levels.[316]

Table 1.3

Elevated FE_{NO}	**Variable changes in FE_{NO} reported**	**Decreased FE_{NO}**
Asthma[146,220-230]	Bronchiectasis[331-333]	Cystic fibrosis[224,300,303-307]
Late asthmatic response[322]	COPD[334-338]	Primary ciliary dyskinesia[308,309,312]
Allergic rhinitis[323]	Systemic sclerosis[339-341]	Pulmonary hypertension[345]
Viral infections[197-199,298]	Chronic lung disease of prematurity[319-321]	HIV-infection[346]
Hepatopulmonary syndrome[324]	Fibrosing alveolitis[342]	ARDS[347]
Liver cirrhosis[325]	Sarcoidosis[343,344]	
Acute/chronic rejection of lung transplant, including bronchiolitis obliterans[326-329]		
Eosinophilic bronchitis[330]		

Secondly, thick mucus may impede passage of NO from the sinuses to the nasal cavity or from epithelial cells to the lumen, or may alter NO metabolism. Levels of nitric oxide metabolites in exhaled breath condensate were not decreased in patients with PCD.[317]

NO is probably involved in stimulating ciliary motility.[318] Nasal NO may also play a role in non-specific host defences, including direct toxic effects on microorganisms.[298] On the other hand, loss of ciliary function may result in reduced NOS output, as NOS is found close to the ciliary basal apparatus in epithelial cells.[318] Reduced endogenous NO production and damage to NO-producing cells may contribute to recurrent airway infections. Measurement of nNO is likely to become the screening tool of choice for PCD.

Chronic lung disease of prematurity

Data on F_{ENO} values for children with chronic lung disease of prematurity (CLD) are conflicting. This may be explained by differences in children's ages, measurement techniques or definitions of CLD.

Baraldi *et al*. found significantly lower levels in school-age children with CLD, compared with full-term, healthy matched controls and preterm children without CLD.[319] These low values were not explained by airway calibre: the children with CLD showed F_{ENO} values four times lower than those in a group of asthmatics with a comparable airflow limitation.[319] A study in younger children (mean age 18 months) revealed values to be higher in patients with CLD (and also CF) compared with controls.[320] Mieskonen *et al*, however, did not find any differences in F_{ENO} between controls and school-age children with CLD.[321]

1.6 Objectives of this Thesis

This two-part thesis first explores several methodological issues of F_{ENO} measurements (Part 1); next, the reader is presented with studies on clinical applications of F_{ENO} measurements in asthmatic children (Part 2).

Basically, the single breath online technique with constant expiratory flow is the preferred method to measure F_{ENO}. However, in young children this technique is not suitable as they are unable to perform the required manoeuvre. One of the major problems for young children is to keep the expiratory flow at a constant level. We developed a device that allowed for offline sampling of exhaled air into a mylar balloon for later analysis. A dynamic flow restrictor within the device makes it possible for the child to maintain a constant flow at minimal effort. We studied feasibility, reproducibility and normal values of F_{ENO} in children 4-8 years old, obtained with this device (Chapter 2).

As we used mylar balloons to store the exhaled air, we analyzed the influence of storage conditions such as temperature, humidity and storage time on NO values in the balloons (Chapter 3).

Several authors studied normal values in children and adults. In general these studies were in low numbers and applied different methodologies, which regrettably makes it difficult to compare findings. Before even considering using F_{ENO} measurements in the management of childhood asthma, we need reliable normal values – obtained in line with ATS/ ERS guidelines. Chapter 4 reports the results of a large multicentre, international reference values study, where more than 400 children were included.

Spirometry and exercise testing performed in adults just before F_{ENO} measurements will fleetingly reduce F_{ENO} values. It is not known whether such testing exerts effects on F_{ENO} in children as well. Yet it would be valuable to know if exercise affects children's F_{ENO} in any significant way, seeing that they are bound to play, jump and run about in the waiting room. Effects of exercise and spirometry on F_{ENO} measurements were evaluated in Chapter 5.

Most methodological aspects of F_{ENO} measurements have been settled by now and measurements are well standardised. As described in section 1.5.3, F_{ENO} is a valid, noninvasive, reproducible marker of eosinophilic airway inflammation. Treatment with ICS reduces F_{ENO} in a dose-dependent way, which makes F_{ENO} a potential guide to anti-inflammatory treatment in asthma. Thus, F_{ENO} may help to rationalize and improve steroid therapy in asthmatic children.

Almost half of asthmatic children seen in our outpatient clinic show elevated F_{ENO} values despite steroid treatment. In Chapter 6 we studied several factors that might explain these elevated values, including faulty inhaler technique and inadequate steroid dosing.

Some children will 'outgrow' their asthma. However, in the absence of objective criteria it is difficult to determine when to reduce or stop ICS. In current practice ICS are withdrawn if children are asymptomatic for a longer – often unspecified – period of time on a low dose of ICS. This practice, however, is known to carry the risk of asthma relapse. We speculated that F_{ENO} could give information that might reduce the risk of relapse after discontinuation of ICS. The results of our explorations of this topic can be found in Chapter 7.

Current asthma therapy in children is mainly adjusted to symptoms, and yet, as discussed in section 1.2, symptoms do not reflect inflammation. We hypothesized that if anti-inflammatory treatment were to be adjusted on F_{ENO} as a marker of asthmatic inflammation, outcome in children with asthma would be better. In the study described in Chapter 8, we treated one group of children on both F_{ENO} and

symptoms, and a second group on symptoms only, and compared outcomes after 1 year.

If 'inflammometry' – or: measuring inflammation – indeed improves asthma management, the doors are open to home measurements of FE_{NO}, offering the possibility to adjust ICS dose according to FE_{NO} in the home situation. New portable and less costly devices are available now. We studied the feasibility and variability of FE_{NO} measured at home with the NIOX MINO portable device. Chapter 9 reports the findings of this study.

REFERENCES

1. Mommers M, Gielkens-Sijstermans C, Swaen GM, van Schayck CP. Trends in the prevalence of respiratory symptoms and treatment in Dutch children over a 12 year period: results of the fourth consecutive survey. Thorax 2005;60:97-9.
2. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). Eur Respir J 1998;12:315-35.
3. Hess J, De Jongste JC. Epidemiological aspects of paediatric asthma. Clin Exp Allergy 2004;34:680-5.
4. von Mutius E. The burden of childhood asthma. Arch Dis Child 2000;82 Suppl 2:II2-5.
5. Milton B, Whitehead M, Holland P, Hamilton V. The social and economic consequences of childhood asthma across the lifecourse: a systematic review. Child Care Health Dev 2004;30:711-28.
6. National Asthma Campaign Asthma Audit Starting as we mean to go on: an audit of children's asthma in the UK. Asthma J 2002;8:1-11.
7. Woolcock AJ, Peat JK. Evidence for the increase in asthma worldwide. Ciba Found Symp 1997;206:122-34; discussion 134-9, 157-9.
8. Toelle BG, Ng K, Belousova E, Salome CM, Peat JK, Marks GB. Prevalence of asthma and allergy in schoolchildren in Belmont, Australia: three cross sectional surveys over 20 years. Bmj 2004;328:386-7.
9. Braun-Fahrlander C, Gassner M, Grize L, Takken-Sahli K, Neu U, Stricker T, Varonier HS, Wuthrich B, et al. No further increase in asthma, hay fever and atopic sensitisation in adolescents living in Switzerland. Eur Respir J 2004;23:407-13.
10. Ronchetti R, Villa MP, Barreto M, Rota R, Pagani J, Martella S, Falasca C, Paggi B, et al. Is the increase in childhood asthma coming to an end? Findings from three surveys of schoolchildren in Rome, Italy. Eur Respir J 2001;17:881-6.
11. Barnes PJ. Pathophysiology of asthma. Br J Clin Pharmacol 1996;42:3-10.
12. Djukanovic R, Roche WR, Wilson JW, Beasley CR, Twentyman OP, Howarth RH, Holgate ST. Mucosal inflammation in asthma. Am Rev Respir Dis 1990;142:434-57.
13. Bousquet J, Chanez P, Lacoste JY, Barneon G, Ghavanian N, Enander I, Venge P, Ahlstedt S, et al. Eosinophilic inflammation in asthma. N Engl J Med 1990;323:1033-9.
14. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med 2000;161:1720-45.
15. Bradley BL, Azzawi M, Jacobson M, Assoufi B, Collins JV, Irani AM, Schwartz LB, Durham SR, et al. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. J Allergy Clin Immunol 1991;88:661-74.
16. Vignola AM, Bousquet J, Chanez P, Gagliardo R, Merendino AM, Chiappara G, Bonsignore G. Assessment of airway inflammation in asthma. Am J Respir Crit Care Med 1998;157:S184-7.
17. Roche WR JP. Remodelling and inflammation. In: M. Silverman, ed. Childhood asthma and other wheezing disorders: Arnold, 2002:93-105.
18. Furuta GT, Nieuwenhuis EE, Karhausen J, Gleich G, Blumberg RS, Lee JJ, Ackerman SJ. Eosinophils alter colonic epithelial barrier function: role for major basic protein. Am J Physiol Gastrointest Liver Physiol 2005;289:G890-7.
19. Ayars GH, Altman LC, Gleich GJ, Loegering DA, Baker CB. Eosinophil- and eosinophil granule-mediated pneumocyte injury. J Allergy Clin Immunol 1985;76:595-604.
20. Tomassini M, Tsicopoulos A, Tai PC, Gruart V, Tonnel AB, Prin L, Capron A, Capron M. Release of granule proteins by eosinophils from allergic and nonallergic patients with eosinophilia on immunoglobulin-dependent activation. J Allergy Clin Immunol 1991;88:365-75.

21. Costa JJ, Matossian K, Resnick MB, Beil WJ, Wong DT, Gordon JR, Dvorak AM, Weller PF, et al. Human eosinophils can express the cytokines tumor necrosis factor-alpha and macrophage inflammatory protein-1 alpha. J Clin Invest 1993;91:2673-84.

22. Kita H, Ohnishi T, Okubo Y, Weiler D, Abrams JS, Gleich GJ. Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. J Exp Med 1991;174:745-8.

23. Wong DT, Elovic A, Matossian K, Nagura N, McBride J, Chou MY, Gordon JR, Rand TH, et al. Eosinophils from patients with blood eosinophilia express transforming growth factor beta 1. Blood 1991;78:2702-7.

24. Okayama Y, Kobayashi H, Ashman LK, Holgate ST, Church MK, Mori M. Activation of eosinophils with cytokines produced by lung mast cells. Int Arch Allergy Immunol 1997;114 Suppl 1:75-7.

25. Rothenberg M. Inflammatory effector cells/ cell migration. In Leung DY, Sampson HA, Geha RS, Szefler SJ., ed. Pediatric allergy. Principles and practice. Mosby, 2003:51-68.

26. Lambrecht BN. Allergen uptake and presentation by dendritic cells. Curr Opin Allergy Clin Immunol 2001;1:51-9.

27. Macaubas C DR, Umetsu DT. Immunology of the asthmatic response. In: Leung DY SH, Geha RS, Szefler SJ., ed. Pediatric allergy. Principles and practice. Mosby, 2003:337-349.

28. Riffo-Vasquez Y, Pitchford S, Spina D. Cytokines in airway inflammation. Int J Biochem Cell Biol 2000;32:833-53.

29. Wilson N. Measurement of airway inflammation in asthma. Curr Opin Pulm Med 2002;8:25-32.

30. Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophilic inflammation in severe persistent asthma. Am J Respir Crit Care Med 1999;160:1532-9.

31. Sur S, Crotty TB, Kephart GM, Hyma BA, Colby TV, Reed CE, Hunt LW, Gleich GJ. Sudden-onset fatal asthma. A distinct entity with few eosinophils and relatively more neutrophils in the airway submucosa? Am Rev Respir Dis 1993;148:713-9.

32. Fahy JV, Kim KW, Liu J, Boushey HA. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. J Allergy Clin Immunol 1995;95:843-52.

33. Carroll N, Carello S, Cooke C, James A. Airway structure and inflammatory cells in fatal attacks of asthma. Eur Respir J 1996;9:709-15.

34. Montefort S, Roche WR, Holgate ST. Bronchial epithelial shedding in asthmatics and non-asthmatics. Respir Med 1993;87 Suppl B:9-11.

35. Sacco O, Silvestri M, Sabatini F, Sale R, Defilippi AC, Rossi GA. Epithelial cells and fibroblasts: structural repair and remodelling in the airways. Paediatr Respir Rev 2004;5 Suppl A:S35-40.

36. British guideline on the management of asthma. Thorax 2003;58 Suppl 1:i1-94.

37. Duiverman EJ. Guideline 'Treating asthma in children' for pediatric pulmonologists (2nd revised edition). II. Medical treatment. Ned Tijdschr Geneeskd 2003;147:2501.

38. Revised GINA guidelines 2002: Global initiative for asthma, National Institutes of Health, National Heart, Lung and Blood Institute. NIH Publication No 02-3659 2002.

39. Bacharier LB, Strunk RC, Mauger D, White D, Lemanske RF Jr, Sorkness CA. Classifying asthma severity in children: mismatch between symptoms, medication use, and lung function. Am J Respir Crit Care Med 2004;170:426-32.

40. Rabe KF, Adachi M, Lai CK, Soriano JB, Vermeire PA, Weiss KB, Weiss ST. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. J Allergy Clin Immunol 2004;114:40-7.

41. Yoos HL, Kitzman H, McMullen A, Sidora-Arcoleo K, Anson E. The language of breathlessness: do families and health care providers speak the same language when describing asthma symptoms? J Pediatr Health Care 2005;19:197-205.

42. Teeter JG, Bleecker ER. Relationship between airway obstruction and respiratory symptoms in adult asthmatics. Chest 1998;113:272-7.

43. Roberts EM. Does your child have asthma? Parent reports and medication use for pediatric asthma. Arch Pediatr Adolesc Med 2003;157:449-55.

44. Halterman JS, McConnochie KM, Conn KM, Yoos HL, Kaczorowski JM, Holzhauer RJ, Allan M, Szilagyi PG. A potential pitfall in provider assessments of the quality of asthma control. Ambul Pediatr 2003;3:102-5.

45. Cabana MD, Slish KK, Nan B, Lin X, Clark NM. Asking the correct questions to assess asthma symptoms. Clin Pediatr (Phila) 2005;44:319-25.

46. Veen JC, Smits HH, Ravensberg AJ, Hiemstra PS, Sterk PJ, Bel EH. Impaired perception of dyspnea in patients with severe asthma. Relation to sputum eosinophils. Am J Respir Crit Care Med 1998;158:1134-41.

47. Kikuchi Y, Okabe S, Tamura G, Hida W, Homma M, Shirato K, Takishima T. Chemosensitivity and perception of dyspnea in patients with a history of near-fatal asthma. N Engl J Med 1994;330:1329-34.

48. Stirling RG, Kharitonov SA, Campbell D, Robinson DS, Durham SR, Chung KF, Barnes PJ. Increase in exhaled nitric oxide levels in patients with difficult asthma and correlation with symptoms and disease severity despite treatment with oral and inhaled corticosteroids. Asthma and Allergy Group. Thorax 1998;53:1030-4.

49. Horvath I, Barnes PJ. Exhaled monoxides in asymptomatic atopic subjects. Clin Exp Allergy 1999;29:1276-80.

50. Van den Toorn LM, Prins JB, Overbeek SE, Hoogsteden HC, De Jongste JC. Adolescents in clinical remission of atopic asthma have elevated exhaled nitric oxide levels and bronchial hyperresponsiveness. Am J Resp Crit Care Med 2000;162:953-7.

51. Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. Am J Respir Crit Care Med 2000;161:9-16.

52. Payne DN, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A. Relationship between Exhaled Nitric Oxide and Mucosal Eosinophilic Inflammation in Children with Difficult Asthma, after Treatment with Oral Prednisolone. Am J Respir Crit Care Med 2001;164:1376-81.

53. Van den Toorn LM, Overbeek SE, De Jongste JC, Leman K, Hoogsteden HC, Prins JB. Airway inflammation is present during clinical remission of atopic asthma. Am J Respir Crit Care Med 2001;164:2107-13.

54. Strunk RC, Szefler SJ, Phillips BR, Zeiger RS, Chinchilli VM, Larsen G, Hodgdon K, Morgan W, et al. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. J Allergy Clin Immunol 2003;112:883-92.

55. Wilson NM, James A, Uasuf C, Payne DN, Hablas H, Agrofioti C, Bush A. Asthma severity and inflammation markers in children. Pediatr Allergy Immunol 2001;12:125-32.

56. Wardlaw AJ, Brightling CE, Green R, Woltmann G, Bradding P, Pavord ID. New insights into the relationship between airway inflammation and asthma. Clin Sci (Lond) 2002;103:201-11.

57. Iredale MJ, Wanklyn SA, Phillips IP, Krausz T, Ind PW. Noninvasive assessment of bronchial inflammation in asthma: no correlation between eosinophilia of induced sputum and bronchial responsiveness to inhaled hypertonic saline. Clin Exp Allergy 1994;24:940-5.

58. Wilson NM, Bridge P, Spanevello A, Silverman M. Induced sputum in children: feasibility, repeatability, and relation of findings to asthma severity. Thorax 2000;55:768-74.

59. Boulet LP, Boulet V, Milot J. How should we quantify asthma control? A proposal. Chest 2002;122:2217-23.

60. de Kluijver J, Evertse CE, Schrumpf JA, van der Veen H, Zwinderman AH, Hiemstra PS, Rabe KF, Sterk PJ. Asymptomatic worsening of airway inflammation during low-dose allergen exposure in asthma: protection by inhaled steroids. Am J Respir Crit Care Med 2002;166:294-300.

61. Roberts G, Hurley C, Bush A, Lack G. Longitudinal study of grass pollen exposure, symptoms, and exhaled nitric oxide in childhood seasonal allergic asthma. Thorax 2004;59:752-6.

62. Sont JK, Han J, van Krieken JM, Evertse CE, Hooijer R, Willems LN, Sterk PJ. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. Thorax 1996;51:496-502.

63. Chang AB, Harrhy VA, Simpson J, Masters IB, Gibson PG. Cough, airway inflammation, and mild asthma exacerbation. Arch Dis Child 2002;86:270-5.

64. Parameswaran K, Pizzichini E, Pizzichini MM, Hussack P, Efthimiadis A, Hargreave FE. Clinical judgement of airway inflammation versus sputum cell counts in patients with asthma. Eur Respir J 2000;15:486-90.

65. Weiss ST, Van Natta ML, Zeiger RS. Relationship between increased airway responsiveness and asthma severity in the childhood asthma management program. Am J Respir Crit Care Med 2000;162:50-6.

66. Woolcock AJ. How does inflammation cause symptoms? Am J Respir Crit Care Med 1996;153:S21-2.

67. Grootendorst DC, Sont JK, Willems LN, Kluin-Nelemans JC, Van Krieken JH, Veselic-Charvat M, Sterk PJ. Comparison of inflammatory cell counts in asthma: induced sputum vs bronchoalveolar lavage and bronchial biopsies. Clin Exp Allergy 1997;27:769-79.

68. Crimi E, Spanevello A, Neri M, Ind PW, Rossi GA, Brusasco V. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. Am J Respir Crit Care Med 1998;157:4-9.

69. Turner MO, Hussack P, Sears MR, Dolovich J, Hargreave FE. Exacerbations of asthma without sputum eosinophilia. Thorax 1995;50:1057-61.

70. Oddera S, Silvestri M, Balbo A, Jovovich BO, Penna R, Crimi E, Rossi GA. Airway eosinophilic inflammation, epithelial damage, and bronchial hyperresponsiveness in patients with mild-moderate, stable asthma. Allergy 1996;51:100-7.

71. Boulet LP, Turcotte H, Brochu A. Persistence of airway obstruction and hyperresponsiveness in subjects with asthma remission. Chest 1994;105:1024-31.

72. Prehn A, Seger RA, Faber J, Torresani T, Molinari L, Gerber A, Sennhauser FH. The relationship of serum-eosinophil cationic protein and eosinophil count to disease activity in children with bronchial asthma. Pediatr Allergy Immunol 1998;9:197-203.

73. Gibson PG, Saltos N, Borgas T. Airway mast cells and eosinophils correlate with clinical severity and airway hyperresponsiveness in corticosteroid-treated asthma. J Allergy Clin Immunol 2000;105:752-9.

74. Haahtela T, Jarvinen M, Kava T, Kiviranta K, Koskinen S, Lehtonen K, Nikander K, Persson T, et al. Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma. N Engl J Med 1994;331:700-5.

75. Pohunek P. Inflammation and airway remodeling. Pediatr Pulmonol Suppl 2004;26:98-9.

76. Djukanovic R. Airway inflammation in asthma and its consequences: implications for treatment in children and adults. J Allergy Clin Immunol 2002;109:S539-48.

77. James A. Airway remodeling in asthma. Curr Opin Pulm Med 2005;11:1-6.

78. Fahy JV, Corry DB, Boushey HA. Airway inflammation and remodeling in asthma. Curr Opin Pulm Med 2000;6:15-20.

79. Baldwin L, Roche WR. Does remodelling of the airway wall precede asthma? Paediatr Respir Rev 2002;3:315-20.

80. Jeffery PK. Remodeling in asthma and chronic obstructive lung disease. Am J Respir Crit Care Med 2001;164:S28-38.

81. Pascual RM, Peters SP. Airway remodeling contributes to the progressive loss of lung function in asthma: An overview. J Allergy Clin Immunol 2005;116:477-86.

82. Cokugras H, Akcakaya N, Seckin, Camcioglu Y, Sarimurat N, Aksoy F. Ultrastructural examination of bronchial biopsy specimens from children with moderate asthma. Thorax 2001;56:25-9.

83. Barbato A, Turato G, Baraldo S, Bazzan E, Calabrese F, Tura M, Zuin R, Beghe B, et al. Airway inflammation in childhood asthma. Am J Respir Crit Care Med 2003;168:798-803.

84. Payne DN, Rogers AV, Adelroth E, Bandi V, Guntupalli KK, Bush A, Jeffery PK. Early thickening of the reticular basement membrane in children with difficult asthma. Am J Respir Crit Care Med 2003;167:78-82.

85. Jenkins HA, Cool C, Szefler SJ, Covar R, Brugman S, Gelfand EW, Spahn JD. Histopathology of severe childhood asthma: a case series. Chest 2003;124:32-41.

86. Pohunek P, Warner JO, Turzikova J, Kudrmann J, Roche WR. Markers of eosinophilic inflammation and tissue re-modelling in children before clinically diagnosed bronchial asthma. Pediatr Allergy Immunol 2005;16:43-51.

87. Saglani S, Malmstrom K, Pelkonen AS, Malmberg LP, Lindahl H, Kajosaari M, Turpeinen M, Rogers AV, et al. Airway remodeling and inflammation in symptomatic infants with reversible airflow obstruction. Am J Respir Crit Care Med 2005;171:722-7.

88. Grol MH, Gerritsen J, Vonk JM, Schouten JP, Koeter GH, Rijcken B, Postma DS. Risk factors for growth and decline of lung function in asthmatic individuals up to age 42 years. A 30-year follow-up study. Am J Respir Crit Care Med 1999;160:1830-7.

89. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med 1995;332:133-8.

90. Corne JM, Marshall C, Smith S, Schreiber J, Sanderson G, Holgate ST, Johnston SL. Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. Lancet 2002;359:831-4.

91. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002;360:1715-21.

92. Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. Am J Respir Crit Care Med 1999;159:1043-51.

93. Sears MR. Increasing asthma mortality--fact or artifact? J Allergy Clin Immunol 1988;82:957-60.

94. Payne D, McKenzie SA, Stacey S, Misra D, Haxby E, Bush A. Safety and ethics of bronchoscopy and endobronchial biopsy in difficult asthma. Arch Dis Child 2001;84:423-6.

95. Faul JL, Demers EA, Burke CM, Poulter LW. The reproducibility of repeat measures of airway inflammation in stable atopic asthma. Am J Respir Crit Care Med 1999;160:1457-61.

96. Haahtela T, Laitinen A, Laitinen LA. Using biopsies in the monitoring of inflammation in asthmatic patients. Allergy 1993;48:65-9.

97. Visner GA, Faro A, Zander DS. Role of transbronchial biopsies in pediatric lung diseases. Chest 2004;126:273-80.

98. Romagnoli M, Vachier I, Vignola AM, Godard P, Bousquet J, Chanez P. Safety and cellular assessment of bronchial brushing in airway diseases. Respir Med 1999;93:461-6.

99. Twaddell SH, Gibson PG, Carty K, Woolley KL, Henry RL. Assessment of airway inflammation in children with acute asthma using induced sputum. Eur Respir J 1996;9:2104-8.

100. Norzila MZ, Fakes K, Henry RL, Simpson J, Gibson PG. Interleukin-8 secretion and neutrophil recruitment accompanies induced sputum eosinophil activation in children with acute asthma. Am J Respir Crit Care Med 2000;161:769-74.

101. Piacentini GL, Vicentini L, Mazzi P, Chilosi M, Martinati L, Boner AL. Mite-antigen avoidance can reduce bronchial epithelial shedding in allergic asthmatic children. Clin Exp Allergy 1998;28:561-7.

102. Oh JW, Lee HB, Kim CR, Yum MK, Koh YJ, Moon SJ, Kang JO, Park IK. Analysis of induced sputum to examine the effects of inhaled corticosteroid on airway inflammation in children with asthma. Ann Allergy Asthma Immunol 1999;82:491-6.

103. Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA, Hargreave FE, Dolovich J. Use of induced sputum cell counts to investigate airway inflammation in asthma. Thorax 1992;47:25-9.

104. Gibson PG, Wlodarczyk JW, Hensley MJ, Gleeson M, Henry RL, Cripps AW, Clancy RL. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. Am J Respir Crit Care Med 1998;158:36-41.

105. Lex C, Payne DN, Zacharasiewicz A, Li AM, Wilson NM, Hansel TT, Bush A. Sputum induction in children with difficult asthma: safety, feasibility, and inflammatory cell pattern. Pediatr Pulmonol 2005;39:318-24.

106. Cai Y, Carty K, Henry RL, Gibson PG. Persistence of sputum eosinophilia in children with controlled asthma when compared with healthy children. Eur Respir J 1998;11:848-53.

107. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. Thorax 1998;53:91-5.

108. Fahy JV, Wong H, Liu J, Boushey HA. Comparison of samples collected by sputum induction and bronchoscopy from asthmatic and healthy subjects. Am J Respir Crit Care Med 1995;152:53-8.

109. Pizzichini E, Pizzichini MM, Kidney JC, Efthimiadis A, Hussack P, Popov T, Cox G, Dolovich J, O'Byrne P, Hargreave FE. Induced sputum, bronchoalveolar lavage and blood from mild asthmatics: inflammatory cells, lymphocyte subsets and soluble markers compared. Eur Respir J 1998;11:828-34.

110. Jatakanon A, Kharitonov S, Lim S, Barnes PJ. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. Thorax 1999;54:108-14.

111. in't Veen JC, Smits HH, Hiemstra PS, Zwinderman AE, Sterk PJ, Bel EH. Lung function and sputum characteristics of patients with severe asthma during an induced exacerbation by double-blind steroid withdrawal. Am J Respir Crit Care Med 1999;160:93-9.

112. Ronchi MC, Piragino C, Rosi E, Stendardi L, Tanini A, Galli G, Duranti R, Scano G. Do sputum eosinophils and ECP relate to the severity of asthma? Eur Respir J 1997;10:1809-13.

113. Park JW, Whang YW, Kim CW, Park YB, Hong CS. Eosinophil count and eosinophil cationic protein concentration of induced sputum in the diagnosis and assessment of airway inflammation in bronchial asthma. Allergy Asthma Proc 1998;19:61-7.

114. Silkoff PE. Noninvasive measurement of airway inflammation using exhaled nitric oxide and induced sputum. Current status and future use. Clin Chest Med 2000;21:345-60.

115. Ulrik CS. Eosinophils and pulmonary function: an epidemiologic study of adolescents and young adults. Ann Allergy Asthma Immunol 1998;80:487-93.

116. Koller DY, Herouy Y, Gotz M, Hagel E, Urbanek R, Eichler I. Clinical value of monitoring eosinophil activity in asthma. Arch Dis Child 1995;73:413-7.

117. Zimmerman B, Lanner A, Enander I, Zimmerman RS, Peterson CG, Ahlstedt S. Total blood eosinophils, serum eosinophil cationic protein and eosinophil protein X in childhood asthma: relation to disease status and therapy. Clin Exp Allergy 1993;23:564-70.

118. Carlsen KH, Halvorsen R, Pettersen M, Carlsen KC. Inflammation markers and symptom activity in children with bronchial asthma. Influence of atopy and eczema. Pediatr Allergy Immunol 1997;8:112-20.

119. Remes S, Korppi M, Remes K, Savolainen K, Mononen I, Pekkanen J. Serum eosinophil cationic protein (ECP) and eosinophil protein X (EPX) in childhood asthma: the influence of atopy. Pediatr Pulmonol 1998;25:167-74.

120. Zimmerman B, Enander I, Zimmerman R, Ahlstedt S. Asthma in children less than 5 years of age: eosinophils and serum levels of the eosinophil proteins ECP and EPX in relation to atopy and symptoms. Clin Exp Allergy 1994;24:149-55.

121. Niimi A, Amitani R, Suzuki K, Tanaka E, Murayama T, Kuze F. Serum eosinophil cationic protein as a marker of eosinophilic inflammation in asthma. Clin Exp Allergy 1998;28:233-40.

122. Adelroth E, Rosenhall L, Johansson SA, Linden M, Venge P. Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage. The effects of antiasthmatic treatment with budesonide or terbutaline. Am Rev Respir Dis 1990;142:91-9.

123. Rao R, Frederick JM, Enander I, Gregson RK, Warner JA, Warner JO. Airway function correlates with circulating eosinophil, but not mast cell, markers of inflammation in childhood asthma. Clin Exp Allergy 1996;26:789-93.

124. Virchow JC, Jr., Holscher U, Virchow C, Sr. Sputum ECP levels correlate with parameters of airflow obstruction. Am Rev Respir Dis 1992;146:604-6.

125. Claman DM, Boushey HA, Liu J, Wong H, Fahy JV. Analysis of induced sputum to examine the effects of prednisone on airway inflammation in asthmatic subjects. J Allergy Clin Immunol 1994;94:861-9.

126. Hoekstra MO. Can eosinophil-derived proteins be used to diagnose or to monitor childhood asthma? Clin Exp Allergy 1999;29:873-4.

127. Twentyman OP, Sams VR, Holgate ST. Albuterol and nedocromil sodium affect airway and leukocyte responses to allergen. Am Rev Respir Dis 1993;147:1425-30.

128. Koshino T, Morita Y, Ito K, Teshima S, Sano Y. Activation of bone marrow eosinophils in asthma. Chest 1993;103:1931-2.

129. Ahlstedt S. Clinical application of eosinophilic cationic protein in asthma. Allergy Proc 1995;16:59-62.

130. Pizzichini E, Pizzichini MM, Efthimiadis A, Dolovich J, Hargreave FE. Measuring airway inflammation in asthma: eosinophils and eosinophilic cationic protein in induced sputum compared with peripheral blood. J Allergy Clin Immunol 1997;99:539-44.

131. Joos GF, O'Connor B, Anderson SD, Chung F, Cockcroft DW, Dahlen B, DiMaria G, Foresi A, et al. Indirect airway challenges. Eur Respir J 2003;21:1050-68.

132. Prieto L, Bruno L, Gutierrez V, Uixera S, Perez-Frances C, Lanuza A, Ferrer A. Airway responsiveness to adenosine 5'-monophosphate and exhaled nitric oxide measurements: predictive value as markers for reducing the dose of inhaled corticosteroids in asthmatic subjects. Chest 2003;124:1325-33.

133. Gronke L, Kanniess F, Holz O, Jorres RA, Magnussen H. The relationship between airway hyper-responsiveness, markers of inflammation and lung function depends on the duration of the asthmatic disease. Clin Exp Allergy 2002;32:57-63.

134. Bavbek S, Demirel YS, Erekul S, Kalayctoglu O, Beder S, Misirligil Z, Gurbuz L. The mechanism of bronchial hyperreactivity in allergic rhinitis patients. A light microscopic study on BAL and bronchial biopsy. Allergol Immunopathol (Madr) 1996;24:45-53.

135. Jöbsis Q, Raatgeep HC, Schellekens SL, Hop WC, Hermans PW, de Jongste JC. Hydrogen peroxide in exhaled air of healthy children: reference values. Eur Respir J 1998;12:483-5.

136. Jöbsis Q, Raatgeep HC, Hermans PW, de Jongste JC. Hydrogen peroxide in exhaled air is increased in stable asthmatic children. Eur Respir J 1997;10:519-21.

137. Horvath I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, Becher G, van Beurden WJ, et al. Exhaled breath condensate: methodological recommendations and unresolved questions. Eur Respir J 2005;26:523-48.

138. Effros RM, Dunning MB, 3rd, Biller J, Shaker R. The promise and perils of exhaled breath condensates. Am J Physiol Lung Cell Mol Physiol 2004;287:L1073-80.

139. Hunt J. Exhaled breath condensate: an evolving tool for noninvasive evaluation of lung disease. J Allergy Clin Immunol 2002;110:28-34.

140. Gaston B, Drazen JM, Loscalzo J, Stamler JS. The biology of nitrogen oxides in the airways. Am J Respir Crit Care Med 1994;149:538-51.

141. Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. Am J Respir Crit Care Med 2001;163:1693-722.

142. Ricciardolo FL, Sterk PJ, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. Physiol Rev 2004;84:731-65.

143. Forstermann U, Schmidt HH, Pollock JS, Sheng H, Mitchell JA, Warner TD, Nakane M, Murad F. Isoforms of nitric oxide synthase. Characterization and purification from different cell types. Biochem Pharmacol 1991;42:1849-57.

144. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci U S A 1987;84:9265-9.

145. Morris SM, Jr., Billiar TR. New insights into the regulation of inducible nitric oxide synthesis. Am J Physiol 1994;266:E829-39.

146. Hamid Q, Springall DR, Riveros-Moreno V, Chanez P, Howarth P, Redington A, Bousquet J, Godard P, Holgate S, Polak JM. Induction of nitric oxide synthase in asthma. Lancet 1993;342:1510-3.

147. Asano K, Chee CB, Gaston B, Lilly CM, Gerard C, Drazen JM, Stamler JS. Constitutive and inducible nitric oxide synthase gene expression, regulation, and activity in human lung epithelial cells. Proc Natl Acad Sci U S A 1994;91:10089-93.

148. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993;329:2002-12.

149. Hamad AM, Clayton A, Islam B, Knox AJ. Guanylyl cyclases, nitric oxide, natriuretic peptides, and airway smooth muscle function. Am J Physiol Lung Cell Mol Physiol 2003;285:L973-83.

150. Ricciardolo FL. Multiple roles of nitric oxide in the airways. Thorax 2003;58:175-82.

151. Barnes PJ, Belvisi MG. Nitric oxide and lung disease. Thorax 1993;48:1034-43.

152. 152. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991;43:109-42.

153. ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005. Am J Respir Crit Care Med 2005;171:912-930.

154. Gustafsson LE, Leone AM, Persson MG, Wiklund NP, Moncada S. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. Biochem Biophys Res Commun 1991;181:852-7.

155. Baraldi E, De Jongste JC. Measurement of exhaled nitric oxide in children-2001. Joint ERS/ATS Task Force on Exhaled NO Measurement in Children. Eur Respir J 2002;20:223-37.

156. Kroesbergen A, Jöbsis Q, Bel EH, Hop WC, de Jongste JC. Flow-dependency of exhaled nitric oxide in children with asthma and cystic fibrosis. Eur Respir J 1999;14:871-5.

157. Silkoff PE, McClean PA, Slutsky AS, Furlott HG, Hoffstein E, Wakita S, Chapman KR, Szalai JP, et al. Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. Am J Respir Crit Care Med 1997;155:260-7.

158. Kimberly B, Nejadnik B, Giraud GD, Holden WE. Nasal contribution to exhaled nitric oxide at rest and during breathholding in humans. Am J Respir Crit Care Med 1996;153:829-36.

159. Lundberg JO, Rinder J, Weitzberg E, Lundberg JM, Alving K. Nasally exhaled nitric oxide in humans originates mainly in the paranasal sinuses. Acta Physiol Scand 1994;152:431-2.

160. Kharitonov SA, Barnes PJ. Nasal contribution to exhaled nitric oxide during exhalation against resistance or during breath holding. Thorax 1997;52:540-4.

161. Piacentini GL, Bodini A, Vino L, Zanolla L, Costella S, Vicentini L, Boner AL. Influence of environmental concentrations of NO on the exhaled NO test. Am J Respir Crit Care Med 1998;158:1299-301.

162. Baraldi E, Azzolin NM, Dario C, Carra S, Ongaro R, Biban P, Zacchello F. Effect of atmospheric nitric oxide (NO) on measurements of exhaled NO in asthmatic children. Pediatr Pulmonol 1998;26:30-4.

163. Franklin PJ, Taplin R, Stick SM. A community study of exhaled nitric oxide in healthy children. Am J Respir Crit Care Med 1999;159:69-73.

164. Avital A, Uwyyed K, Berkman N, Bar-Yishay E, Godfrey S, Springer C. Exhaled nitric oxide is age-dependent in asthma. Pediatr Pulmonol 2003;36:433-8.

165. Jilma B, Kastner J, Mensik C, Vondrovec B, Hildebrandt J, Krejcy K, Wagner OF, Eichler HG. Sex differences in concentrations of exhaled nitric oxide and plasma nitrate. Life Sci 1996;58:469-76.

166. Franklin PJ, Stick SM, Le Souef PN, Ayres JG, Turner SW. Measuring exhaled nitric oxide levels in adults: the importance of atopy and airway responsiveness. Chest 2004;126:1540-5.

167. Grasemann H, Storm van's Gravesande K, Buscher R, Drazen JM, Ratjen F. Effects of sex and of gene variants in constitutive nitric oxide synthases on exhaled nitric oxide. Am J Respir Crit Care Med 2003;167:1113-6.

168. Wong GW, Liu EK, Leung TF, Yung E, Ko FW, Hui DS, Fok TF, Lai CK. High levels and gender difference of exhaled nitric oxide in Chinese schoolchildren. Clin Exp Allergy 2005;35:889-93.

169. Silkoff PE, Wakita S, Chatkin J, Ansarin K, Gutierrez C, Caramori M, McClean P, Slutsky AS, Zamel N, Chapman KR. Exhaled nitric oxide after beta2-agonist inhalation and spirometry in asthma. Am J Respir Crit Care Med 1999;159:940-4.

170. Persson MG, Wiklund NP, Gustafsson LE. Endogenous nitric oxide in single exhalations and the change during exercise. Am Rev Respir Dis 1993;148:1210-4.

171. Phillips CR, Giraud GD, Holden WE. Exhaled nitric oxide during exercise: site of release and modulation by ventilation and blood flow. J Appl Physiol 1996;80:1865-71.

172. Deykin A, Massaro AF, Coulston E, Drazen JM, Israel E. Exhaled nitric oxide following repeated spirometry or repeated plethysmography in healthy individuals. Am J Respir Crit Care Med 2000;161:1237-40.

173. Deykin A, Halpern O, Massaro AF, Drazen JM, Israel E. Expired nitric oxide after bronchoprovocation and repeated spirometry in patients with asthma. Am J Respir Crit Care Med 1998;157:769-75.

174. Kissoon N, Duckworth LJ, Blake KV, Murphy SP, Lima JJ. Effect of beta2-agonist treatment and spirometry on exhaled nitric oxide in healthy children and children with asthma. Pediatr Pulmonol 2002;34:203-8.

175. Piacentini GL, Bodini A, Peroni DG, Miraglia del Giudice M Jr, Costella S, Boner AL. Reduction in exhaled nitric oxide immediately after methacholine challenge in asthmatic children. Thorax 2002;57:771-3.

176. Beier J, Beeh KM, Kornmann O, Buhl R. Sputum induction leads to a decrease of exhaled nitric oxide unrelated to airflow. Eur Respir J 2003;22:354-7.

177. Garnier P, Fajac I, Dessanges JF, Dall'Ava-Santucci J, Lockhart A, Dinh-Xuan AT. Exhaled nitric oxide during acute changes of airways calibre in asthma. Eur Respir J 1996;9:1134-8.

178. de Gouw HW, Hendriks J, Woltman AM, Twiss IM, Sterk PJ. Exhaled nitric oxide (NO) is reduced shortly after bronchoconstriction to direct and indirect stimuli in asthma. Am J Respir Crit Care Med 1998;158:315-9.

179. Yates DH, Kharitonov SA, Barnes PJ. Effect of short- and long-acting inhaled beta2-agonists on exhaled nitric oxide in asthmatic patients. Eur Respir J 1997;10:1483-8.

180. Ho LP, Wood FT, Robson A, Innes JA, Greening AP. The current single exhalation method of measuring exhaled nitric oxide is affected by airway calibre. Eur Respir J 2000;15:1009-1013.

181. Terada A, Fujisawa T, Togashi K, Miyazaki T, Katsumata H, Atsuta J, Iguchi K, Kamiya H, et al. Exhaled nitric oxide decreases during exercise-induced bronchoconstriction in children with asthma. Am J Respir Crit Care Med 2001;164:1879-84.

182. Persson MG, Gustafsson LE. Ethanol can inhibit nitric oxide production. Eur J Pharmacol 1992;224:99-100.

183. Jones AW, Fransson M, Maldonado-Holmertz E. Does consumption of ethanol distort measurements of exhaled nitric oxide? Respir Med 2005;99:196-9.

184. Persson MG, Cederqvist B, Wiklund CU, Gustafsson LE. Ethanol causes decrements in airway excretion of endogenous nitric oxide in humans. Eur J Pharmacol 1994;270:273-8.

185. Yates DH, Kharitonov SA, Robbins RA, Thomas PS, Barnes PJ. The effect of alcohol ingestion on exhaled nitric oxide. Eur Respir J 1996;9:1130-3.

186. Taylor ES, Smith AD, Cowan JO, Herbison GP, Taylor DR. Effect of caffeine ingestion on exhaled nitric oxide measurements in patients with asthma. Am J Respir Crit Care Med 2004;169:1019-21.

187. Bruce C, Yates DH, Thomas PS. Caffeine decreases exhaled nitric oxide. Thorax 2002;57:361-3.

188. Warke TJ, Shields MD, Finnegan J. Caffeine and exhaled nitric oxide. Thorax 2003;58:281.

189. Kharitonov SA, Robbins RA, Yates D, Keatings V, Barnes PJ. Acute and chronic effects of cigarette smoking on exhaled nitric oxide. Am J Respir Crit Care Med 1995;152:609-12.

190. Maniscalco M, Di Mauro V, Farinaro E, Carratu L, Sofia M. Transient decrease of exhaled nitric oxide after acute exposure to passive smoke in healthy subjects. Arch Environ Health 2002;57:437-40.

191. Yates DH, Breen H, Thomas PS. Passive smoke inhalation decreases exhaled nitric oxide in normal subjects. Am J Respir Crit Care Med 2001;164:1043-6.

192. Warke TJ, Mairs V, Fitch PS, Ennis M, Shields MD. Possible association between passive smoking and lower exhaled nitric oxide in asthmatic children. Arch Environ Health 2003;58:613-6.

193. Hoyt JC, Robbins RA, Habib M, Springall DR, Buttery LD, Polak JM, Barnes PJ. Cigarette smoke decreases inducible nitric oxide synthase in lung epithelial cells. Exp Lung Res 2003;29:17-28.

194. ten Hacken NH, van der Vaart H, van der Mark TW, Koeter GH, Postma DS. Exhaled nitric oxide is higher both at day and night in subjects with nocturnal asthma. Am J Respir Crit Care Med 1998;158:902-7.

195. Mattes J, van's Gravesande KS, Moeller C, Moseler M, Brandis M, Kuehr J. Circadian variation of exhaled nitric oxide and urinary eosinophil protein X in asthmatic and healthy children. Pediatr Res 2002;51:190-4.

196. Georges G, Bartelson BB, Martin RJ, Silkoff PE. Circadian variation in exhaled nitric oxide in nocturnal asthma. J Asthma 1999;36:467-73.

197. de Gouw HW, Grunberg K, Schot R, Kroes AC, Dick EC, Sterk PJ. Relationship between exhaled nitric oxide and airway hyperresponsiveness following experimental rhinovirus infection in asthmatic subjects. Eur Respir J 1998;11:126-32.

198. Murphy AW, Platts-Mills TA, Lobo M, Hayden F. Respiratory nitric oxide levels in experimental human influenza. Chest 1998;114:452-6.

199. Kharitonov SA, Yates D, Barnes PJ. Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. Eur Respir J 1995;8:295-7.

200. Jöbsis Q, Schellekens SL, Kroesbergen A, Hop WC, de Jongste JC. Sampling of exhaled nitric oxide in children: end-expiratory plateau, balloon and tidal breathing methods compared. Eur Respir J 1999;13:1406-10.

201. Baraldi E, Dario C, Ongaro R, Scollo M, Azzolin NM, Panza N, Paganini N, Zacchello F. Exhaled nitric oxide concentrations during treatment of wheezing exacerbation in infants and young children. Am J Respir Crit Care Med 1999;159:1284-8.

202. Wildhaber JH, Hall GL, Stick SM. Measurements of exhaled nitric oxide with the single-breath technique and positive expiratory pressure in infants. Am J Respir Crit Care Med 1999;159:74-8.

203. Baraldi E, Scollo M, Zaramella C, Zanconato S, Zacchello F. A simple flow-driven method for online measurement of exhaled NO starting at the age of 4 to 5 years. Am J Respir Crit Care Med 2000;162:1828-32.

204. Artlich A, Jonsson B, Bhiladvala M, Lonnqvist PA, Gustafsson LE. Single breath analysis of endogenous nitric oxide in the newborn. Biol Neonate 2001;79:21-6.

205. Jöbsis Q, Raatgeep HC, Hop WC, de Jongste JC. Controlled low flow off line sampling of exhaled nitric oxide in children. Thorax 2001;56:285-9.

206. Ratjen F, Kavuk I, Gartig S, Wiesemann HG, Grasemann H. Airway nitric oxide in infants with acute wheezy bronchitis. Pediatr Allergy Immunol 2000;11:230-5.

207. Buchvald F, Bisgaard H. FE_{NO} measured at fixed exhalation flow rate during controlled tidal breathing in children from the age of 2 yr. Am J Respir Crit Care Med 2001;163:699-704.

208. Daniel PF, Klug B, Valerius NH. Measurement of exhaled nitric oxide in young children during tidal breathing through a facemask. Pediatr Allergy Immunol 2005;16:248-53.

209. Jöbsis Q, Schellekens SL, Kroesbergen A, Hop WC, de Jongste JC. Offline sampling of exhaled air for nitric oxide measurement in children: methodological aspects. Eur Respir J 2001;17:898-903.

210. Kharitonov SA, Gonio F, Kelly C, Meah S, Barnes PJ. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. Eur Respir J 2003;21:433-8.

211. Buchvald F, Bisgaard H. Comparisons of the complementary effect on exhaled nitric oxide of salmeterol vs montelukast in asthmatic children taking regular inhaled budesonide. Ann Allergy Asthma Immunol 2003;91:309-13.

212. Kissoon N, Duckworth LJ, Blake KV, Murphy SP, Taylor CL, DeNicola LR, Silkoff PE. Exhaled nitric oxide concentrations: Online versus offline values in healthy children. Pediatr Pulmonol 2002;33:283-92.

213. Ekroos H, Karjalainen J, Sarna S, Laitinen LA, Sovijarvi AR. Short-term variability of exhaled nitric oxide in young male patients with mild asthma and in healthy subjects. Respir Med 2002;96:895-900.

214. Gabbay E, Fisher AJ, Small T, Leonard AJ, Corris PA. Exhaled single-breath nitric oxide measurements are reproducible, repeatable and reflect levels of nitric oxide found in the lower airways. Eur Respir J 1998;11:467-72.

215. Olin AC, Alving K, Toren K. Exhaled nitric oxide: relation to sensitization and respiratory symptoms. Clin Exp Allergy 2004;34:221-6.

216. Pedroletti C, Zetterquist W, Nordvall L, Alving K. Evaluation of exhaled nitric oxide in schoolchildren at different exhalation flow rates. Pediatr Res 2002;52:393-8.

217. Baraldi E, Azzolin NM, Cracco A, Zacchello F. Reference values of exhaled nitric oxide for healthy children 6-15 years old. Pediatr Pulmonol 1999;27:54-8.

218. Maniscalco M, Vatrella A, Sofia M. Passive smoke and exhaled nitric oxide. Am J Respir Crit Care Med 2002;165:1188; author reply 1188.

219. Mills PC, Marlin DJ, Demoncheaux E, Scott C, Casas I, Smith NC, Higenbottam T. Nitric oxide and exercise in the horse. J Physiol 1996;495:863-74.

220. Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. Eur Respir J 1993;6:1368-70.

221. Byrnes CA, Dinarevic S, Shinebourne EA, Barnes PJ, Bush A. Exhaled nitric oxide measurements in normal and asthmatic children. Pediatr Pulmonol 1997;24:312-8.

222. Baraldi E, Azzolin NM, Zanconato S, Dario C, Zacchello F. Corticosteroids decrease exhaled nitric oxide in children with acute asthma. J Pediatr 1997;131:381-5.

223. Lundberg JO, Nordvall SL, Weitzberg E, Kollberg H, Alving K. Exhaled nitric oxide in paediatric asthma and cystic fibrosis. Arch Dis Child 1996;75:323-6.

224. Dotsch J, Demirakca S, Terbrack HG, Huls G, Rascher W, Kuhl PG. Airway nitric oxide in asthmatic children and patients with cystic fibrosis. Eur Respir J 1996;9:2537-40.

225. Artlich A, Hagenah JU, Jonas S, Ahrens P, Gortner L. Exhaled nitric oxide in childhood asthma. Eur J Pediatr 1996;155:698-701.

226. Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. Lancet 1994;343:133-5.

227. Kharitonov SA, Chung KF, Evans D, O'Connor BJ, Barnes PJ. Increased exhaled nitric oxide in asthma is mainly derived from the lower respiratory tract. Am J Respir Crit Care Med 1996;153:1773-80.

228. Nelson BV, Sears S, Woods J, Ling CY, Hunt J, Clapper LM, Gaston B. Expired nitric oxide as a marker for childhood asthma. J Pediatr 1997;130:423-7.

229. Hunt J, Gaston B. Airway nitrogen oxide measurements in asthma and other pediatric respiratory diseases. J Pediatr 2000;137:14-20.

230. Persson MG, Zetterstrom O, Agrenius V, Ihre E, Gustafsson LE. Single-breath nitric oxide measurements in asthmatic patients and smokers. Lancet 1994;343:146-7.

231. Mattes J, Storm van's Gravesande K, Reining U, Alving K, Ihorst G, Henschen M, Kuehr J. NO in exhaled air is correlated with markers of eosinophilic airway inflammation in corticosteroid-dependent childhood asthma. Eur Respir J 1999;13:1391-5.

232. Piacentini GL, Bodini A, Costella S, Vicentini L, Mazzi P, Sperandio S, Boner AL. Exhaled nitric oxide and sputum eosinophil markers of inflammation in asthmatic children. Eur Respir J 1999;13:1386-90.

233. Lim S, Jatakanon A, John M, Gilbey T, O'Connor B J, Chung KF, Barnes PJ. Effect of inhaled budesonide on lung function and airway inflammation. Assessment by various inflammatory markers in mild asthma. Am J Respir Crit Care Med 1999;159:22-30.

234. Piacentini GL, Bodini A, Costella S, Suzuki Y, Zerman L, Peterson CG, Boner AL. Exhaled nitric oxide, serum ECP and airway responsiveness in mild asthmatic children. Eur Respir J 2000;15:839-43.

235. Tsujino I, Nishimura M, Kamachi A, Makita H, Munakata M, Miyamoto K, Kawakami Y. Exhaled Nitric Oxide - Is It Really a Good Marker of Airway Inflammation in Bronchial Asthma? Respiration 2000;67:645-651.

236. Berlyne GS, Parameswaran K, Kamada D, Efthimiadis A, Hargreave FE. A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation. J Allergy Clin Immunol 2000;106:638-44.

237. Gibson PG, Henry RL, Thomas P. Noninvasive assessment of airway inflammation in children: induced sputum, exhaled nitric oxide, and breath condensate. Eur Respir J 2000;16:1008-15.

238. Lim S, Jatakanon A, Meah S, Oates T, Chung KF, Barnes PJ. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in mild to moderately severe asthma. Thorax 2000;55:184-188.

239. Turktas H, Oguzulgen K, Kokturk N, Memis L, Erbas D. Correlation of exhaled nitric oxide levels and airway inflammation markers in stable asthmatic patients. J Asthma 2003;40:425-30.

240. Saito J, Inoue K, Sugawara A, Yoshikawa M, Watanabe K, Ishida T, Ohtsuka Y, Munakata M. Exhaled nitric oxide as a marker of airway inflammation for an epidemiologic study in schoolchildren. J Allergy Clin Immunol 2004;114:512-6.

241. Franklin PJ, Turner SW, Le Souef PN, Stick SM. Exhaled nitric oxide and asthma: complex interactions between atopy, airway responsiveness, and symptoms in a community population of children. Thorax 2003;58:1048-52.

242. Cardinale F, de Benedictis FM, Muggeo V, Giordano P, Loffredo MS, Iacoviello G, Armenio L. Exhaled nitric oxide, total serum IgE and allergic sensitization in childhood asthma and allergic rhinitis. Pediatr Allergy Immunol 2005;16:236-42.

243. Jouaville LF, Annesi-Maesano I, Nguyen LT, Bocage AS, Bedu M, Caillaud D. Interrelationships among asthma, atopy, rhinitis and exhaled nitric oxide in a population-based sample of children. Clin Exp Allergy 2003;33:1506-11.

244. Barreto M, Villa MP, Martella S, Ronchetti F, Darder MT, Falasca C, Pagani J, Massa F, et al. Exhaled nitric oxide in asthmatic and non-asthmatic children: influence of type of allergen sensitization and exposure to tobacco smoke. Pediatr Allergy Immunol 2001;12:247-56.

245. Silvestri M, Spallarossa D, Frangova Yourukova V, Battistini E, Fregonese B, Rossi GA. Orally exhaled nitric oxide levels are related to the degree of blood eosinophilia in atopic children with mild-intermittent asthma. Eur Respir J 1999;13:321-6.

246. Brussee JE, Smit HA, Kerkhof M, Koopman LP, Wijga AH, Postma DS, Gerritsen J, Grobbee DE, et al. Exhaled nitric oxide in 4-year-old children: relationship with asthma and atopy. Eur Respir J 2005;25:455-61.

247. Pedroletti C, Lundahl J, Alving K, Hedlin G. Exhaled nitric oxide in asthmatic children and adolescents after nasal allergen challenge. Pediatr Allergy Immunol 2005;16:59-64.

248. Silvestri M, Sabatini F, Spallarossa D, Fregonese L, Battistini E, Biraghi MG, Rossi GA. Exhaled nitric oxide levels in non-allergic and allergic mono- or polysensitised children with asthma. Thorax 2001;56:857-62.

249. Steerenberg PA, Janssen NA, de Meer G, Fischer PH, Nierkens S, van Loveren H, Opperhuizen A, Brunekreef B, et al. Relationship between exhaled NO, respiratory symptoms, lung function, bronchial hyperresponsiveness, and blood eosinophilia in school children. Thorax 2003;58:242-5.

250. Artlich A, Busch T, Lewandowski K, Jonas S, Gortner L, Falke KJ. Childhood asthma: exhaled nitric oxide in relation to clinical symptoms. Eur Respir J 1999;13:1396-401.

251. Sippel JM, Holden WE, Tilles SA, O'Hollaren M, Cook J, Thukkani N, Priest J, Nelson B, et al. Exhaled nitric oxide levels correlate with measures of disease control in asthma. J Allergy Clin Immunol 2000;106:645-50.

252. Warke TJ, Mairs V, Fitch PS, McGovern V, Ennis M, Shields MD. Exhaled nitric oxide in relation to the clinical features of childhood asthma. J Asthma 2004;41:751-7.

253. Nordvall SL, Janson C, Kalm-Stephens P, Foucard T, Toren K, Alving K. Exhaled nitric oxide in a population-based study of asthma and allergy in schoolchildren. Allergy 2005;60:469-75.

254. Rosias PP, Dompeling E, Dentener MA, Pennings HJ, Hendriks HJ, Van Iersel MP, Jöbsis Q. Childhood asthma: exhaled markers of airway inflammation, asthma control score, and lung function tests. Pediatr Pulmonol 2004;38:107-14.

255. al-Ali MK, Eames C, Howarth PH. Exhaled nitric oxide; relationship to clinicophysiological markers of asthma severity. Respir Med 1998;92:908-13.

256. Colon-Semidey AJ, Marshik P, Crowley M, Katz R, Kelly HW. Correlation between reversibility of airway obstruction and exhaled nitric oxide levels in children with stable bronchial asthma. Pediatr Pulmonol 2000;30:385-92.

257. Latzin P, Beck J, Griese M. Exhaled nitric oxide in healthy children: variability and a lack of correlation with atopy. Pediatr Allergy Immunol 2002;13:37-46.

258. del Giudice MM, Brunese FP, Piacentini GL, Pedulla M, Capristo C, Decimo F, Capristo AF. Fractional exhaled nitric oxide (FE_{NO}), lung function and airway hyperresponsiveness in naive atopic asthmatic children. J Asthma 2004;41:759-65.

259. van Amsterdam JG, Janssen NA, de Meer G, Fischer PH, Nierkens S, van Loveren H, Opperhuizen A, Steerenberg PA, et al. The relationship between exhaled nitric oxide and allergic sensitization in a random sample of school children. Clin Exp Allergy 2003;33:187-91.

260. Gratziou C, Lignos M, Dassiou M, Roussos C. Influence of atopy on exhaled nitric oxide in patients with stable asthma and rhinitis. Eur Respir J 1999;14:897-901.

261. Chng SY, Van Bever HP, Lian D, Lee SX, Xu XN, Wang XS, Goh DY. Relationship between exhaled nitric oxide and atopy in Asian young adults. Respirology 2005;10:40-5.

262. Gratziou C, Rovina N, Lignos M, Vogiatzis I, Roussos C. Exhaled nitric oxide in seasonal allergic rhinitis: influence of pollen season and therapy. Clin Exp Allergy 2001;31:409-16.

263. Moody A, Fergusson W, Wells A, Bartley J, Kolbe J. Increased nitric oxide production in the respiratory tract in asymptomatic pacific islanders: an association with skin prick reactivity to house dust mite. J Allergy Clin Immunol 2000;105:895-9.

264. Frank TL, Adisesh A, Pickering AC, Morrison JF, Wright T, Francis H, Fletcher A, Frank PI, et al. Relationship between exhaled nitric oxide and childhood asthma. Am J Respir Crit Care Med 1998;158:1032-6.

265. Henriksen AH, Linghaas-Holmen T, Sue-Chu M, Bjermer L. Combined use of exhaled nitric oxide and airway hyperresponsiveness in characterizing asthma in a large population survey. Eur Respir J 2000;15:849-55.

266. Menz G, Ying S, Durham SR, Corrigan CJ, Robinson DS, Hamid Q, Pfister R, Humbert M, et al. Molecular concepts of IgE-initiated inflammation in atopic and nonatopic asthma. Allergy 1998;53:15-21.

267. Malmberg LP, Turpeinen H, Rytila P, Sarna S, Haahtela T. Determinants of increased exhaled nitric oxide in patients with suspected asthma. Allergy 2005;60:464-8.

268. Leung TF, Wong GW, Ko FW, Lam CW, Fok TF. Clinical and atopic parameters and airway inflammatory markers in childhood asthma: a factor analysis. Thorax 2005.

269. Leuppi JD, Downs SH, Downie SR, Marks GB, Salome CM. Exhaled nitric oxide levels in atopic children: relation to specific allergic sensitisation, AHR, and respiratory symptoms. Thorax 2002;57:518-23.

270. Djukanovic R, Lai CK, Wilson JW, Britten KM, Wilson SJ, Roche WR, Howarth PH, Holgate ST. Bronchial mucosal manifestations of atopy: a comparison of markers of inflammation between atopic asthmatics, atopic nonasthmatics and healthy controls. Eur Respir J 1992;5:538-44.

271. Baraldi E, Carra S, Dario C, Azzolin N, Ongaro R, Marcer G, Zacchello F. Effect of natural grass pollen exposure on exhaled nitric oxide in asthmatic children. Am J Respir Crit Care Med 1999;159:262-6.

272. Baur X, Barbinova L. Latex allergen exposure increases exhaled nitric oxide in symptomatic healthcare workers. Eur Respir J 2005;25:309-16.

273. Chatkin JM, Ansarin K, Silkoff PE, McClean P, Gutierrez C, Zamel N, Chapman KR. Exhaled nitric oxide as a noninvasive assessment of chronic cough. Am J Respir Crit Care Med 1999;159:1810-3.

274. Deykin A, Massaro AF, Drazen JM, Israel E. Exhaled nitric oxide as a diagnostic test for asthma: online versus offline techniques and effect of flow rate. Am J Respir Crit Care Med 2002;165:1597-601.

275. Smith AD, Cowan JO, Filsell S, McLachlan C, Monti-Sheehan G, Jackson P, Taylor DR. Diagnosing asthma: comparisons between exhaled nitric oxide measurements and conventional tests. Am J Respir Crit Care Med 2004;169:473-8.

276. Dupont LJ, Demedts MG, Verleden GM. Prospective evaluation of the validity of exhaled nitric oxide for the diagnosis of asthma. Chest 2003;123:751-6.

277. Malmberg LP, Pelkonen AS, Haahtela T, Turpeinen M. Exhaled nitric oxide rather than lung function distinguishes preschool children with probable asthma. Thorax 2003;58:494-9.

278. Thomas PS, Gibson PG, Wang H, Shah S, Henry RL. The relationship of exhaled nitric oxide to airway inflammation and responsiveness in children. J Asthma 2005;42:291-5.

279. Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. Am J Respir Crit Care Med 1996;153:454-7.

280. Yates DH, Kharitonov SA, Robbins RA, Thomas PS, Barnes PJ. Effect of a nitric oxide synthase inhibitor and a glucocorticosteroid on exhaled nitric oxide. Am J Respir Crit Care Med 1995;152:892-6.

281. Silkoff PE, McClean P, Spino M, Erlich L, Slutsky AS, Zamel N. Dose-response relationship and reproducibility of the fall in exhaled nitric oxide after inhaled beclomethasone dipropionate therapy in asthma patients. Chest 2001;119:1322-8.

282. Tsai YG, Lee MY, Yang KD, Chu DM, Yuh YS, Hung CH. A single dose of nebulized budesonide decreases exhaled nitric oxide in children with acute asthma. J Pediatr 2001;139:433-7.

283. Jones SL, Herbison P, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, Taylor DR. Exhaled NO and assessment of anti-inflammatory effects of inhaled steroid: dose-response relationship. Eur Respir J 2002;20:601-8.

284. Beck-Ripp J, Griese M, Arenz S, Koring C, Pasqualoni B, Bufler P. Changes of exhaled nitric oxide during steroid treatment of childhood asthma. Eur Respir J 2002;19:1015-9.

285. Kharitonov SA, Donnelly LE, Montuschi P, Corradi M, Collins JV, Barnes PJ. Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma. Thorax 2002;57:889-96.

286. Spallarossa D, Battistini E, Silvestri M, Sabatini F, Biraghi MG, Rossi GA. Time-dependent changes in orally exhaled nitric oxide and pulmonary functions induced by inhaled corticosteroids in childhood asthma. J Asthma 2001;38:545-53.

287. Xie QW, Kashiwabara Y, Nathan C. Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase. J Biol Chem 1994;269:4705-8.

288. Guo FH, De Raeve HR, Rice TW, Stuehr DJ, Thunnissen FB, Erzurum SC. Continuous nitric oxide synthesis by inducible nitric oxide synthase in normal human airway epithelium in vivo. Proc Natl Acad Sci U S A 1995;92:7809-13.

289. Robbins RA, Springall DR, Warren JB, Kwon OJ, Buttery LD, Wilson AJ, Adcock IM, Riveros-Moreno V, et al. Inducible nitric oxide synthase is increased in murine lung epithelial cells by cytokine stimulation. Biochem Biophys Res Commun 1994;198:835-43.

290. Redington AE, Meng QH, Springall DR, Evans TJ, Creminon C, Maclouf J, Holgate ST, Howarth PH, et al. Increased expression of inducible nitric oxide synthase and cyclo-oxygenase-2 in the airway epithelium of asthmatic subjects and regulation by corticosteroid treatment. Thorax 2001;56:351-7.

291. Jones SL, Kittelson J, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, Taylor DR. The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med 2001;164:738-43.

292. Bisgaard H. Leukotriene modifiers in pediatric asthma management. Pediatrics 2001;107:381-90.

293. Straub DA, Minocchieri S, Moeller A, Hamacher J, Wildhaber JH. The effect of montelukast on exhaled nitric oxide and lung function in asthmatic children 2 to 5 years old. Chest 2005;127:509-14.

294. Straub DA, Moeller A, Minocchieri S, Hamacher J, Sennhauser FH, Hall GL, Wildhaber JH. The effect of montelukast on lung function and exhaled nitric oxide in infants with early childhood asthma. Eur Respir J 2005;25:289-94.

295. Bisgaard H, Loland L, Oj JA. NO in exhaled air of asthmatic children is reduced by the leukotriene receptor antagonist montelukast. Am J Respir Crit Care Med 1999;160:1227-31.

296. Overbeek SE, Mulder PG, Baelemans SM, Hoogsteden HC, Prins JB. Formoterol added to low-dose budesonide has no additional antiinflammatory effect in asthmatic patients. Chest 2005;128:1121-7.

297. Silkoff PE, Romero FA, Gupta N, Townley RG, Milgrom H. Exhaled nitric oxide in children with asthma receiving Xolair (omalizumab), a monoclonal anti-immunoglobulin E antibody. Pediatrics 2004;113:e308-12.

298. Sanders SP, Proud D, Permutt S, Siekierski ES, Yachechko R, Liu MC. Role of nasal nitric oxide in the resolution of experimental rhinovirus infection. J Allergy Clin Immunol 2004;113:697-702.

299. Karupiah G, Xie QW, Buller RM, Nathan C, Duarte C, MacMicking JD. Inhibition of viral replication by interferon-gamma-induced nitric oxide synthase. Science 1993;261:1445-8.

300. Grasemann H, Michler E, Wallot M, Ratjen F. Decreased concentration of exhaled nitric oxide (NO) in patients with cystic fibrosis. Pediatr Pulmonol 1997;24:173-7.

301. Franklin PJ, Hall GL, Moeller A, Horak F, Brennan S, Stick SM. Exhaled nitric oxide is not reduced in infants with cystic fibrosis. Eur Respir J 2006;27:350-4.

302. Moeller A, Horak FJ, Lane C, Knight D, Kicic A, Brennan S et al. Inducible NO synthase expression is low in airway epithelium from young children with cystic fibrosis. Thorax 2006 (epub ahead of print, doi:10.1136/thx.2005.054643).

303. Kelley TJ, Drumm ML. Inducible nitric oxide synthase expression is reduced in cystic fibrosis murine and human airway epithelial cells. J Clin Invest 1998;102:1200-7.

304. Downey D, Elborn JS. Nitric oxide, iNOS, and inflammation in cystic fibrosis. J Pathol 2000;190:115-6.

305. Grasemann H, Ioannidis I, Tomkiewicz RP, de Groot H, Rubin BK, Ratjen F. Nitric oxide metabolites in cystic fibrosis lung disease. Arch Dis Child 1998;78:49-53.

306. Ojoo JC, Mulrennan SA, Kastelik JA, Morice AH, Redington AE. Exhaled breath condensate pH and exhaled nitric oxide in allergic asthma and in cystic fibrosis. Thorax 2005;60:22-6.

307. Ho LP, Innes JA, Greening AP. Nitrite levels in breath condensate of patients with cystic fibrosis is elevated in contrast to exhaled nitric oxide. Thorax 1998;53:680-4.

308. Narang I, Ersu R, Wilson NM, Bush A. Nitric oxide in chronic airway inflammation in children: diagnostic use and pathophysiological significance. Thorax 2002;57:586-9.

309. Karadag B, James AJ, Gultekin E, Wilson NM, Bush A. Nasal and lower airway level of nitric oxide in children with primary ciliary dyskinesia. Eur Respir J 1999;13:1402-5.

310. Lundberg JO, Weitzberg E, Nordvall SL, Kuylenstierna R, Lundberg JM, Alving K. Primarily nasal origin of exhaled nitric oxide and absence in Kartagener's syndrome. Eur Respir J 1994;7:1501-4.

311. Corbelli R, Bringolf-Isler B, Amacher A, Sasse B, Spycher M, Hammer J. Nasal nitric oxide measurements to screen children for primary ciliary dyskinesia. Chest 2004;126:1054-9.

312. Horvath I, Loukides S, Wodehouse T, Csiszer E, Cole PJ, Kharitonov SA, Barnes PJ. Comparison of exhaled and nasal nitric oxide and exhaled carbon monoxide levels in bronchiectatic patients with and without primary ciliary dyskinesia. Thorax 2003;58:68-72.

313. Wodehouse T, Kharitonov SA, Mackay IS, Barnes PJ, Wilson R, Cole PJ. Nasal nitric oxide measurements for the screening of primary ciliary dyskinesia. Eur Respir J 2003;21:43-7.

314. Grasemann H, Gartig SS, Wiesemann HG, Teschler H, Konietzko N, Ratjen F. Effect of L-arginine infusion on airway NO in cystic fibrosis and primary ciliary dyskinesia syndrome. Eur Respir J 1999;13:114-8.

315. Loukides S, Kharitonov S, Wodehouse T, Cole PJ, Barnes PJ. Effect of arginine on mucociliary function in primary ciliary dyskinesia. Lancet 1998;352:371-2.

316. Baraldi E, Pasquale MF, Cangiotti AM, Zanconato S, Zacchello F. Nasal nitric oxide is low early in life: case study of two infants with primary ciliary dyskinesia. Eur Respir J 2004;24:881-3.

317. Csoma Z, Bush A, Wilson NM, Donnelly L, Balint B, Barnes PJ, Kharitonov SA. Nitric oxide metabolites are not reduced in exhaled breath condensate of patients with primary ciliary dyskinesia. Chest 2003;124:633-8.

318. Jain B, Rubinstein I, Robbins RA, Leise KL, Sisson JH. Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. Biochem Biophys Res Commun 1993;191:83-8.

319. Baraldi E, Bonetto G, Zacchello F, Filippone M. Low exhaled nitric oxide in school-age children with bronchopulmonary dysplasia and airflow limitation. Am J Respir Crit Care Med 2005;171:68-72.

320. Storme L, Riou Y, Leclerc F, Dubois A, Deschildre A, Pierre MH, Logier R, Robin H, Lequien P. Exhaled nitric oxide (NO) and respiratory function measured with body plethysmography in children. Arch Pediatr 1998;5:389-96.

321. Mieskonen ST, Malmberg LP, Kari MA, Pelkonen AS, Turpeinen MT, Hallman NM, Sovijarvi AR. Exhaled nitric oxide at school age in prematurely born infants with neonatal chronic lung disease. Pediatr Pulmonol 2002;33:347-55.

322. Kharitonov SA, O'Connor BJ, Evans DJ, Barnes PJ. Allergen-induced late asthmatic reactions are associated with elevation of exhaled nitric oxide. Am J Respir Crit Care Med 1995;151:1894-9.

323. Henriksen AH, Sue-Chu M, Lingaas Holmen T, Langhammer A, Bjermer L. Exhaled and nasal NO levels in allergic rhinitis: relation to sensitization, pollen season and bronchial hyperresponsiveness. Eur Respir J 1999;13:301-6.

324. Cremona G, Higenbottam TW, Mayoral V, Alexander G, Demoncheaux E, Borland C, Roe P, Jones GJ. Elevated exhaled nitric oxide in patients with hepatopulmonary syndrome. Eur Respir J 1995;8:1883-5.

325. Rolla G, Brussino L, Colagrande P, Scappaticci E, Morello M, Bergerone S, Ottobrelli A, Cerutti E, et al. Exhaled nitric oxide and impaired oxygenation in cirrhotic patients before and after liver transplantation. Ann Intern Med 1998;129:375-8.

326. Fisher AJ, Gabbay E, Small T, Doig S, Dark JH, Corris PA. Cross sectional study of exhaled nitric oxide levels following lung transplantation. Thorax 1998;53:454-8.

327. Gabbay E, Walters EH, Orsida B, Whitford H, Ward C, Kotsimbos TC, Snell GI, Williams TJ. Post-lung transplant bronchiolitis obliterans syndrome (BOS) is characterized by increased exhaled nitric oxide levels and epithelial inducible nitric oxide synthase. Am J Respir Crit Care Med 2000;162:2182-7.

328. Verleden GM, Dupont LJ, Van Raemdonck DE, Vanhaecke J. Accuracy of exhaled nitric oxide measurements for the diagnosis of bronchiolitis obliterans syndrome after lung transplantation. Transplantation 2004;78:730-3.

329. Brugiere O, Thabut G, Mal H, Marceau A, Dauriat G, Marrash-Chahla R, Castier Y, Leseche G, et al. Exhaled NO may predict the decline in lung function in bronchiolitis obliterans syndrome. Eur Respir J 2005;25:813-9.

330. Brightling CE, Symon FA, Birring SS, Bradding P, Wardlaw AJ, Pavord ID. Comparison of airway immunopathology of eosinophilic bronchitis and asthma. Thorax 2003;58:528-32.

331. Kharitonov SA, Wells AU, O'Connor BJ, Cole PJ, Hansell DM, Logan-Sinclair RB, Barnes PJ. Elevated levels of exhaled nitric oxide in bronchiectasis. Am J Respir Crit Care Med 1995;151:1889-93.

332. Tsang KW, Leung R, Fung PC, Chan SL, Tipoe GL, Ooi GC, Lam WK. Exhaled and sputum nitric oxide in bronchiectasis: correlation with clinical parameters. Chest 2002;121:88-94.

333. Ho LP, Innes JA, Greening AP. Exhaled nitric oxide is not elevated in the inflammatory airways diseases of cystic fibrosis and bronchiectasis. Eur Respir J 1998;12:1290-4.

334. Maziak W, Loukides S, Culpitt S, Sullivan P, Kharitonov SA, Barnes PJ. Exhaled nitric oxide in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998;157:998-1002.

335. Rutgers SR, Meijer RJ, Kerstjens HA, van der Mark TW, Koeter GH, Postma DS. Nitric oxide measured with single-breath and tidal-breathing methods in asthma and COPD. Eur Respir J 1998;12:816-9.

336. Clini E, Bianchi L, Vitacca M, Porta R, Foglio K, Ambrosino N. Exhaled nitric oxide and exercise in stable COPD patients. Chest 2000;117:702-7.

337. Delen FM, Sippel JM, Osborne ML, Law S, Thukkani N, Holden WE. Increased exhaled nitric oxide in chronic bronchitis: comparison with asthma and COPD. Chest 2000;117:695-701.

338. Papi A, Romagnoli M, Baraldo S, Braccioni F, Guzzinati I, Saetta M, Ciaccia A, Fabbri LM. Partial reversibility of airflow limitation and increased exhaled NO and sputum eosinophilia in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000;162:1773-7.

339. Rolla G, Colagrande P, Scappaticci E, Chiavassa G, Dutto L, Cannizzo S, Bucca C, Morello M, et al. Exhaled nitric oxide in systemic sclerosis: relationships with lung involvement and pulmonary hypertension. J Rheumatol 2000;27:1693-8.

340. Kharitonov SA, Cailes JB, Black CM, du Bois RM, Barnes PJ. Decreased nitric oxide in the exhaled air of patients with systemic sclerosis with pulmonary hypertension. Thorax 1997;52:1051-5.

341. Moodley YP, Lalloo UG. Exhaled nitric oxide is elevated in patients with progressive systemic sclerosis without interstitial lung disease. Chest 2001;119:1449-54.

342. Paredi P, Kharitonov SA, Loukides S, Pantelidis P, du Bois RM, Barnes PJ. Exhaled nitric oxide is increased in active fibrosing alveolitis. Chest 1999;115:1352-6.

343. Moodley YP, Chetty R, Lalloo UG. Nitric oxide levels in exhaled air and inducible nitric oxide synthase immunolocalization in pulmonary sarcoidosis. Eur Respir J 1999;14:822-7.

344. Ziora D, Kaluska K, Kozielski J. An increase in exhaled nitric oxide is not associated with activity in pulmonary sarcoidosis. Eur Respir J 2004;24:609-14.

345. Riley MS, Porszasz J, Miranda J, Engelen MP, Brundage B, Wasserman K. Exhaled nitric oxide during exercise in primary pulmonary hypertension and pulmonary fibrosis. Chest 1997;111:44-50.

346. Loveless MO, Phillips CR, Giraud GD, Holden WE. Decreased exhaled nitric oxide in subjects with HIV infection. Thorax 1997;52:185-6.

347. Brett SJ, Evans TW. Measurement of endogenous nitric oxide in the lungs of patients with the acute respiratory distress syndrome. Am J Respir Crit Care Med 1998;157:993-7

part 1

METHODOLOGY

2 Chapter

EXHALED NITRIC OXIDE MEASUREMENTS WITH DYNAMIC FLOW RESTRICTION IN CHILDREN AGED 4 - 8 YEARS

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ABSTRACT

Fractional exhaled nitric oxide concentration (F_{ENO}) depends on exhalation flow; however, children often are unable to perform controlled flow procedures. Therefore, a device was developed for offline F_{ENO} sampling, with dynamic flow restriction (DFR).

The authors compared offline with online F_{ENO}, assessed feasibility, and obtained normal values for F_{ENO} in children aged 4 - 8 yrs. Subjects inhaled nitric oxide (NO)-free air and exhaled into the device, where DFR kept exhalation flow constant at 50 $ml \cdot s^{-1}$. Dead space air was discarded. Exhaled air was collected in a 150 ml mylar balloon. Online measurements were performed and values compared with offline F_{ENO} in 19 adult volunteers. Seventy-nine children performed offline sampling. All samples were analysed with a chemiluminescence NO-analyser. Normal values were obtained in 34 healthy children.

There was an excellent correlation between on- and offline values. Bland and Altman plots showed good agreement between on- and offline F_{ENO}. Seventy-four out of 79 children were able to perform a correct offline procedure. Geometric mean ± SEM F_{ENO} in healthy children was 4.9 ± 1.2 parts per billion (ppb) for male children and 7.6 ± 1.1 ppb for female children.

It can be concluded that offline fraction of exhaled nitric oxide measurements with dynamic flow restriction are feasible in young children and correspond to online values.

INTRODUCTION

Fractional exhaled nitric oxide concentration (F_{ENO}) is a noninvasive marker of eosinophilic airway inflammation in asthma.[1,2] The American Thoracic Society (ATS) published guidelines for the sampling of F_{ENO} in adults and older children.[3] In addition, a joint European Respiratory Society (ERS)/ATS task force on exhaled nitric oxide (NO) measurements in children has summarised its recommendations.[4] F_{ENO} values are highly dependent on exhalation flow.[5] Hence, ATS guidelines recommend measurements during a single, slow exhalation with constant, low flow. However, 4 - 8-yr-old children are often unable to perform the required controlled flow procedures needed for standardised sampling.[6,7] In children unable to cooperate, tidal breathing methods or exhalations with uncontrolled flow have been used, but these are ill standardised and allow for contamination with ambient and nasal NO.[3] A dynamic flow restrictor (DFR) that automatically varies resistance depending on blowing pressure overcomes these problems. The ERS/ATS Task Force 2001 is the first to give recommendations on measuring F_{ENO} in children, and it mentions DFR as method of choice.[4] However, published data on DFR concern techniques where manual adjustments are made and this limits their applicability. Especially in young children F_{ENO} as a noninvasive, simple, reproducible inflammatory marker would be very useful as a diagnostic and monitoring tool in respiratory disease. Therefore, the authors developed a new, flow-constant device for offline measurement of F_{ENO} in children aged ≥ 4 yrs, who can cooperate but have difficulty in performing controlled expiratory manoeuvres. In the present study, the authors first validated the device against online F_{ENO} in young adult volunteers. Next, F_{ENO} was measured in schoolchildren aged 4 - 8 yrs to assess feasibility and obtain normal values.

SUBJECTS AND METHODS

Subjects

The offline technique was validated in 19 adult volunteers, selected to provide a wide range of F_{ENO}. Children aged 4 - 8 yrs were recruited from a primary school. The parents of 86 out of 125 children gave written informed consent for their children to participate in the study. All parents completed a modified, validated International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire about wheezing, rhinitis and eczema.[8] Questions about parental smoking, recent colds and use of medication were added. For 1 week, a diary card on respiratory symptoms and use of medication was completed. Height and weight were measured. The study was approved by the Medical Ethical Committee of the Erasmus MC-Sophia Children's Hospital.

Offline exhaled nitric oxide measurements

Exhaled air was collected with a new, custom-built device, which contained a mechanical variable flow restrictor that kept exhalation flow constant at 50 $ml \cdot s^{-1}$ over a pressure range of 5 - 20 cmH_2O (*fig 2.1*).

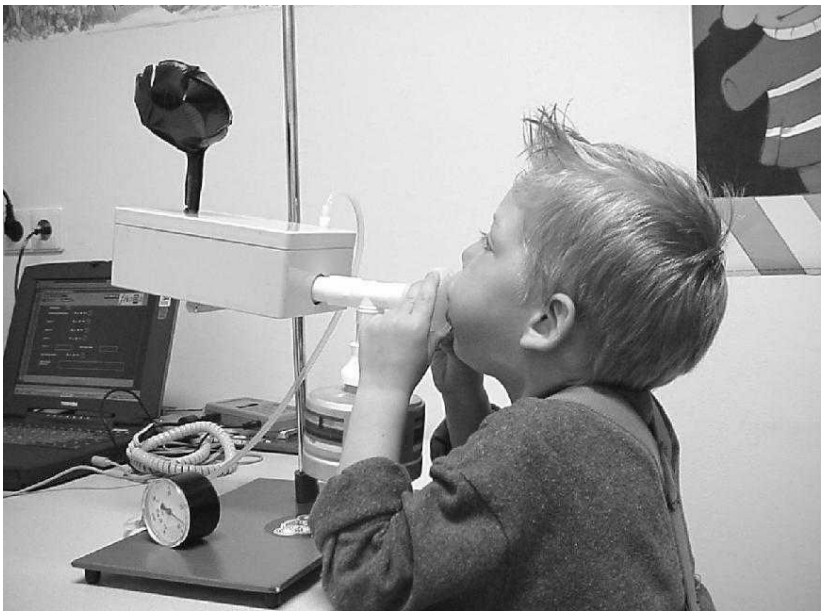

Figure 2.1 A *4-yr-old male performing the single-breath offline procedure for fractional exhaled nitric oxide measurement with dynamic flow restriction (published with permission from Baraldi and De Jongste[4]).*

Preliminary experiments with calibration gas of 115 parts per billion (ppb) indicated that the device was NO inert. Subjects were asked to take a deep breath through a mouthpiece attached to a bag with NO-free medical air *via* a nonrebreathing valve. They immediately exhaled into the DFR. A manometer checked mouth pressure and manoeuvres were accepted when pressures were 5 - 10 cmH_2O. A whistling feedback signal indicated a pressure of > 5 cmH_2O. The dead space air of the subject and device was discarded by a side tube. Air exhaled during the last 3 - 4 s was collected in a 150 ml NO-impermeable mylar balloon (Jurjen de Vries BV, Leeuwarden, The Netherlands). Total duration of the measurement was ~ 10 s. All subjects performed five manoeuvres with a varying exhalation time, discarding exhaled air during 3, 4, 6, 8 and 10 s corresponding to 150 - 500 ml, in random order. Between measurements, there was a minimum of 30 s rest. All balloons were sealed, and analysed within 2 h. Samples of ambient air were collected and analysed. A chemiluminescence analyser (Sievers 280 NOA, Boulder CO, USA) with a sensitivity of < 0.1 ppb and a detection range of < 0.1 - 500,000 ppb was used for measuring NO. The analyser was checked once daily before the measurements, using certified NO-free gas and certified 115 ppb gas (BOC, Herenthout, Belgium). The sampling flow was 200 $ml \cdot min^{-1}$ and the response time 200 ms.

Online exhaled nitric oxide measurements

Three online manoeuvres were performed on the NIOX NO-analyser (Aerocrine, Stockholm, Sweden) according to ATS guidelines.[3] Subjects inspired NO-free air and exhaled for a minimum of 7. A FE_{NO} plateau of ≥ 3 s was required to approve the measurement. Exhalation flow was kept constant through visual feedback and a DFR at 50 $ml \cdot s^{-1}$. Mouth pressure was checked.

Offline exhaled nitric oxide measurements in children

Exhaled air was collected while children attended school, during the morning hours of 1 week. The first 4 s of exhaled air (200 ml) were discarded, thus the total duration of the measurement was ~ 7 s. All balloons were analysed within 6 h, a period in which NO stays stable in balloons.[9] Samples of ambient air were collected every hour.

Data analysis

The method of BLAND and ALTMAN was used to assess reproducibility of duplicate measurements and agreement between offline and online values.[10] For correlations between F_{ENO} and diary card data, age, height, sex and ambient NO-values, bivariate correlations analysis was used (Pearson's correlation coefficient). Data are presented as geometric means (log transformed, processed and backtransformed) and standard error of the mean (SEM). The level of significance was set at $P = 0.05$.

RESULTS

Validation of offline device

Nineteen subjects (seven male) with a mean age of 29.5 yrs (range 21.9 - 39.5 yrs) participated. Geometric mean F_{ENO} was 21.9 ppb (6.0 - 109.6) for online values and 20.0 ppb (7.6 - 79.6) for offline values, with 150 ml discarded dead space volume. With higher discarded volumes offline, F_{ENO} was similar (*table 2.1*). There was an excellent correlation between on- and offline values, irrespective of the discarded dead space volume. Pearson correlation coefficients varied between 0.95 and 0.98 ($P < 0.001$) (*table 2.1*). BLAND and ALTMAN[10] plots for agreement between on- and offline values for discarded volumes of 200 and 500 ml are shown in *figure 2.2*.

Table 2.1

	Discarded volume ml	F_{ENO} ppb*	Pearson r**	mean ± SD difference on-/offline ppb
Online	-	21.9	-	-
Offline 3 s	150	20.0	0.95	1.1 ± 1.3
Offline 4 s	200	20.9	0.96	1.0 ± 1.3
Offline 6 s	300	21.9	0.97	1.0 ± 1.2
Offline 8 s	400	21.9	0.98	1.0 ± 1.2
Offline 10s	500	21.7	0.98	1.0 ± 1.2

Effect of discarded dead space volume on offline fractional exhaled nitric oxide concentration (F_{ENO}) and correlation with online values.
* *Geometric mean F_{ENO}*
** *Pearson correlation coefficient for online and offline values*
n = 19

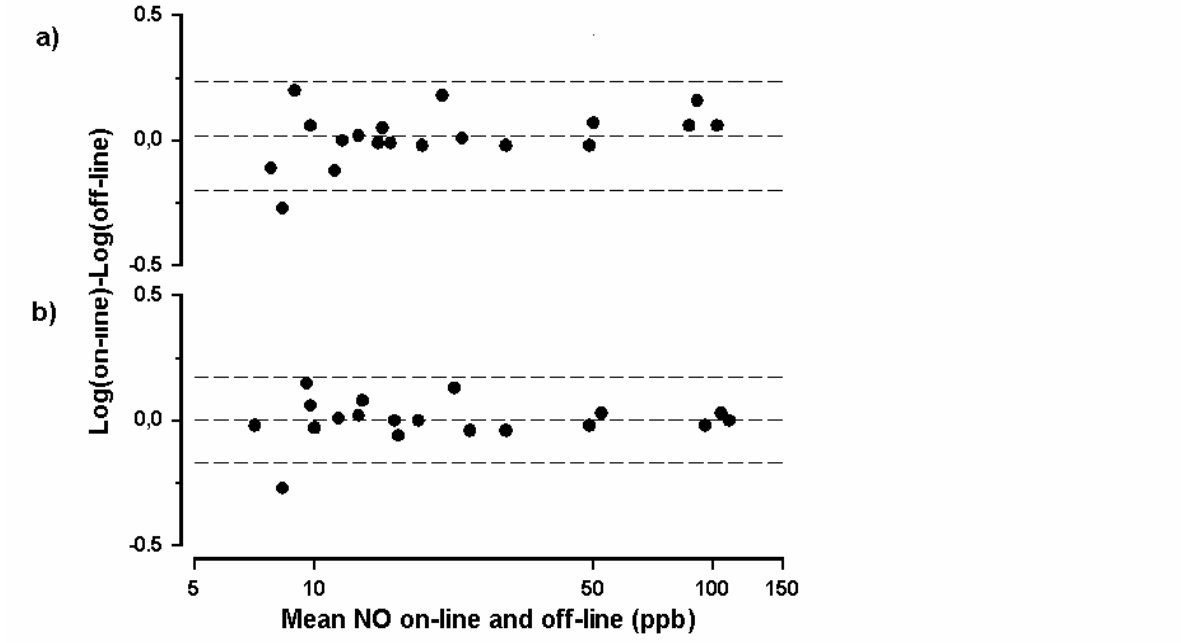

Figure 2.2 *Bland and Altman[10] analysis of agreement between offline and online fractional exhaled nitric oxide concentration values in adults after discarding*
a) *200 (4 s)*
b) *500 (10 s) ml of air*
NO *nitric oxide*
Dashed lines indicate ± 2 SD

Offline measurements in children

Eighty-six children entered the study, but five children were excluded because they could not perform the manoeuvres (mean age 57 months, range 50 - 64 months). Two children were absent during the days of the measurements. Hence, data were obtained from 79 children (46 male, 33 female) with a mean age of 67 months (range 44 - 89 months) (*table 2.2*). Symptom scores were low, only one child had a symptom score > 7 and 73 % had a symptom score of 0. There was no correlation between FE_{NO} values and diary card scores or between any of the individual questions of the ISAAC questionnaire and FE_{NO}. No correlation was found between FE_{NO} and age or height. However, the authors found significantly higher FE_{NO} values in female compared with male children. Geometric mean values were 5.0 ± 1.1 ppb for males and 6.9 ± 1.1 ppb for females (P = 0.006). Male and female children did not differ in height, age or symptom scores (*table 2.2*).

Ambient air and exhaled nitric oxide

Ambient NO levels were low and varied between 0.0 - 19.9 ppb, median 3.7 ppb. Despite the inhalation of NO-free air, a significant correlation between FE_{NO} and ambient NO was found (r = 0.529, P < 0.001). Every 10 ppb elevation of ambient air resulted in a 2.1 ppb increase in FE_{NO}. No correlation was found between FE_{NO} and ambient NO < 7 ppb.

Table 2.2 Data for the study population

	Whole group *	**Male +**	**Female #**
F_{ENO} ppb	5.8 ± 1.1	5.0 ± 1.1	6.9 ± 1.1¶
age months	68 (44 - 89)	67 (44 - 84)	71 (52 - 89)
weight kg	20.6 (15.1 - 31.3)	20.5 (16.6 - 31.3)	20.9 (15.1 - 30.3)
height cm	117.0 (105.3 - 130.9)	115.3 (107.0 - 129.5)	119.2 (105.3 - 130.9)
symptom scores	0.0 (0 - 105)	0.0 (0 - 105)	0.0 (0 - 7)

Data are presented as median (range) or geometric mean ± SEM.
F_{ENO} fractional exhaled nitric oxide concentration
* *n = 79*
\+ *n = 46*
\# *n = 33*
¶ *P = 0.006*

Duplicate measurements

BLAND and ALTMAN[10] analysis showed no difference and a high reproducibility of duplicate measurements (*fig. 2.3*). The intraclass correlation coefficient was 0.84.

Normal values

Thirty-four children (20 males) had a negative ISAAC questionnaire for asthma and allergy, did not have any airway symptoms and were steroid-naive. These children were used to determine normal values. Their mean age was 69 months (range 52 - 84 months). Geometric mean F_{ENO} was 4.9 ± 1.2 ppb for males and 7.6 ± 1.1 ppb for females (P = 0.02). There was no correlation between age, weight or height and F_{ENO}.

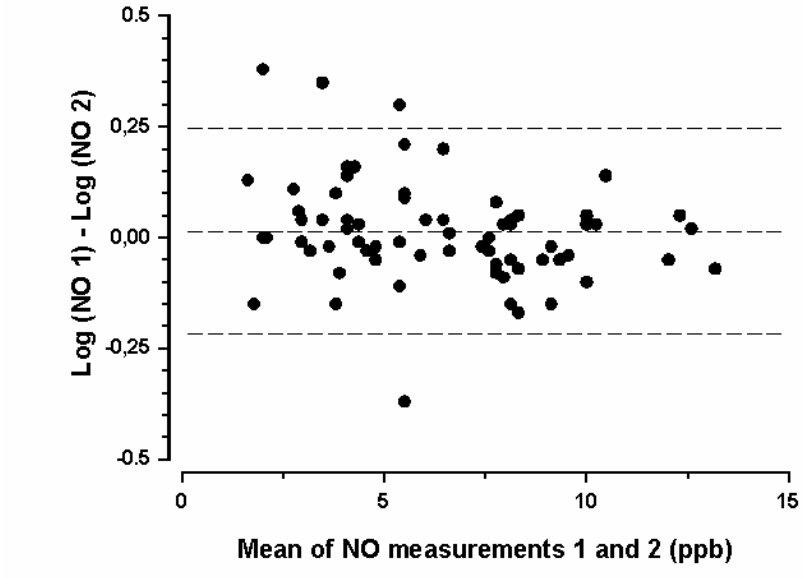

Figure 2.3 *Bland and Altman[10] analysis of agreement between duplicate fractional exhaled nitric oxide concentration values.*
NO *nitric oxide.*
Dashed lines indicate ± 2 SD

DISCUSSION

The authors found an excellent correlation between online values and F_{ENO} obtained offline employing DFR. In addition, there was good agreement between the two methods. Increasing the volume of discarded dead space air did not improve agreement. Reproducibility of duplicate offline measurements was good.

Correlations of different on- and offline sampling techniques have been reported in the literature.[7,11-14] Several authors found an excellent correlation between on- and offline values; however agreement between both methods differed in different studies. While Silkoff *et al.*[12] found lower offline values compared to online values, Paredi *et al.*[11] found that agreement was flow dependent and best with expiratory flows of ~ 150 ml·s^{-1}. At the recommended expiratory flow of 50 ml·s^{-1} these authors found low agreement. In contrast, the present findings show that with a single-breath, low-flow exhalation according to ATS guidelines, controlled by dynamic flow restriction and discarding dead space air, both good correlation and agreement between on- and offline values can be obtained. As the NIOX NO-analyser (Aerocrine) works with DFR as well, this finding is not surprising.

Several groups described different offline techniques for sampling exhaled NO.[7,11-19] These include tidal breathing techniques and uncontrolled or controlled single exhalations into a reservoir. Tidal breathing techniques require no active cooperation and are feasible down to the newborn age. On the other hand, exhalation flow cannot easily be controlled and it is difficult to prevent nasal contamination adequately, leading to increased variation in F_{ENO} values. Uncontrolled single exhalations against a resistance are easy to perform and reproducible even in young children, and there is no need for expensive or complicated equipment.[7,14,16] However, in the absence of flow control, F_{ENO} values may vary and be unsuitable to monitor individual patients. Controlled single exhalation manoeuvres improve reproducibility and agreement with online procedures. Biofeedback signals can be used to facilitate the procedure. Nevertheless, many young children will still have difficulty in performing these manoeuvres. Dynamic flow restriction overcomes this problem. The majority of all children in the present study successfully completed the sampling procedure with the offline device with dynamic flow restriction. Hence, it is now feasible to obtain reliable F_{ENO} values at a constant, low flow in children ≥ 4 yrs, using a simple mechanical sampling device.

The present data are the first on 4 - 8-yr-old children, measured with a constant flow of 50 ml·s^{-1}. Although the number of children was relatively small, the values found are in agreement with values from previous studies in older children.[9,15,20] Moreover, symptom scores in the whole study population were low, and F_{ENO} values in children with and without symptoms were not significantly different. This suggests that, in this young age group, airway symptoms are less likely to be related to eosinophilic airway inflammation than in older subjects, thus confirming

earlier observations.[21] Future studies using larger groups should establish reference values.

The authors found significantly higher FE_{NO} values in female compared to male children. Only in one previous study was a difference in FE_{NO} between males and females found. However, in this study higher FE_{NO} levels as well as higher plasma nitrate levels were found in males.[22] Male and female children in the present study did not differ in weight, height or age. Female children were not expected to have a higher prevalence of atopy or mild asthma and symptom scores did not differ between males and females (P = 0.48).[23,24] As atopy was not measured specifically, a difference in atopic status between males and females may still be possible in this relatively small sample. The authors could not confirm the correlation between age and FE_{NO} that was reported for older children, nor was a correlation with height found.[9]

No correlation between diary card symptom score or questionnaire answers and FE_{NO} was shown. However, symptom scores in the authors' group (measured in summer) were very low and only four children were treated for respiratory symptoms with inhaled steroids. Therefore, lack of correlation may be due to the relatively healthy population that was included.

The authors found a significant correlation between FE_{NO} and ambient NO levels > 7 ppb. Several studies investigated the correlation between FE_{NO} and ambient NO using different sampling techniques, resulting in conflicting data.[25-27] Ambient air can have NO values as high as several hundred ppb and may contaminate dead space of the airways, thus giving false high FE_{NO} values after expiration in a reservoir. Inspiration of NO-free air or discarding dead space air have been proposed as methods to eliminate this disturbing factor.[3] However, despite these measures, the authors still found a correlation between FE_{NO} and ambient NO. Several explanations for the present findings are possible. According to ATS recommendations, children took only one deep breath of NO free air, which may be not enough to effectively wash out dead space NO. Although special attention was paid to closure of the mouth around the mouthpiece during inspiration, some children may have inhaled ambient air along the mouthpiece. Based on the authors' findings in adult volunteers where agreement did not improve with higher discarded volumes, the first 150 to 200 ml of each exhalation was discarded before exhaled air was sampled. Because the anatomical dead space of children is smaller than in adults, discarding this amount of exhaled air should be sufficient to avoid contamination with dead space air. The low FE_{NO} in these healthy children may reveal any contamination more readily than higher levels in asthmatics. The authors recommend to record ambient NO levels when measuring FE_{NO}.

In conclusion, the authors found an excellent agreement and correlation between on- and offline fractional exhaled nitric oxide values, with a new constant low-flow offline device for single-breath measurement with dynamic flow restriction. With

this device, fractional exhaled nitric oxide can be reproducibly measured in children 4 - 8 yrs, with a high success rate. Normal fractional exhaled nitric oxide values for healthy male and female children are reported. Furthermore, there was a weak correlation between ambient nitric oxide > 7 parts per billion and fractional exhaled nitric oxide levels, despite the inhalation of nitric oxide-free air and dead space discarding. Thus, the authors propose this offline technique and dynamic flow restriction as a suitable method to collect samples for fractional exhaled nitric oxide measurements in children too young to cooperate with recommended online procedures.

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REFERENCES

1. Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. Lancet 1994;343:133-135.
2. Barnes PJ, Kharitonov SA. Exhaled nitric oxide: a new lung function test. Thorax 1996;51:233-237.
3. Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide in Adults and Children-1999. Am J Respir Crit Care Med 1999;160:2104-2117.
4. Baraldi E, de Jongste JC. Measurement of exhaled nitric oxide in children-2001. Joint ERS/ATS Task Force on Exhaled NO Measurement in Children. Eur Respir J 2001 (in press).
5. Kroesbergen A, Jöbsis Q, Bel EH, Hop WC, de Jongste JC. Flow-dependency of exhaled nitric oxide in children with asthma and cystic fibrosis. Eur Respir J 1999;14:871-875.
6. Baraldi E, Scollo M, Zaramella C, Zanconato S, Zacchello F. A simple flow-driven method for online measurement of exhaled NO starting at the age of 4 to 5 years. Am J Respir Crit Care Med 2000;162:1828-1832.
7. Jöbsis Q, Schellekens SL, Kroesbergen A, Hop WC, de Jongste JC. Sampling of exhaled nitric oxide in children: end-expiratory plateau, balloon and tidal breathing methods compared. Eur Respir J 1999;13:1406-1410.
8. Asher MI, Keil U, Anderson HR et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-491.
9. Jöbsis Q, Raatgeep HC, Hop WC, de Jongste JC. Controlled low flow off line sampling of exhaled nitric oxide in children. Thorax 2001;56:285-289.
10. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307-310.
11. Paredi P, Loukides S, Ward S et al. Exhalation flow and pressure-controlled reservoir collection of exhaled nitric oxide for remote and delayed analysis. Thorax 1998;53:775-779.
12. Silkoff PE, Stevens A, Pak J, Bucher-Bartelson B, Martin RJ. A method for the standardized offline collection of exhaled nitric oxide. Chest 1999;116:754-759.
13. Kissoon N, Duckworth LJ, Blake KV, Murphy SP, Taylor CL, Silkoff PE. F_{ENO}: relationship to exhalation rates and online versus bag collection in healthy adolescents. Am J Respir Crit Care Med 2000;162:539-45.
14. Canady RG, Platts-Mills T, Murphy A, Johannesen R, Gaston B. Vital capacity reservoir and online measurement of childhood nitrosopnea are linearly related. Am J Respir Crit Care Med 1999;159:311-314.
15. Baraldi E, Azzolin NM, Cracco A, Zacchello F. Reference values of exhaled nitric oxide for healthy children 6-15 years old. Pediatr Pulmonol 1999;27:54-58.
16. Nelson BV, Sears S, Woods J, Yee Ling C, Hunt J, Clapper LM et al. Expired nitric oxide as a marker for childhood asthma. J Pediatr 1997;130:423-427.
17. Jöbsis Q, Raatgeep HC, Hop WCJ, De Jongste JC. Controlled low flow offline sampling of exhaled nitric oxide in children. Thorax 2001;56:285-289.
18. Jöbsis Q, Schellekens SL, Kroesbergen A, Hop WC, de Jongste JC.Offline sampling of exhaled air for nitric oxide measurement in children: methodological aspects. Eur Respir J 2001:17:898-903.
19. Baraldi E, Dario C, Ongaro R, Scollo M, Azzolin NM, Panza N et al. Exhaled nitric oxide concentrations during treatment of wheezing exacerbation in infants and young children. Am J Respir Crit Care Med 1999;159:1284-1288.
20. Daniel PF, Klug B, Valerius NH. Collection and measurement of exhaled nitric oxide in young children during tidal breathing. Eur Respir J 2001;18 (suppl.33):38s.
21. Fitch PS, Brown V, Schock BC, Taylor R, Ennis M, Shields MD. Chronic cough in children: bronchoalveolar lavage findings. Eur Respir J 2000;16:1109-1114.
22. Jilma B, Kastner J, Mensik C et al. Sex differences in concentrations of exhaled nitric oxide and plasma nitrate. Life Sci 1996;58:469-76.

23. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med 1995;332:133-138.

24. Sears MR, Burrows B, Flannery EM, Herbison GP, Holdaway MD. Atopy in childhood. I. Gender and allergen related risks for development of hay fever and asthma. Clin Exp Allergy 1993;23:941-948.

25. Baraldi E, Azzolin NM, Dario C et al. Effect of atmospheric nitric oxide (NO) on measurements of exhaled NO in asthmatic children. Pediatr Pulmonol 1998;26:30-34.

26. Piacentini GL, Bodini A, Vino L et al. Influence of environmental concentrations of NO on the exhaled NO test. Am J Respir Crit Care Med 1998;158:1299-1301.

27. Therminarias A, Flore P, Favre-Juvin A, Oddou MF, Delaire M, Grimbert F. Air contamination with nitric oxide: effect on exhaled nitric oxide response. Am J Respir Crit Care Med 1998;157:791-795.

Chapter 3

Exhaled Nitric Oxide in Mylar Balloons: Influence of Storage Time, Humidity and Temperature

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ABSTRACT

Background: Mylar balloons are used to collect exhaled air for analysis of fractional nitric oxide concentration (F_{ENO}).

Aim: We studied the effect of storage conditions on the stability of nitric oxide (NO) in mylar balloons.

Methods: Exhaled air samples and calibration gases were stored in mylar balloons at 4, 21 and 37 °C, with or without silica gel. NO was measured after 0, 6, 9, 24 and 48 h. Scheffe F-tests were used to compare NO values.

Results: NO remained stable in balloons for 9 h at all temperatures, without silica gel. NO increased between 9 and 48 h, but only with low initial F_{ENO}. Silica gel increased variability.

Conclusions: F_{ENO} in mylar balloons is stable for at least 9 h. The storage temperature is not critical, but silica gel increases variability.

INTRODUCTION

Exhaled nitric oxide (eNO) has been proposed as a noninvasive marker to evaluate airway inflammation in atopic asthma.[1,2] The fractional concentration of exhaled NO (F_{ENO}) can be measured online by direct exhalation into a chemiluminescence analyser. Recently, the American Thoracic Society and European Respiratory Society published guidelines for standardised F_{ENO} measurements in adults and children.[3,4]

Offline sampling of eNO and storage in a reservoir offers the possibility of remote and delayed analysis of F_{ENO}, independent of the immediate presence of a nitric oxide (NO) analyser, which offers advantages for epidemiological field studies and home monitoring of asthma.[5-10] American Thoracic Society guidelines suggest that mylar balloons are suitable for the collection of eNO.[3] However, little is known about the effect of storage conditions on the stability of NO in such balloons. The aim of this study was to evaluate the influence of storage time, temperature and air humidity on F_{ENO}, collected offline in mylar balloons.

MATERIALS AND METHODS

Exhaled NO measurements and study design

Exhaled air was collected from three adult volunteers, two healthy and one atopic asthmatic (F_{ENO} 12.4, 21.0 and 34.0 ppb respectively), with an offline method according to American Thoracic Society guidelines.[3] Subjects inhaled NO-free air and exhaled through a one-way valve into a device in which a dynamic flow restrictor limits flow at 50 ml/s.[6] Mouth pressure was checked by a manometer and accepted between 5 and 20 cm H_2O. Dead space air (250 ml) was discarded by a manually operated switch and exhaled air was collected in 150 ml mylar balloons. Total duration of the measurement was 8 - 12 s. Between exhalations there was a minimum of 30 s rest. Ambient NO levels were recorded during collection. Each volunteer filled 15 balloons with and 15 without silicagel. To assess whether NO levels were affected by possible factors in exhaled air, 20 balloons were filled with dry calibration gas containing < 1 ppb or 115 ppb NO.

Balloons containing exhaled air with and without silica gel were sealed and stored at temperatures of 4, 21 or 37 ºC. Balloons containing dry calibration gases were stored at 21 ºC (< 1 ppb) and at 4, 21 and 37 ºC (115 ppb). Balloons were sampled immediately after the exhalation and 6, 9, 24 and 48 h later. The NO concentration in balloons was measured with a chemiluminescence NO-analyser (Sievers 280, Boulder, CO, USA), with a sensitivity of < 0.1 ppb and a detection range of 0.1 – 500.000 ppb. The analyser was checked once daily, before the measurements, using certified NO-free gas and 115 ppb calibration gas, and calibrated when needed. Samples were obtained by passing the sample tube into the balloons, using a 175 ml/min sample flow for 10 s.

Statistical analysis

FE_{NO} levels immediately after collection and after 6, 9, 24, and 48 h were calculated as geometric means of five balloons with and five balloons without silica gel for each subject at each different ambient temperature. Data were compared by analysis of variance (ANOVA) for repeated measurements and the levels of significance were verified by Scheffe *F*-test. A two-tailed $P < 0.05$ was considered significant.

RESULTS

FE_{NO} remained stable in balloons for 9 h at all temperatures when no silica gel was added and irrespective of initial values (*Fig. 3.1a-c*); no significant differences were found between values at 6 and 9 h compared with 0 h. In balloons with low initial FE_{NO}, NO concentrations increased significantly between 9 and 48 h, compared with 0 h ($P < 0.05$). Under all storage conditions, silica gel increased variability (*Fig. 3.1d-f*) ($P < 0.05$). Stability was worst at 37 ºC, in the presence of silica gel (*Fig. 3.1f*).

DISCUSSION

We explored the stability of NO in exhaled air during storage in a mylar balloon, and studied the influence of temperature and humidity. Our results indicate that FE_{NO} levels remain stable in mylar balloons for the first 9 h after collection, but may rise over 24 - 48 h. These findings are in agreement with previous work by Barreto *et al.* and Silkoff *et al.,* who showed that FENO levels in balloons present a progressive rise at 24 h and 48 h after collection.[9,10] These studies, however, did not evaluate the influence of environmental temperature and the possible effects of humidity, using silica gel to absorb water vapour, or comparing NO in exhaled air with NO in dry gas. We showed that ambient temperature did not influence the stability of NO in balloons for the first 9 h after collection.

Paredi and coworkers demonstrated that eNO levels of 15 and 60 ppb were stable for 24 h in mylar reservoirs containing silica gel.[8] However, control experiments without silicagel were lacking.

The source of the increase in NO during long-term storage is unclear. Selective leakage of non-NO gases seems unlikely, as this would require considerable volume changes that were not observed. We speculate that NO is released from the balloon during storage, depending on intraluminal NO levels.

We conclude that offline assessment of FE_{NO} in samples of exhaled air collected in mylar balloons is reliable, provided that samples are analysed within 9 h. The storage temperature is not critical, but silica gel should not be added as it reduces FE_{NO} stability.

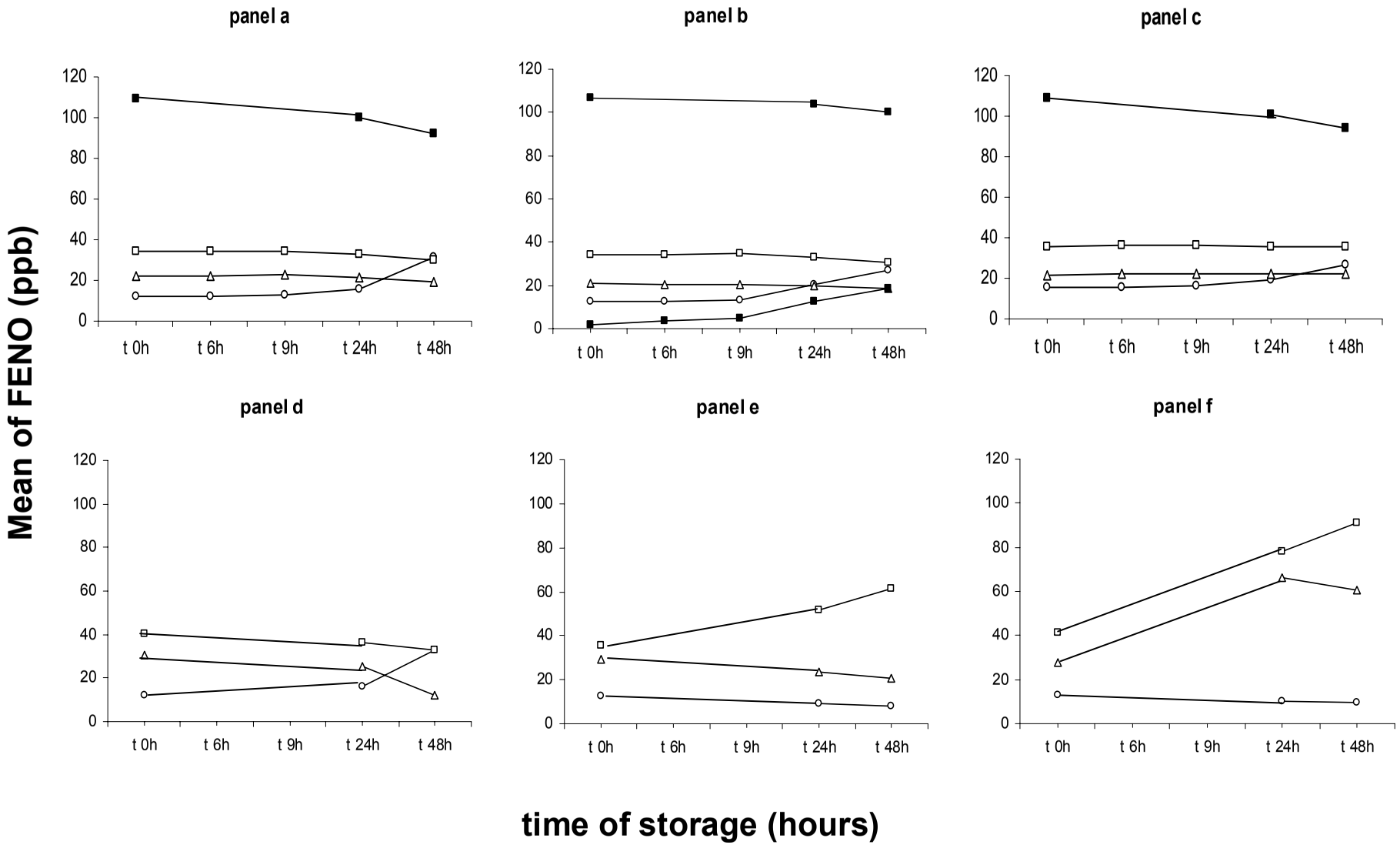

Figure 3.1 *Changes of different NO levels (ppb) in mylar balloons without silica gel, stored at (a) 4 ºC, (b) 21 ºC and (c) 37 ºC, and in balloons with silica gel, stored at (d) 4 ºC, (e) 21º C and (f) 37 ºC during 48 h. Open symbols, exhaled air samples (O, low; Δ, medium healthy; □, high (asthmatic)); closed symbols, calibration gas. Note the non-normal x axis.*

ACKNOWLEDGMENTS

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REFERENCES

1. Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. Am J Respir Crit Care Med 2001;163:1693-722.
2. Kharitonov SA, Barnes PJ. Exhaled nitric oxide: a marker of airway inflammation? Curr Opin Anesthesiol 1996;9:542-8.
3. American Thoracic Society. Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children. Am J Respir Crit Care Med 1999;160:2104-17.
4. Baraldi E, de Jongste JC. Measurement of exhaled nitric oxide in children. A joint ERS/ATS Task Force report. Eur Respir J 2002;20:223-37.
5. Jöbsis Q, Raatgeep HC, Hop WC, de Jongste JC. Controlled low flow offline sampling of exhaled nitric oxide in children. Thorax 2001;56:285-9.
6. Pijnenburg MW, Hofhuis W, Lissenberg ET et al. Exhaled nitric oxide measurements with dynamic flow restriction in children 4-8 years. Eur Respir J 2002;20:919-24.
7. Jöbsis Q, Schellekens SL, Kroesbergen A, Hop WC, de Jongste JC. Sampling of exhaled nitric oxide in children: end-expiratory plateau, balloon and tidal breathing methods compared. Eur Respir J 1999;13:1406-10.
8. Paredi P, Loukides S, Ward S et al. Exhalation flow and pressure-controlled reservoir collection of exhaled nitric oxide for remote and delayed analysis. Thorax 1998;53:775-9.
9. Silkoff PE, Stevens A, Pak J, Bucher-Bartelson B, Martin RJ. A method for the standardized offline collection of exhaled nitric oxide. Chest 1999;116:754-9.
10. Barreto M, Villa MP, Martella S et al. Offline exhaled nitric oxide measurements in children. Pediatr Pulmonol 2001;32:159-67

4 Chapter

Measurements of Exhaled Nitric Oxide in Healthy Subjects Age 4 to 17 Years

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ABSTRACT

Background: Fractional exhaled nitric oxide (FE_{NO}) is used in monitoring of asthma.

Objectives: The aim of this multicenter study was to establish normal values of FE_{NO} and assess feasibility in children with a standardized method and equipment approved for clinical use.

Methods: FE_{NO} was measured in healthy subjects of 4 to 17 years according to American Thoracic Society guidelines (single breath online, exhalation flow 50 ml/s) with a chemiluminescense analyzer (NIOX Exhaled Nitric Oxide Monitoring system, Aerocrine, Sweden) in 3 European and 2 US centers. Each child performed 3 acceptable nitric oxide measurements within 6 attempts and completed an extended International Study of Asthma and Allergy in Children questionnaire.

Results: Measurement of FE_{NO} was attempted in 522 children. Four hundred five children completed the study according to the protocol. Geometric mean FE_{NO} in 405 children was 9.7 ppb, and the upper 95% confidence limit was 25.2 ppb. FE_{NO} increased significantly with age, and higher FE_{NO} was seen in children with self-reported rhinitis/conjunctivitis or hay fever. The success rate was age-dependent and improved from 40% in the children 4 years old to almost 100 % from the age of 10 years. The repeatability of 3 approved measurements was 1.6 ppb (95% CI, 1.49 - 1.64 ppb).

Conclusions: FE_{NO} in healthy children is below 15 to 25 ppb depending on age and self-reported atopy. Measurement of FE_{NO} by NIOX® is simple and safe and has a good repeatability. Feasibility depends on age and may be difficult in the preschool child.

INTRODUCTION

The fractional concentration of nitric oxide (NO) in exhaled air (F_{ENO}) is generally higher in individuals with asthma than in healthy subjects,[1] although reported values of F_{ENO} in healthy subjects vary from 3 ppb to 88 ppb.[2-7] These variations may partly be attributed to different measurement techniques but also to low numbers studied. In addition, confounding factors such as age, sex, and atopic symptoms have not always been taken into account.

Increasing use of F_{ENO} measurement in the diagnosis and monitoring of asthma has urged the need for reference values of F_{ENO} measured with commercially available equipment.

In 1999, guidelines on the measurement of F_{ENO} were issued by the American Thoracic Society (ATS),[8] updated for children in 2001 by an European Respiratory Society/ATS task force.[9] Recently commercial equipment (NIOX, Aerocrine, Sweden) obtained Food and Drug Administration clearance, allowing dissemination of its clinical application.

The primary objective of this study was to establish reference values of F_{ENO} according to international guidelines in children of 4 to 17 years. Secondary objectives were to determine the short-term repeatability of F_{ENO} levels; to investigate the effect of possible confounders (ambient NO level, age, sex, ethnicity, height, weight, and self-reported information on passive smoking, eczema, and atopic symptoms in the children and family); to determine the success rate for the procedure for each age group; and to report any possible discomfort or adverse events during the measurements.

METHODS

Study subjects and protocol

Five centers, 3 in Europe (Copenhagen, Denmark, Rotterdam, The Netherlands and Padova, Italy) and 2 centers in the US (Denver, Colo and Charlottesville, Va) participated in this open, multicenter study. We planned to recruit 450 children equally distributed between boys and girls and divided into 9 age groups (4, 5, 6, 7, 8 - 9, 10 - 11, 12 - 13, 14 - 15, 16 - 17 years).

The children were recruited from kindergartens and public schools. Written informed consent was provided by parents and the child (if 12 years or older). Exclusion criteria were a history of asthma or related respiratory symptoms defined by the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire;[10] history of treatment with asthma-specific medication (ß-agonists; steroids; leukotriene receptor antagonists); other chronic pulmonary diseases or recent airway infection (cold, flu, sore throat within the last 7 days), active smoking, or inability to comply with the study procedure.

The study was approved by the local ethics committee in each country and local health authorities where needed.

FE_{NO} measurements

FE_{NO} was measured using the online single breath method with NIOX (Nitric Oxide Monitoring System; Aerocrine, Sweden) according to ATS guidelines.[8] The subject was seated comfortably, with the instrument at a convenient position in front of the subject. No nose clip was used.

The subject inserted the mouth piece and inhaled NO-free air to total lung capacity over a period of 2 to 3 seconds through the mouthpiece of the NIOX instrument. The subject then started exhalation against a positive mouthpiece counter pressure of 10 to 20 cm H_2O to ensure that the soft palate was closed against the nasal cavity, thus preventing contamination of exhaled NO with NO of nasal origin. Nose clip was not used.[11] Exhalation flow was 50 ml/s. Children less than 10 years of age performed a 6-second exhalation and FE_{NO} was calculated during the last 2 seconds of the exhalation. Children ≥ 10 years performed an exhalation of 10 seconds, and FE_{NO} was calculated during the last 3 seconds of the exhalation. A measurement was accepted if (1) the mean flow was 0.045 to 0.055 l/s, (2) the instant flow was 0.0375 to 0.0625 l/s, and (3) the instantaneous mouth pressure was 5 to 20 cm H_20. Exhalations were approved if they did not deviate more than 2.5 ppb or 10% and were completed within a 15-minute period. The interval between exhalations was at least 30 sec. Each subject performed no more than a total of 6 exhalations, and the total number of exhalations performed to obtain 3 acceptable FE_{NO} values was recorded. FE_{NO} was calculated as the mean of 3 correct exhalations.

All measurements were performed between 8:00 AM and 5:00 PM. Information on eating or any strenuous physical activity during the last 60 minutes before testing was obtained. The subject rested in the sitting position for 5 minutes prior to the measurement procedure.

Nitric oxide-free air was used in all measurements to minimize the influence of ambient NO on the results.
After the measurements children were asked if they had any adverse event in association with the procedure.

Questionnaire

Each subject completed the ISAAC core questionnaires for asthma, rhinitis and eczema.[10] Atopy was defined as any positive answer regarding rhinitis/conjunctivitis and/or hay fever. Additional information was obtained on recent infection, passive smoking, parental disposition to asthma or atopy, and ethnicity. The questionnaire was completed by the parents. When data were missing, these were filled in by a telephone interview afterward. The parents were

asked to contact the investigator in case a subject developed a respiratory tract infection within 3 days after the FE_{NO} measurement.

Data analyses

Analyses were performed using data from all subjects who completed the study according to the protocol (n = 405), except feasibility analyses, which were performed on a data set consisting of the total number of subjects having performed or tried to perform FE_{NO} measurements (n = 522).

Because the distribution of FE_{NO} was skewed, all analyses were performed with log-transformed data (e^{ln}), and results are presented as point estimates and 1-sided CIs with back-transformed values. Reference values of FE_{NO} are presented as geometric means and upper 95% 1-sided confidence limits for individuals. Individual upper 95% confidence limits were calculated as: $e^{(LN(mean) + t \times LN(SD))}$, where *t* is the Student *t* with appropriate number of degrees of freedom (n -1) and *n* the number of observations in a group.

All analyses were performed with and without outliers, and both results were presented if different. Outliers were defined as FE_{NO} values above arithmetic mean + 2 SD. Factors possibly affecting FE_{NO} were analyzed by linear regression multivariate analyses and Pearson correlation analysis. Group comparisons were analyzed by independent *t* tests and 4-field tables by Fisher exact test.

Short-term repeatability (intraindividual SD) was calculated as the SD of 3 measurements within a subject. The CIs for repeatability were calculated by using the χ^2 distribution, because the variance of repeatability was distributed according to this distribution.

Success rates were calculated as the frequencies of children of different ages that were able to perform the measurement procedure according to the protocol. Multivariate regression and partial correlation analyses were performed in order to investigate possible influence on FE_{NO} from confounding factors such as age, sex, and other factors.

RESULTS

Subject flow chart

Five hundred thirty-four children were recruited for the study. Four hundred five children (191 boys) completed the study with approved measurements. Subject flow diagram and demographic data are presented in *Fig. 4.1* and *Table 4.1*.

Within 1 hour before the FE_{NO} measurement, 16% of the subjects had food or liquid intake (other than water) and 1.5% had performed strenuous physical activity.

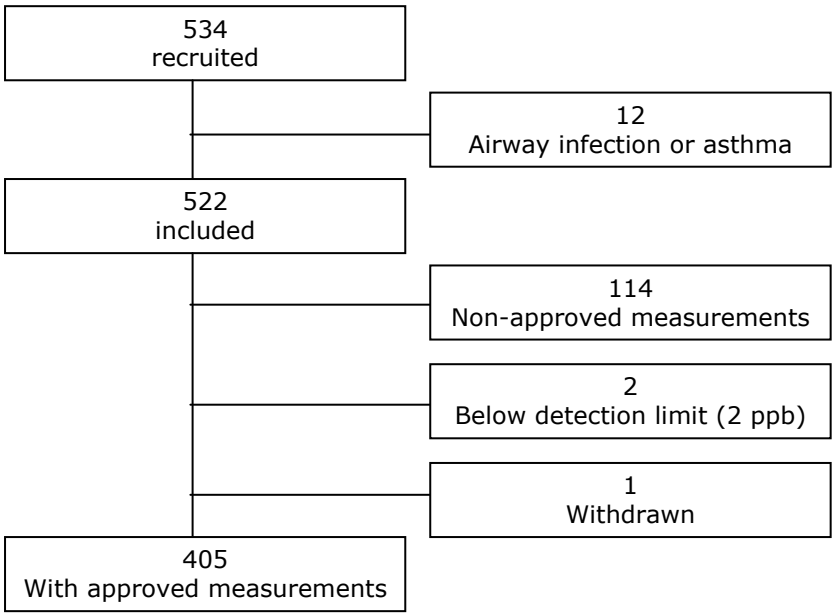

Figure 4.1 *Subject flow diagram*

Table 4.1 Anthropometric and demographic characteristics

n	**405**	
Male	191	(47 %)
Height, mean (range)	140.9 cm	(91.0 – 193.0)
Weight, mean (range)	37.4 kg	(14.0 – 115.0)
Ethnic background		
Caucasian	374	(92.3%)
Black	23	(5.7%)
Asian	5	(1.2%)
Hispanic	3	(0.8%)

Reference FE_{NO} values for age and sex

Table 4.2 presents geometric means and upper 95% limits of FE_{NO} reference values according to age for all children (n = 405). Sixteen children had extreme FE_{NO} values (> 34.9 ppb) and were considered outliers. Data are also presented without these 16 outliers, and without outliers and 57 atopic children (rhinitis/conjunctivitis and hay fever; n = 332).

There was no difference in FE_{NO} between boys and girls (geometric mean = 10.0 ppb *vs* 9.4 ppb, respectively; *P* = .27). Individual values are presented in *Fig. 4.2* (without outliers). FE_{NO} was significantly and positively related to age in both sexes with an increase (slope) of FE_{NO} of 5% (95% CI, 3% to 6%) per annum.

On the basis of simple linear regression analysis, estimates of geometric means and upper 1-sided 95% limits for the reference values according to age with and without outliers are presented in *Fig 4.3*.

Table 4.2 Reference values for FE_{NO}

Age, y	n	Geometric mean (ppb)	Individual 95% upper limit	n	Geometric mean (ppb)	Individual 95% upper limit	n	Geometric mean (ppb)	Individual 95% upper limit
	With outliers			Without outliers			Without outliers and atopics*		
4	29	7.1	15.7	29	7.1	15.7	27	7.0	15.0
5	35	7.9	16.6	35	7.9	16.6	33	8.0	17.1
6	49	8.2	19.3	48	8.0	17.1	42	7.5	15.5
7-9	107	8.1	18.4	106	8.0	17.2	89	7.8	17.1
10-13	105	11.2	28.2	98	10.1	19.2	85	9.8	18.0
14 -17	80	13.7	39.2	73	11.9	24.2	56	11.6	22.4
Total	405	9.7	25.2	389	9.0	19.4	332	8.8	18.5

* *Positive answer to rhinitis / conjunctivitis or hay fever symptoms*

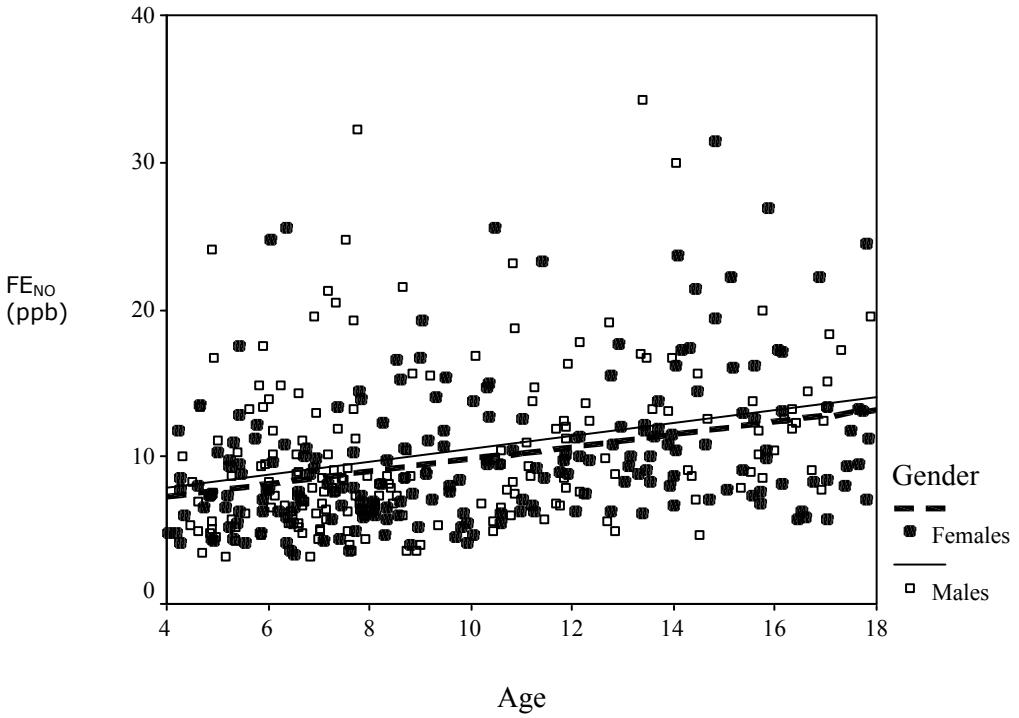

Figure 4.2 *Individual FE_{NO} values broken down by sex and age for all included children except outliers (> 34.9 ppb)*

Outliers

Extremely high FE_{NO} levels (> 34.9 ppb) were found in 16 subjects (10 boys), predominately older children (88% > 10 years) The outliers were characterized by having more reports of rhinitis symptoms ever (37.5% *vs* 11.9%) or within the last 12 months (31.3% *vs* 8.2%) or itchy-watery eyes (25.0% *vs* 5.1%; Fisher exact test, $P < .01$), whereas reporting of hay fever was similar (6.3% *vs* 6.7%) to the rest of the sample (n = 389). Among non-Caucasian children, 22% were outliers, in contrast with only 3% of Caucasian children. The outliers were older ($P < .002$), and in a multivariate regression analysis controlling for age, they were also

significantly heavier (P < .005) and taller (P < .037) but did not have a significantly higher BMI (P < .24).

Height and weight

In partial correlation analyses, adjusted for age, anthropometric variables height (r = 0.115; P < .02), weight (r= 0.139; P < .005), and body surface area (r = 0.145;P < .004) were all significantly positively related to natural logarithm FE_{NO} when including all children (n = 405). After removing the outliers, the correlations were no longer statistically significant, with the exception of body surface area (r = 0.102; P < .05). Body mass indexes were not significant correlated to LN FE_{NO}.

ISAAC questionnaire

Fourteen percent (n = 57) of the children reported 1 or more of the following symptoms: running nose/conjunctivitis (n = 52), itching eyes (n = 24), and hay fever (n=27); they were defined as atopics. Children with symptoms of rhinitis had significantly higher FE_{NO} values than children without (12.7 ppb *vs* 9.3 ppb; P < .004). The same was true for children reporting itching eyes (13.5 ppb *vs* 9.5 ppb; P < .019) or hay fever (13.9 ppb *vs* 9.5 ppb; P < .001). These self-reported manifestations were all significantly positively related to FE_{NO} (P < .01), even when controlling for age.

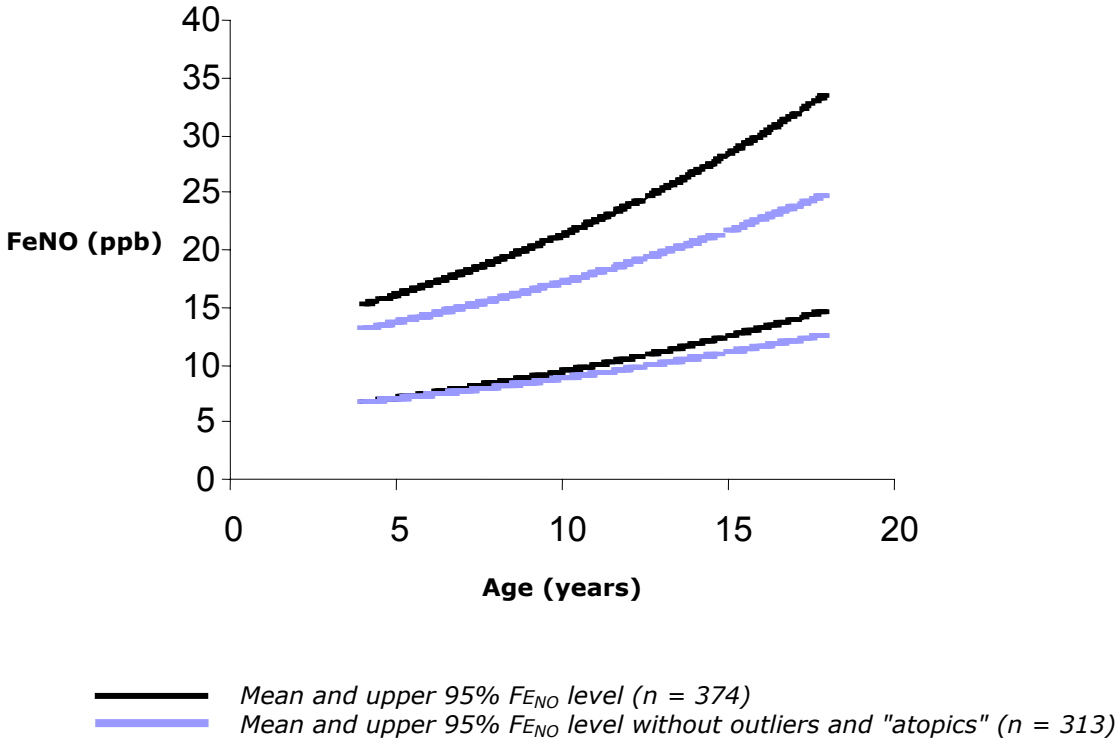

Figure 4.3 *FE_{NO} values in healthy children*

History of atopy in the family is significant more frequent in atopic children compared with nonatopic (24% *vs* 12%; $P < .003$).

A history of eczema (11%), ambient NO level, time of day, atopy in family (34%), and passive smoking within the last 6 months (32%) had no significant influence on FE_{NO} level.

Ethnic background

Non-Caucasian subjects (n = 31: African American subjects, Asian subjects, and others) had significantly higher FE_{NO} ($P < .002$) mean values (mean difference = 9.1 ppb [95% CI, 3.6 – 14.7 ppb]) compared with Caucasian subjects. However, when controlled for age, sex, and self-reported atopy in a stepwise forward linear regression analysis, ethnic origin had only a borderline significant effect on FE_{NO} ($P < .076$).

Country difference

When controlling for age, sex, ethnicity, and self-reported atopy, there was a significant effect of geographic location: the site in Virginia had significantly higher arithmetic FE_{NO} levels (mean FE_{NO} difference = 6.5 ppb; 95% CI, 2.7 – 10.3 ppb; $P < .001$) compared with the other centres, also after excluding outliers.

Diurnal variation

There was a trend of increased FE_{NO} in the afternoon ($r = 0.11$; $P < .03$), but when performing a regression analysis including time point and age as independent variables, the variation of FE_{NO} was explained by the age rather than the time point. This was because older children more often performed their measurement in the afternoon.

Success rate

One hundred forty-three children violated the protocol because they could not cooperate or needed more than 6 attempts to obtain 3 approved measurements. Twenty-nine were still included in the analyses as they had apparently been interrupted by other children during the procedure. Success rates improved with age and increased from 40% at 4 years and 60 % at 5 years to 100% at school age.

Repeatability

The within-subject SD estimated from individuals with 3 approved measurements was 1.6 ppb (95%CI, 1.49 – 1.64).

Safety

One 5-year-old boy was withdrawn after the first measurement because he started crying. No serious or nonserious adverse events were reported.

DISCUSSION

This is the first report on normal values of exhaled NO in healthy children from preschool age to adolescence performed according to current ATS guidelines by using commercially available equipment and the online single breath technique.

We report that the upper normal level of F_{ENO} in children from 4 to 17 years ranges from 15 ppb in the young children to 25 ppb in adolescents, with a mean increase of 1 ppb per year. We established this age dependency, which has been suggested in other smaller studies.[3,12,13] The reason F_{ENO} increases with age is not known, but there is no age-related F_{ENO} trend in adulthood. [14] The age dependency in children may be related to the developmental and maturational changes; increased lung volume and airway surface area;[12] changes in airway NO diffusion coefficients, which may be dependent on surface area;[3] or age-dependent induction of inducible nitric oxide synthase secondary to recurrent immunological stimulation.[15] The use of a constant exhalation flow rate (50 ml/s) in children of different airway sizes may also be a factor explaining the age dependency of exhaled NO, because the same flow is relatively higher for young children.[16]

Age per se may not influence F_{ENO}, because variables like body weight, height, and body surface area all were related to F_{ENO} when controlling for age. However, the partial correlation coefficients were rather low (0.115 - 0.139). Studies in children have not previously reported any correlation between F_{ENO} and height or weight, contrary to healthy adults, in whom F_{ENO} has been reported to depend on height, weight, body mass index, and body surface area.[14] Interestingly, when outliers were excluded, the correlations were not significant. Whether differences in body size explain the F_{ENO} difference between US and Europe is not clear, but the US subjects were significantly taller and heavier when controlled for age, atopy, and ethnicity compared with the European children. In addition, 50% of the outliers came from the US site (Virginia), which to some extent could explain the increased F_{ENO} in this group.

Upper limits of normal F_{ENO} values between 15 and 25 ppb in the current study are comparable with findings of the few earlier studies in children[11,12,17,18] using the same exhalation flow of 50 ml/s as recommended by the ATS.[8] However, children younger than 6 to 7 years have not been included in any of the previous studies with similar methodology. An earlier study in children between 4 and 7 years measured F_{ENO} using a single breath and 50 ml/s exhalation flow reported normal values of 4.9 and 7.6 ppb for boys and girls, respectively.[7]

A significantly higher F_{ENO} has been reported in healthy men compared with women[11] and in girls compared with boys.[7] We found no sex effect in children, nor has that been found in other previous studies in children.[3,12,15]

Children who reported allergic symptoms in the nose or eyes or hay fever had higher FE_{NO} levels than children without. Previous studies have reported that adolescents and adults with allergic rhinitis have increased $FE_{NO,}$[19,20] and that sensitized but asymptomatic children and adults have higher FE_{NO} than asymptomatic non-sensitized individuals.[12,21,22] Allthough data are still conflicting, sensitization, rhinitis, and conjunctivitis may be independent determinants of FE_{NO} in subjects without asthma of all ages.[3,23]

Excluding subjects with a suspected history of allergic rhinitis causes only a minor change of the upper 95% FE_{NO} levels (*Table 4.2*). Still, suspicion of allergic rhinitis should be considered when measuring $FE_{NO,}$ because acute allergen exposure might influence FE_{NO}.[19,24] Because this study included healthy children from public institutions, unfortunately it was not feasible to measure atopy objectively by skin prick tests or total specific IgE, so we cannot rule out that atopic subjects have higher FE_{NO} levels. However, because the ISAAC questionnaire is simple and validated, we believe that these reports are reliable.

A history of eczema or passive smoking was not correlated to FE_{NO}, and previous reports have been conflicting.[12,25,26]
Our study was a cross-sectional study with 1 visit during the daytime; hence, diurnal variation could not be investigated. In 6 nonatopic school children, a circadian rhythm of the inflammatory markers (FE_{NO} and urine eosinophilic protein X) has been demonstrated with a peak in the early morning.[27] However, the majority of the children in our study were measured within a rather narrow period of time during the day (9 AM to 3 PM). Indeed, other studies have not been able to prove a significant variation during the day.[3,11,28]

Most subjects were Caucasians, and this study was not powered to detect any effect from ethnic background on FE_{NO}. Indeed, 43% of the extreme outliers were non-Caucasian subjects, but they accounted for only 7% of all included children. It can be speculated whether genetic differences might explain the high prevalence of non-Caucasian outliers. Second, the significantly increased mean FE_{NO} level in 1 US center (Virginia) compared with the other participating centers may partly reflect genetic differences, because this US center contributed 67% of the non-Caucasian subjects. Significant differences in allele frequencies between Caucasians subjects and African American subjects in the neuronal nitric oxide synthase (NOS1) gene have been described, and this enzyme is involved in the endogenous NO production.[29] Further studies may reveal the need for ethnicity-specific reference FE_{NO} values.

Even though FE_{NO} is assumed to be unaffected by ambient NO when using the single breath technique,[16,30] it is generally recommended to use NO-free air if ambient NO is in excess of 50 ppb.[8] We therefore used NO-free air for all measurements. Exposure during the morning hours to high levels of outdoor pollution has been associated with increased FE_{NO}, which persisted as long as 5

hours,[31] but because ambient NO levels in general were higher in Europe compared with the United States, such a phenomenon cannot explain the difference in mean F_{ENO} between the United States and Europe.

Food intake or drinking (not water) and extreme physical activity within an hour before the measurement may confound the F_{ENO} measurement. Previous studies have shown that nitrate-rich food before measurement might increase F_{ENO} by denitrifying microbiological organisms in the mouth,[32,33] and intake of caffeine, which is found in coffee, tea and cola, has been associated with a brief reduction of F_{ENO} in healthy adults over 4 hours.[34] Sixteen per cent of our children had been eating or drinking shortly before measurements, but any effect of this consumption on F_{ENO} could not be demonstrated, because their F_{ENO} level was measured only once. Exercise might increase[35] or decrease[36] F_{ENO} in healthy subjects, but very few subjects had performed physical activity within 60 minutes before the measurement procedure without any significant effect on the results. F_{ENO} values were similar to the other children when controlled for confounders such as age and atopic symptoms.

Nitric oxide measurement was possible in 40% of 4-year-old children. The success rate increased to 80 to 100% in late school age. The NIOX has a built-in dynamic flow restrictor, which helps the child to maintain constant flow despite small variations in mouth pressure. To increase feasibility in the youngest age groups, we reduced exhalation time to 6 seconds for children younger than 10 years, which deviates from the current guidelines.[8] However, in the ATS guidelines of 1999, a plateau of 2 seconds and minimal exhalation of 4 seconds are recommended for children younger than 12 years.

Short-term repeatability (intrasubject variation) of 3 approved measurements was 1.6 ppb. This is even better than in the study by Kharitonov *et al*.[11] using the same NO analyzer, in which the mean pooled SD of all measurements from 59 children and adults was 2.1 ppb.

In conclusion, F_{ENO} measurement with NIOX is safe, has excellent short-term repeatability and is feasible in school-age children. The upper limit of normal F_{ENO} level in healthy children significantly increases with age from 15 ppb at the age of 4 to 25 ppb in adolescence. F_{ENO} was higher in children with self-reported atopic manifestations.

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REFERENCES

1. Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. Eur Respir J 1993;6:1368-1370.
2. Dotsch J, Demirakca S, Terbrack HG, Huls G, Rascher W, Kuhl PG. Airway nitric oxide in asthmatic children and patients with cystic fibrosis. Eur Respir J 1996;9:2537-2540.
3. Latzin P, Beck J, Griese M. Exhaled nitric oxide in healthy children: variability and a lack of correlation with atopy. Pediatr Allergy Immunol 2002;13:37-46.
4. Baraldi E, Azzolin NM, Cracco A, Zacchello F. Reference values of exhaled nitric oxide for healthy children 6-15 years old. Pediatr Pulmonol 1999;27:54-58.
5. Scollo M, Zanconato S, Ongaro R, Zaramella C, Zacchello F, Baraldi E. Exhaled nitric oxide and exercise-induced bronchoconstriction in asthmatic children. Am J Respir Crit Care Med 2000;161:1047-1050.
6. Kharitonov SA, Yates D, Barnes PJ. Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. Eur Respir J 1995;8:295-297.
7. Pijnenburg MW, Lissenberg ET, Hofhuis W, Ghiro L, Hop WC, Holland WP, De Jongste JC. Exhaled nitric oxide measurements with dynamic flow restriction in children aged 4-8 yrs. Eur Respir J 2002;20:919-924.
8. Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. Am J Respir Crit Care Med 1999;160:2104-2117.
9. Baraldi E, De Jongste JC. Measurement of exhaled nitric oxide in children, 2001. Eur Respir J 2002;20:223-37.
10. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-491.
11. Kharitonov SA, Gonio F, Kelly C, Meah S, Barnes PJ. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. Eur Respir J 2003;21:433-438.
12. Fraklin PJ, Taplin R, Stick SM. A community study of exhaled nitric oxide in healthy children. Am J Respir Crit Care Med 1999;159:69-73.
13. Kissoon N, Duckworth LJ, Blake KV, Murphy SP, Taylor CL, DeNicola LR, et al. Exhaled nitric oxide concentrations: online versus offline values in healthy children. Pediatr Pulmonol 2002;33:283-292.
14. Tsang KW, Ip SK, Leung R, Tipoe GL, Chan SL, Shum IH, et al. Exhaled nitric oxide: the effects of age, gender and body size. Lung 2001;179:83-91.
15. Avital A, Uwyyed K, Berkman N, Bar-Yishay E, Godfrey S, Springer C. Exhaled nitric oxide is age-dependent in asthma. Pediatr Pulmonol 2003;36:433-438.
16. Sikoff PE, McClean PA, Slutsky AS, Furlott HG, Hoffstein E, Wakita S, et al. Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. Am J Respir Crit Care Med 1997;155:260-267.
17. Jöbsis Q, Schellekens SL, Kroesbergen A, Hop WC, De Jongste JC. Offline sampling of exhaled air for nitric oxide measurement in children: methodological aspects. Eur Respir J 2001;17:898-903.
18. Buchvald F, Eiberg H, Bisgaard H. Heterogeneity of F_{ENO} response to inhaled steroid in asthmatic children. Clin Exp Allergy 2003;33:1735-1740.
19. Henriksen AH, Sue-Chu M, Lingaas HT, Langhammer A, Bjermer L. Exhaled and nasal NO levels in allergic rhinitis: relation to sensitization, pollen season and bronchial hyperresponsiveness. Eur Respir J 1999;13:301-306.
20. Gratziou CM, Lignos M, Dassiou M, Roussos C. Influence of atopy on exhaled nitric oxide in patients with stable asthma and rhinitis. Eur Respir J 1999;14:897-901.
21. Van Amsterdam JG, Janssen NA, De Meer G, Fischer PH, Nierkens S, Van Loveren H, et al.The relationship between exhaled nitric oxide and allergic sensitization in a random sample of school children. Clin.Exp.Allergy 2003;33:187-191.
22. Horvath I, Barnes PJ. Exhaled monoxides in asymptomatic atopic subjects. Clin Exp Allergy 1999;29:1276-1280.

23. Berlyne GS, Parameswaran K, Kamada D, Efthimiadis A, Hargreave FE. A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation. J Allergy Clin Immunol 2000;106:638-644.

24. Baraldi E, Carra S, Dario C, Azzolin N, Ongaro R, Marcer G, Zacchello F. Effect of natural grass pollen exposure on exhaled nitric oxide in asthmatic children. Am J Respir Crit Care Med 1999;159:262-266.

25. Yates DH, Breen H, Thomas PS. Passive smoke inhalation decreases exhaled nitric oxide in normal subjects. Am J Respir Crit Care Med 2001;164:1043-1046.

26. Spergel JM, Paller AS. 2003. Atopic dermatitis and the atopic march. J Allergy Clin Immunol 2003;112:S118-S127.

27. Mattes J, Storm van's Gravenzande GK, Moeller C, Moseler M, Brandis M, Kuehr J. Circadian variation of exhaled nitric oxide and urinary eosinophil protein X in asthmatic and healthy children. Pediatr Res 2002;51:190-194.

28. Ten Hacken NH, Van der Vaart H, Van der Mark TW, Koeter GH, Postma DS. Exhaled nitric oxide is higher both at day and night in subjects with nocturnal asthma. Am J Respir Crit Care Med 1998;158:902-907.

29. Togashi H, Sasaki M, Frohman E, Taira E, Ratan RR, Dawson TM, Dawson VL. Neuronal (type I) nitric oxide synthase regulates nuclear factor kappaB activity and immunologic (type II) nitric oxide synthase expression. Proc Natl Acad Sci U S A 1997;94:2676-2680.

30. Piacentini GL, Bodini A, Vino L, Zanolla L, Costella S, Vicentini L, Boner AL. Influence of environmental concentrations of NO on the exhaled NO test. Am J Respir Crit Care Med 1998;158:1299-1301.

31. Van Amsterdam JG, Verlaan BP, van Loveren H, Elzakker BG, Vos SG, Opperhuizen A, Steerenberg PA. Air pollution is associated with increased level of exhaled nitric oxide in nonsmoking healthy subjects. Arch Environ Health 1999;54:331-335.

32. Olin AC, Aldenbratt A, Ekman A, Ljungkvist GL, Jungersten L, Alving K, Toren K. Increased nitric oxide in exhaled air after intake of a nitrate-rich meal. Respir Med 2001;95:153-158.

33. Zetterquist W, Pedroletti C, Lundberg JO, Alving K. Salivary contribution to exhaled nitric oxide. Eur Respir J 1999;13:327-333.

34. Bruce C, Yates DH, Thomas PS. Caffeine decreases exhaled nitric oxide. Thorax 2002;57:361-363.

35. Jungersten L, Ambring A, Wall B, Wennmalm A. Both physical fitness and acute exercise regulate nitric oxide formation in healthy humans. J Appl Physiol 1997;82:760-764.

36. Terada A, Fujisawa T, Togashi K, Miyazaki T, Katsumata H, Atsuta J, et al. Exhaled nitric oxide decreases during exercise-induced bronchoconstriction in children with asthma. Am J Respir Crit Care Med 2001;164:1879-1884.

Chapter 5

The Effect of Spirometry and Exercise on Exhaled Nitric Oxide in Children

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ABSTRACT

American Thoracic Society (ATS) guidelines recommend to refrain from spirometry or exercise before measuring fractional exhaled nitric oxide (F_{ENO}) because forced breathing maneuvers might influence F_{ENO} values. However, the few studies already reported in children have given conflicting results.

The aim of the study was to observe to what extent spirometry or exercise could affect F_{ENO} asthmatic children.

Twenty-four asthmatic children (mean age 12.8 years) were enrolled. F_{ENO} was measured with a chemiluminescence analyzer. Measurements of F_{ENO} were performed before and 5, 15, 30, 45 and 60 minutes after spirometry or a 6-min walk test, on two separate days in random order.

Geometric mean F_{ENO} at baseline was 25.6 parts per billion (ppb) before spirometry and 23.5 ppb before exercise. A small drop of F_{ENO} to 24.2 and 23.7 ppb was found 5 and 15 min after spirometry (both $P = 0.04$). After exercise, F_{ENO} values showed a larger drop to 18.5 ppb after 5 min and 20.7 ppb after 15 min ($P < 0.001$; $P = 0.004$ respectively). Changes in F_{ENO} occured after exercise irrespective of baseline F_{ENO} and returned to baseline within 30 minutes.

We conclude that both spirometry and exercise affect F_{ENO} in asthmatic children. As the changes after exercise may lead to erroneous interpretations, children should refrain from physical exercise during at least 30 min before F_{ENO} measurement.

INTRODUCTION

Fractional exhaled nitric oxide (F_{ENO}) is an easy, repeatable, safe and noninvasive marker of bronchial inflammation in allergic asthma. Standardized guidelines for the measurement of F_{ENO} have been published, and analyzers that meet those requirements are commercially available for clinical application.[1,2] Guidelines from the joint European Respiratory Society (ERS)/ American Thoracic Society (ATS) task force recommend to perform F_{ENO} analysis before spirometric tests, because it has been shown that forced expiratory maneuvers reduce F_{ENO} levels in healthy and asthmatic adults.[3-5] However, only one study by Kissoon *et al*.[6] is available on the effect of spirometric tests on F_{ENO} in children and the results are in contrast to those found in adults by Silkoff *et al*. and Deykin *et al*.[3,5] Exercise may reduce F_{ENO} values as well and guidelines recommend to refrain from strenuous exercise for 1 h before the F_{ENO} test.[7-13] Again, the few data that are available in children reached conflicting results.[12,13] In particular, while a study by Scollo *et al*.[12] reported no change in F_{ENO} values after a treadmill exercise challenge, Terada *et al*.[13] reported that F_{ENO} values significantly decreased after exercise in children with and without exercise-induced bronchoconstriction (EIB) and in healthy subjects. F_{ENO} is increasingly used next to spirometry, hence it seems of practical importance to understand whether forced expiratory maneuvers influence F_{ENO} values in children. Also, children are likely to run and play in the waiting room, thus it is important to understand to what extent physical exercise can influence their F_{ENO} values. The duration of the effects of both spirometry and exercise on F_{ENO} values is not yet known, therefore we established the timing of the measurements in relation to such maneuvers. The aim of the present study was to observe whether or not spirometry or exercise immediately preceding F_{ENO} measurement could influence F_{ENO} values in asthmatic children.

MATERIALS AND METHODS

Subjects

We recruited 24 asthmatic children (12 boys) aged 7.9 – 17.7 yr (mean 12.8 yr) with a range of F_{ENO} values [6.5 – 176.2 parts per billion (ppb)]. A diagnosis of asthma was based on a history of recurrent episodes of wheezing, coughing and shortness of breath, reversible bronchoconstriction and/or airway hyperresponsiveness to metacholine.[14]

Seventeen subjects had RAST class 2 or higher for at least one airborne allergen and all patients were on controller medication at the moment of the study (*table 5.1*). All subjects used inhaled salbutamol as needed. Bronchodilators were withheld for 8 h (short acting) or 48 h (long acting) before the tests.
None of the patients had a history of upper respiratory tract infection or asthma exacerbation during 2 wk before the study. Parents and children, if older than 12 yr, gave their written informed consent. The study was approved by the Medical Ethical Committee of the Erasmus MC–Sophia Children's Hospital.

Table 5.1 Baseline characteristics of study subjects

Subject	Age years	Sex	FE_{NO} baseline pre-spirometry *	FE_{NO} baseline pre-exercise *	FEV_1 % of predicted	FVC % of predicted	Allergy (RAST class 2 or higher)	Controller medication mcg/day
1	9	M	11.2	15.4	112	126	No	Budesonide 400 Formoterol 12
2	11	M	18.0	12.8	81	83	Dust mite	Beclometasone 400
3	11	F	36.2	25.5	116	108	No	Budesonide 400
4	15	M	14.8	9.9	85	104	Dust mite, Pollens	Budesonide 400
5	11	M	8.5	6.9	100	100	Dust mite, Pollens	Budesonide 400 Formoterol 12
6	17	F	46.7	29.4	109	116	Pollens	Budesonide 400 Formoterol 9
7	11	F	7.2	8.3	113	111	Dust mite	Fluticasone 200
8	12	M	45.8	95.8	94	93	Dust mite	Budesonide 100
9	9	F	14.1	13.0	105	117	No	Budesonide 400 Formoterol 12
10	12	M	33.0	45.9	77	96	Grass Pollens	Fluticasone 200 Salmeterol 50
11	14	M	28.6	19.3	93	80	Dust mite	Budesonide 400
12	14	F	58.8	105.3	92	107	Dust mite	Budesonide 400
13	13	M	27.2	10.1	99	108	Dust mite, Pollens	Budesonide 400 Formoterol 12
14	12	F	176.2	62.9	105	104	No	Fluticasone 200
15	15	M	140.3	85.3	103	98	Dust mite, Pollens	Budesonide 400 Formoterol 12
16	11	F	6.5	8.2	92	78	No	Fluticasone 200 Salmeterol 50
17	10	M	14.8	13.5	99	108	Dust mite	Beclometasone 400
18	14	F	78.4	77.4	77	68	Dust mite, Pollens	Fluticasone 250
19	14	F	35.0	19.4	105	100	Dust mite, Pollens	Beclometasone 400 Formoterol 12
20	16	F	32.2	39.0	109	97	No	Beclometasone 400
21	13	F	17.9	14.7	107	101	Dust mite, Cat, Dog	Beclometasone 400
22	7	M	17.6	35.1	75	77	Dust mite, Pollens	Beclometasone 400
23	16	F	27.5	32.0	103	107	No	Budesonide 400 Formoterol 9
24	10	M	11.1	14.2	76	112	Dust mite	Beclometasone 400

* *FE_{NO} is expressed as geometric mean of two triplicate measurements*

Study design

The study was performed on 2 days within 2 wk. In random order, children performed spirometry (day A) or a 6-min walk test (day B). On both days baseline FE_{NO} was measured twice during a resting period of 30 min, at $t = -30$ min and $t = -15$ min. Then either exercise or spirometry was performed ($t = 0$), followed by FE_{NO} measurements at $t = 5$, 15, 30, 45 and 60 min.

Pulmonary function testing

Spirometry was performed with a dry rolling seal spirometer (Jaeger, Würzburg, Germany), according to ATS criteria.[15] Three forced vital capacity (FVC) maneuvers were performed and the best value of FVC and forced expiratory volume in 1 s (FEV_1) was recorded.

Exercise testing

A 6-min walk test was performed indoors along a long, flat, straight, enclosed corridor with a hard surface.[16] The test was modified and adapted to the study needs; children walked between two 8-m points for 6 min. Heart rate was recorded at the start and at the end of the exercise; the total number of rounds and the total meters covered were recorded.

FE_{NO} measurements

The FE_{NO} measurements were carried out with the NIOX NO-analyzer (Aerocrine, Stockholm, Sweden) according to ERS/ATS guidelines.[2] Children inspired NO-free air and exhaled for a minimum of 7 s. Exhalation flow was kept constant at 50 ml/s through a visual feedback mechanism and dynamic flow restrictor. Any exhalation, which did not meet the ERS/ATS requirements, was not accepted by the NIOX system and the subjects were asked to perform a new exhalation maneuver. At each session three correctly executed exhalations were recorded. FE_{NO} values were expressed in ppb.

Statistical analysis

Intraclass correlation coefficients were calculated for both days in order to assess the reproducibility of FE_{NO} at $t = -30$ and $t = -15$. Differences in baseline values of the two study days were analyzed by paired *t*-test. Changes in FE_{NO} from baseline at the various time points were analyzed with repeated measurements ANOVA; two-tailed *P*-values of < 0.05 were considered significant.

The FE_{NO} data were log-transformed before analysis in order to obtain a normal distribution, and expressed as geometric mean. Regression analysis was used to investigate the relation between FEV_1 and FE_{NO}.

RESULTS

Baseline FE_{NO} before spirometry and exercise were not significantly different and the two baseline measurement were highly reproducible within children (intraclass correlation coefficient = 0.99 on both days). Hence, the geometric mean of the two baseline values was calculated and used as the individual baseline for the analysis. Baseline geometric mean FE_{NO} values were 25.6 ppb (range 6.5 – 176.2 ppb) before spirometry and 23.5 ppb (range 7.0 – 105.3 ppb) before exercise.

All subjects successfully performed the spirometric tests. Mean FEV_1 was 97% of predicted (range 75 – 116%), and mean FVC was 100% of predicted (range 68 – 126%). A small but significant drop of FE_{NO} to 24.2 ppb and to 23.7 ppb was found, respectively, 5 and 15 min after spirometry (both $P = 0.04$; *Fig. 5.1*). Values of FEV_1 did not significantly correlate either with the baseline FE_{NO} or with changes in FE_{NO}.

Children covered an average distance of 473 m (range 272 – 848 m), leading to a mean increase in heart rate of 87 beats per minute (range 31 – 133). We found a consistent and significant drop in FE_{NO} values after exercise to 18.5 ppb after 5 min and 20.7 ppb after 15 min ($P < 0.001$ and $P = 0.004$ compared with baseline respectively) (*Fig. 5.1*). Nineteen subjects (79%) showed the maximum drop in FE_{NO} values within 5 min after exercise. A fall in FE_{NO} after exercise occurred irrespective of baseline FE_{NO} and independently of the amount of exercise as reflected by the increase in heart rate (*Fig. 5.2*). Only two subjects did not show a recovery of FE_{NO} values within 80% of baseline within 30 min after exercise. The mean changes in FE_{NO} from baseline at 5 min after spirometry and exercise were significantly larger after exercise than after spirometry ($P < 0.001$, paired *t*-test).

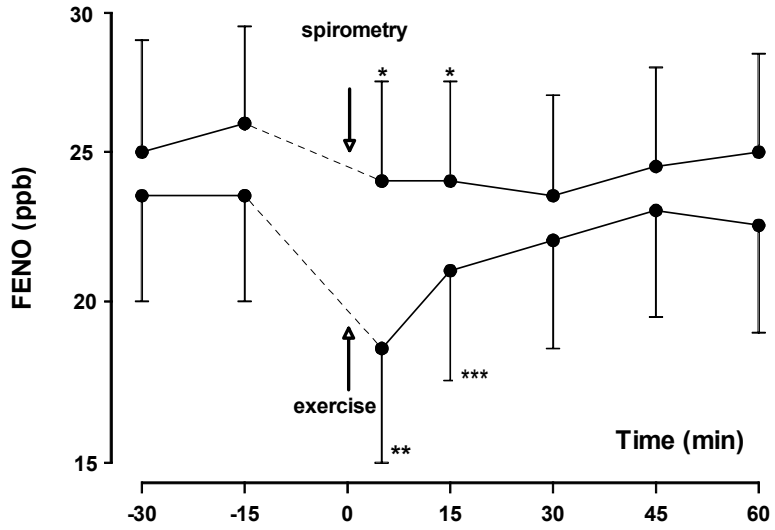

Figure 5.1 *FE_{NO} before and after spirometry (upper curve) and exercise (lower curve) in asthmatic children (n = 24). FE_{NO} is shown as geometric means and SEM. The changes in log-transformed FE_{NO} values 5 and 15 min. after spirometry and exercise, compared to baseline values, are significant (*P = 0.04, **P < 0.001, ***P = 0.004).*

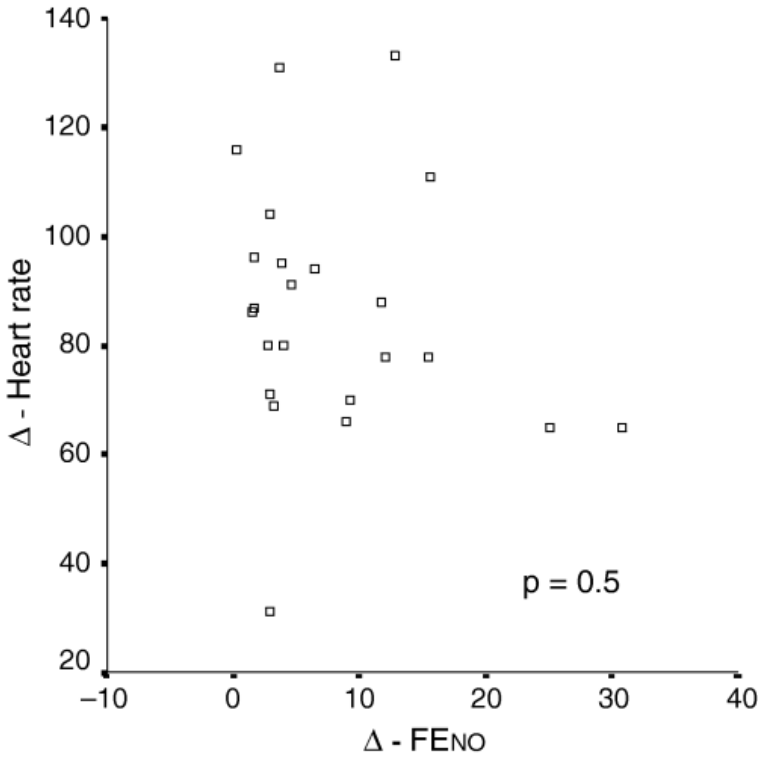

Figure 5.2 *Changes in FE_{NO} values (Δ - FE_{NO}) after exercise in relation to the amount of exercise, expressed as difference in heart rate post and pre-exercise (Δ - heart rate). There is no significant correlation.*

DISCUSSION

This is the first study comparing the effects of spirometry and exercise on FE_{NO} in asthmatic children. Our results show a consistent, significant reduction of FE_{NO} both after exercise and after spirometry. The effect of exercise on FE_{NO} values is larger than that of spirometry and the changes of FE_{NO} values are seen in the first 30 min after these maneuvers.

Previous studies about the influence of either spirometry or exercise on FE_{NO} values reached different conclusions. Deykin *et al*.[3] studied asthmatic adults and found that spirometry induced a significant drop in FE_{NO} values that was maximal 30 min after spirometry. The authors speculated that repeated spirometric maneuvers reduced NO production through an effect on bronchodilating non-adrenergic non-cholinergic nerves within the airways.[3] Silkoff *et al*.[4] found a fall in FE_{NO} values 1 min after spirometry in normal and asthmatic adults. In both groups, FE_{NO} returned to baseline within 1 h. In another study, Deykin *et al*.[5] documented a reduction in FE_{NO} after FVC maneuvers in non-asthmatic adults, while no change was found after repeated plethysmography, and suggested that the change in FE_{NO} values after spirometry is related to the pressure and volume history of the lung. Surprisingly, the only study on the effect of spirometry on FE_{NO} in children reached the opposite conclusion. Kissoon *et al*.[6] observed that FE_{NO} values slightly increased within 8 – 18 min after spirometry in asthmatic children. In this study

five of 10 asthmatic children used inhaled steroids, and in children on steroids, the mean increase of FE_{NO} was lower than in children not on steroids. Authors also evaluated the effect of spirometry and metered dose inhaler (MDI)-placebo on FE_{NO} and found that in poorly controlled asthmatics (not on inhaled steroids) there was a significant increase of FE_{NO} values 18 min after the study maneuvers. They speculated that the rapid and substantial changes in lung volume that occur during spirometry may trigger neural mechanisms leading to increased NO release from the lower airway.[6]

Our results are in agreement with the findings previously reported in adults, while different from the results of the study of Kissoon *et al*. The reasons for the discrepancies are not clear. The use of inhaled corticosteroids by the participants of our study might have suppressed the NO production from the source of nitric oxide, which may be sensitive to forced breathing. This could explain, on the one hand, the opposite results from the study of Kissoon *et al*., and also the relatively small decrease of FE_{NO} levels after the forced expiratory maneuvers, which seems unlikely to be of clinical relevance in most situations, particularly as the fall in FE_{NO} was observed to the greatest extent in children with normal levels.

Our findings regarding the effect of exercise on FE_{NO} are consistent with earlier observations. Data reported by Terada *et al*.[13] showed a drop in FE_{NO} values after exercise in asthmatic and healthy children. These authors also observed that patients with EIB were more likely to need a longer recovery than children without EIB before FE_{NO} values came back to baseline.[13] In contrast, Scollo *et al*.[12] evaluated the effects of exercise on FE_{NO} levels in 24 asthmatic children and 18 healthy controls; they concluded that FE_{NO} values did not change after exercise challenge in either healthy controls or in asthmatic children with and without EIB.[12] Exercise may lead to hyperventilation and reduced airway caliber in asthmatic children; both increase airflow velocity. We speculate that changes in bronchial diameter are responsible for reduced FE_{NO} levels after exercise. To avoid repeated forced breathing, we did not measure lung function after exercise. Despite a constant exhalation flow during FE_{NO} measurements, the reduced airway surface area might have led to lower nitric oxide diffusion through the airways. We found a recovery to baseline FE_{NO} values after 30 min post-exercise, when there was no clinical evidence of hyperventilation or bronchoconstriction.

What are the clinical implications of our findings? Children play and run in the waiting room. They should however refrain from exercise before performing FE_{NO} measurements, or FE_{NO} values would be underestimated, depending on the amount of exercise performed. We also defined the duration of the effect of exercise on FE_{NO} values and we suggest that asthmatic children refrain from strenuous exercise for 30 min before the FE_{NO} measurements, rather than the recommended 60 min. In fact, beyond 15 min there is only a small variation in FE_{NO} values, which can be attributed to random variation with time. Although the effect of spirometry on FE_{NO}

appears small, we suggest that future guidelines recommend to refrain from spirometry for 30 min before F_{ENO} measurements.

We conclude that exercise and, to a lesser extent, repeated spirometric maneuvers influence F_{ENO} values in children. As the changes after exercise may lead to erroneous interpretations, children should refrain from physical exercise during at least 30 min before F_{ENO} measurements.

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REFERENCES

1. American Thoracic Society. Recommendation for standardized procedures for the Online and Offline measurement of Exhaled lower respiratory Nitric Oxide and Nasal Nitric Oxide in Adults and Children. Am J Respir Crit Care Med 1999;160:2104-2117.
2. Baraldi E, de Jongste JC. Measurement of exhaled nitric oxide in children, 2001. Joint ERS/ATS task force on Exhaled Nitric Oxide in Children. Eur Respir J 2002;20:223-237.
3. Deykin A, Halpern O, Massaro F, Drazen JM, Israel E. Expired nitric oxide after bronchoprovocation and repeated spirometry in patients with asthma. Am J Respir Crit Care Med 1998;157:69-75.
4. Silkoff PE, Wakita S, Chatkin J, Ansarin K, Gutierrez C, Caramori M, McClean P, Slutsky AS, Zamel N, Chapman KR. Exhaled nitric oxide after beta-2 agonists inhalation and spirometry in asthma. Am J Respir Crit Care Med 1999;159:940-944.
5. Deykin A, Massaro AF, Coulston E, Drazen JM, Israel E. Exhaled nitric oxide following repeated spirometry or repeated plethysmography in healthy individuals. Am J Respir Crit Care Med 2000;161:1237-1240.
6. Kissoon N, Duckworth LJ, Blake KV, Murphy SP, Lima JJ. Effect of beta-2 agonist treatment and spirometry on exhaled nitric oxide in healthy children and children with asthma. Pediatric Pulmonology 2002;34:203-208.
7. Maroun MJ, Mehta S, Turcotte R, Cosio MG, Hussain SN. Effects of physical conditioning on endogenous nitric oxide output during exercise. J Appl Physiol 1995;79:1219-1225.
8. Iwamoto J, Pendergast DR, Suzuki H, Krasney JA. Effect of graded exercise on nitric oxide in expired air in humans. Respir Physiol 1994;97:333-345.
9. Bauer JA, Wald JA, Doran S, Soda D. Endogenous nitric oxide in expired air: effects of acute exercise in humans. Life Sci 1994;55:1903-1909.
10. Phillips CR, Giraud GD, Holden WE. Exhaled nitric oxide during exercise: site of release and modulation by ventilation and blood flow. J Appl Physiol 1996;80:1865-1871.
11. Persson MG, Wiklund NP, Gustaffson LE. Endogenous nitric oxide in single exhalations and the change during exercise. Am Rev Respir Dis 1993;148:1210-1214.
12. Scollo S, Zanconato S, Onagro R, Zaramella C, Zacchello F, Baraldi E. Exhaled nitric oxide and exercise-induced bronchoconstriction in asthmatic children. Am J Respir Crit Care Med 2000;161:1047-1050.
13. Terada A, Fujisawa T, Togashi K, Miyazaki T, Katsumata H, Atsuta J, Iguchi K, Kamiya H, Togari H. Exhaled nitric oxide decreases during exercise-induced bronchoconstriction in children with asthma. Am J Respir Crit Care Med 2001;164:1879-1884.
14. GINA: Global Initiative for Asthma Management and Prevention. Available at: http://www.ginasthma.com 2002.
15. Gardner RM. Standardization of spirometry: a summary of recommendation from the American Thoracic Society. The 1987 update. Ann Intern Med 1988;108:217-220.
16. ATS Statement: Guidelines for the Six-Minute Walk Test. Am J Respir Crit Care Med 2002;166:111-117.

part 2

Clinical Applications

6 chapter

High Fractional Concentration of Nitric Oxide in Exhaled Air Despite Steroid Treatment in Asthmatic Children

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ABSTRACT

Background: The fractional concentration of nitric oxide in exhaled air (F_{ENO}) is elevated in atopic asthma and typically responds to treatment with inhaled corticosteroids (ICS). However, some patients have persistently high F_{ENO} levels despite treatment.

Objective: We studied how optimizing the inhalation technique and increasing ICS doses would affect F_{ENO} in stable atopic asthmatic children who had elevated F_{ENO} while using ICS.

Methods: In 41 stable asthmatic children who were treated with ICS (median daily dose 800 µg budesonide equivalent, range 100-1600 µg) and maintained $F_{ENO} \geq 20$ ppb, we optimized the inhalation technique by thorough instruction and measured F_{ENO} 2 weeks later. Then, if F_{ENO} remained ≥ 20 ppb, we increased the ICS dose and reassessed F_{ENO} 2 weeks later.

Results: Improving the inhalation technique did not reduce F_{ENO}. Increasing ICS from a daily median dose of 800 µg to 1200 µg budesonide had no significant effect on F_{ENO}. F_{ENO} correlated positively with symptom scores in the following 2 and 4 weeks (P = 0.001, 0.002) and β_2-agonist use the 2 and 4 weeks following F_{ENO} measurement (P = 0.02, 0.004).

Conclusions: We conclude that common steps in asthma treatment, i.e. inhalation instruction and increasing ICS dose, were both ineffective in reducing F_{ENO} in atopic asthmatic children with elevated F_{ENO} values despite treatment with ICS. This implies that F_{ENO} cannot simply be incorporated in current treatment guidelines.

INTRODUCTION

The fractional nitric oxide concentration in exhaled air (FE_{NO}) is a marker of eosinophilic airway inflammation that reliably distinguishes steroid-free atopic asthmatics from normals.[1-5] Increased FE_{NO} in asthma has been attributed to upregulation of inducible NO-synthase (iNOS) by pro-inflammatory cytokines in airway epithelial cells.[6] Accordingly, treatment with anti-inflammatory drugs like inhaled corticosteroids (ICS) reduces FE_{NO} by directly effecting iNOS transcription and by reducing airway inflammation.[7-10] Even a single dose of ICS can already decrease FE_{NO} and multiple low doses of ICS may normalize FE_{NO}, with other markers of inflammation still remaining elevated.[11-13] The extent of FE_{NO} reduction by ICS is dose dependent.[9,14-16] This suggests that FE_{NO} is a sensitive and fast-responding marker, which could be useful as a monitoring tool in asthma management. However, FE_{NO} was also shown to remain elevated despite ICS treatment.[7] In our paediatric tertiary care setting almost half of the atopic asthmatic patients using ICS show elevated FENO values.[17] In an experimental setting, the effect of ICS on FE_{NO} closely correlated with treatment adherence, suggesting that poor adherence may partly explain persistently high FE_{NO} in ICS-treated asthmatics.[17-19] Alternatively, inhalation of drugs by children is prone to errors, and poor inhalation technique could well explain elevated FE_{NO}.[20,21] Finally, a recent biopsy study suggested that high FE_{NO} in children with severe asthma who used systemic steroids could be because of steroid-insensitive airway inflammation.[15] In the present study, we addressed inadequate inhalation technique and under medication as possible explanatory factors for elevated NO despite ICS. In a two-step approach, we aimed to reduce FE_{NO} first by instructing and checking the optimal inhalation technique. Second, those patients with persistently high FE_{NO} levels despite adequate inhalation technique were given higher doses of ICS in a further attempt to normalize FE_{NO}.

PATIENTS AND METHODS

Patients

Children aged 6 - 18 years who fulfilled American Thoracic Society (ATS) criteria for asthma and were clinically stable for at least 3 months were recruited from the outpatient clinic of the Sophia Children's Hospital. Atopy was defined as RAST class 2 or higher for at least one airborne allergen. Children with atopic asthma who had used stable doses of ICS during the preceding month and had FE_{NO} values exceeding 20 ppb were selected. Children with symptoms suggestive of respiratory tract infection or with oral steroid use during the preceding 4 weeks or during the study, children on leukotriene receptor antagonists and children who smoked were excluded. Written informed consent was obtained. The study was approved by the Medical Ethical Committee of Erasmus University Medical Centre-Sophia Children's Hospital.

Study design (*Fig. 6.1*)
At inclusion (visit one), children performed a flow-volume curve preceded by FE_{NO} measurement. The inhalation technique was scored on various items (*Table 6.1*). They were randomly allocated to one of two groups: one group (group 1) was first given thorough inhalation instruction according to the guidelines of the Dutch Asthma Fund, until a satisfactory performance was obtained.[22] Symptom scores and use of β_2-agonists were recorded during 2 weeks, and FE_{NO} was measured and the inhalation technique was re-assessed at t = 2 weeks (visit two). Patients whose FE_{NO} was still ≥ 20 ppb at t = 2 weeks were prescribed higher doses of ICS, after ascertaining that they followed good inhalation technique. By protocol, we doubled ICS if the initial dose was 400 μg or less budesonide or equivalent; initial doses of 400 - 800 μg were increased by 50% and doses above 800 μg by one-third. FE_{NO} was measured again at t = 4 weeks (visit three). The total study duration for this group was 4 weeks. The other group (group 2) was not instructed initially and served as a control for group 1. Patients in this group were merely asked to record symptom scores and rescue β_2-agonist use during 2 weeks, to rule out a possible effect of enrolment, which, as such could improve compliance and the inhalation technique. At t = 2 weeks (visit two), these children were instructed as well, and followed the same protocol as the others. The total study duration for these children was 6 weeks.

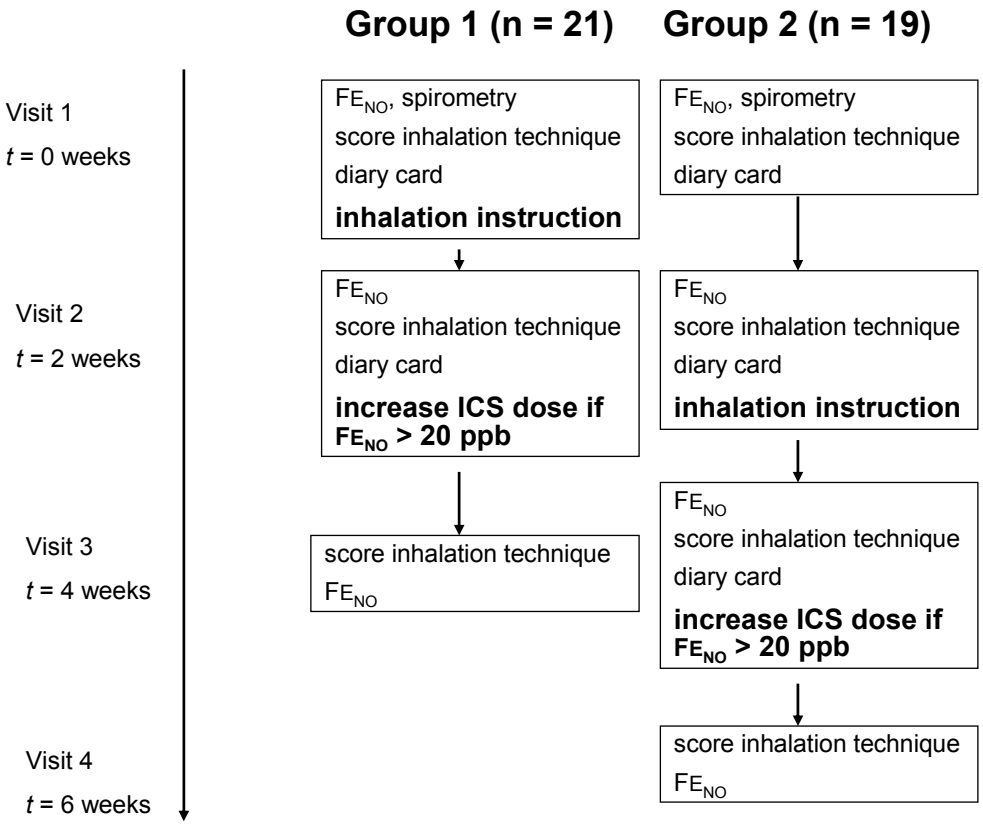

Figure 6.1 *Study design*

Table 6.1 Inhalation technique checklist, as provided by The Dutch Asthma Fund

	Dry powder inhaler
1	remove cap or open inhaler*
2	rotate before usage*
3	exhale to residual volume
4	place mouthpiece between teeth and lips*
5	inhale forcefully and deeply*
6	take inhaler out of mouth
7	hold breath for 5 s
	MDI plus spacer
1	shake the inhaler*
2	correct assembly of the spacer device and MDI*
3	activation of the canister*
4	one puff at a time
5	place mouthpiece between teeth and lips*
6	inhale at least five times
7	check that spacer valve is moving*

* *essential items*
MDI *metered dose inhaler*

Fractional concentration of nitric oxide in exhaled air measurements
Children performed three online manoeuvres on the NIOX NO analyser (Aerocrine, Stockholm, Sweden) according to ATS and ERS guidelines.[23] They inspired NO-free air and exhaled for a minimum of 7 s. Exhalation flow was kept constant at 50 ml/s through a visual feedback mechanism and a dynamic flow restrictor.

Pulmonary function tests
Flow-volume curves were obtained using dry rolling seal spirometers (Masterscope, Jaeger, Würzburg, Germany). Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) were recorded before and after administration of 1 mg of terbutalin and expressed as percentage predicted.

Inhalation instruction
Both the parents and the child were instructed by the investigators (E.B., M.P.).[22] First, children were asked to demonstrate how they used their medication. The investigator observed the performance and scored this on a checklist.[24] Seven items were scored for metered dose-inhaler/ spacer combinations (MDIs) and for dry powder inhalers (DPI), 4 and 5 of which, respectively, were considered essential for reliable drug delivery to the lungs (*Table 6.1*). If one or more essential items were missed the technique was considered insufficient. In that case the investigators instructed the use of a placebo inhaler, until a satisfactory performance was observed. The importance of compliance with prescribed treatment was emphatically pointed out. The child or the parents were asked to record symptoms and use of medication daily on diary cards.

Symptom scores

Symptoms were scored on a diary card as used in previous studies. Coughing, wheezing and dyspnoea were each scored twice daily on a four-point scale (0 - 3) for day and night separately. The maximum weekly symptom score was therefore 126. β_2-agonist use was recorded once daily.

Data analysis

FE_{NO} values were log transformed before all calculations. We used Spearman's correlation test to assess the relations of FE_{NO} with symptom scores, use of rescue medication and pulmonary function tests. Values in group 1 (instruction at first visit) were compared with values in group 2 (no initial instruction) by unpaired *t*-tests. Moreover, FE_{NO} before and after inhalation instruction or increasing the ICS dose were compared by paired *t*-tests. FE_{NO} data are presented as ratio and 95% confidence intervals (CIs) of the ratio of geometric means. Two-sided *P*-values < 0.05 were considered significant.

RESULTS

Forty-two atopic asthmatic children with $FE_{NO} \geq 20$ ppb were recruited. Two, one in each group, dropped out because their condition exacerbated during the study. One of them needed oral steroids between visit one and two and was excluded from the study. The second, (randomized to group 2) required an oral course between visit three and four. This patient's data till visit three were still used in the analyses. Of the 41 analysed patients (27 boys), 21 had been randomized to receive inhalation instruction first, the remaining 20 were instructed 2 weeks later. Patient data are summarized in *Table 6.2*.

Table 6.2 Patient data (n = 41 atopic asthmatic children, 27 boys)

	Mean	**Range**	**SD**
Age (years)	12.4	6.8 - 17.8	2.7
Weight (kg)	43.1	20.2 - 65.7	13.5
Height (cm)	151.0	117.0 – 177.6	16.0
FVC (% pred)	103	71 – 123	14
FEV_1 (% pred)	97	73 – 122	13
FVC after bronchodilator (% pred)	103	74 – 129	15
FEV_1 after bronchodilator (% pred)	104	79 – 128	13
β_2 response of FEV_1 (%)	6	-5 to 20	6
Initial ICS dose, μg (budesonide equivalent)	800*	100 – 1600	385

* *median*
FVC *forced vital capacity*
FEV_1 *forced exoiratory volume in 1 s*
ICS *inhaled corticosteroids*
SD *standard deviation*

Effect of inhalation instruction on FE_{NO}

Baseline geometric mean FE_{NO} (FE_{NO} at visit one) did not differ between the groups: 46.4 *vs*. 43.1 ppb, respectively.

In group 1 (immediate instruction) FE_{NO} before and 2 weeks after instruction was 46.4 and 42.2, the ratio of the geometric mean being 0.9 (95% CI 0.7 – 1.2) (*P* = 0.31) (*Fig. 6.2a*).

In group 2 (delayed instruction) FE_{NO} did not change significantly during the next 2 weeks, 43.1 and 46.7 ppb, respectively (ratio of geometric mean 1.1, 95% CI 0.9 - 1.4) (*P* = 0.48), indicating no effect of enrolment on FE_{NO} (*Fig. 6.2b*). The mean change in FE_{NO} between visits one and two did not differ between the groups (*P* = 0.26).

Considering all 41 children together, the ratio of geometric mean FE_{NO} before and 2 weeks after inhalation instruction was 0.9 (95% CI 0.8 – 1.1) (*P* = 0.31), absolute values being, respectively, 46.6 and 43.2 ppb. Seventeen of them missed one or more essential items when first demonstrating their inhalation technique. Sixteen of them could be instructed to inhale properly, and continued to show adequate inhalation technique 2 weeks later.

In this subgroup (n = 16) the geometric mean FE_{NO} decreased from 51.2 ppb to 43.6 ppb, ratio of geometric mean 0.9 (95% CI 0.7 - 1.0, *P* = 0.10).
The changes in FE_{NO} proved not to be associated with initial steroid dose, lung function or demographic data.

Effect of increased inhaled corticosteroids dose on fractional concentration of nitric oxide in exhaled air

Despite adequate inhalation technique, 37 out of the 41 children still had FE_{NO} values ≥ 20 ppb. Two weeks after instruction in an open extension, the ICS dose in these children was increased with a median dose of 400 μg (range 100 - 800) to a median daily dose of 1200 μg budesonide or equivalent. However, one patient refused to take the increased dose and was excluded from analysis. Two weeks after increasing the dose, FE_{NO} had decreased from 49.2 ppb to 43.1 ppb, the ratio of the geometric mean FE_{NO} 0.9 (95% CI 0.8 – 1.0) (*P* = 0.08) (*Fig. 6.3*). There was no correlation between the change in FE_{NO} after increasing ICS and initial steroid dose (*r* = 0.17, *P* = 0.26) or the absolute increase in steroid dose (*r* = 0.26, *P* = 0.12). Although this study was not designed for subgroup analysis, we established that doubling the dose in children with a daily dose of inhaled steroids of 400 μg budesonide equivalent or less (n = 15), significantly reduced FE_{NO} from 46.6 to 35.6 ppb, ratio geometric mean 0.8 (95% CI 0.6 – 0.9), *P* = 0.01. The effects of dose changes on FE_{NO} did not depend on which steroid was taken: children on ultrafine particle beclomethasone (n = 18) showed similar findings as those using conventional aerosols (data not shown).

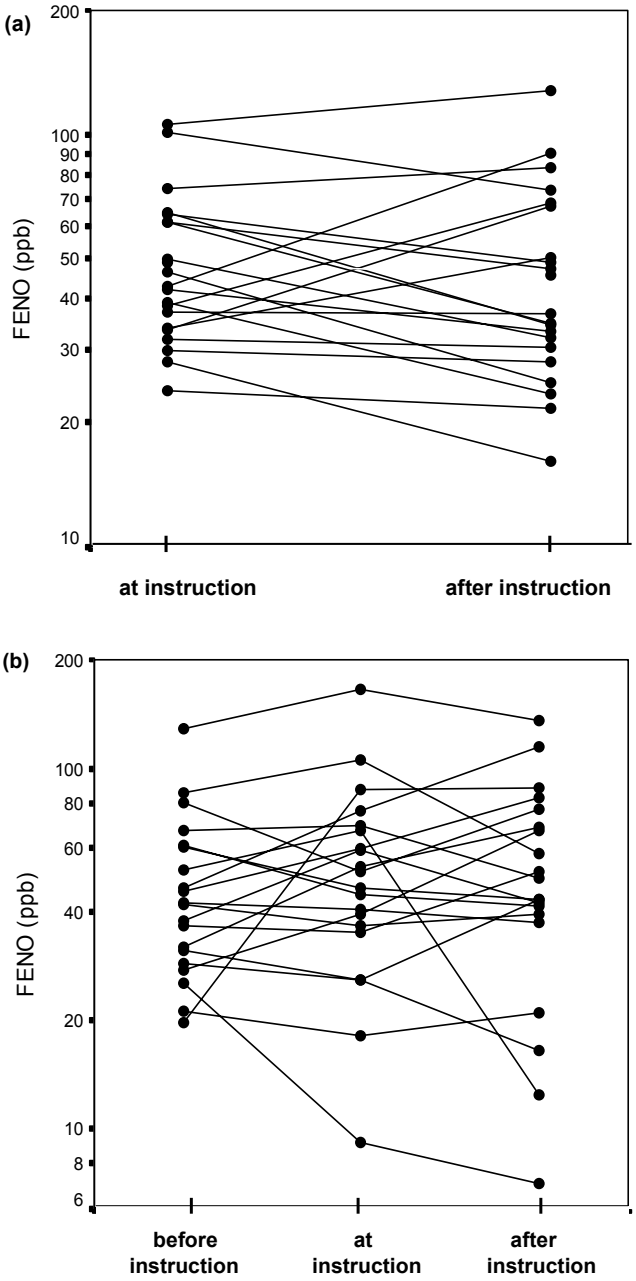

Figure 6.2 *Change in fractional concentration of nitric oxide in exhaled air (F_{ENO}) in group 1 (a) and in group 2 (b). In group 1 (instruction during the first visit) F_{ENO} decreased from 46.4 to 42.2 (P = 0.31). In group 2 (delayed instruction) F_{ENO} increased from 43.1 to 46.7 ppb during the first 2 weeks (P = 0.48). Two weeks after inhalation instruction the geometric mean F_{ENO} in this group was 44.3 ppb (P = 0.65). The mean difference in F_{ENO} between visits one and two did not differ between the groups (P = 0.28).*

Fractional concentration of nitric oxide in exhaled air, symptom scores and β_2-agonist use

We found a significant positive correlation between F_{ENO} at the time of instruction and symptom scores in the following 2 and 4 weeks (r = 0.51, P = 0.001 and r = 0.47, P = 0.002 respectively), indicating that F_{ENO} was predictive of symptoms during the coming weeks. The same was true for use of β_2-agonists (r = 0.39, P = 0.02 and r = 0.46, P = 0.004 at t = 2 and 4 weeks after instruction, respectively). *Figure 6.4* shows data for correlations after 2 weeks.

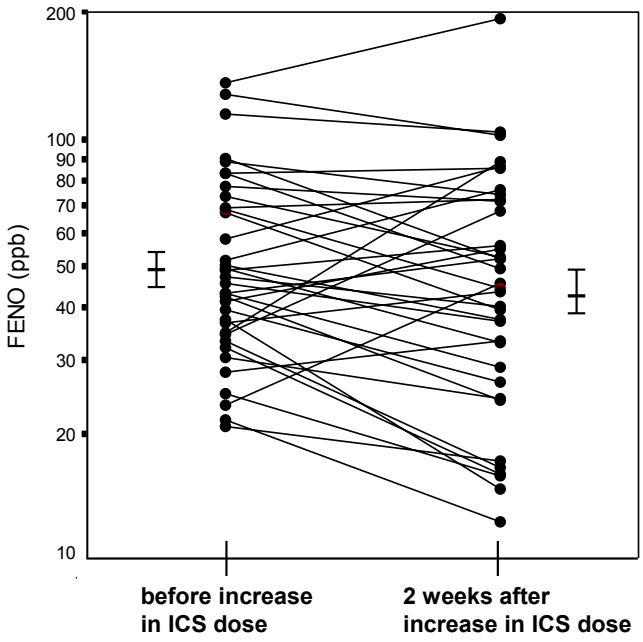

Figure 6.3 *Fractional concentration of nitric oxide in exhaled air (FE_{NO}) values before and 2 weeks after increasing inhaled corticosteroids dose in 36 atopic asthmatic children with $FE_{NO} \geq 20$ ppb. All were able to demonstrate optimal inhalation technique. The geometric mean FE_{NO} fell from 49.2 to 43.1 ppb (P = 0.08). Bars represent the geometric means plus or minus 1 SEM.*

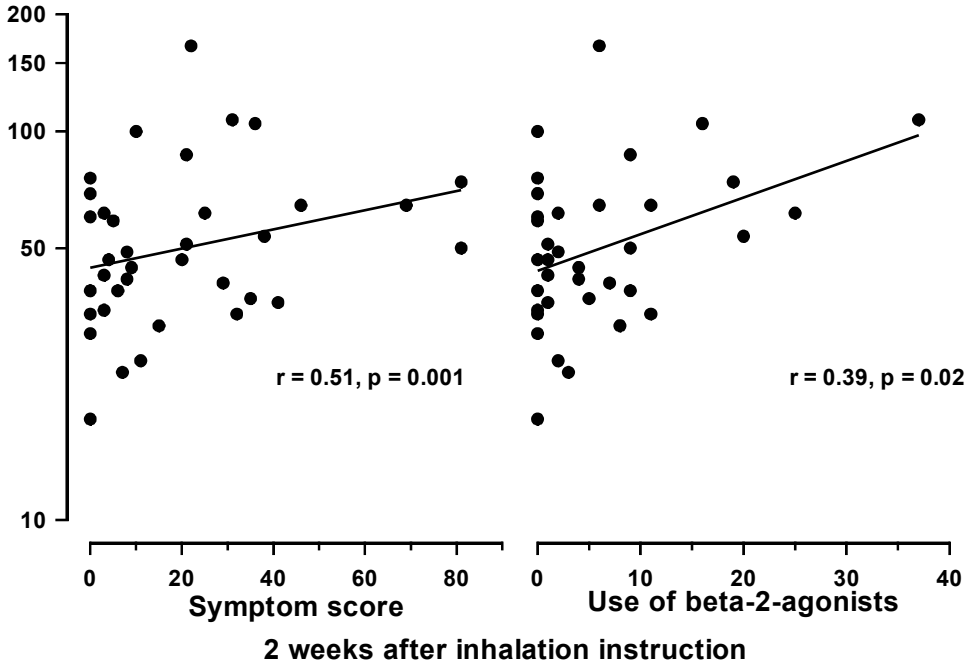

Figure 6.4 *(a) Correlation between fractional concentration of nitric oxide in exhaled air (FE_{NO}) at the time of instruction and symptom scores in the following 2 weeks (r = 0.51, P = 0.001). (b) Correlation between FE_{NO} and β_2-agonist use 2 weeks after instruction (r = 0.39, P = 0.02).*

DISCUSSION

We studied plausible factors that could explain persistently high FE_{NO} in atopic asthmatic children using ICS. Without intervention, FE_{NO} was stable over 2 weeks. Neither improvement of inhalation technique nor increasing ICS dose reduced FE_{NO} in this group of selected patients.

Earlier studies found that changes in ICS dose were mirrored by changes in FE_{NO}, and that the effect of ICS on FE_{NO} occurs within days or weeks.[7,9,11,13,14,18] Hence, FE_{NO} as a rapid marker of airway inflammation seems sufficiently sensitive to detect the influence of recent ICS treatment. However, in our tertiary care setting as many as half of all steroid-treated atopic asthmatics had FE_{NO} values $\geq$ 20 ppb.[17] The cut-off level for FE_{NO} of 20 ppb in this study was chosen based on previous findings using similar methodology in our own clinic (unpublished data) and normal values from an international study in a large group of children, including our own population, which showed age-dependent upper 95% confidence levels of 15 - 25 ppb. Hence, the present cut-off level seems appropriate to define normality.[24]

As many as 30 - 70% of asthmatic children at school age have been shown to have a flawed inhalation technique, and yet the correct technique is crucial for delivery of steroids in the bronchial tree.[20,21,25] This is why we studied whether inhalation instruction would bring about FE_{NO} reduction. Nevertheless, in those who showed satisfactory inhalation technique 2 weeks after instruction, we found no reduction of FE_{NO} as compared with those who did not receive initial instruction. A possible explanation is that children who improved their performance while under observation may have reverted to a poor technique unobserved at home. Alternatively, despite a non-optimal inhalation technique, some children might still have been able to deposit some steroid in their lungs, especially those using high doses of ICS. In such cases, poor inhaler technique cannot simply explain FE_{NO}. Moreover, the 16 children who showed inadequate inhaler technique that could be fully corrected may be insufficient to draw conclusions on whether FE_{NO} is useful in assessing the inhaler technique.

We strongly encouraged good adherence, and participation in a trial may be expected to improve adherence in the selected population, but obviously we cannot exclude that some children may not have taken the prescribed dose. However, FE_{NO} did not improve in those who were not instructed initially, the ones that acted as controls for those who did get instructed. Hence it seems unlikely that children improved adherence just because of enrolment in the study. Two recent studies found that treatment compliance strongly correlated with FE_{NO}, which is also our experience.[17-19] We did not attempt to quantify adherence in the present study, and hence poor adherence may have accounted for elevated FE_{NO} in children with optimal inhalation technique while under observation and with adequate steroid dose.

Although in some children FE_{NO} decreased after ICS dose was increased, normal values below 20 ppb were only achieved in 6 children. This is consistent with findings from Baraldi *et al*. showing that asthmatic children with acute exacerbation who were treated with oral steroids did not achieve normal FE_{NO} values.[7] Regression to the mean may account for the decrease in FE_{NO} after increasing the ICS dose. However, this decrease did not reach statistical significance. As our tertiary care centre mainly treats children with severe asthma, median steroid doses were quite high. High initial ICS doses might explain why FE_{NO} did not decrease in our population as the beneficial effects of ICS tend to plateau at higher doses.[11,26,27] Our patients might already have been optimally treated regarding their FE_{NO}. Moreover, dose increments in the higher dose ranges were relatively small. Although this study was not designed for subgroup analysis, the hypothesis that a dose-response effect on FE_{NO} is lacking is supported by the significant decrease that we found in a subgroup of children receiving a daily ICS dose of 400 μg budesonide equivalent or less. Further research in asthmatic children on relatively low doses of ICS, as seen in primary and secondary clinics, is needed to validate this finding.

Findings from Jatakanon *et al*. are consistent with our finding that children on high steroid doses in this study do not show a dose-response effect of ICS on FE_{NO}.[11] We cannot, however, exclude that very high ICS doses or systemic steroids could have further reduced FE_{NO}.

The 2-week time span in our study may arguably have been too short to detect a significant effect of ICS dose change on FE_{NO}. A recent study describes an decrease of FE_{NO} between 1 and 8 weeks after introducing ICS.[14] However, FE_{NO} was not measured in the intervening period and may well have stabilized earlier. Others have shown steroid effects on FE_{NO} well within 2 weeks.[9,13] Hence, we consider it unlikely that we missed the effects of our interventions on FE_{NO} in spite of the short time span.

As asthma affects the peripheral airways, it might be argued that ICS may not reach their target in children, who have smaller peripheral airways than adults but are yet treated with similar aerosols.[28] Reduced peripheral deposition of ICS could well explain the lack of steroid effect on FE_{NO}, which is generated in the peripheral airways. However, half of our patients received extrafine particle beclomethason, which is inducive to peripheral lung deposition. Although the numbers were too small for a formal subgroup analysis, these patients did not show different effects of ICS dose increment on FE_{NO}. Hence, we think that bad peripheral airway deposition of ICS is an unlikely explanation for high FE_{NO} in ICS treated asthmatic children.

As most children in the present study had been using ICS for years, no data were available on FE_{NO} before the start of ICS. Thus, there might have been children in whom FE_{NO} has responded well after starting ICS, and children in whom high FE_{NO}

levels persisted after starting ICS. These might represent different subgroups of asthma with different NOS genotypes, perhaps accounting for different responses of F_{ENO} to steroids.[29,30] Heterogeneity of the response of F_{ENO} to ICS was demonstrated by Buchvald *et al*., in a study in which elevated F_{ENO} was significantly related to bronchial hyper-responsiveness (BHR).[31] Intersubject variability in ICS response was also shown by others, independent of the ICS used.[32] It is possible that treatment effects on F_{ENO} are more distinct in genetically homogeneous groups.

True steroid insensitivity is considered extremely rare, especially in children, and hence is an unlikely explanation for high F_{ENO} under steroid treatment.[33] However, Payne *et al*. demonstrated that children with difficult, severe asthma with high F_{ENO} despite an oral steroid course often show persisting airway inflammation in bronchial biopsies.[15,34] The prevalence of such steroid insensitivity in stable asthmatics seen in a tertiary care setting like ours is not known. There may be subgroups of children requiring more steroids or with persisting inflammation despite adequate steroid dosing.

We confirmed that F_{ENO} was predictive of symptoms and use of β_2-agonists in the coming 2 weeks.[35] This suggests that F_{ENO} may be useful as an early, objective indicator of loss of asthma control. Longer term studies are needed to evaluate the merits of ICS dose adaptation guided by F_{ENO}.

In conclusion, we found that correcting a faulty inhalation technique in atopic asthmatic children on ICS with persistently elevated F_{ENO}, does not seem to reduce F_{ENO}. Increasing the ICS dose had a minor effect, but did not normalize F_{ENO} either. It remains to be shown whether and how treatment should be adapted in order to normalize elevated F_{ENO}. Until this is clarified, F_{ENO} cannot merely be incorporated in existing treatment guidelines.

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REFERENCES

1. Artlich A, Hagenah JU, Jonas S, Ahrens P, Gortner L. Exhaled nitric oxide in childhood asthma. Eur J Pediatr 1996; 155:698-701.
2. Barnes PJ, Kharitonov SA. Exhaled nitric oxide: a new lung function test. Thorax 1996;51:233-237.
3. Byrnes CA, Dinarevic S, Shinebourne EA, Barnes PJ, Bush A. Exhaled nitric oxide measurements in normal and asthmatic children. Pediatr Pulmonol 1997;24:312-318.
4. Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. Lancet 1994;343:133-135.
5. Nelson BV, Sears S, Woods J et al. Expired nitric oxide as a marker for childhood asthma. J Pediatr 1997;130:423-427.
6. Hamid Q, Springall DR, Riveros-Moreno V et al. Induction of nitric oxide synthase in asthma. Lancet 1993;342:1510-1513.
7. Baraldi E, Azzolin NM, Zanconato S, Dario C, Zacchello F. Corticosteroids decrease exhaled nitric oxide in children with acute asthma. J Pediatr 1997;131:381-385.
8. Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. Am J Respir Crit Care Med 1996;153:454-457.
9. Kharitonov SA, Yates DH, Chung KF, Barnes PJ. Changes in the dose of inhaled steroid affect exhaled nitric oxide levels in asthmatic patients. Eur Respir J 1996;9:196-201.
10. Lim S, Jatakanon A, John M et al. Effect of inhaled budesonide on lung function and airway inflammation. Assessment by various inflammatory markers in mild asthma. Am J Respir Crit Care Med 1999;159:22-30.
11. Jatakanon A, Kharitonov S, Lim S, Barnes PJ. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. Thorax 1999;54:108-114.
12. Piacentini GL, Bodini A, Costella S et al. Exhaled nitric oxide, serum ECP and airway responsiveness in mild asthmatic children. Eur Respir J 2000;15:839-843.
13. Tsai YG, Lee MY, Yang KD, Chu DM, Yuh YS, Hung CH. A single dose of nebulized budesonide decreases exhaled nitric oxide in children with acute asthma. J Pediatr 2001;139:433-437.
14. Jones SL, Herbison P, Cowan JO et al. Exhaled NO and assessment of anti-inflammatory effects of inhaled steroid: dose-response relationship. Eur Respir J 2002;20:601-608.
15. Payne DN, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. Am J Respir Crit Care Med 2001;164:1376-1381.
16. Silkoff PE, McClean P, Spino M, Erlich L, Slutsky AS, Zamel N. Dose-response relationship and reproducibility of the fall in exhaled nitric oxide after inhaled beclomethasone dipropionate therapy in asthma patients. Chest 2001;119:1322-1328.
17. Pijnenburg MW, Ghiro L, Baraldi E, De Jongste JC. Exhaled nitric oxide in atopic asthmatic children on inhaled steroids correlates with self-reported compliance. Am J Respir Crit Care Med 2002;165:A796.
18. Beck-Ripp J, Griese M, Arenz S, Koring C, Pasqualoni B, Bufler P. Changes of exhaled nitric oxide during steroid treatment of childhood asthma. Eur Respir J 2002;19:1015-1019.
19. Delgado-Corcoran C, Kissoon N, Murphy SP, Duckworth LJ. Exhaled nitric oxide reflects asthma severity and asthma control. Pediatr Crit Care Med 2004;5:48-52.
20. Janssens HM, Devadason SG, Hop WC, LeSouef PN, De Jongste JC, Tiddens HA. Variability of aerosol delivery via spacer devices in young asthmatic children in daily life. Eur Respir J 1999;13:787-791.
21. Janssens HM, Heijnen EM, de Jong VM et al. Aerosol delivery from spacers in wheezy infants: a daily life study. Eur Respir J 2000;16:850-856.
22. Manual inhalation-instruction, Dutch asthma foundation, Leusden, 1999.

23. Baraldi E, De Jongste JC on behalf of the task force. ERS/ATS statement. Measurement of exhaled nitric oxide in children, 2001. Eur Respir J 2002;20:223-237.

24. Buchvald F, Baraldi E, Gaston B, De Jongste JC, Silkoff P, Bisgaard H. Feasibility and normal values of exhaled nitric oxide in healthy children and adolescence between 4-17 years measured with NIOX. Eur Respir J 2003;22:568s.

25. Kamps AW, van Ewijk B, Roorda RJ, Brand PL. Poor inhalation technique, even after inhalation instructions, in children with asthma. Pediatr Pulmonol 2000;29:39-42.

26. Masoli M, Holt S, Weatherall M, Beasley R. Dose-response relationship of inhaled budesonide in adult asthma: a meta-analysis. Eur Respir J 2004;23:552-8.

27. Kharitonov SA, Donnelly LE, Montuschi P, Corradi M, Collins JV, Barnes PJ. Dose-dependent onset and cessation of action of inhaled budesonide on exhaled NO and symptoms in mild asthma.Thorax 2002;57:889-96.

28. Martin RJ. Therapeutic significance of distal airway inflammation in asthma. J Allergy Clin Immunol 2002;109:S447-460.

29. Wechsler ME, Grasemann H, Deykin A et al. Exhaled nitric oxide in patients with asthma: association with NOS1 genotype. Am J Respir Crit Care Med 2000;162:2043-7.

30. van 's Gravesande KS, Wechsler ME, Grasemann H et al. Association of a missense mutation in the NOS3 gene with exhaled nitric oxide levels. Am J Respir Crit Care Med 2003;168:228-31.

31. Buchvald F, Eiberg H, Bisgaard H. Heterogeneity of $F_{E_{NO}}$ response to inhaled steroid in asthmatic children. Clin Exp Allergy 2003;33:1735-40

32. Szefler SJ, Martin RJ, King TS et al. Significant variability in response to inhaled corticosteroids for persistent asthma. J Allergy Clin Immunol. 2002;109:410-8.

33. Wamboldt FS, Spahn JD, Klinnert MD et al. Clinical outcomes of steroid-insensitive asthma. Ann Allergy Asthma Immunol 1999;83:55-60.

34. Payne DN, Wilson NM, James A, Hablas H, Agrafioti C, Bush A. Evidence for different subgroups of difficult asthma in children. Thorax 2001;56:345-350.

35. Jones SL, Kittelson J, Cowan JO et al. The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med 2001;164:738-743

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7 Chapter

Exhaled Nitric Oxide Predicts Asthma Relapse in Children with Clinical Asthma Remission

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ABSTRACT

Background: Nitric oxide in exhaled air (FE_{NO}) is a marker of eosinophilic airway inflammation. A study was undertaken to determine whether FE_{NO} predicts asthma relapse in asymptomatic asthmatic children in whom inhaled corticosteroids are discontinued.

Methods: Forty children (21 boys) of mean age 12.2 years on a median dose of 400 µg budesonide or equivalent (range 100 - 400) were included. FE_{NO} was measured before and 2, 4, 12 and 24 weeks after withdrawal of steroids. A relapse was defined as more than one exacerbation per month, or need for β agonist treatment on 4 days per week for at least 2 weeks, or diurnal peak flow variability of > 20%. FE_{NO} measurements were performed online with an expiratory flow of 50 ml/s.

Results: Nine patients relapsed. Two and 4 weeks after withdrawal of steroids geometric mean FE_{NO} in children who were about to relapse was higher than in those who did not relapse: 35.3 *v* 15.7 ppb at 2 weeks (ratio 2.3; 95% CI 1.2 to 4.1; *P* = 0.01) and 40.8 *v* 15.9 ppb at 4 weeks (ratio 2.6; 95% CI 1.3 to 5.1). An FE_{NO} value of 49 ppb at 4 weeks after discontinuation of steroids had the best combination of sensitivity (71%) and specificity (93%) for asthma relapse.

Conclusion: FE_{NO} 2 and 4 weeks after discontinuation of steroids in asymptomatic asthmatic children may be an objective predictor of asthma relapse.

INTRODUCTION

Remission of asthma is common in children.[1,2] It is difficult to determine the time point at which to reduce or stop inhaled corticosteroids (ICS). ICS are currently adapted on clinical grounds with dose reduction based on patient history, sometimes influenced by parental or doctor's fears of side effects. However, there are few if any objective means to guide parents or children. According to current practice, ICS are discontinued in asthmatic children who are symptom-free for at least 6 months on a low dose of inhaled steroids. Follow up is discontinued soon after withdrawal of ICS. However, some of these children will have a relapse, and there is currently no objective parameter to predict the probability of asthma relapse following steroid withdrawal. Measurement of the fractional nitric oxide concentration in exhaled air (F_{ENO}) has been proposed as a marker of eosinophilic airway inflammation.[3,4] F_{ENO} levels can be obtained easily and repeatedly, with minimal discomfort to the patient, and measurement techniques have been well standardised.[4,5] Treatment with ICS reduces F_{ENO} by a direct effect on transcription of inducible NO synthase and by reducing airway inflammation.[6-8] Even low doses of ICS may decrease F_{ENO} to normal levels.[6,9-13] If F_{ENO} could give additional information on the risk of asthma relapse, this could potentially modify current treatment strategies. Only a few studies have addressed the use of F_{ENO} as a predictor of loss of asthma control related to changes in ICS dose in adults, and the results are unequivocal.[11,14,15] The aim of this study was to evaluate whether or not F_{ENO} predicts the probability of asthma relapse in children in whom ICS are discontinued because of clinical remission.

METHODS

Forty children aged 6 - 18 years with asthma according to ATS criteria were enrolled at the moment when discontinuation of ICS was considered because of lack of symptoms for more than 6 months at a stable dose of ICS (100 - 400 µg/day of budesonide or equivalent). Children on leukotriene receptor antagonists were excluded. Atopy was defined as RAST class 2 or higher for at least one airborne allergen. Written informed consent was obtained and the study was approved by the medical ethical committee of the Erasmus Medical Centre.

Study design

This prospective study lasted 26 weeks. During a run-in period of 2 weeks and 2 weeks before every visit, children recorded cough, wheezing and dyspnoea twice daily on a 4 point scale (0 - 3) on a diary card as used in previous studies.[16] The maximum possible cumulative symptom score was 252. Medication use and peak expiratory flow (PEF) were recorded twice daily. Children used a peak flow meter at home (Glaxo Wellcome, Zeist, The Netherlands) and recorded the personal best value of three attempts in the morning and evening. Diurnal PEF variability was defined as the difference between evening and morning values, divided by the mean of both measurements. At the start of the run-in period, the inhalation

technique was checked and adherence to ICS treatment was strongly encouraged. Baseline FE_{NO} was measured at t = -2 and t = 0 weeks. Forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) before and after bronchodilation were measured at t = -2, 12 and 24 weeks. At t = 0 weeks, treatment with ICS was discontinued in patients with low symptom scores (below 14), mean PEF variability within 20%, and not needing rescue medication. FE_{NO} was monitored 2, 4, 12 and 24 weeks after withdrawal of ICS. The study design is shown in *table 7.1.*

Table 7.1 study design

	time (weeks)					
	t = -2	t = 0	t = 2	t = 4	t = 12	t = 24
FE_{NO}	x	x	x	x	x	x
diary card		x	x	x	x	x
spirometry	x				x	x

Symptom scores were obtained during the 2 weeks before each visit. At t = 0 inhaled steroids were discontinued.

In cases in whom an upper respiratory tract infection occurred, follow up visits were postponed for 2 weeks. Patients were asked to contact the investigator if an exacerbation occurred, which was defined as asthma symptoms not responding to the bronchodilator. Subsequently, children were seen and treated by their own paediatric pulmonologist, not the investigator. A relapse was defined as more than one exacerbation per month and/or exacerbations requiring oral or inhaled steroid use and/or need for rescue bronchodilators on four or more days per week for at least two consecutive weeks and/or mean diurnal PEF variability of > 20% according to the guidelines of the Dutch Paediatric Respiratory Group.[17] The primary endpoint was relapse. At the point at which relapse occurred, children dropped out of the study.

FE_{NO} measurements

FE_{NO} was measured online with an expiratory flow of 50 ml/s according to ATS and ERS guidelines.[4,5] NO was continuously sampled with a sampling flow of 175 ml/min and analysed by a chemiluminescence analyser (Sievers 280 NOA, Boulder CO, USA). The analyser was calibrated weekly using 0 and 115 ppb NO certified gases (BOC, Herenthout, Belgium).

Lung function testing

Flow-volume curves were obtained with a dry rolling seal spirometer (Jaeger, Würzburg, Germany) according to ATS guidelines. After maximal inspiration, three reproducible loops with a maximum variability in FVC of 10% were obtained. FVC and FEV_1 are expressed as percentage predicted.[18]

Statistical analysis

FE_{NO} values were logarithmically transformed before statistical analyses and the results expressed as geometric means. FE_{NO} at t = 0 weeks was used as baseline. For each interval between two measurements we assessed whether FE_{NO} at the beginning of the interval was predictive for the occurrence of relapse during the interval. Subsequently, the information of these four analyses were combined using conditional logistic regression which relates the probability of relapse in each period to FE_{NO} at the start of this period. Multivariate analysis was repeated using either FE_{NO} or the ratio of FE_{NO} to baseline FE_{NO}. FE_{NO} at baseline and at the various time points was compared in patients with and without clinical relapse using Mann-Whitney U tests. ROC curves for FE_{NO} 2 and 4 weeks after discontinuation of ICS were constructed. The correlation of FE_{NO} and clinical and lung function parameters was assessed using Spearman's correlation coefficient.

RESULTS

Of the 40 children included in the study, one dropped out of the study because of a high symptom score during the run-in period, and two were lost to follow up. The remaining 37 patients (21 boys) had a mean age of 12.2 years (range 7.3 – 16.9). Data on the study population are shown in *table 7.2*. Twenty nine were atopic; these children did not differ from non-atopic children in age, height and weight, nor in pulmonary function tests or baseline FE_{NO}. None of the children used long acting β_2 agonists or leukotriene antagonists.

Table 7.2 Baseline anthropometric and lung function data of study population (n = 37 children, 21 boys)

	without relapse (n = 28)	**with relapse (n = 9)**
age (years)	12.2 (7.3 – 16.9)	12.3 (10.0 – 15.8)
atopy (n)	21	8
daily dose ICS (budesonide equivalent) µg	400 (100 – 400)	200 (100 – 400)
height (m)	1.53 (1.30 – 1.81)	1.52 (1.44 – 1.66)
weight (kg)	10.2 (7.3 – 14.2)	14.8 (8.5 - 25.8)
FEV1 (% pred)	100 (73 – 134)	99 (88 – 109)
FVC (% pred)	102 (66 – 126)	105 (87 –118)
FEV1 (% pred) post *	106 (80 – 139)	107 (91 – 119)
FVC (% pred) post*	103 (66 – 127)	105 (78 – 118)
PEF (l/s)	383 (195 - 600)	426 (270 – 800)
PEF diurnal variabilty (%)	5.6 (1-10)	6.3 (3 – 11)
Baseline FE_{NO} (ppb)	10.5 (7.3 – 14.2)	14.8 (8.5 – 25.8)

ICS *inhaled corticosteroid*
FEV_1 *forced expiratory volume in 1 second*
FVC *forced vital capacity*
PEF *peak expiratory flow*
FE_{NO} *fractional nitric oxide concentration in exhaled air*
Data are given as mean (range) except for the dose of ICS which is given as median. FE_{NO} is given as geometric mean with 95% confidence interval. None of the items differed significantly between children with and without relapse.
* *After bronchodilation with 1000 µg terbutaline.*

Baseline geometric mean FE_{NO} at t = 0 was 11.2 ppb (95% CI 8.5 to15.3). This did not differ significantly from FE_{NO} at t = -2, the start of the run-in period (*P* = 0.67). Intra-individual variability between values at t = -2 and t = 0 weeks was considerable (intraclass correlation coefficient 0.52).

FE_{NO} and relapse of asthma

Nine patients (24%), one of whom was non-atopic, had a clinical relapse after a median of 36 days (range 14 - 141). Of these nine patients, five had two exacerbations within 1 month or a single exacerbation requiring oral or inhaled steroids and four used their bronchodilator as rescue therapy for ≥ 4 days a week during at least two consecutive weeks. Six children relapsed between 4 and 12 weeks after withdrawal of ICS; in the periods 0 - 2 weeks, 2 - 4 weeks, and 12 - 24 weeks, one patient relapsed in each period (*fig. 7.1*). Children who experienced an asthma relapse did not differ in baseline demographic or pulmonary function data (*table 7.2*). There was no difference in initial steroid dose of children who did or did not relapse (Mann-Whitney U test, *P* = 0.28), nor was there a significant difference in baseline geometric FE_{NO} between the two groups of patients (14.8 ppb *v* 10.5 ppb, respectively; ratio 1.4; 95% CI 0.7 to 2.8, *P* = 0.32). Two weeks after withdrawal of ICS the geometric mean FE_{NO} in children who relapsed thereafter (n = 8) was significantly higher than in those who did not relapse (35.3 ppb *v* 15.7 ppb; ratio 2.3; 95% CI 1.2 to 4.1, *P* = 0.01). The same was true for FE_{NO} after 4 weeks without steroids for the seven children who relapsed after 4 weeks (40.8 ppb and 15.9 ppb; ratio 2.6; 95% CI 1.3 to 5.1, *P* = 0.009; *fig. 7.1*).

FE_{NO} at 4 weeks after withdrawal of ICS predicted relapse in the forthcoming period (4 - 12 weeks after withdrawal, *P* = 0.025). Multivariate logistic regression combining results of all periods showed that FE_{NO} was a better predictor of asthma relapse (*P* = 0.001) than the FE_{NO} ratio (actual FE_{NO} divided by baseline FE_{NO}) (*P* = 0.04). For each doubling of FE_{NO} the relapse rate increased by a factor 3.0 (95% CI 1.5 to 7.1). The results were similar when only atopic patients were analysed.

Two patients were included who later admitted to having used more than 400 µg budesonide regularly before enrolment. One of them relapsed. If both children were excluded, multivariate logistic regression still showed that FE_{NO} predicted asthma relapse in the remainder (*P* = 0.003).

ROC curves indicated that a FE_{NO} value of 49 ppb 4 weeks after stopping steroids had the best combination of sensitivity and specificity for predicting relapse (sensitivity: 71% (95% CI 29 to 96) and specificity 93% (95% CI 76 to 99); *fig. 7.2*). The positive and negative predictive values of FE_{NO} of 49 ppb were 71% and 93%, respectively.

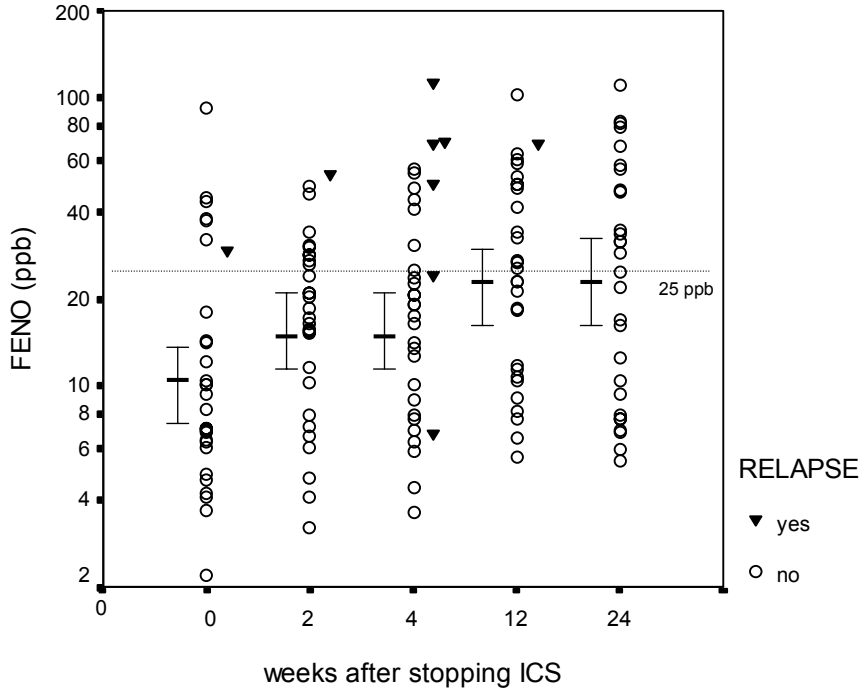

Figure 7.1 *FE_{NO} values in patients with (▼) and without (○) an asthma relapse. For each period (0 - 2, 2 - 4, 4 - 12 and 12 - 24 weeks) patients were classified according to whether they relapsed or not in the period indicated. FE_{NO} values were obtained at the start of each period. For patients without a relapse geometric mean FE_{NO} and 95% confidence intervals are given. The x axis depicts number of weeks after withdrawal of ICS. One patient relapsed in the first period (0 - 2 weeks), one between 2 and 4 weeks, six between 4 and 12 weeks and one after 12 weeks.*

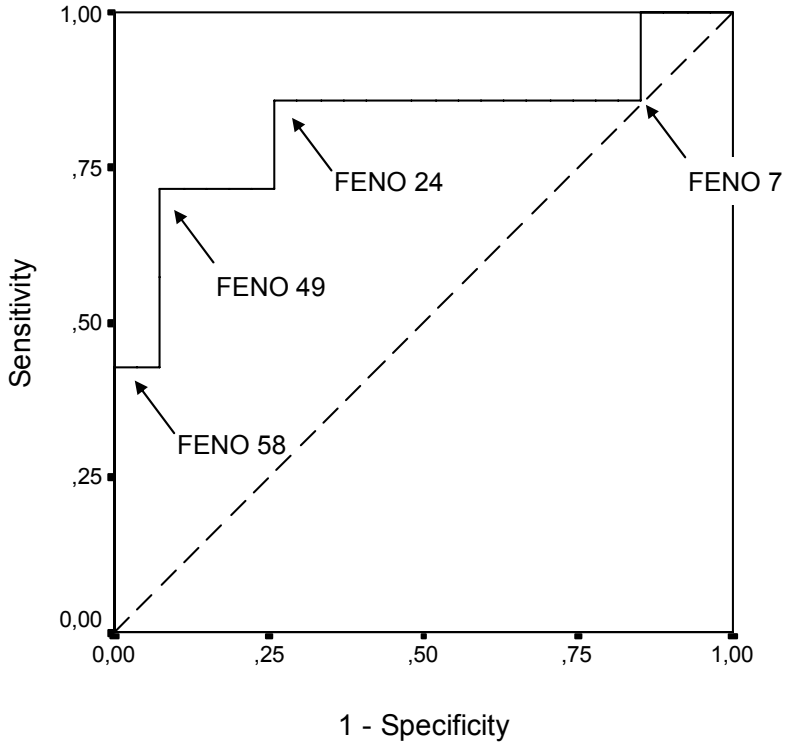

Figure 7.2 *ROC curve for FE_{NO} 4 weeks after discontinuation of inhaled corticosteroids. The optimal combination of sensitivity and specificity for identifying children with relapse was for FE_{NO} 49 ppb (71% and 93% respectively).*

The course of FE_{NO} in all individuals who experienced a relapse is shown in *fig. 7.3*. In patients who did not relapse there was a general trend for FE_{NO} to rise with time from a geometric mean of 10.2 ppb at the start of the study to 22.2 ppb after 26 weeks of follow up (*fig. 7.1*). FE_{NO} showed an overall tracking pattern in those who did not relapse.

Correlations between FE_{NO}, clinical symptoms and pulmonary function tests

The results of pulmonary function tests, atopic state, PEF variability, cumulative symptom score and rescue medication during the run-in period did not differ between children with or without a relapse. Cumulative symptom scores, spirometric data, PEF values and PEF variability did not correlate with geometric mean FE_{NO} at any time point.

Other parameters and relapse of asthma

Symptom scores, peakflow, diurnal variability in peak flow, or other lung function tests performed during the run-in period did not predict asthma relapse.

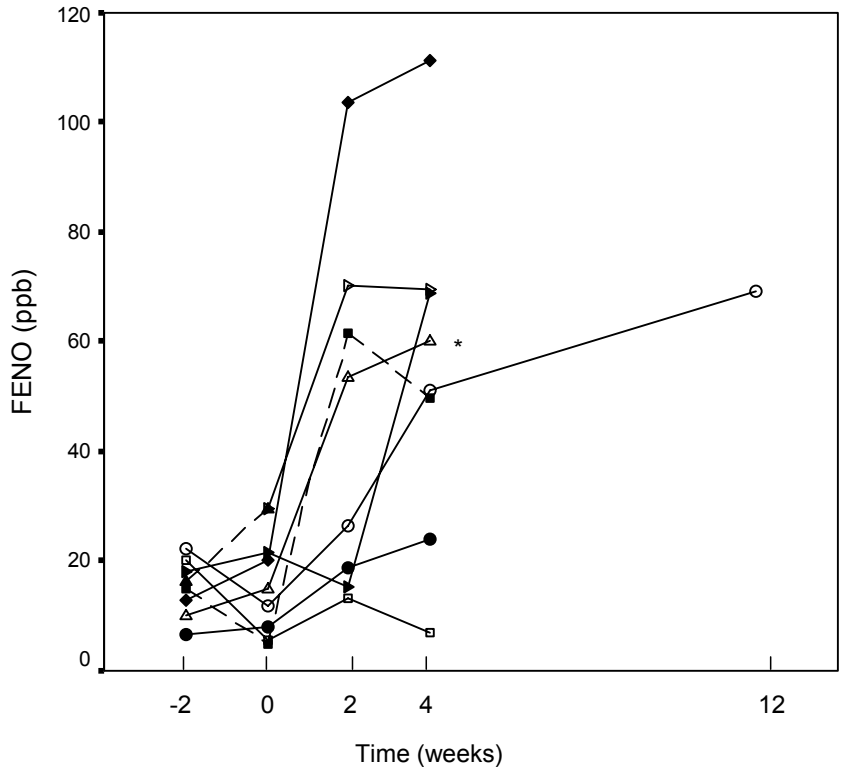

Figure 7.3 *Course of FE_{NO} values in nine patients who relapsed. The only non-atopic patient who experienced a relapse is marked with an asterisk*

DISCUSSION

We found that F_{ENO} at 2 and 4 weeks after discontinuing ICS predicted asthma relapse in asthmatic children who were taken off ICS because of clinical remission. Initial F_{ENO} levels measured while patients were still on ICS were not predictive of relapse. An F_{ENO} of 49 ppb or higher 4 weeks after discontinuation of ICS had the best combination of sensitivity (71%) and specificity (93%).

Few other studies have assessed F_{ENO} longitudinally after discontinuation or reduction of ICS, and none of these included children. Our results are in agreement with the study by Jones *et al* in which adult asthmatics treated with a mean daily ICS dose of up to 1600 µg were forced off steroids and followed for loss of asthma control.[11] In this study, in contrast to ours, discontinuing steroids was not clinically indicated, and loss of control occurred earlier, after a median of 17 days. Their F_{ENO} values were much lower, probably due to the higher flow rate of 250 ml/s used to obtain exhaled air samples. Lim *et al* performed a similar study in adults and their preliminary report states that NO in mixed nasal/oral exhaled air did not predict asthma relapse.[14] However, mixed expired air is contaminated by high nasal levels of NO which makes interpretation impossible. Moreover, they defined relapse as a recurrence of asthma symptoms requiring either β_2 agonists or ICS. This might well explain any discrepancy between their findings and ours.

Jatakanon *et al* studied several noninvasive markers of airway inflammation in asthma exacerbations induced by forced reduction of ICS doses from more than 800 µg to 200 µg budesonide in adults.[6] F_{ENO} at baseline did not predict loss of asthma control. However, there was a rapid increase in F_{ENO} before exacerbations 2 - 4 weeks after decreasing inhaled steroids. Only 15 patients were included in the study, and this small number could easily lead to nonsignificant findings. Furthermore, ICS were not completely withdrawn, which reduces the possibility of finding a difference between the groups.

The increases in F_{ENO} over time in children who relapsed were consistent, and larger than within-subject baseline fluctuations. However, the intra-individual variability of F_{ENO} measurements at the beginning and at end of our 2 weeks run-in period was quite high. Few data are available on long-term within-subject reproducibility of F_{ENO} in asthmatic children. Earlier reports have focused on short-term reproducibility, which is excellent. Kharitonov *et al* found intraclass correlation coefficients better than 0.90 in adults and children with and without asthma, with 95% limits of agreement of about 4 ppb when children were tested repeatedly within 4 days.[19] Jones et al reported a within-subject coefficient of variation of F_{ENO} measured with a 1 week interval of 10.5%.[11] The variability in our study may be due to the long interval of 2 weeks between F_{ENO} assessments. We also reasoned that inclusion in the study as such might affect F_{ENO} because of better compliance with ICS during the run-in. This seems unlikely as increased compliance would lead to a reduction in F_{ENO} whereas we found no significant

difference between FE_{NO} at t = -2 and t = 0, with a trend towards higher levels at t = 0. In addition, ambient NO levels can be a source of variability. However, we found no relation between ambient NO and FE_{NO}, so we think ambient NO levels do not explain the variability of FE_{NO}.

We included both atopic and non-atopic asthmatic subjects, reflecting the asthma population in daily practice. The numbers are too small for a subgroup analysis; only one non-atopic child relapsed. The patients who did not relapse without medication showed a wide range of FE_{NO} values (*fig. 7.1*). In these children no correlation was found between FE_{NO} and symptom scores. As our follow up was 6 months, we cannot exclude that some of the children might relapse later. The possible clinical relevance of an increased FE_{NO} in asymptomatic children therefore remains unclear.

What are the implications of these results for clinical practice? Our findings in this relatively small group of asthmatic children strongly suggest that FE_{NO} measurements at 2 and 4 weeks after cessation of steroids are helpful for identifying children in whom relapse of asthma is more likely to occur and who might benefit from a close follow up. However, patient numbers in this study are small and more children who did not relapse had raised FE_{NO} than those who did relapse. Larger studies are needed to confirm the role of FE_{NO} in decision making on ICS in asthmatic children and to calculate more accurately the sensitivity and specificity of different cut-off levels of FE_{NO}

In conclusion, this is the first study in children showing that FE_{NO} is an early predicting marker of relapse in asthma after cessation of ICS. Larger studies are now warranted to substantiate this finding to further define the role of FE_{NO} in this aspect of asthma management.

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REFERENCES

1. Strachan DP. The epidemiology of childhood asthma. Allergy 1999;54:7-11.
2. Barbee RA, Murphy S. The natural history of asthma. J Allergy Clin Immunol 1998;102:S65-72.
3. Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. Am J Respir Crit Care Med 2001;163:1693-722.
4. Baraldi E, De Jongste JC on behalf of the task force. ERS/ ATS statement. Measurement of exhaled nitric oxide in children. Eur Respir J 2002;20:223-37.
5. Recommendations for standardised procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children –1999. Am J Respir Crit Care Med 1999;160:2104-17.
6. Jatakanon A, Kharitonov SA, Lim S et al. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. Thorax 1999;54:108-14.
7. Baraldi E, Azzolin NM, Zanconato S et al. Corticosteroids decrease exhaled nitric oxide in children with acute asthma. J Pediatr 1997;131:381-5.
8. Hamid Q, Springall DR, Riveros-Moreno V et al. Induction of nitric oxide synthase in asthma. Lancet 1993;342:1510-3.
9. Kharitonov SA, Yates DH, Chung KF et al. Changes in the dose of inhaled steroid affect exhaled nitric oxide levels in asthmatic patients. Eur Respir J 1996;9:196-201.
10. Lim S, Jatakanon A, John M et al. Effect of inhaled budesonide on lung function and airway inflammation. Assessment by various inflammatory markers in mild asthma. Am J Respir Crit Care Med 1999;159:22-30.
11. Jones SL, Kittelson J, Cowan JO et al. The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med 2001;164:738-43.
12. Silkoff PE, McClean P, Spino M et al. Dose-response relationship and reproducibility of the fall in exhaled nitric oxide after inhaled beclomethasone dipropionate therapy in asthma patients. Chest 2001;119:1322-8.
13. Beck-Ripp J, Griese M, Arenz S et al. Changes of exhaled nitric oxide during steroid treatment of childhood asthma. Eur Respir J 2002;19:1015-9.
14. Lim TK, Ngerng WJ, Phillip R et al. Exhaled nitric oxide levels did not predict asthma relapse during the discontinuation of inhaled corticosteroid therapy. Am J Respir Crit Care Med 2001;163: A26.
15. Leuppi JD, Salome CM, Jenkins CR et al. Predictive markers of asthma exacerbation during stepwise dose reduction of inhaled corticosteroids. Am J Respir Crit Care Med 2001;163:406-12.
16. Verberne AA, Frost C, Duiverman EJ et al. Addition of salmeterol versus doubling the dose of beclomethasone in children with asthma. Am J Respir Crit Care Med 1998;158:213-9.
17. Hoekstra MO. Treatment of asthma in children; revised guidelines from paediatric pneumologists. Ned Tijdschr Geneesk 1997;141:2223-9.
18. Zapletal AS, Samanek M, Paul T. Lung function in children and adolescents. Methods, reference values. In: Progress in respiration research. Series ed Herzog H. Vol. 22 Basel: Karger, 1987:114-218.
19. Kharitonov SA, Gonio F, Kelly C, Meah S, Barnes PJ. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. Eur Respir J 2003; 21: 433-438.

8 Chapter

TITRATING STEROIDS ON EXHALED NITRIC OXIDE IN CHILDREN WITH ASTHMA: A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Rationale: Corticosteroids are the antiinflammatory treatment of choice in asthma. Treatment guidelines are mainly symptom-driven but symptoms are not closely related to airway inflammation. The fraction of nitric oxide in exhaled air (FE_{NO}) is a marker of airway inflammation in asthma.

Objective: We evaluated whether titrating steroids on FE_{NO} improved asthma management in children.

Methods: Eighty-five children with atopic asthma, using inhaled steroids, were allocated to a FE_{NO} group (n = 39) in which treatment decisions were made on both FE_{NO} and symptoms, or to a symptom group (n = 46) treated on symptoms only. Children were seen every 3 months over a 1-year period.

Measurements: Symptoms were scored during 2 weeks before visits and 4 weeks before the final visit. FE_{NO} was measured at all visits, and airway hyperresponsiveness and FEV_1 were measured at the start and end of the study. Primary endpoint was cumulative steroid dose.

Results: Changes in steroid dose from baseline did not differ between groups. In the FE_{NO} group, hyperresponsiveness improved more than in the symptom group (2.5 *vs*. 1.1 doubling dose, $P = 0.04$). FEV_1 in the FE_{NO} group improved, and the change in FEV_1 was not significantly different between groups. The FE_{NO} group had 8 severe exacerbations versus 18 in the symptom group. The change in symptom scores did not differ between groups. FE_{NO} increased in the symptom group; the change in FE_{NO} from baseline differed between groups ($P = 0.02$).

Conclusion: In children with asthma, 1 year of steroid titration on FE_{NO} did not result in higher steroid doses and did improve airway hyperresponsiveness and inflammation.

INTRODUCTION

Chronic airway inflammation is a key feature of asthma, and inhaled corticosteroids (ICS) are the cornerstone of asthma treatment. Decisions to start ICS or change the dose are now mainly based on symptoms reported by the child or parents.[1] Symptoms, however, are nonspecific and not closely related to the presence and severity of airway inflammation.[2] Symptom-based treatment may thus easily lead to excessive ICS doses, whereas fear of ICS side effects may lead to underdosing, especially in children.[3] Lung function tests show no or only marginal correlation with airway inflammation.[4,5] Airway hyperresponsiveness does correlate weakly with airway inflammation, and its incorporation in treatment decision making has shown beneficial effects in a single study in adults, albeit at the cost of high ICS doses.[6] The question is whether asthma treatment in children should be targeted at symptoms or at airway inflammation. The airways produce nitric oxide (NO), and its fraction in exhaled air (F_{ENO}) is elevated in steroid-naive atopic asthma.[7-11] In adults and children with atopic asthma, F_{ENO} correlates with eosinophils in induced sputum and with eosinophil infiltration of the airway wall, and this makes F_{ENO} the first noninvasive "inflammometer" for asthma.[2,12-15] Treatment with corticosteroids reduces F_{ENO} levels in patients with asthma in a dose-dependent manner.[16,17] The hypothesis of this study was that titrating ICS on both F_{ENO} and symptoms, compared with titrating on symptoms only, would result in lower ICS doses and better asthma management. Part of this study has been reported in the form of an abstract.[18]

METHODS

Patients

Children aged 6 - 18 years who fulfilled ATS criteria for asthma were recruited from the outpatient clinic of Erasmus MC-Sophia Children's Hospital. Patients had been using ICS at a constant dose for at least 3 months preceding the study. All patients were atopic, defined as RAST class 2 or higher for at least 1 airborne allergen ever. Written informed consent was obtained. The study was approved by the hospital medical ethical committee (Erasmus University Medical Center, Rotterdam, the Netherlands).

Study design

After a 2-week run-in period, children were randomly allocated to one of two groups stratified for baseline F_{ENO} (≥ 30 or < 30 ppb) and dose of ICS (≥ 400 or < 400 µg budesonide or equivalent daily dose) (*Figure 8.1*). In one group (F_{ENO} group), ICS doses were determined by F_{ENO} and symptoms according to the algorithm in *Figure 8.2* and *Table 8.1*; in the other group (symptom group), only symptoms influenced ICS dosing. The study duration was 12 months, with five visits at 3-month intervals. F_{ENO} was measured at each visit, and the ICS dose was then adapted to F_{ENO} and/or symptom scores recorded during the previous 2 weeks. Cut-off levels for dose adaptation were a cumulative symptom score of 14

(in 2 weeks) and an FE_{NO} of 30 ppb. Cut-off levels were chosen using the results of a previous study, where a mean symptom score of 14 corresponded to clinical stability; a FE_{NO} of 30 ppb is the + 2SD limit of normality in our population.[19,20] Patients and physicians were blinded for FE_{NO}; the investigators (M.P., M.B.) provided the physicians with written advice on ICS dose according to the algorithm in *Figure 8.2*. Throughout the study, 2 mg budesonide (or equivalent dose of other ICS) was the maximum allowed daily dose. The study design was such that the patient's physician was allowed to deviate from the recommended ICS dose; this should be motivated.

Pulmonary function tests and bronchoprovocation tests with methacholine were performed during Visits 1 and 5. At all visits, inhaler technique was checked and optimized.

We reasoned that, by using FE_{NO}, steroid doses could be downtitrated in a considerable proportion of children who were overtreated on the basis of their symptoms.[21] Hence, primary endpoint was the cumulative steroid dose (sum of mean daily steroid doses of Visits 1 to 5); secondary endpoints were mean daily symptom score, mean daily number of bronchodilator doses taken, percentage of symptom-free days during the last 4 weeks of the study, number of oral prednisone courses during the study, and provocative dose of methacholine causing a 20% fall in FEV_1 (PD_{20}), FVC, FEV_1, and maximal expiratory flow at 25% of vital capacity (MEF_{25}) during the final visit.

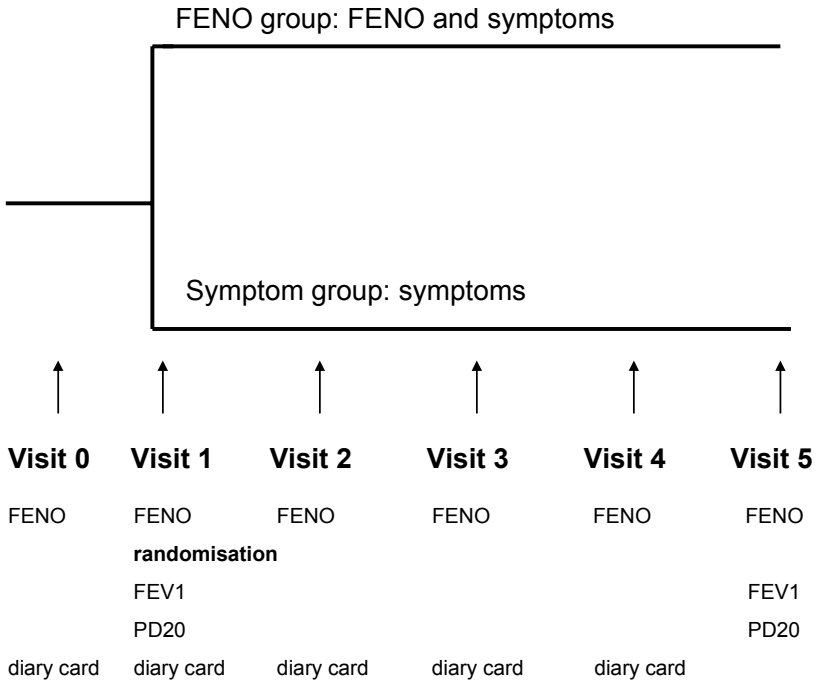

Figure 8.1 *Study design*

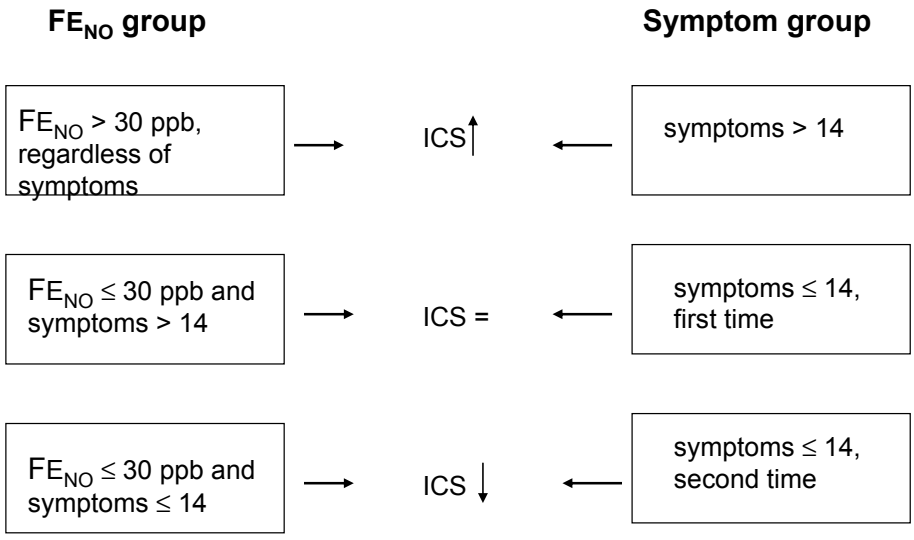

Figure 8.2 *Treatment algorithm*
ICS inhaled corticosteroids

Lung function and FE_{NO} testing

Lung function testing included measurement of PD_{20} methacholine and spirometry. FVC, FEV_1, and MEF_{25} were measured on a Masterscreen electronic spirometer (Jaeger, Würzburg, Germany). FVC and FEV_1 were recorded and expressed as percentage predicted. Bronchial provocation tests with methacholine were performed according to the dosimeter method. Calibrated DeVilbiss 646 nebulizers (DeVilbiss Health Care Inc, Somerset, USA) and Rosenthal dosimeters (Lab for Applied Immunology, Fairfax, USA) were used. Provocative doses causing a 20% fall in FEV_1 (PD20) from baseline were calculated by means of linear interpolation of the logarithmic dose-response curve. At the end of the bronchial provocation 1 mg of terbutaline was administered and after a 20-minute pause FVC and FEV_1 were measured again. In patients with a FEV_1/ FVC < 70% bronchoprovocation was not performed.

Table 8.1 Changes in inhaled corticosteroid dose

Initial dose*	increase if indicated (µg)	Decrease if indicated (µg)
100	100	100
200	200	100
400	400	200
500	500	250
800	400	400
1000	500	500
1200	400	400
1600	400	400
2000	0	1000

* *budesonide (µg) or equivalent dose of other inhaled corticosteroid*

FE_{NO} was measured online according to guidelines from the European Respiratory Society (ERS) and American Thoracic Society (ATS) using the NIOX analyzer (Aerocrine, Solna, Sweden).[22] Patients performed 3 online maneuvers and the mean of these 3 measurements was recorded.

Symptom scores

Symptom scores were obtained from diary cards validated in previous studies.[19] Coughing, wheezing and dyspnea were scored twice daily (day and night) on a four-point scale (0 - 3), with 3 as a maximal score. The maximum possible 2-weekly symptom score therefore was 252 (or maximal daily score of 18). Cut-off for dose adaptation was a 2-weekly score of 14 or higher. *Table 8.2* shows the translated diary questions. The use of β-2 agonists and the percentage of symptom-free days were calculated over the 2 weeks preceding each visit. At the final visit, the same parameters over the 4 weeks preceding the visit were calculated.

Table 8.2 Translated diary questions

During the day, did you experience:	coughing wheezing dyspnea
0	*no coughing/ wheezing/ dyspnea*
1	*coughing/ wheezing/ dyspnea during one short period of the day*
2	*coughing/ wheezing/ dyspnea during 2 or more short periods of the day*
3	*coughing/ wheezing/ dyspnea during (almost) the whole day*
During the night, did you experience:	coughing wheezing dyspnea
0	*no coughing/ wheezing/ dyspnea*
1	*waking up once due to coughing/ wheezing/ dyspnea*
2	*waking up two or more times due to coughing/ wheezing/ dyspnea*
3	*disturbed sleep during most of the night due to coughing/ wheezing/ dyspnea*

Adherence

In children using dry powder fluticasone or fluticasone/salmeterol combination, adherence with treatment was assessed by asking children to return all used and unused diskus inhalers at every visit. Actually used doses divided by prescribed doses defined adherence.

Statistical analysis

The power calculation of this study was based on data of a pilot study in a similar population of 68 asthmatic children prescribed inhaled steroids (data on file). In these children geometric mean daily steroid dose was 417 μg budesonide or equivalent, and a reduction of 30% was considered relevant. Taking the observed

between patients variability of daily doses into account, 38 patients per group were required to demonstrate this 30% decrease in daily steroid dose with a power of 80% at alpha (two-sided) equal to 0.05.

FE_{NO} values were log transformed in analysis and presented as geometric means. For within group comparisons the Wilcoxon test or paired *t*-test was used. Changes during treatment of ICS, logarithmically transformed FE_{NO}, symptoms, bronchodilator use and lung function at visit 5 were compared using analysis of covariance (ANCOVA), with baseline values as covariates. Spearman rank correlation was used to evaluate correlations. Data were analyzed with SPSS for Windows, version 10.1.

Changes of ^{2}logPD20 from baseline were compared using the t-test. In this analysis the change from baseline was considered a right (left) censored observation if a patient had not reached a 20% drop in FEV_1 after the maximal provocative dose of methacholine of 1285 micrograms at the last (first) visit. STATA software (procedure CNREG) was used in this calculations.[23] Patients who did not reach the 20% drop in FEV_1 at the first and final visit were excluded as no conclusion can be drawn for such children. Changes are expressed as doubling doses. The same method was used for the evaluation of the relation between FE_{NO} and PD20. $P = 0.05$ (two-sided) was considered the limit of significance.

RESULTS

The patient flow is shown in *Figure 8.3*. A total of 108 children were approached to participate in the study. Of those, 16 refused, in the majority of cases because they were too busy with school, and 3 children could not be randomized after run in. In the end, 89 children were included, 42 in the FE_{NO} group and 47 in the symptom group. They were well matched for age, sex, FE_{NO}, lung function tests, and initial ICS dose (*Table 8.3*). Four patients dropped out, three in the FE_{NO} group and one in the symptom group. Of the dropouts in the FE_{NO} group, one was admitted to the intensive care unit for a severe asthma attack. At the visit 5 weeks before this admission, FE_{NO} in this patient had increased from 46.6 to 84.5 ppb and the ICS dose had been increased from 1,000 to 1,500 µg budesonide equivalent. Other dropouts were related to non-compliance.

Steroid doses

Overall, mean (SEM) cumulative ICS doses (sum of doses of Visits 1 – 5) did not differ between groups: 4,407 (367) µg for the FE_{NO} group and 4,332 (383) µg for the symptom group ($P = 0.73$). In both groups, mean daily ICS dose increased between Visits 1 and 2 by 169 µg (95% confidence interval [CI], 80 – 259; $P < 0.001$) in the FE_{NO} group and 172 µg (95% CI, 92 – 251; $P < 0.001$) in the symptom group. The dose increase between Visits 1 and 5 was not significant within groups and did not differ between groups (*Figure 8.4*). Eight prednisone

courses were prescribed in 7 patients in the FE_{NO} group versus 18 in 10 patients in the symptom group. This difference did not reach significance, perhaps because, in the symptom group, six children received multiple courses (P = 0.60).

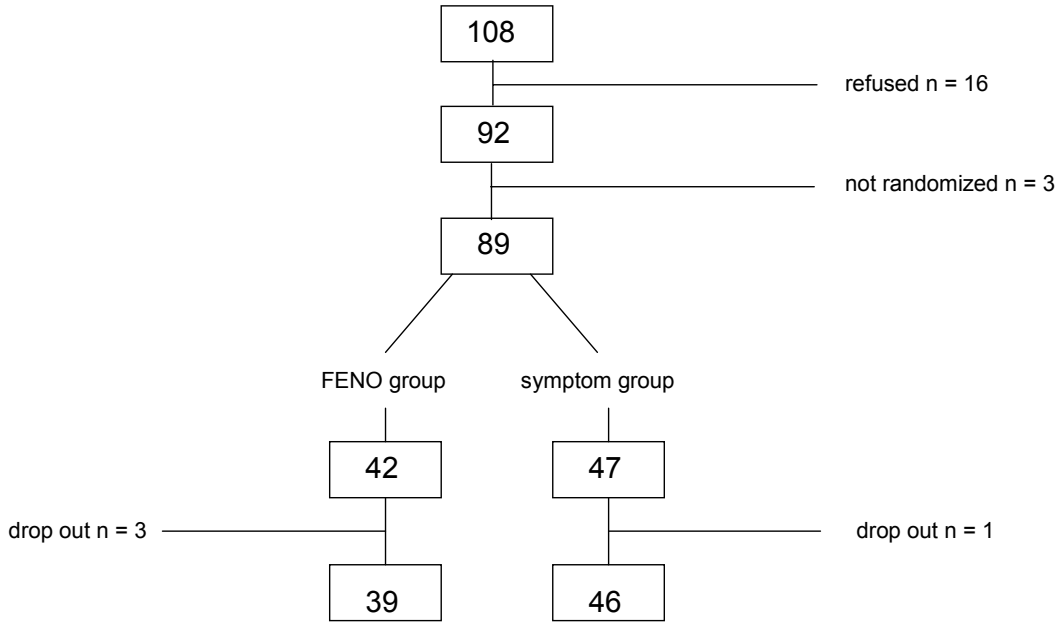

Figure 8.3 *Patient flow.*
A total of 108 children were approached to participate in the study, of whom 16 refused. Three children could not be randomized: one was not stable, one was nonadherent to the study protocol, and one was not randomized for reasons not related to the child. In the FE_{NO} group, three children dropped out; in the symptom group, one dropped out.

Table 8.3 Patient characteristics

	FE_{NO} group (n = 39)	**Symptom group (n = 46)**
Age, yr	11.9 (2.9, 6.9 - 17.7)	12.6 (2.8, 6.5 - 18.5)
Male sex, n	25	30
Initial ICS dose, μg	762 (median 800) (335, 200 - 1,600)	746 (median 800) (410, 200 - 2,000)
FE_{NO} (ppb)*	26.4 (5.6 - 134.9)	29.8 (3.1 - 117.5)
FEV_1, % pred	96 (14, 65 - 121)	99 (20, 64 - 150)
FVC, % pred	100 (13, 77 - 125)	104 (14, 74 - 137)
$MEF_{25,}$ % pred	65 (27, 17 - 131)	68 (36, 20 - 171)
$PD_{20,}$** μg methacholine	245 (12, > 1,285)	225 (9, > 1,285)
Weight, kg	43.2 (15.0, 20.5 - 80.5)	48.5 (18.8, 19.6 - 106.6)
Height, cm	148.8 (18.0, 113.1 - 177.0)	152.4 (17.2,109.3 - 181.4)
Mean daily symptom score	1.4 (2.0, 0 - 7.6)	2.0 (2.4, 0 - 9.9)
Mean daily β-2 agonists, puffs	0.4 (0.6, 0 - 2.4)	0.4 (0.5, 0 - 2)
Symptom-free days, %	58 (34, 0 - 100)	44 (40, 0 - 100)

ICS *inhaled corticosteroids*
MEF_{25} *maximal expiratory flow at 25% of vital capacity.*
Mean data (SD, range) at start of the study are shown; there are no clinically relevant differences between groups.
* *Geometric mean (range)*
** *Median (range)*

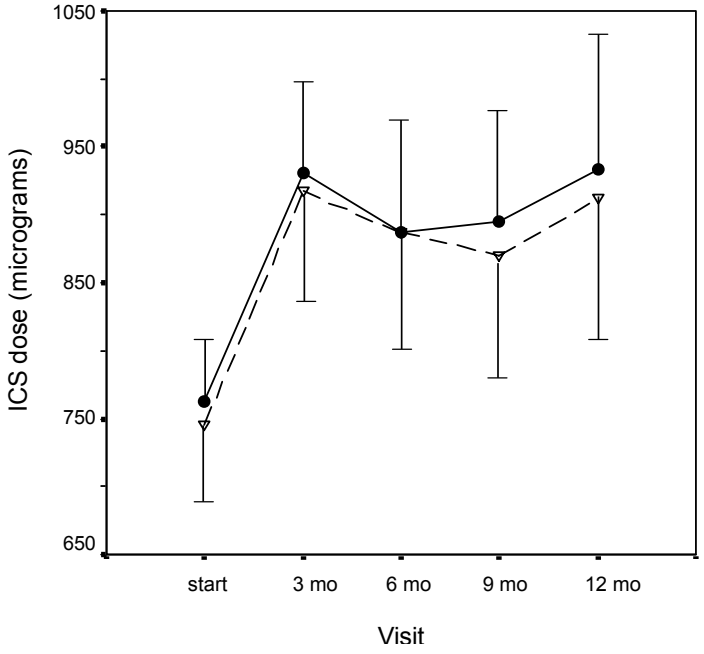

Figure 8.4 *Median daily steroid dose (SEM) in both groups at different visits. Differences between both groups are all nonsignificant. Within groups, ICS dose at Visit 1 differed from the dose at Visit 2 (both P < 0.001). ICS doses at Visits 1 and 5 were not significantly different.*
Closed circles FE_{NO} *group*
open triangles symptom group

Lung function

In 10 children (4 in the FE_{NO} and 6 in the symptom group), bronchoprovocation could not be performed due to FEV_1 being less than 70% at Visit 1 and/or Visit 5. In one child, the PD_{20} at Visit 5 was missing. *Figure 8.5* shows geometric means of PD_{20} methacholine in the remaining 74 patients who had measurements at both occasions. In 16 children (7 in the FE_{NO} group and 9 in the symptom group), no PD_{20} was reached after the maximal provocative dose of methacholine at both Visits 1 and 5. Because no conclusion could be drawn on change in PD_{20} between Visits 1 and 5 in these 27 children, they were excluded from the analyses of changes in PD_{20}. The proportion of children who did not reach a PD_{20} at 12 months, adjusted for baseline, did not differ significantly between the two groups (P = 0.11). In the remaining 58 children, the mean increase in PD_{20} was 2.5 doubling doses in the FE_{NO} group and 1.1 doubling doses in the symptom group. The difference of these changes between the two groups was significant (1.3 doubling doses; 95% CI, 0.1 – 2.5; P = 0.04).

In the FE_{NO} group, FEV_1 increased between Visits 1 and 5, from 95.8 to 100.3% (P = 0.008), whereas FEV_1 in the symptom group remained stable (*Figure 8.6*). At Visit 5, the change in FEV_1 was larger in the FE_{NO} group than in the symptom group, but the difference was not significant (baseline-adjusted difference, 2.3%; 95% CI, –1.8 to +6.3%; P = 0.27). Analyses of FVC and $MEF_{25\%}$ did not reveal significant differences between groups.

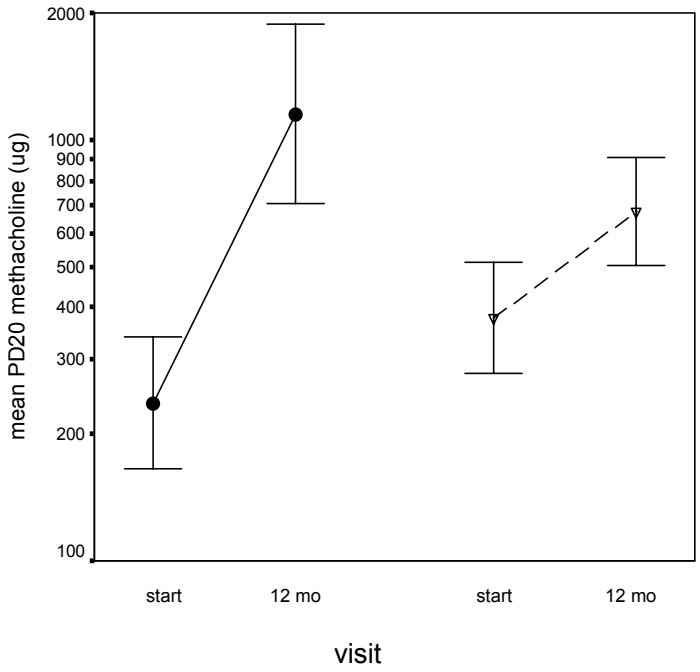

Figure 8.5 *Airway hyperresponsiveness (SEM) on the y axis at Visits 1 and 5 (x axis). Children who could not perform the test due to baseline obstruction at either occasion were excluded (paired measurements remaining, n = 74). Excluding patients who did not reach a decrease of 20% in FEV_1 at both occasions, the mean increase in PD_{20} was 2.5 doubling doses in the F_{ENO} group and 1.1 doubling doses in the symptom group. The difference in change of PD_{20} between both groups was significant (1.3 doubling doses; 95% CI, 0.1–2.5; P = 0.04).*
closed circles the F_{ENO} group
open triangles the symptom group

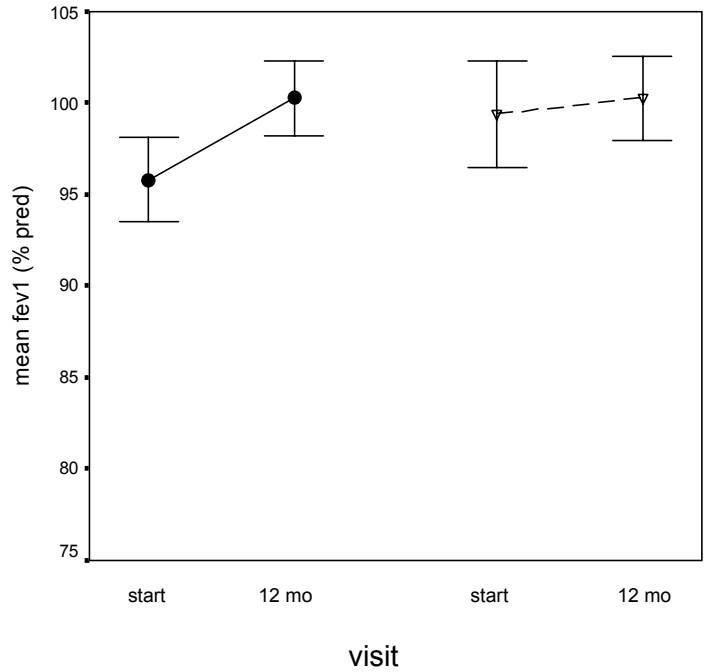

Figure 8.6 *Results of spirometry. FEV_1 (SEM) at Visits 1 and 5.*
closed circles the F_{ENO} group
open triangles the symptom group.
There was a significant difference in FEV_1 at Visit 5 for the F_{ENO} group (P = 0.008).

FE_{NO}

Initially, geometric mean (SD) FE_{NO} was similar in both groups: 26.4 (2.1) ppb in the FE_{NO} group and 29.8 (2.3) ppb in the symptom group (P = 0.48). FE_{NO} did not differ between start and end of the run-in: the ratio of geometric mean FE_{NO} was 1.0 (95% CI, 0.9 – 1.1). The intraclass correlation coefficient of both measurements was 0.87. These outcomes indicate a stable baseline FE_{NO} and good reproducibility. At the end of the study, geometric mean FE_{NO} was 32% higher in the symptom group. The change in FE_{NO} during the study was significantly different between the groups; the ratio of geometric means, adjusted for baseline, was 1.32 (95% CI, 1.04 – 1.68, P = 0.023). Within the FE_{NO} group, no significant change was found, whereas in the symptom group there was a significant increase in FE_{NO}, from 30.8 to 36.7 ppb (P = 0.035; *Figure 8.7*). At Visit 5, but not at Visit 1, FE_{NO} correlated with PD_{20}: a doubling of FE_{NO} corresponded with a decrease in PD_{20} of 0.7 doubling dose (P = 0.03).

Symptom Scores, Use of β-2 Agonists, and Symptom-free Days

The change in mean daily symptom scores between Visits 1 and 5 did not differ between the FE_{NO} and symptom group (0.1 and 0.6, respectively; P = 0.40). The same was true for the mean daily use of β-2 agonists (P = 0.28) and the fraction of symptom-free days during the last 4 weeks of the study (P = 0.69). However, in the symptom group, mean daily symptom scores during the study decreased from 1.6 at Visit 1 to 1.0 at Visit 5 (P = 0.02).

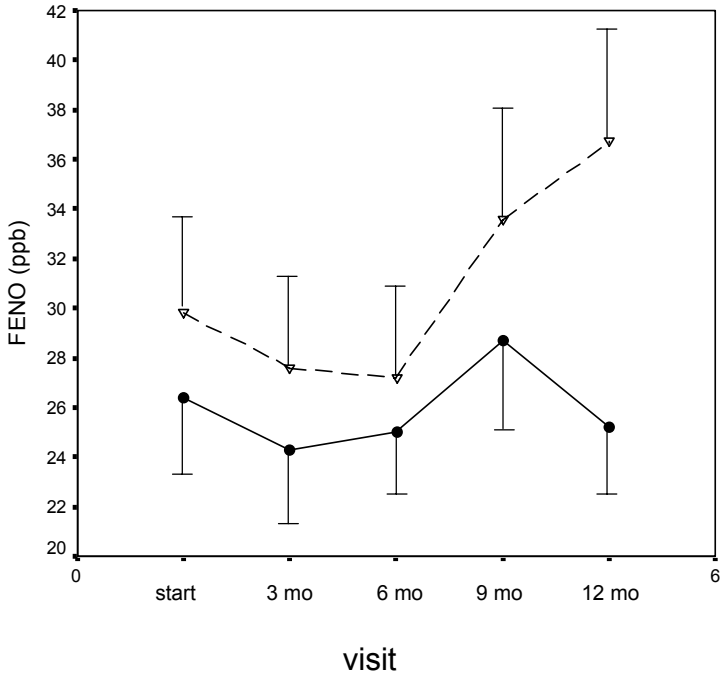

Figure 8.7 *Results of FE_{NO} measurements at all clinic visits. Vertical axis depicts FE_{NO}; horizontal axis is time. P = 0.02 for the change in FE_{NO} from baseline at Visit 5. FE_{NO} increased significantly in the symptom group (P = 0.035).*
closed circles *the FE_{NO} group*
open triangles *the symptom group.*

FE_{NO} and Asthma Severity

FE_{NO} at Visit 1 correlated with symptom scores and use of β-2 agonists in the 2 preceding weeks (r = 0.28, *P* = 0.02, and r = 0.28, *P* = 0.01, respectively). The same was true for FE_{NO} at Visit 2 (r = 0.22, *P* = 0.05, and r = 0.24, *P* = 0.03, respectively). FE_{NO} did not predict symptom scores in the 2 weeks before the next visit. FE_{NO} did not correlate with lung function parameters at the same visits. At all time points, FE_{NO} correlated strongly with later FE_{NO} values in the same subjects (all *P* < 0.001).

Decision Making

For both groups separately, at all visits, we conducted analyses to determine if the advised ICS dose would have been different had patients been allocated to the other group. For the whole study population, in 36% of cases the ICS dose would have been the same; in 29 and 35%, the dose would have been higher or lower, respectively. These proportions did not differ between the groups.

Adherence of Patients and Physicians

Median adherence in the subgroup using dry powder fluticasone dipropionate or fluticasone dipropionate/salmeterol (n = 14) was 97% (range, 55 – 124%).

The study design allowed the patient's physician to deviate from the recommended ICS dose. This happened at 42 occasions (between 1 and 10 children per group at each visit). The distribution over the groups was equal (19 occasions in the FE_{NO} group, 23 in the symptom group [NS]). The main reasons for deviations were suspected airway infection and preference for other therapeutic interventions.

Discussion

This study shows that in children with allergic asthma, titration of ICS on FE_{NO} every 3 months for 1 year did not increase steroid doses and did improve airway hyperresponsiveness compared with titrating on symptoms only. Children treated on symptoms demonstrated an increase of FE_{NO}, which is suggestive of more airway inflammation.

This is the first prospective, double-blind, randomized controlled trial in children with atopic asthma incorporating FE_{NO} in treatment algorithms. Conventional measures of asthma control, such as symptoms, use of rescue β-2 agonists, and lung function testing, do not accurately reflect ongoing airway inflammation and thus can be considered suboptimal for guiding ICS treatment.[2,24,25]

Although studies in children are limited, there is some evidence that airway inflammation may already result in remodelling at a young age.[26,27] Indeed, asthma is associated with reduced growth of lung function, and lung function at a young age is a determinant of lung function in adult life.[28] Early detection and

treatment of inflammation might, therefore, be important in strategies to improve asthma control and long-term prognosis. Two studies in adults support the concept that monitoring of airway inflammation can improve asthma treatment. Green and colleagues[29] conducted a trial in which 68 patients with asthma were managed on induced sputum eosinophil counts or on British Thoracic Society (BTS) guidelines. Patients treated on eosinophils had fewer asthma exacerbations and hospital admissions compared with the BTS group, with similar average daily doses of ICS. Interestingly, after 12 months of treatment, F_{ENO} was 48% lower in the sputum eosinophils group. Sont and colleagues[6] demonstrated that better asthma control could be achieved in adult patients who received ICS, based on their level of airway hyperresponsiveness, at the cost of higher steroid doses. Hyperresponsiveness correlates weakly with airway inflammation.[30] Only a few longitudinal studies have examined the possible clinical relevance of F_{ENO} in asthma management. Roberts and coworkers[31] have demonstrated that F_{ENO} relates to previous allergen exposure and asthma control. We demonstrated that F_{ENO} is helpful in predicting asthma relapse in children who discontinue ICS because of clinical remission[32] and, similarly, Zacharasiewicz and colleagues[21] found that F_{ENO} predicted loss of asthma control in children with asthma in whom ICS were tapered irrespective of F_{ENO}. In a recent single-blind controlled trial in adults with asthma, Smith and colleagues showed that using F_{ENO} for dose adjustments of ICS led to similar asthma control with less ICS in the F_{ENO}-treated group compared with the group treated on conventional parameters.[33] This study showed an increase in ICS dose in the control group, rather than a dose reduction in the F_{ENO} group, perhaps due to the study design, in which multiple factors could lead to higher ICS doses in the control group but not in the F_{ENO} group. Hence, the interpretation of these findings is difficult. We now show that airway hyperresponsiveness improved substantially in the F_{ENO} group compared with the symptom group. In the present study, the ICS doses increased in both groups and were similar at the end of the study (*Figure 8.4*). The dose increase is explained by the increase after Visit 1 in both groups. The relatively high median steroid doses in our population probably reflect the tertiary care character of our center.

Our treatment algorithm was based on previous findings, clinical experience, and current treatment guidelines on asthma in children.[1] In the symptom group, we decided to decrease ICS dose only after 6 months of a stable clinical condition. One could argue that due to this algorithm, patients in the F_{ENO} group were more prone to receiving lower ICS doses, as they were allowed a decrease in ICS dose after a single low symptom score. On the other hand, ICS doses in the F_{ENO} group were increased in case F_{ENO} was above 30 ppb, even when symptoms were low. More frequent visits to measure F_{ENO} and adjust ICS, or a longer follow-up, might have resulted in better outcomes. As this is the first prospective, long-term, NO-driven dose titration study, we could not be sure about the best algorithm concerning the frequency of follow-up visits, F_{ENO} measurements, and cut-offs for dose adjustments.

It can be argued that the chosen cut-offs for FE_{NO} and symptoms in our algorithm were too low. We based our symptom cut-off on experience from a previous study.[19] Children in the present study may have had somewhat more severe asthma, as 43% exceeded this cut-off at Visit 1, and this resulted in an increase in ICS dose. In the present algorithm, symptom scores affected treatment decision in both groups, and we cannot know how an alternative cut-off would have affected the outcome.

Our FE_{NO} cut-off level was based on the + 2SD limit of normality from a recent large reference value study in children using the same equipment and population.[20] Therefore, we believe that the 30 ppb cut-off was appropriate. However, it is not known whether efforts to normalize FE_{NO} with ICS are necessarily feasible and effective. Arguably, the ICS dose increments in our algorithm might have been too small to reduce FE_{NO}. However, there is no evidence that very high ICS doses produce additional clinical benefit, although they can cause systemic side effects.[34] We therefore believe that administering higher doses with the purpose of normalizing FE_{NO} was not warranted.

Symptom scores were low in both groups throughout the study. There was a significant, small improvement in symptom scores in the symptom group only. This is not surprising, as symptoms were not an inclusion criterion and all children had been stable on ICS for at least 3 months before enrollment. Given the limited room for improvement, we did not consider reduction in symptoms as a suitable endpoint, and we feel that the small difference between both strategies is hardly clinically relevant.

The changes in hyperresponsiveness as a result of the FE_{NO} strategy, however, are substantial. Airway hyperresponsiveness is a major determinant of asthma prognosis, and is associated with reduced growth of airway caliber in childhood and an accelerated decline of lung function in adulthood.[35-39] Hence, we speculate that the FE_{NO} strategy has the potential to improve the long-term outcome of childhood asthma more than the symptom-based strategy.

Adherence to treatment strongly correlates with FE_{NO}.[40,41] In the present study, poor adherence may have accounted for elevated FE_{NO} in some children. However, in the children on dry powder fluticasone or fluticasone/salmeterol, median adherence was 97% (range, 55 – 124%), which is relatively high compared with average adherence rates of between 63 and 92% reported in the literature.[42] Children displaying poor adherence are likely to be distributed equally over both groups. Hence, we feel confident that poor adherence did not affect our comparisons.

What are the practical implications of our findings? ICS dose titration using FE_{NO} was shown to be feasible and improved important objective endpoints in children with moderate to severe allergic asthma. We feel that the time has come to

introduce FE_{NO} in to the routine assessment of children with asthma in specialist practice, and to take FE_{NO} into account when treatment decisions are made. Further studies might bring to light whether outcome could be further improved by choosing other cut-off levels for symptoms and FE_{NO}, or by more frequent dose adjustments.

In conclusion, we have shown that a treatment algorithm using FE_{NO} for ICS dose titration every 3 months for 1 year is superior to conventional treatment guided by symptoms, and leads to similar clinical asthma control and less airway hyperresponsiveness, obstruction, and inflammation with a similar ICS dose

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FOOTNOTE

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REFERENCES

1. Revised GINA guidelines 2002: Global initiative for asthma, National Institutes of Health, National Heart, Lung and Blood Institute. NIH Publication No 02-3659 2002.
2. Van den Toorn LM, Overbeek SE, De Jongste JC, Leman K, Hoogsteden HC, Prins JB. Airway inflammation is present during clinical remission of atopic asthma. Am J Respir Crit Care Med 2001;164:2107-13.
3. van Grunsven PM. The magnitude of fear of adverse effects as a reason for nonparticipation in drug treatment: a short review. J Asthma 2001;38:113-9.
4. Silvestri M, Sabatini F, Sale R, Defilippi AC, Fregonese L, Battistini E, Biraghi MG, Rossi GA. Correlations between exhaled nitric oxide levels, blood eosinophilia, and airway obstruction reversibility in childhood asthma are detectable only in atopic individuals. Pediatr Pulmonol 2003;35:358-63.
5. Strunk RC, Szefler SJ, Phillips BR, Zeiger RS, Chinchilli VM, Larsen G, Hodgdon K, Morgan W, Sorkness CA, Lemanske RF. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. J Allergy Clin Immunol 2003;112:883-92.
6. Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. Am J Respir Crit Care Med 1999;159:1043-51.
7. Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. Eur Respir J 1993;6:1368-70.
8. Nelson BV, Sears S, Woods J, Ling CY, Hunt J, Clapper LM, Gaston B. Expired nitric oxide as a marker for childhood asthma. J Pediatr 1997;130:423-7.
9. Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. Am J Respir Crit Care Med 2001;163:1693-722.
10. Hunt J, Gaston B. Airway nitrogen oxide measurements in asthma and other pediatric respiratory diseases. J Pediatr 2000;137:14-20.
11. Kharitonov SA, Barnes PJ. Does Exhaled Nitric Oxide Reflect Asthma Control?. Yes, it does! Am J Respir Crit Care Med 2001;164:727-8.
12. Van den Toorn LM, Prins JB, Overbeek SE, Hoogsteden HC, De Jongste JC. Adolescents in clinical remission of atopic asthma have elevated exhaled nitric oxide levels and bronchial hyperresponsiveness. Am J Resp Crit Care Med 2000;162:953-7.
13. Payne DN, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. Am J Respir Crit Care Med 2001;164:1376-81.
14. Piacentini GL, Bodini A, Costella S, Vicentini L, Mazzi P, Sperandio S, Boner AL. Exhaled nitric oxide and sputum eosinophil markers of inflammation in asthmatic children. Eur Respir J 1999;13:1386-90.
15. Berlyne GS, Parameswaran K, Kamada D, Efthimiadis A, Hargreave FE. A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation. J Allergy Clin Immunol 2000;106:638-44.
16. Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. Am J Respir Crit Care Med 1996;153:454-7.
17. Tsai YG, Lee MY, Yang KD, Chu DM, Yuh YS, Hung CH. A single dose of nebulized budesonide decreases exhaled nitric oxide in children with acute asthma. J Pediatr 2001;139:433-7.
18. Pijnenburg MW, Bakker EM, Hop WC, De Jongste JC. Titrating inhaled steroids on exhaled nitric oxide improves FEV1 in allergic asthmatic children. Am J Respir Crit Care Med 2005;2(abstract issue):A690.
19. Verberne AA, Frost C, Roorda RJ, van der Laag H, Kerrebijn KF. One year treatment with salmeterol compared with beclomethasone in children with asthma. The Dutch Paediatric Asthma Study Group. Am J Respir Crit Care Med 1997;156:688-95.

20. Buchvald F, Baraldi E, Carraro S, Gaston B, De Jongste JC, Pijnenburg MW, Silkoff P, Bisgaard H. Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years. J Allergy Clin Immunol 2005;115:1130-6.

21. Zacharasiewicz A, Wilson N, Lex C, Erin EM, Li AM, Hansel T, Khan M, Bush A. Clinical use of noninvasive measurements of airway inflammation in steroid reduction in children. Am J Respir Crit Care Med. 2005;171:1077-82.

22. Baraldi E, De Jongste JC. Measurement of exhaled nitric oxide in children-2001. Joint ERS/ATS Task Force on Exhaled NO Measurement in Children. Eur Respir J 2002;20:223-7.

23. Statacorp. Stata Statistical Software: Release 8.2. College Station, TX, USA. Stata Corporation, 2003.

24. Spallarossa D, Battistini E, Silvestri M, Sabatini F, Fregonese L, Brazzola G, Rossi GA. Steroid-naive adolescents with mild intermittent allergic asthma have airway hyperresponsiveness and elevated exhaled nitric oxide levels. J Asthma 2003;40:301-10.

25. Warke TJ, Fitch PS, Brown V, Taylor R, Lyons JD, Ennis M, Shields MD. Outgrown asthma does not mean no airways inflammation. Eur Respir J 2002;19:284-7.

26. Djukanovic R. Airway inflammation in asthma and its consequences: implications for treatment in children and adults. J Allergy Clin Immunol 2002;109:S539-48.

27. Barbato A, Turato G, Baraldo S, Bazzan E, Calabrese F, Tura M, Zuin R, Beghe B, Maestrelli P, Fabbri LM et al. Airway inflammation in childhood asthma. Am J Respir Crit Care Med 2003;168:798-803.

28. Grol MH, Gerritsen J, Vonk JM, Schouten JP, Koeter GH, Rijcken B, Postma DS. Risk factors for growth and decline of lung function in asthmatic individuals up to age 42 years. A 30-year follow-up study. Am J Respir Crit Care Med 1999;160:1830-7.

29. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002;360:1715-21.

30. Ward C, Pais M, Bish R, Reid D, Feltis B, Johns D, Walters EH. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. Thorax 2002;57:309-16.

31. Roberts G, Hurley C, Bush A, Lack G. Longitudinal study of grass pollen exposure, symptoms, and exhaled nitric oxide in childhood seasonal allergic asthma. Thorax. 2004;59:752-6.27.

32. Pijnenburg MW, Hofhuis W, Hop WC, De Jongste JC. Exhaled nitric oxide predicts asthma relapse in children with clinical asthma remission. Thorax. 2005;60:215-8.

33. Smith AD, Cowan JO, Brassett KP, Herbison GP, Taylor DR. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. N Engl J Med. 2005;352:2163-73.

34. Powell H, Gibson PG. High dose versus low dose inhaled corticosteroid as initial starting dose for asthma in adults and children. Cochrane Database Syst Rev 2004:CD004109.

35. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, Cowan JO, Herbison GP, Silva PA, Poulton R. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med 2003;349:1414-22.

36. Rasmussen F, Taylor DR, Flannery EM, Cowan JO, Greene JM, Herbison GP, Sears MR. Risk factors for airway remodelling in asthma manifested by a low postbronchodilator FEV1/vital capacity ratio: a longitudinal population study from childhood to adulthood. Am J Respir Crit Care Med 2002;165:1480-8.

37. Rijcken B, Schouten JP, Xu X, Rosner B, Weiss ST. Airway hyperresponsiveness to histamine associated with accelerated decline in FEV1. Am J Respir Crit Care Med 1995;151:1377-82.

38. Xuan W, Peat JK, Toelle BG, Marks GB, Berry G, Woolcock AJ. Lung function growth and its relation to airway hyperresponsiveness and recent wheeze. Results from a longitudinal population study. Am J Respir Crit Care Med 2000;161:1820-4.

39. Sherrill D, Sears MR, Lebowitz MD, Holdaway MD, Hewitt CJ, Flannery EM, Herbison GP, Silva PA. The effects of airway hyperresponsiveness, wheezing, and atopy on longitudinal pulmonary function in children: a 6-year follow-up study. Pediatr Pulmonol 1992;13:78-85.

40. Delgado-Corcoran C, Kissoon N, Murphy SP, Duckworth LJ. Exhaled nitric oxide reflects asthma severity and asthma control. Pediatr Crit Care Med 2004;5:48-52.

41. Beck-Ripp J, Griese M, Arenz S, Koring C, Pasqualoni B, Bufler P. Changes of exhaled nitric oxide during steroid treatment of childhood asthma. Eur Respir J 2002;19:1015-9.

42. Cochrane MG, Bala MV, Downs KE, Mauskopf J, Ben-Joseph RH. Inhaled corticosteroids for asthma therapy: patient compliance, devices, and inhalation technique. Chest 2000;117:542-50.

Chapter 9

Daily Ambulatory Exhaled Nitric Oxide Measurements in Asthma

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ABSTRACT

Background: Exhaled NO (FE_{NO}) is a noninvasive, validated marker for asthmatic airway inflammation. Recently, a new hand-held NO-analyzer has been developed which makes it possible to monitor FE_{NO} at home.

Aim of the study: We assessed feasibility and analyzed variability of daily FE_{NO} home measurements.

Methods: Twenty-one asthmatics (mean age 14.5 yr; range 8 - 25 yr) participated. Nineteen used a stable dose of inhaled corticosteroids and all of them were in a stable clinical condition. FE_{NO} was measured twice daily for 14 consecutive days. Measurements and symptom scores were recorded on a smart card in the analyzer. Symptom score items included well-being, wheeze, activity and nocturnal symptoms.

Results: Measurements showed a success rate of 93%. We found a significant diurnal variation in FE_{NO} with geometric mean morning levels 14% higher than evening levels (95% CI: 4% - 25%; $P = 0.013$). Individual subjects showed marked fluctuation of FE_{NO}. The mean intrasubject coefficient of variation of FE_{NO} was 40% for morning and 36% for evening values. FE_{NO} and cumulative symptom scores did not correlate.

Conclusion: Home FE_{NO} measurements are feasible, and offer the possibility to asses airway inflammation on a daily basis. Further study is needed to interpret and evaluate possible benefits of FE_{NO} home monitoring.

INTRODUCTION

The fraction of nitric oxide in exhaled air (F_{ENO}) correlates with eosinophils in induced sputum and with eosinophil infiltration of the airway wall in atopic asthmatics.[1-5] This makes F_{ENO} a candidate marker to monitor asthmatic airway inflammation. In the clinic, F_{ENO} is routinely measured by chemiluminescence analyzers, which are expensive, not easily transportable, require frequent calibration and thus are not suitable for home monitoring. Recently, a new hand-held NO-analyzer (NIOX MINO™, Aerocrine, Solna, Sweden) was developed, which offers the possibility to measure F_{ENO} in the home situation. A good agreement between F_{ENO} tests with a conventional, approved NO-analyzer (NIOX, Aerocrine, Solna, Sweden) and NIOX MINO™ was observed in a recent study.[6] Home monitoring of F_{ENO} has the potential to detect inflammation at an early stage and adapt treatment with inhaled steroids accordingly. This study was performed to evaluate feasibility and variability of F_{ENO} measurements with NIOX MINO™ at home. Second, we investigated the correlation between symptoms and F_{ENO} during the two weeks of the study.

METHODS

Patients with doctor's diagnosed asthma, aged 6 - 25 yr were recruited during their outpatient clinic visit at the hospital. Patients should be in a stable clinical condition as judged by their own pediatric pulmonologist. Patients who had an exacerbation or used oral prednisone in the 3 months preceding the study, or who used rescue beta-2 agonists more than four times a week in the 2 wk preceding the study, patients who smoked and patients with a concurrent disease affecting F_{ENO} were excluded. Atopy was defined as RAST class 2 or higher for at least one inhalation allergen ever. The study was approved by the Medical Ethical Committee of the Erasmus University Medical Centre. In an open, observational, prospective study, patients measured F_{ENO} twice daily for 2 wk at home. The NIOX MINO™ measures NO by an electrochemical sensor (*figure 9.1*). The measurement range is between 0 and 300 ppb with an accuracy of ± 5 ppb or ± 10% if F_{ENO} is > 30 ppb. Deep inhalation through the mouthpiece provides NO-free air via an NO-scrubber. Next, exhalation for 10 s with a constant flow of 50 ml/s is required. A visual and audible feedback system helps to maintain pressure within pre-set limits to ensure constant flow. Measurements are approved if exhaled pressure is between 10 and 20 cm H_2O with deviation allowed during the first 3 s of the measurement. For this study, there was no maximum for the number of allowed attempts. In previous studies NIOX MINO™ was validated and compared with the conventional NIOX NO-analyzer and showed good agreement.[6,7] NIOX MINO™ is provided with a smart card, which records all measurement results and time of measurements. The product we used also has a diary function where symptom scores can be recorded. Well-being, wheeze, activity and nocturnal symptoms are scored on a 4-point scale (maximal daily score 12) and entered in an electronic diary. Data on F_{ENO} levels are available within minutes after the maneuver; symptom scores can be entered

during these minutes. Patients were instructed by one researcher (SF) and were provided with a hand-held analyzer in case of satisfactory performance.

Mixed Model ANOVA was used to compare morning and evening FE_{NO} values and to investigate the correlation between FE_{NO} and cumulative symptom scores. In these analyses FE_{NO} values were log transformed. Spearman non-parametric correlation was used to assess the correlation of age with feasibility. Significance was assumed at $P < 0.05$. SPSS for Windows version 10.1 and SAS (procedure PROC MIXED) were used for statistical analyses.

RESULTS

Twenty-one asthmatics (11 male) were included. Their mean (range) age was 14.5 yr (8.1 - 25.8), 18 of them were atopic. Lung function measurements showed a mean (range) FEV1 of 93% of predicted (57% - 120%) and a mean FVC of 100% (75% - 124%). Nineteen used an inhaled steroid, median dose (range) 400 µg/day budesonide or equivalent (200 - 1600). Of those, 12 used a long-acting beta agonist as well.

Of the maximum number of 588 FE_{NO} measurements (21 subjects, twice daily for 2 wk) 44 values were missing, which gives a success rate of 93%. Non-adherence was the main cause of missing values. The success rate was age-dependent, in the way that older age was associated with worse adherence ($r = -0.56$, $P = 0.008$). The only technical problem requiring intervention of the researchers was sensor failure in one analyzer.

Figure 9.1 *Six year old patient performing a measurement of FE_{NO} with the NIOX MINO™*

Only 3 patients were non-atopic and as atopy might affect variability of FE_{NO} and the correlation with symptoms, these patients were excluded from further analyses. Two of them used a combination of inhaled steroids and a long-acting beta-2 agonist, the other one did not use any medication. Geometric mean morning FE_{NO} in the remaining 18 patients was 14% higher compared to evening levels (95% CI: 4% - 25%; *P* = 0.013), (*figure 9.2*). There was marked fluctuation of FE_{NO} in individual patients with a mean coefficient of variation (CV) of 40% for morning values (range 18% - 93%) and 36% for evening values (range 15 - 72). The difference in coefficient of variation for morning and evening values was not significant (*P* = 0.35, Wilcoxon Signed Ranks test).

Overall, no significant correlation between the mean daily symptom scores and geometric mean FE_{NO} levels was found (*figure 9.3*). In the same way, within-patients between-days changes of FE_{NO} did not correlate with changes of cumulative symptoms on following days.
Analysis of the results within individuals showed a number of patterns, of which examples are shown in *figure 9.4*. Most children showed stable, low FE_{NO} with either stable, low symptom scores (n = 8) (*figure 9.4a*) or high, sometimes widely varying symptom scores (n = 7) (*figure 9.4b*). Five showed evident parallel changes of FE_{NO} and symptoms over several days: one of them started measuring immediately upon returning from a school camp and showed a gradual normalization of extremely high FE_{NO} within 2 wk, paralleled by a reduction in symptoms (*figure 9.4c*). Another child visited a similar camp during the 2 wk of monitoring; afterwards both FE_{NO} and symptoms had risen dramatically (*figure 9.4d*). One patient showed high stable FE_{NO} with low symptoms (*figure 9.4e*), and one high stable FE_{NO} with high symptom scores (*figure 9.4f*).

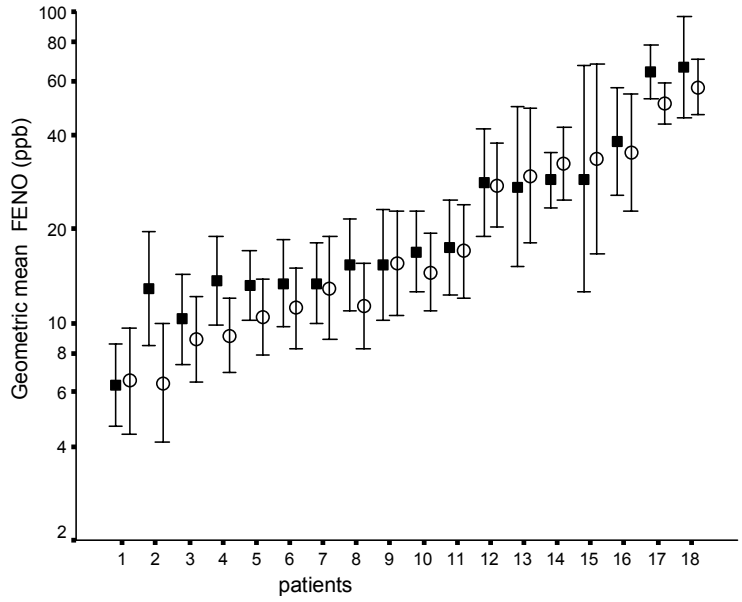

Figure 9.2 *Individual diurnal variability of geometric mean FE_{NO} for each patient. Morning values (closed symbols) and afternoon values (open symbols) ± 1 SD for each patient during two wk. The mean diurnal variation of all patients was 14% (95% CI 4 – 25%, P = 0.013).*

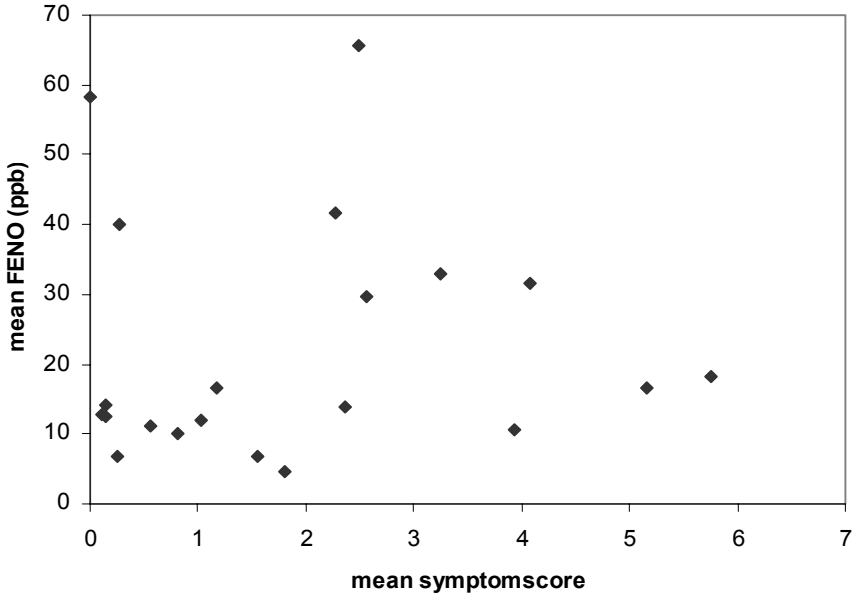

Figure 9.3 *Mean daily cumulative symptom scores (horizontal axis) as a function of geometric mean FE_{NO}-levels. Each symbol represents a patient. The correlation was not significant.*

DISCUSSION

This is the first report on FE_{NO} measurements taken by asthmatic patients in their homes. Until now, the bulky and complicated NO chemiluminescence analyzers have limited the applicability of FE_{NO} as an instrument for frequent monitoring of airway inflammation in asthma. The new hand-held analyzer was successfully used for daily home measurements of FE_{NO} during 2 wk, without professional support. We found significant diurnal FE_{NO} variability with highest values in the morning, and considerable day-to-day variation. Mean FE_{NO} values and changes in FE_{NO} between days did not correlate with mean symptom scores within the time frame of the study; individual results were suggestive of parallel changes in some children as a result of external factors.

Others and we have shown that FE_{NO} correlates with eosinophilic airway inflammation, assessed in induced sputum or airway biopsies in atopic asthmatic adults and children.[1-5] Treatment with corticosteroids reduces FE_{NO} levels in asthmatic patients in a dose-dependent manner.[8,9] Measuring FE_{NO} at home on a daily basis offers the possibility to detect changes in inflammation in an early stage and to adjust treatment accordingly.

We demonstrated a marked diurnal FE_{NO} variation. This is in line with findings by Mattes *et al*, who found a statistical trend towards higher morning FE_{NO} values and a cosine like circadian rhythm of FE_{NO}, for asthmatic as well as a small number of healthy children.[10] Ten Hacken *et al* found higher FE_{NO} at 4 a.m. compared to 4 p.m. in adult patients with nocturnal asthma and peak flow variability of > 15%.[11]

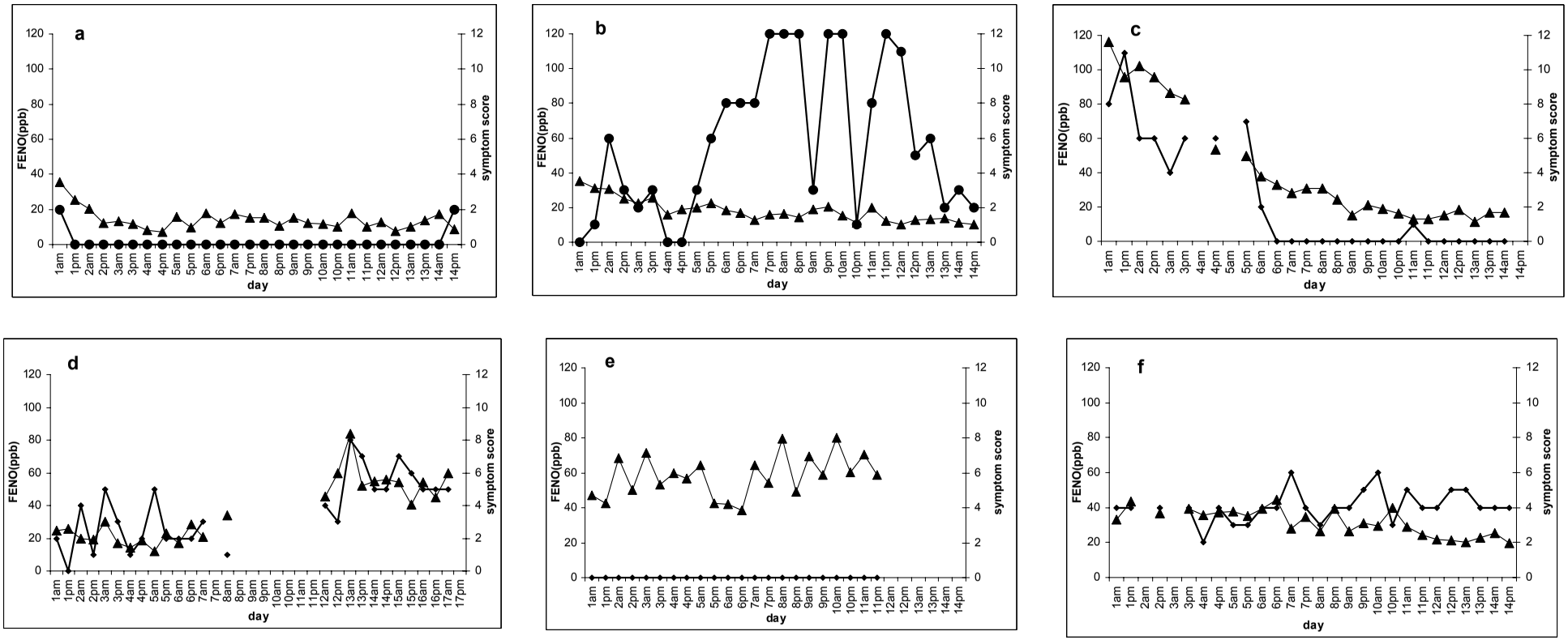

Figure 9.4 *Six examples of individual FE_{NO} and symptom scores. On the x-axis time in days, on the y-axis FE_{NO} (left, triangles) and cumulative symptom scores (right, circles).*

a *low FE_{NO} with low symptom scores.*

b *low FE_{NO} with varying symptom scores.*

c *parallel change of FE_{NO} and symptoms: this child started measurements after returning from a camp. Both FE_{NO} and symptoms start high and normalize within 2 weeks.*

d *parallel change of FE_{NO} and symptoms: a child who went to a school camp during the 2 weeks of measurement, missed some measurements for that reason and showed a marked increase in both symptoms and FE_{NO} upon return home.*

e *high FE_{NO} with low symptoms.*

f *high FE_{NO} with high symptom scores.*

In contrast, Kharitonov *et al* found no diurnal or day-to-day variation in healthy and asthmatic adults and children during a 5-day follow-up.[12] The mechanism of diurnal F_{ENO} variation is still unclear. Both variation in the inflammatory process and external factors like sleep and food intake might play a role. For the moment, it seems important to perform serial F_{ENO} measurements within patients at the same time of the day.

In the present study, day-to-day variation of F_{ENO} was common, however, especially in the lower (normal) F_{ENO} ranges this variability is clinically not relevant. Although we tried to include patients with stable asthma, almost half of them experienced clinical instability during the study and in some cases an external trigger seemed responsible for parallel changes in F_{ENO} and symptoms (*figure 9.4*). This clinical instability may at least partly explain the high day-to-day variation we found in this study. On the contrary, some individuals showed marked instability of symptoms with stable, low F_{ENO}.

There is a good agreement between F_{ENO} measurements with NIOX MINO™ and a conventional chemiluminescence analyzer. In this study, an average disagreement of 0.5 ppb with a mean standard deviation of 3.8 ppb was found from 251 measurements in 19 subjects.[6] This makes it very unlikely that the diurnal variability may be explained by variability of the analyzer itself.

Our study was not designed to answer the question if home monitoring of F_{ENO} might predict clinical instability. Earlier studies on the correlation of F_{ENO} and asthma severity and control show conflicting results. These may be due to different measuring techniques, patient selection criteria and the use of ICS.[13-20] However, some recent studies including our own showed that F_{ENO} might be used as a 'loss of control' marker, predicting asthma exacerbations or relapse after cessation or reduction of ICS.[21-23] In the study of Jones, single measurements and changes of F_{ENO} from baseline had positive predictive values of 80% - 90% for predicting and diagnosing loss of asthma control.[22] In a previous study, we followed children with clinical remission of asthma who were taken off ICS; F_{ENO} at 2 and 4 wk after withdrawal predicted asthma relapse in the forthcoming months.[23] We speculate that the lack of a correlation between symptoms and F_{ENO} in the present study may be due to its limited time frame. The question is whether F_{ENO} might be used to adapt ICS doses, and whether or not frequent home monitoring has advantages above infrequent assessment at clinic visits. The relevance of F_{ENO} fluctuations for asthma management is clearly important to elucidate.

We conclude that ambulatory F_{ENO} measurements with NIOX MINO™ are feasible, and offer the possibility to measure asthmatic inflammation frequently at home. Morning values are significantly higher than evening values and considerable day-to-day variability exists, partly due to clinical instability. Longer follow-up studies are now warranted to evaluate whether and how frequent F_{ENO} measurements may be used to improve asthma management.

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REFERENCES

1. Piacentini GL, Bodini A, Costella S, et al. Exhaled nitric oxide and sputum eosinophil markers of inflammation in asthmatic children. Eur Respir J 1999;13:1386-90.
2. Van den Toorn LM, Prins JB, Overbeek SE, Hoogsteden HC, De Jongste JC. Adolescents in clinical remission of atopic asthma have elevated exhaled nitric oxide levels and bronchial hyperresponsiveness. Am J Resp Crit Care Med 2000;162:953-7.
3. Berlyne GS, Parameswaran K, Kamada D, Efthimiadis A, Hargreave FE. A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation. J Allergy Clin Immunol 2000;106:638-44.
4. Payne DN, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. Am J Respir Crit Care Med 2001;164:1376-81.
5. Van den Toorn LM, Overbeek S, De Jongste JC, Leman K, Hoogsteden HC, Prins JB. Airway inflammation is present during clinical remission of atopic asthma. Am J Respir Crit Care Med 2001;164:2107-13.
6. Hemmingsson T, Linnarsson D, Gambert R. Novel hand-held device for exhaled nitric oxide analysis in research and clinical applications. J Clin Monit 2004;18:379-87.
7. Alving K, Nordvall SL, Janson C, Pedroletti C. Agreement between a stationary device (NIOX) and a new hand-held device (NIOX MINO) for fractional exhaled nitric oxide (FE_{NO}) measurements in adults and children. Eur Respir J 2004;24:163S.
8. Tsai YG, Lee MY, Yang KD, Chu DM, Yuh YS, Hung CH. A single dose of nebulized budesonide decreases exhaled nitric oxide in children with acute asthma. J Pediatr 2001;139:433-7.
9. Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. Am J Respir Crit Care Med 1996;153:454-7.
10. Mattes J, Storm van 's Gravesande K, Moeller C, Moseler M, Brandis M, Kuehr J. Circadian variation of exhaled nitric oxide and urinary eosinophil protein X in asthmatic and healthy children. Pediatr Res 2002;51:190-4.
11. ten Hacken NH, van der Vaart H, van der Mark TW, Koeter GH, Postma DS. Exhaled nitric oxide is higher both at day and night in subjects with nocturnal asthma. Am J Respir Crit Care Med 1998;158:902-7.
12. Kharitonov SA, Gonio F, Kelly C, Meah S, Barnes PJ. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. Eur Respir J 2003;21:433-8.
13. Artlich A, Busch T, Lewandowski K, Jonas S, Gortner L, Falke KJ. Childhood asthma: exhaled nitric oxide in relation to clinical symptoms. Eur Respir J 1999;13:1396-401.
14. Sippel JM, Holden WE, Tilles SA, et al. Exhaled nitric oxide levels correlate with measures of disease control in asthma. J Allergy Clin Immunol 2000;106:645-50.
15. Roberts G, Hurley C, Bush A, Lack G. Longitudinal study of grass pollen exposure, symptoms, and exhaled nitric oxide in childhood seasonal allergic asthma. Thorax 2004;59:752-6.
16. Payne DN, Qiu Y, Zhu J, et al. Airway inflammation in children with difficult asthma: relationships with airflow limitation and persistent symptoms. Thorax 2004;59:862-9.
17. Meyts I, Proesmans M, De Boeck K. Exhaled nitric oxide corresponds with office evaluation of asthma control. Pediatr Pulmonol 2003;36:283-9.
18. Olin AC, Alving K, Toren K. Exhaled nitric oxide: relation to sensitization and respiratory symptoms. Clin Exp Allergy 2004;34:221-6.
19. Delgado-Corcoran C, Kissoon N, Murphy SP, Duckworth LJ. Exhaled nitric oxide reflects asthma severity and asthma control. Pediatr Crit Care Med 2004;5:48-52.
20. Warke TJ, Mairs V, Fitch PS, McGovern V, Ennis M, Shields MD. Exhaled nitric oxide in relation to the clinical features of childhood asthma. J Asthma 2004;41:745-751.

21. Jatakanon A, Lim S, Barnes PJ. Changes in sputum eosinophils predict loss of asthma control. Am J Respir Crit Care Med 2000;161:64-72.

22. Jones SL, Kittelson J, Cowan JO, et al. The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med 2001;164:738-43.

23. Pijnenburg MW, Hofhuis W, Hop WC, De Jongste JC. Exhaled nitric oxide predicts asthma relapse in children with clinical asthma remission. Thorax 2005: 60:215-8.

Chapter 10

Discussion

partly based on:
Exhaled Nitric Oxide Measurements:
Clinical Application and Interpretation

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Andrew D. Smith
Johan C. de Jongste

Thorax, in press

The first scientific papers on elevated FE_{NO} levels as a marker of eosinophilic airway inflammation in asthmatic patients raised great expectations for a potential role of 'inflammometry' in the management of asthma. As measuring FE_{NO} is noninvasive, easy, repeatable, and reproducible and provides valuable additional information, this test has the potential to improve asthma management, especially in children. As comparison between the early studies was difficult in view of the different methodologies used, studies in the 1990s began to focus on harmonizing the methodology of measuring FE_{NO}. This has resulted in standardized guidelines for the measurement of FE_{NO} in adults and children.[1,2] Some methodological issues that remained unresolved were investigated in this thesis.

With most methodological issues having been settled, the doors were wide open to studies on the clinical applications of FE_{NO} in asthma management, and these were presented in the second part of this thesis. An algorithm will be presented on the use of FE_{NO} in asthmatic children for everyday paediatric practice.

10.1 Methodological Issues

In 1997 the European Respiratory Society Task Force issued the first recommendations on the measurement of exhaled and nasal nitric oxide,[3] followed by updated guidelines in 1999 and 2005.[2-4] Most methodological issues have been sorted out by now, but yet some questions and problems remain unsolved. Measuring FE_{NO} in preschool children forms a challenge and is discussed in section 10.1.1. Some remaining issues on normal values are touched upon in section 10.1.2.

10.1.1 Preschool children

An ERS/ATS statement on the measurement of FE_{NO} in children – with special attention to preschool children – appeared in 2002.[1] This addressed the phenomenon of young children having difficulty in performing a single breath online (SBOL) FE_{NO} measurement.[5-7] Maintaining a constant low flow, for example, may be troublesome. Dynamic flow restrictors, which vary their resistance with the blowing pressure, may allow children to exhale at a constant rate while mouth pressure is varying. In chapter 2 we demonstrated that this method is feasible in the vast majority of 4 to 8-year-old children.[8] Another possible modification to the SBOL method is the use of a variable expiratory resistance that is manually adjusted by the technician to maintain constant flow.[7] If children do not cooperate at all, cannot exhale long enough to reach a plateau or refuse to grip the mouthpiece, collecting exhaled air samples during spontaneous breathing manoeuvres may be possible. FE_{NO} may be measured online during tidal breathing with expiratory flow adjusted by changing the expiratory resistance, if they are able to breath slowly but regularly through a mouthpiece.[9] Two offline methods are available that are suitable for young children. First, children accepting a mouthpiece may exhale via a mouthpiece, against a resistance, into a reservoir.[5,10]

FE_{NO} values obtained with this method correlate well with online values and differentiate between children with and without airway disease.[5,10,11] Second, tidal breathing via a mouthpiece or mask covering the mouth or mouth and nose, with or without expiratory resistance, is applicable in children who do not cooperate at all (*figure 1.6*).[5,12,13] In sedated infants and neonates a single breath technique during raised-volume rapid thoraco-abdominal compression has been used to determine FE_{NO} values.[14]

In summary, several techniques to measure FE_{NO} in preschool children are in use, which correlate well with the SBOL method and may offer valuable information on the nature of respiratory symptoms in this age group. In section 10.2.4 clinical applications of FE_{NO} measurements in young children will be discussed.

10.1.2 Normal values

Section 1.5.2 scrutinized particular factors that may influence FE_{NO} measurements. For one, it appears that FE_{NO} is highly dependent on the expiratory flow and that ambient NO and NO from the nose or paranasal sinuses may contaminate FE_{NO}.[15-21] Then, in our reference values study (chapter 4) we found that patient-related factors such as age and ethnicity may influence FE_{NO}. Several such factors are discussed below.

Age. We found FE_{NO} to increase with age, at a rate of about 1 ppb per year.[22] This finding is in line with data from other, much smaller studies.[23-25] It has been suggested that growth of the lungs and airway surface area may be responsible for this age-dependent increase.[23] A plausible explanation is that the expiratory flow of 50 ml/s for all ages is relatively high for the younger children, and higher expiratory flows give rise to lower FE_{NO} values. Then, it was hypothesized that increased induction of iNOS due to a higher cumulative rate of infections in older children may account for the age-dependency of FE_{NO}.[26] This, however, is unlikely, as the infection load will decrease with age.

Genetics. Variability of FE_{NO} may, at least in part, be explained by genetic predisposition, especially due to sequence variations in the neuronal NOS (NOS1) gene, which is involved in the endogenous NO production.[27] Significant differences between Caucasian subjects and African American subjects in allele frequencies in the NOS1 gene have been described.[28] This may explain the higher FE_{NO} values in non-Caucasian children compared with Caucasian children in our reference value study, which Wong *et al*, reported as well.[22,29] Further studies may reveal the need for ethnicity-specific reference FE_{NO} values.

Gender. Like in several other studies, FE_{NO} in boys and girls did not differ in our reference values study.[22,23,25,26] This is in contrast with the results of the study in chapter 2, where FE_{NO} in girls was higher than in boys.[8] However, this study shows a limitation in sample size, which was rather small (20 boys and 14 girls).

Food. Although caffeine and alcohol intake may influence FE_{NO} levels, this phenomenon is likely to be absent in (young) children.[30-36] The effect of chocolate or soft drinks intake has not been studied, but is potentially more important in children.

10.2 Clinical Applications

Various lines of evidence converge to provide the rationale for using FE_{NO} measurements in the assessment and management of respiratory disease in children. Firstly, FE_{NO} is highly correlated with eosinophilic airway inflammation, as discussed in section 1.5.3. Secondly, eosinophilic airway inflammation is associated with a positive response to steroid treatment.[37-40] Thirdly, elevated FE_{NO} levels predict steroid responsiveness in patients with non-specific respiratory symptoms.[39,41-43] And lastly, ICS treatment in asthma results in a dose-dependent fall in FE_{NO}.[13,37,44-52]

Taken together, these data provide foundational evidence that FE_{NO} measurements have a potentially important role in evaluating patients with airway disease, in that they may predict responses to steroid treatment and may monitor responses to ICS therapy. The full range of possible applications of FE_{NO} measurements is summarized in *Table 10.1*.

Table 10.1 Possible applications of FE_{NO} measurements in paediatric asthma

Screening for asthma in epidemiological studies
Diagnosis of eosinophilic airway inflammation
Predicting response to steroids
Evaluation of response to:
Steroids (inhaled or systemic)
LTRA
Other
Selection of treatment modalities additional to ICS (e.g. LABA or LTRA)
Predicting asthma exacerbations
Predicting asthma relapse after clinical remission
Adherence check
Dose titration of ICS

10.2.1 FE_{NO} in the diagnosis of asthma

FE_{NO} may play a role as a diagnostic tool in epidemiological studies or in selected patients with respiratory symptoms.[43,53-59]

Three studies examined the value of FE_{NO} for diagnostic purposes in epidemiological surveys in children.[54,55,59] Thomas *et al*. found a 47% sensitivity and a 93% specificity for FE_{NO} > 7 ppb for the diagnosis of asthma in a cohort of 107 school children.[59] This cut-off value seems very low, but FE_{NO} was measured

offline, without controlling the expiratory flow. Evidence for the overall diagnostic utility of F_{ENO} measurements in young children is mixed. Brussee *et al*. in an unselected population of preschool children too young to perform spirometry, found F_{ENO} to perform poorly in distinguishing asthma and non-asthma in individual subjects.[54] Differences in F_{ENO} between atopics, children with doctor-diagnosed asthma, and healthy children were less pronounced than in older subjects.[54] These data were in line with findings by Prasad *et al*. in 368 school children.[55] Contrastingly, in a *selected* group of 96 young children with asthmatic symptoms or history, F_{ENO} discriminated between probable asthma or healthy control with 86% sensitivity and 92% specificity.[60] In this study, F_{ENO} performed better than baseline lung function and bronchodilator response as measured using impulse oscillometry. Narang *et al*. studied F_{ENO} levels in children with various respiratory disorders – including asthma – and in healthy controls. At expiratory flows ranging from 200 - 280 ml/s the negative and positive predictive value for F_{ENO} > 25 ppb as a predictor of asthma were 80 and 100%, respectively.[61]

The sensitivities and specificities quoted here are comparable or even better than those obtained with current diagnostic tools such as bronchoprovocation testing and spirometry.[53,56,62] Clearly the different methods are often complementary and may be detecting different aspects of the disease in asthma.

Epidemiological studies in adults are lacking, but F_{ENO} measurements were found to be helpful in discriminating asthma from non-asthma in patients with respiratory complaints, at least in those whose underlying inflammation was eosinophilic.[53,56,57,63] For example, Dupont *et al* evaluated 240 non-smoking steroid-naïve individuals of whom 160 (67%) fulfilled criteria for the diagnosis of asthma, and found that F_{ENO} levels were highly predictive of asthma with 85% sensitivity and 90% specificity.[57] Smith *et al*. in a smaller study demonstrated comparable sensitivity (88%) and specificity (79%) and predictive values were almost identical to those obtained using induced sputum cell counts.[53] A striking feature in this study was the poor performance of almost all 'conventional' diagnostic tests against which F_{ENO} measurements were compared. This reflects the fact that groups of unselected patients will show predominance of mild disease without abnormal lung function. Hence in this setting, F_{ENO} measurements may be more relevant than traditional lung function tests.

It is important to realize that patients may fulfil conventional clinical criteria for the diagnosis of asthma and yet have normal F_{ENO} levels, particularly non-atopic subjects. Normal F_{ENO} values do not exclude the diagnosis of asthma. Measuring AHR may reveal a positive, clinically relevant result. Thus F_{ENO} measurements complement rather than substitute for measuring AHR.[56,64] This highlights the heterogeneity of the asthma phenotype, as well as the fact that F_{ENO} measurements provide a perspective on only one, important, aspect of the 'asthma syndrome'.

FE_{NO} in the differential diagnosis of children with non-specific chronic respiratory symptoms. FE_{NO} measurements may have a role in the differential diagnosis of non-specific chronic respiratory symptoms (see table in section 1.5.5). Especially in young children there is a broad differential diagnosis , including post-viral bronchial hyperresponsiveness, recurrent wheeze due to small airways, post-nasal drip and other ENT problems, cough variant asthma, gastro-oesophageal reflux disease, cystic fibrosis, congenital abnormalities of the airways or lungs and primary ciliary dyskinesia.

10.2.2 Predicting the response to steroids

Treatment with corticosteroids was found to reduce airway eosinophilia in asthma and to simultaneously improve clinical parameters.[37-40] In contrast, in asthma not characterized by eosinophilia (at least absent in sputum), the response to steroid therapy is likely to be poor.[65,66] One study, which included both adults and children, addressed the question if elevated FE_{NO} levels can predict steroid responsiveness in patients with non-specific respiratory symptoms.[41] FE_{NO} appeared a much better predictor than spirometry, bronchodilator response and AHR measurements. This study identified as optimum cut-off point for predicting steroid response a FE_{NO} of 47 ppb.

In a study set up to characterize the within-subject responses to fluticasone and the leukotriene receptor antagonist montelukast, children with elevated FE_{NO} values were more likely to respond to fluticasone than to montelukast.[39] Moreover, in the same study, children were better controlled on fluticasone than on montelukast when FE_{NO} was high.[40]

Little *et al*. demonstrated that the clinical benefit of oral steroids in patients with asthma on ICS is greatest in patients with elevated FE_{NO} levels.[42]

On the other hand, for other diagnoses – e.g. tracheomalacia presenting as 'asthma', it may be just as helpful to have a low-normal FE_{NO} level, indicating a condition which is not characterized by eosinophilic airway inflammation, and which in turn is less likely to be steroid-responsive.

In conclusion, these studies indicate that FE_{NO} is helpful in predicting responses to steroid treatment, and that its use potentially improves the cost-benefit ratio of a trial of inhaled or oral steroids.

10.2.3 FE_{NO} in the management of childhood asthma

Two important questions have emerged regarding FE_{NO} measurements in the management of asthma. Firstly, can they be used to predict exacerbations? Secondly, can they guide decisions relating to anti-inflammatory treatment?

Predicting exacerbations. Asthma is characterized by relapses and remissions, with deterioration in control provoked by particular triggers or by poor compliance with anti-inflammatory therapy. There is a need for an objective measurement tool that will alert to impending deterioration. Of limited help are the PEF

measurements that are currently used to fulfil this role. In children, changes in PEF poorly reflect changes in asthma activity, PEF diaries are kept very unreliably, and self management programs including PEF monitoring are no more effective than programs based on education and monitoring of symptoms.[67-69]

Overall, there is evidence that FE_{NO} has prognostic value to predict deteriorating asthma.[70,71] Harkins *et al*. in a study with 22 patients noted that elevated FE_{NO} in routine practice predicted an exacerbation within the next two weeks.[71] In a study by Jones *et al*, measurements of AHR to hypertonic saline, sputum eosinophils and FE_{NO} measurements all ranked similarly as predictors of loss of control in 78 asthma patients following ICS withdrawal, with positive predictive values ranging from 80 to 90%.[70] In a small study involving a steroid reduction protocol, Jatakanon *et al* reported that both changes in sputum eosinophils and FE_{NO} measurements predicted loss of control.[72] All these studies, except the one by Harkins *et al*, employed a steroid-withdrawal protocol to induce a clinical exacerbation, which however is an artificial strategy. The only study in children investigated FE_{NO} levels in sensitized patients every 4 weeks during the pollen season. Thirty-two asthma exacerbations were observed over the period, and although data suggested that FE_{NO} rose before an exacerbation, this could not be proven.[73] With the advent of home monitoring programs, the inclusion of daily FE_{NO} measurements may prove to be beneficial in anticipating deteriorating asthma.

Predicting the outcome of ICS withdrawal in stable asthma. A relevant question is whether markers of airway inflammation can be used to predict successful reduction or withdrawal of ICS treatment. In chapter 7 we reported that following steroid withdrawal, FE_{NO} levels in currently asymptomatic children 2 and 4 weeks later were highly predictive of relapse during the subsequent 24 weeks. A cut-off point for FE_{NO} of 49 ppb provided the best predictive accuracy – i.e. a FE_{NO} value above this threshold predicted likely asthma relapse.[74] Zacharasiewicz *et al*. concurrently performed a slightly different study in 40 children with stable asthma eligible for steroid reduction.[75] They halved ICS doses every 8 weeks and found that 15 children experienced loss of asthma control. A negative predictive value of 92% was obtained for FE_{NO} at a cut-off point of 22 ppb or less. The negative predictive value of sputum eosinophil counts (cut-off point 0%) was 100% – i.e. treatment reduction or even withdrawal was 100% successful during the subsequent 8 weeks when sputum eosinophilia was absent.[75]

In studies in adults, no prognostic significance could be derived from FE_{NO} measurements, whereas sputum eosinophil counts (> 0.8%) were highly predictive of subsequent loss of asthma control, over 6 months in one study and over 16 weeks in the other.[76,77] One of these studies used baseline rather than sequential FE_{NO} values in the calculations.[77] In the second, the number of patients in whom FE_{NO} values were obtained was small, making valid comparisons difficult.[76]

All in all, we can conclude that high FE_{NO} levels (> 50 ppb) are likely to predict asthma relapse, and that low FE_{NO} levels (< 20 ppb in children and 25 ppb in adults) are likely to predict asthma stability, if measured at least 4 weeks after ICS reduction or withdrawal in currently asymptomatic patients. Outcomes among those showing intermediate levels (FE_{NO} 20 - 50 ppb) are less certain. At least in adults, sputum eosinophil counts may offer superior prognostic accuracy for ICS treatment decisions. In (young) children, however, sputum induction is not very successful, carries a risk of bronchoconstriction, is time consuming and requires skilled investigators.[78-80] FE_{NO} measurements are much more feasible in children.

Adjustment of inhaled corticosteroid dose. Several studies have recently explored whether targeting anti-inflammatory treatment on AHR,[81] sputum eosinophils[82] or FE_{NO}[83,84] can be used to optimize ICS dosing, including our study discussed in chapter 8.[83] Notwithstanding differences in design, populations and endpoints, all studies measuring inflammation in the management of asthma point in the same direction: targeting inflammation improves asthma outcome[81-83] or reduces the need for ICS without compromising clinical outcome.[84]

The underlying rationale for each of these studies is both plausible and desirable i.e. anti-inflammatory therapy should be adjusted to ensure minimum airway inflammation. Clinicians now tend to base dosing on uncontrolled symptoms or impaired lung function assuming that these result from uncontrolled airway inflammation. Yet, correlations between airway inflammation and either symptoms or lung function are weak, as discussed in section 1.2. Thus the use of these end-points to guide treatment can only be regarded as second best, as it is far more rational to adjust ICS dosing in an attempt to control airway inflammation.

The two randomized controlled trials in which FE_{NO} measurements have been used to guide long-term treatment with ICS showed significant but differing benefit.[83,84] One, Smith *et al*. followed 94 adult asthmatics who completed a dose titration phase and then for 12 months were assigned to either treatment on the basis of FE_{NO} measurements or the conventional Global Initiative for Asthma (GINA) guidelines.[84] The primary outcome was the frequency of exacerbations of asthma; the secondary outcome was the mean daily dose of ICS. After a run-in period during which patients used a daily dose of 500 or 750 µg of fluticasone, ICS dose in the FE_{NO} group was down titrated at 4-weekly intervals in case FE_{NO} was low (< 15 ppb, corresponding to < 35 ppb at an expiratory flow of 50 ml/s). Down titration in the control group was only allowed if asthma was controlled according to the GINA guidelines. The optimal dose was fixed at a level one step higher than the one that showed loss of control (control group) or FE_{NO} > 15 ppb (FE_{NO} group). In the following 12 months, patients were kept on this optimal dose, and stepped up when loss of control or FE_{NO} > 15 ppb, respectively, was noted. Down titration below the optimal dose established in the initial phase was not possible. In this study a 40% reduction in ICS dose requirements was achieved in the FE_{NO} group,

without significant difference in the rate of asthma exacerbations between groups.[84]

The second trial was the study by our group (Chapter 8) in which we set the cut-point for F_{ENO} levels similarly at 30 ppb. The F_{ENO} group showed a significant reduction in the severity of AHR, with a concomitant (but non-significant) reduction in exacerbations requiring oral prednisone. Cumulative ICS use did not differ between the F_{ENO} and control groups.[83] Two possible explanations for the dissimilar outcomes present themselves. First, study designs were different: Smith *et al*. applied a design that did not allow for ICS dose reduction over the full 12 months, whereas we made treatment decisions every 3 months. Second, patients in the 'symptom group' in our study were managed on their symptoms only, whereas Smith *et al*. based treatment decisions on four items – i.e. symptoms, PEF variability, bronchodilator use and FEV_1 –, making it more likely to step up ICS treatment in the 'GINA guidelines' group.

What are the pitfalls in these studies? First, both used a single cut-off level for F_{ENO} to prompt either an increase or a decrease in ICS dose. Although single cut-points are appropriate in 'back-titration' studies with high ICS starting doses, *two* cut-points defining *three* management choices – i.e. increase, no change or decrease in dose – may be more effective. Clearly this is an area that requires further investigation.

Second, the 'one size fits all' approach used in both studies may not be appropriate in regular clinical practice. An alternative method of dealing with F_{ENO} in individual patients might be using 'personal best values' as baseline or target F_{ENO} levels.

Third, the two studies applied substantially different criteria to guide ICS dose adjustment in the control groups. Cut-points will significantly determine the outcome in any dose-adjustment strategy.

Upwards and downwards titration. An important and as yet unresolved issue is whether F_{ENO} measurements should be used for both upwards and downwards ICS dose titration. Clearly, the withdrawal of unnecessary ICS treatment or reducing excessive doses is an important goal of F_{ENO} monitoring. This was the primary endpoint in our study, and although in the study by Smith *et al*. ICS dose reduction was a secondary endpoint, reduction indeed proved to be the principal outcome.[84] Thus, data to date suggest that dose reduction is an achievable objective. However, it has been argued that F_{ENO} may be too sensitive to detect changes in inflammation. Steroids have an anti-inflammatory effect, but also a direct effect on iNOS expression and thus on NO production. Hence a fall in F_{ENO} within a few days would not necessarily mean that eosinophilic inflammation is completely controlled.[45,46,85-89] This was confirmed by Jatakanon *et al*, who showed a further decrease in sputum eosinophil counts and an improvement in AHR after 4 weeks' administration of 1600 µg versus 400 µg budesonide, whereas maximal

reduction in FE_{NO} was reached with the 400 µg dose.[45] It is well known that after starting ICS treatment, different endpoints will change over different time spans. For example, while FE_{NO} responds to steroid treatment within 1 - 4 weeks,[37,51] it may take months before optimal FEV_1 is achieved, or even months to years before optimal AHR is reached.[90-92] In our dose titration study follow up was 12 months, and even over this relatively short period of time AHR improved significantly in the FE_{NO} group compared with the symptom group.[83] Therefore, ICS dose titration guided by FE_{NO} is likely to be effective in improving AHR on the long term.

For patients with persistently high FE_{NO} (> 50 ppb), it is as yet unknown whether *increasing* the dose of ICS is justified, particularly if a patient is asymptomatic. Higher doses reduce the frequency and severity of asthma exacerbations, and persistently elevated FE_{NO} levels may be the signal to prescribe higher ICS doses with this objective in mind. This issue is a controversial one. On the one hand, ICS dose increments in the higher range often have little effect on FE_{NO}. Also, as we showed in chapter 6, it is often not possible to normalize levels if patients show persistently high FE_{NO} levels. This may be the case even when they are given maximum ICS doses, which, for that matter, may have significant side effects.[93,94] Anecdotally it would appear that even when asthma is well controlled, 'normal' FE_{NO} levels are rarely achieved. Thus, FE_{NO} levels measured when asthma is stable may be taken as the baseline reference points for individual patients against which subsequent measurements are weighed.

Until further studies have been completed, it seems prudent to put the emphasis on dose reduction when FE_{NO} levels are low (less than 15 to 25 ppb depending on age). We propose that high FE_{NO} levels should prompt an increase in ICS dose only if asthma is poorly controlled and issues of poor compliance and/or poor inhaler technique have been addressed.

Asthma severity and FE_{NO}. Our dose titration study (chapter 8) included only children with moderate to severe asthma, which obviously restricts conclusions to these particular patients. We cannot be confident, therefore, of the beneficial effect of including FE_{NO} in decision-making in patients with mild asthma on low doses of ICS. However, both the study of Zacharasiewicz *et al*. and our own 'withdrawal' study (chapter 7) included children on low doses of ICS, and in both FE_{NO} was found to be effective in tapering or stopping ICS.[74,75] So, it seems that FE_{NO} may also prove to be beneficial in titrating ICS dose in children irrespective of disease severity.

Role of FE_{NO} in managing non-atopic asthmatic patients. Most of the studies on the utility of FE_{NO} measurements in asthma management concerned allergic patients. However, the study by Smith *et al*. included 16 non-atopic patients as well and these patients showed a heterogeneous picture. In some of these FE_{NO} increased after ICS down titration, whereas in others ICS tapering or even withdrawal was not associated with rise in FE_{NO} (A. Smith, personal

communication).[84] To date, insufficient data are available on the utility of FE_{NO} in non-atopic asthmatic patients.

Long term benefits. As follow up in the two dose titration trials reported was no longer than one year, we can only speculate on the longer term benefits of titrating steroids on FE_{NO}.[83,84] We feel it is promising that AHR significantly improved in patients managed on FE_{NO} versus those on symptoms, even within this relatively short period. As AHR is one of the most important determinants of asthma prognosis, and is associated with reduced growth of airway calibre in childhood, this finding seems to point at improved treatment and better prognosis of childhood asthma on the longer term as well.[95-98]

10.2.4 Use of FE_{NO} in preschool children

In preschool children respiratory symptoms are extremely common, and there is a broad differential diagnosis with varying prognosis and response to treatment.[99-101] The majority of children presenting with wheezing have transient conditions associated with diminished airway function, and are unlikely to respond to inhaled steroid treatment.[101-103] Given that spirometry and sputum induction cannot be easily performed at this age, a test that might inform on expected response to treatment would be very helpful. As the single breath technique for measuring FE_{NO} is not feasible in this age group, several alternatives have been developed, varying from modifications of the standard online technique to offline tidal breathing methods without flow control.[5,7,9,11,12,14,104-108] In general these techniques seem less sensitive in discriminating asthmatics from non-asthmatics.[1]

However, Avital *et al*, applying an offline tidal breathing technique in children aged from 2 - 7 years, showed this technique to discriminate between asthmatic children and healthy controls as well as nonasthmatic children with chronic cough, with sensitivities and specificities up to 80%.[109] Meyts *et al*. were able to differentiate 4-year-old children with recurrent wheeze from healthy controls with similar methodology, however there was considerable overlap between the groups.[110] Baraldi *et al*. compared FE_{NO} values of 13 young children with recurrent wheeze with those of 9 healthy controls and 6 children with a first episode of wheezing.[12] Exhaled air was collected offline during tidal breathing without flow control. During acute episodes, the children with recurrent wheeze showed significantly higher FE_{NO} levels than controls, whereas FE_{NO} levels in children with a first episode of wheezing did not differ from normals. These data are in keeping with those from Ratjen *et al*, who measured peak FE_{NO} online in mixed exhaled air from mouth and nose.[106] Elphick *et al*. demonstrated lower levels of FE_{NO} in infants with cystic fibrosis compared with healthy controls, but a recent study could not confirm this finding.[111,112] Corticosteroids treatment in infants and young children with recurrent wheeze and increased FE_{NO} was found to reduce FE_{NO} to normal or near-normal values.[12,113] Also, montelukast reduced FE_{NO} values in young children with early onset asthma.[114,115]

Table 10.2

FE_{NO} (ppb)	Range	Eosinophilic airway inflammation	Interpretation	
			ICS naive	ICS treated
< 5	Low	Unlikely	Consider: PCD, CF, CLD (?)	
5 - 25	Normal	Unlikely	Consider: • wheezy bronchitis • ENT disorders • gastro-oesophageal reflux • neutrophilic asthma • Congenital abnormalities • Immunodeficiencies • Sinusitis • Vocal cord dysfunction • Anxiety/ hyperventilation	IF SYMPTOMATIC: Review diagnosis (see steroid naive). IF ASYMPTOMATIC: Implies good adherence to treatment. Reduce dose, or in case of low ICS, withdraw ICS.
25 - 35	Intermediate	Present, but mild	Consider ICS, consider viral infection	IF SYMPTOMATIC, consider: • infection • ongoing allergen exposure • poor adherence • poor inhaler technique • MDI plus spacer instead of DPI • Adding LABA or LTRA • Increasing ICS dose IF ASYMPTOMATIC: No change in ICS if patient is stable
> 35	High	Significant	Asthma is very likely, positive response to ICS is likely.	IF SYMPTOMATIC: see intermediate values. PLUS: • imminent exacerbation or relapse (FE_{NO} > 50 ppb) • steroid resistance (rare) IF ASYMPTOMATIC: No change in ICS if patient is stable

PCD	*primary ciliary dyskinesia*	DPI	*dry powder inhaler*
CF	*cystic fibrosis*	LABA	*long acting β_2-agonist*
CLD	*chronic lung disease*	LTRA	*leukotriene receptor antagonist*
MDI	*metered dose inhaler*		

These studies suggest that FE_{NO} may be useful in the differential diagnosis of respiratory symptoms in young children, and may potentially allow for better targeting of anti-inflammatory treatment in this age group.

10.2.5 Interpretation of FE_{NO} values

Based on currently available data, we developed an algorithm for interpreting FE_{NO} results in paediatric practice (*Table 10.2*). This algorithm does not purport to fit all asthmatic children, as we are fully aware that asthma is a heterogeneous disease and that there is no 'one size fits all approach'. Some considerations relevant to the interpretation of this algorithm follow below.

The algorithm is based on normal values in children, obtained with the international reference values study discussed in chapter 4.[22] In this study the geometric mean FE_{NO} (upper limit of the 95% confidence interval) ranged from 7.1 ppb (15.7) at age 4 years, to 13.7 ppb (25.2) for adolescents.

Normal FE_{NO}. A normal FE_{NO} value implies the absence of eosinophilic airway inflammation (section 1.5.3). Thus, in steroid-naive, or steroid-treated *symptomatic* children with FE_{NO} values < 15 to 25 ppb depending on age, an alternative or additional diagnosis to atopic asthma should be considered, such as ENT disorders, gastro-oesophageal reflux, neutrophilic asthma, immunodeficiencies or congenital abnormalities. In steroid-treated children who are asymptomatic we suggest to reduce ICS, or to withdraw treatment if doses are low.[74,75]

Low FE_{NO}. If FE_{NO} is below normal, cystic fibrosis and primary ciliary dyskinesia are amongst the differential diagnoses to exclude (see section 1.5.5).

Intermediate FE_{NO}. Intermediate FE_{NO} values (25 - 35 ppb) in children using ICS may reflect inadequate treatment – usually a matter of poor compliance with anti-inflammatory treatment rather than inadequate ICS dosing.[50,116,117] We feel that poor inhaler technique resulting in inadequate drug delivery is a plausible reason for elevated FE_{NO} values, which however we could not prove for asthmatic children on a median dose of 800 µg of budesonide (chapter 6).[93] Switching to metered dose inhaler plus spacer may be considered in patients using a powder inhaler. Ongoing allergen exposure or (viral) infections may account for intermediate FE_{NO} values.[73,118-125] Options are then to add a LTRA or LABA, or to increase the ICS dose. For asymptomatic children with intermediate FE_{NO} values we recommend not to alter treatment.

Elevated FE_{NO}. Elevated FE_{NO} values (> 35 ppb) in symptomatic children indicate uncontrolled eosinophilic airway inflammation. Asthma is then very likely in steroid-naive children, in particular if there is evidence of reversible airway obstruction, and we may expect them to respond positively to inhaled or oral steroids. The issues discussed above for intermediate values – such as inhalation technique, compliance, allergen exposure and infections – should also be

considered for ICS treated children showing high FE_{NO} values. Moreover, imminent exacerbation or asthma relapse, inadequate ICS dose or steroid resistance (rare in children) are possible causes as well. Based on the currently available data, we advise not to increase ICS doses for asymptomatic patients with elevated FE_{NO} values.

Limitations of the algorithm. Our algorithm is built on group mean data, and may not always be helpful in determining clinical relevant changes in individual patients. In the study by Jones *et al*, the median change in FE_{NO} occurring between stability and 'loss of control' after withdrawal of ICS treatment was 16.9 ppb, but values ranged very widely, from -10 ppb to +141 ppb.[70] The predictive accuracy of a 60% or greater change from baseline was limited. Further work is required to shed more light on this issue, particularly with regard to the changes (absolute and percentage) that might be anticipated in patients experiencing an asthma exacerbation while regular controller therapy is being continued.

Phenotypes and 'FE_{NO}'- types. Asthma being a heterogeneous disease, patients will show different clinical phenotypes each with their specific baseline values and specific 'target' FE_{NO} levels. Even when asthma is stable, FE_{NO} levels may remain high. However, the strategy of 'normalizing' FE_{NO} levels has not yet been proven to result in clinical benefit documented. Patients would perhaps be better off from individualized 'FE_{NO}-typing' and cut-off levels. Defining all components of the 'asthma syndrome' in individual children (symptoms, airway calibre, AHR and inflammation) will enable to discern several phenotypes. Phenotype-specific treatment could well be of greater benefit to an individual child than is treatment according to guidelines derived from findings in large heterogeneous groups.[126] This may be in particular true for children with severe asthma, and preschool children with wheeze.[127]

10.3 Future Research

There are several questions that deserve further scrutiny.

First, there is the issue of FE_{NO} cut-off values. In our dose titration study one single cut-off point – 30 ppb – guided treatment decisions for all patients. We recommend future research efforts to focus on the question whether individual cut-offs, based on a patient's baseline values – as a 'personal best' FE_{NO} – would perhaps yield better individual outcomes.

Then, the value of FE_{NO} measurements in the management of non-atopic asthmatic patients or in patients with neutrophilic inflammation remains to be established. We believe there is a vital role for 'FE_{NO}-typing' in the phenotyping of asthmatic patients.

Our patients form a selected group of patients with moderate to severe asthma, i.e. those treated in our hospital, a tertiary care centre. Clearly, it would be worthwhile to study the value of F_{ENO} measurements in children with milder disease or difficult-to-treat asthma as well.

Frequent monitoring of F_{ENO} at home is a promising development, as it could help prevent exacerbations of asthma by adjusting the steroid dose as soon as baseline F_{ENO} is increasing. Reversely, a dose decrease in periods of suppressed inflammation may prevent overdosing with its risk of adverse side effects.

The asymptomatic patient with persistently elevated F_{ENO} despite ICS, yet thought to show good compliance and inhaler technique, presents a dilemma.

Ongoing inflammation and remodelling may contribute to irreversible airway obstruction and AHR. It is unclear, however, if patients diagnosed with ongoing inflammation will benefit from higher doses of ICS and if treating inflammation may prevent airway remodelling.[128] Although AHR, F_{ENO}, eosinophilic inflammation and RBM thickness in asymptomatic subjects with a history of asthma all were found to improve during treatment with ICS, quality of life did not.[129] This suggests that patients will not be very motivated to take higher steroid doses.

Lastly, evidence is needed for the usefulness of F_{ENO} measurements in preschool children.

10.4 IN CONCLUSION

Asthma is a common disease in children and the work presented in this thesis has direct practical relevance to asthma management in children. The use of F_{ENO} measurements enables to administer ICS more effectively and more efficiently. F_{ENO} provides us with a practical tool to distinguish patients who will benefit from ICS from those who will not, and patients who require additional therapy from those whose medication dose could feasibly be reduced.

As an 'inflammometer', F_{ENO} provides the clinician with hitherto unavailable information regarding the nature of underlying airway inflammation, thus complementing conventional physiological testing, including the measurement of AHR.

The studies presented and discussed in this thesis pave the way towards better treatment of childhood asthma using inflammometry.

REFERENCES

1. Baraldi E, De Jongste JC. Measurement of exhaled nitric oxide in children-2001. Joint ERS/ATS Task Force on Exhaled NO Measurement in Children. Eur Respir J 2002;20:223-37.
2. ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005. Am J Respir Crit Care Med 2005;171:912-930.
3. Kharitonov S, Alving K, Barnes PJ. Exhaled and nasal nitric oxide measurements: recommendations. The European Respiratory Society Task Force. Eur Respir J 1997;10:1683-93.
4. Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide in Adults and Children-1999. Am J Respir Crit Care Med 1999;160:2104-2117.
5. Jöbsis Q, Schellekens SL, Kroesbergen A, Hop WC, de Jongste JC. Sampling of exhaled nitric oxide in children: end-expiratory plateau, balloon and tidal breathing methods compared. Eur Respir J 1999;13:1406-10.
6. Kissoon N, Duckworth L, Blake K, Murphy S, Silkoff PE. Exhaled nitric oxide measurements in childhood asthma: techniques and interpretation. Pediatr Pulmonol 1999;28:282-96.
7. Baraldi E, Scollo M, Zaramella C, Zanconato S, Zacchello F. A simple flow-driven method for online measurement of exhaled NO starting at the age of 4 to 5 years. Am J Respir Crit Care Med 2000;162:1828-32.
8. Pijnenburg MW, Lissenberg ET, Hofhuis W, Ghiro L, Hop WC, Holland WP, de Jongste JC. Exhaled nitric oxide measurements with dynamic flow restriction in children aged 4-8 yrs. Eur Respir J 2002;20:919-24.
9. Buchvald F, Bisgaard H. FE_{NO} measured at fixed exhalation flow rate during controlled tidal breathing in children from the age of 2 yr. Am J Respir Crit Care Med 2001;163:699-704.
10. Canady RG, Platts-Mills T, Murphy A, Johannesen R, Gaston B. Vital capacity reservoir and online measurement of childhood nitrosopnea are linearly related. Clinical implications. Am J Respir Crit Care Med 1999;159:311-4.
11. Jöbsis Q, Schellekens SL, Kroesbergen A, Hop WC, de Jongste JC. Offline sampling of exhaled air for nitric oxide measurement in children: methodological aspects. Eur Respir J 2001;17:898-903.
12. Baraldi E, Dario C, Ongaro R, Scollo M, Azzolin NM, Panza N, Paganini N, Zacchello F. Exhaled nitric oxide concentrations during treatment of wheezing exacerbation in infants and young children. Am J Respir Crit Care Med 1999;159:1284-8.
13. Baraldi E, Azzolin NM, Zanconato S, Dario C, Zacchello F. Corticosteroids decrease exhaled nitric oxide in children with acute asthma. J Pediatr 1997;131:381-5.
14. Wildhaber JH, Hall GL, Stick SM. Measurements of exhaled nitric oxide with the single-breath technique and positive expiratory pressure in infants. Am J Respir Crit Care Med 1999;159:74-8.
15. Baraldi E, Azzolin NM, Dario C, Carra S, Ongaro R, Biban P, Zacchello F. Effect of atmospheric nitric oxide (NO) on measurements of exhaled NO in asthmatic children. Pediatr Pulmonol 1998;26:30-4.
16. Kharitonov SA, Barnes PJ. Nasal contribution to exhaled nitric oxide during exhalation against resistance or during breath holding. Thorax 1997;52:540-4.
17. Kimberly B, Nejadnik B, Giraud GD, Holden WE. Nasal contribution to exhaled nitric oxide at rest and during breathholding in humans. Am J Respir Crit Care Med 1996;153:829-36.
18. Kroesbergen A, Jöbsis Q, Bel EH, Hop WC, de Jongste JC. Flow-dependency of exhaled nitric oxide in children with asthma and cystic fibrosis. Eur Respir J 1999;14:871-5.
19. Lundberg JO, Rinder J, Weitzberg E, Lundberg JM, Alving K. Nasally exhaled nitric oxide in humans originates mainly in the paranasal sinuses. Acta Physiol Scand 1994;152:431-2.

20. Piacentini GL, Bodini A, Vino L, Zanolla L, Costella S, Vicentini L, Boner AL. Influence of environmental concentrations of NO on the exhaled NO test. Am J Respir Crit Care Med 1998;158:1299-301.

21. Silkoff PE, McClean PA, Slutsky AS, Furlott HG, Hoffstein E, Wakita S, Chapman KR, Szalai JP, Zamel N. Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. Am J Respir Crit Care Med 1997;155:260-7.

22. Buchvald F, Baraldi E, Carraro S, Gaston B, De Jongste J, Pijnenburg MW, Silkoff PE, Bisgaard H. Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years. J Allergy Clin Immunol 2005;115:1130-6.

23. Franklin PJ, Taplin R, Stick SM. A community study of exhaled nitric oxide in healthy children. Am J Respir Crit Care Med 1999;159:69-73.

24. Kissoon N, Duckworth LJ, Blake KV, Murphy SP, Taylor CL, DeNicola LR, Silkoff PE. Exhaled nitric oxide concentrations: Online versus offline values in healthy children. Pediatr Pulmonol 2002;33:283-92.

25. Latzin P, Beck J, Griese M. Exhaled nitric oxide in healthy children: variability and a lack of correlation with atopy. Pediatr Allergy Immunol 2002;13:37-46.

26. Avital A, Uwyyed K, Berkman N, Bar-Yishay E, Godfrey S, Springer C. Exhaled nitric oxide is age-dependent in asthma. Pediatr Pulmonol 2003;36:433-8.

27. Wechsler ME, Grasemann H, Deykin A, Silverman EK, Yandava CN, Israel E, Wand M, Drazen JM. Exhaled nitric oxide in patients with asthma. Association with nos1 genotype. Am J Respir Crit Care Med 2000;162:2043-7.

28. Togashi H, Sasaki M, Frohman E, Taira E, Ratan RR, Dawson TM, Dawson VL. Neuronal (type I) nitric oxide synthase regulates nuclear factor kappaB activity and immunologic (type II) nitric oxide synthase expression. Proc Natl Acad Sci U S A 1997;94:2676-80.

29. Wong GW, Liu EK, Leung TF, Yung E, Ko FW, Hui DS, Fok TF, Lai CK. High levels and gender difference of exhaled nitric oxide in Chinese schoolchildren. Clin Exp Allergy 2005;35:889-93.

30. Yates DH, Kharitonov SA, Robbins RA, Thomas PS, Barnes PJ. The effect of alcohol ingestion on exhaled nitric oxide. Eur Respir J 1996;9:1130-3.

31. Taylor ES, Smith AD, Cowan JO, Herbison GP, Taylor DR. Effect of caffeine ingestion on exhaled nitric oxide measurements in patients with asthma. Am J Respir Crit Care Med 2004;169:1019-21.

32. Jones AW, Fransson M, Maldonado-Holmertz E. Does consumption of ethanol distort measurements of exhaled nitric oxide? Respir Med 2005;99:196-9.

33. Bruce C, Yates DH, Thomas PS. Caffeine decreases exhaled nitric oxide. Thorax 2002;57:361-3.

34. Warke TJ, Shields MD, Finnegan J. Caffeine and exhaled nitric oxide. Thorax 2003;58:281.

35. Persson MG, Cederqvist B, Wiklund CU, Gustafsson LE. Ethanol causes decrements in airway excretion of endogenous nitric oxide in humans. Eur J Pharmacol 1994;270:273-8.

36. Persson MG, Gustafsson LE. Ethanol can inhibit nitric oxide production. Eur J Pharmacol 1992;224:99-100.

37. Lim S, Jatakanon A, John M, Gilbey T, O'Connor B J, Chung KF, Barnes PJ. Effect of inhaled budesonide on lung function and airway inflammation. Assessment by various inflammatory markers in mild asthma. Am J Respir Crit Care Med 1999;159:22-30.

38. Djukanovic R, Homeyard S, Gratziou C, Madden J, Walls A, Montefort S, Peroni D, Polosa R, et al. The effect of treatment with oral corticosteroids on asthma symptoms and airway inflammation. Am J Respir Crit Care Med 1997;155:826-32.

39. Szefler SJ, Phillips BR, Martinez FD, Chinchilli VM, Lemanske RF, Strunk RC, Zeiger RS, Larsen G, et al. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. J Allergy Clin Immunol 2005;115:233-42.

40. Zeiger RS, Szefler SJ, Phillips BR, Schatz M, Martinez FD, Chinchilli VM, Lemanske RF Jr, Strunk RC, et al. Response profiles to fluticasone and montelukast in mild-to-moderate persistent childhood asthma. J Allergy Clin Immunol 2006;117:45-52.

41. Smith AD, Cowan JO, Brassett KP, Filsell S, McLachlan C, Monti-Sheehan G, Herbison GP, Taylor DR. Exhaled nitric oxide: a predictor of steroid response. Am J Respir Crit Care Med 2005;172:453-9.

42. Little SA, Chalmers GW, MacLeod KJ, McSharry C, Thomson NC. Noninvasive markers of airway inflammation as predictors of oral steroid responsiveness in asthma. Thorax 2000;55:232-4.

43. Chatkin JM, Ansarin K, Silkoff PE, McClean P, Gutierrez C, Zamel N, Chapman KR. Exhaled nitric oxide as a noninvasive assessment of chronic cough. Am J Respir Crit Care Med 1999;159:1810-3.

44. Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. Am J Respir Crit Care Med 1996;153:454-7.

45. Jatakanon A, Kharitonov S, Lim S, Barnes PJ. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. Thorax 1999;54:108-14.

46. Yates DH, Kharitonov SA, Robbins RA, Thomas PS, Barnes PJ. Effect of a nitric oxide synthase inhibitor and a glucocorticosteroid on exhaled nitric oxide. Am J Respir Crit Care Med 1995;152:892-6.

47. Silkoff PE, McClean P, Spino M, Erlich L, Slutsky AS, Zamel N. Dose-response relationship and reproducibility of the fall in exhaled nitric oxide after inhaled beclomethasone dipropionate therapy in asthma patients. Chest 2001;119:1322-8.

48. Tsai YG, Lee MY, Yang KD, Chu DM, Yuh YS, Hung CH. A single dose of nebulized budesonide decreases exhaled nitric oxide in children with acute asthma. J Pediatr 2001;139:433-7.

49. Jones SL, Herbison P, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, Taylor DR. Exhaled NO and assessment of anti-inflammatory effects of inhaled steroid: dose-response relationship. Eur Respir J 2002;20:601-8.

50. Beck-Ripp J, Griese M, Arenz S, Koring C, Pasqualoni B, Bufler P. Changes of exhaled nitric oxide during steroid treatment of childhood asthma. Eur Respir J 2002;19:1015-9.

51. Kharitonov SA, Donnelly LE, Montuschi P, Corradi M, Collins JV, Barnes PJ. Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma. Thorax 2002;57:889-96.

52. Spallarossa D, Battistini E, Silvestri M, Sabatini F, Biraghi MG, Rossi GA. Time-dependent changes in orally exhaled nitric oxide and pulmonary functions induced by inhaled corticosteroids in childhood asthma. J Asthma 2001;38:545-53.

53. Smith AD, Cowan JO, Filsell S, McLachlan C, Monti-Sheehan G, Jackson P, Taylor DR. Diagnosing asthma: comparisons between exhaled nitric oxide measurements and conventional tests. Am J Respir Crit Care Med 2004;169:473-8.

54. Brussee JE, Smit HA, Kerkhof M, Koopman LP, Wijga AH, Postma DS, Gerritsen J, Grobbee DE, et al. Exhaled nitric oxide in 4-year-old children: relationship with asthma and atopy. Eur Respir J 2005;25:455-61.

55. Prasad A, Langford B, Stradling JR, Ho LP. Exhaled nitric oxide as a screening tool for asthma in school children. Respir Med 2006;100:167-73.

56. Berkman N, Avital A, Breuer R, Bardach E, Springer C, Godfrey S. Exhaled nitric oxide in the diagnosis of asthma: comparison with bronchial provocation tests. Thorax 2005;60:383-8.

57. Dupont LJ, Demedts MG, Verleden GM. Prospective evaluation of the validity of exhaled nitric oxide for the diagnosis of asthma. Chest 2003;123:751-6.

58. Jouaville LF, Annesi-Maesano I, Nguyen LT, Bocage AS, Bedu M, Caillaud D. Interrelationships among asthma, atopy, rhinitis and exhaled nitric oxide in a population-based sample of children. Clin Exp Allergy 2003;33:1506-11.

59. Thomas PS, Gibson PG, Wang H, Shah S, Henry RL. The relationship of exhaled nitric oxide to airway inflammation and responsiveness in children. J Asthma 2005;42:291-5.

60. Malmberg LP, Pelkonen AS, Haahtela T, Turpeinen M. Exhaled nitric oxide rather than lung function distinguishes preschool children with probable asthma. Thorax 2003;58:494-9.

61. Narang I, Ersu R, Wilson NM, Bush A. Nitric oxide in chronic airway inflammation in children: diagnostic use and pathophysiological significance. Thorax 2002;57:586-9.

62. Dundas I, McKenzie S. Spirometry in the diagnosis of asthma in children. Curr Opin Pulm Med 2006;12:28-33.

63. Deykin A, Massaro AF, Drazen JM, Israel E. Exhaled nitric oxide as a diagnostic test for asthma: online versus offline techniques and effect of flow rate. Am J Respir Crit Care Med 2002;165:1597-601.

64. Henriksen AH, Lingaas-Holmen T, Sue-Chu M, Bjermer L. Combined use of exhaled nitric oxide and airway hyperresponsiveness in characterizing asthma in a large population survey. Eur Respir J 2000;15:849-55.

65. Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. Thorax 2002;57:875-9.

66. Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Non-eosinophilic corticosteroid unresponsive asthma. Lancet 1999;353:2213-4.

67. Brand PL, Roorda RJ. Usefulness of monitoring lung function in asthma. Arch Dis Child 2003;88:1021-5.

68. Kamps AW, Roorda RJ, Brand PL. Peak flow diaries in childhood asthma are unreliable. Thorax 2001;56:180-2.

69. Kamps AW, Brand PL. Education, self-management and home peak flow monitoring in childhood asthma. Paediatr Respir Rev 2001;2:165-9.

70. Jones SL, Kittelson J, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, Taylor DR. The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med 2001;164:738-43.

71. Harkins MS, Fiato KL, Iwamoto GK. Exhaled nitric oxide predicts asthma exacerbation. J Asthma 2004;41:471-6.

72. Jatakanon A, Lim S, Barnes PJ. Changes in sputum eosinophils predict loss of asthma control. Am J Respir Crit Care Med 2000;161:64-72.

73. Roberts G, Hurley C, Bush A, Lack G. Longitudinal study of grass pollen exposure, symptoms, and exhaled nitric oxide in childhood seasonal allergic asthma. Thorax 2004;59:752-6.

74. Pijnenburg MW, Hofhuis W, Hop WC, De Jongste JC. Exhaled nitric oxide predicts asthma relapse in children with clinical asthma remission. Thorax. 2005;60:215-8.

75. Zacharasiewicz A, Wilson N, Lex C, Erin EM, Li AM, Hansel T, Khan M, Bush A. Clinical use of noninvasive measurements of airway inflammation in steroid reduction in children. Am J Respir Crit Care Med 2005;171:1077-82.

76. Deykin A, Lazarus SC, Fahy JV, Wechsler ME, Boushey HA, Chinchilli VM, Craig TJ, Dimango E, et al. Sputum eosinophil counts predict asthma control after discontinuation of inhaled corticosteroids. J Allergy Clin Immunol 2005;115:720-7.

77. Leuppi JD, Salome CM, Jenkins CR, Anderson SD, Xuan W, Marks GB, Koskela H, Brannan JD, et al. Predictive Markers of Asthma Exacerbation during Stepwise Dose Reduction of Inhaled Corticosteroids. Am J Respir Crit Care Med 2001;163:406-412.

78. Wilson NM, Bridge P, Spanevello A, Silverman M. Induced sputum in children: feasibility, repeatability, and relation of findings to asthma severity. Thorax 2000;55:768-74.

79. Lex C, Payne DN, Zacharasiewicz A, Li AM, Wilson NM, Hansel TT, Bush A. Sputum induction in children with difficult asthma: safety, feasibility, and inflammatory cell pattern. Pediatr Pulmonol 2005;39:318-24.

80. Gibson PG. Use of induced sputum to examine airway inflammation in childhood asthma. J Allergy Clin Immunol 1998;102:S100-1.

81. Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. Am J Respir Crit Care Med 1999;159:1043-51.

82. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002;360:1715-21.

83. Pijnenburg MW, Bakker EM, Hop WC, De Jongste JC. Titrating Steroids on Exhaled Nitric Oxide in Asthmatic Children: a Randomized Controlled Trial. Am J Respir Crit Care Med 2005;172:831-6.

84. Smith AD, Cowan JO, Brassett KP, Herbison GP, Taylor DR. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. N Engl J Med 2005;352:2163-73.

85. Hamid Q, Springall DR, Riveros-Moreno V, Chanez P, Howarth P, Redington A, Bousquet J, Godard P, et al. Induction of nitric oxide synthase in asthma. Lancet 1993;342:1510-3.

86. Xie QW, Kashiwabara Y, Nathan C. Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase. J Biol Chem 1994;269:4705-8.

87. Guo FH, De Raeve HR, Rice TW, Stuehr DJ, Thunnissen FB, Erzurum SC. Continuous nitric oxide synthesis by inducible nitric oxide synthase in normal human airway epithelium in vivo. Proc Natl Acad Sci U S A 1995;92:7809-13.

88. Robbins RA, Springall DR, Warren JB, Kwon OJ, Buttery LD, Wilson AJ, Adcock IM, Riveros-Moreno V, et al. Inducible nitric oxide synthase is increased in murine lung epithelial cells by cytokine stimulation. Biochem Biophys Res Commun 1994;198:835-43.

89. Redington AE, Meng QH, Springall DR, Evans TJ, Creminon C, Maclouf J, Holgate ST, Howarth PH, et al. Increased expression of inducible nitric oxide synthase and cyclo-oxygenase-2 in the airway epithelium of asthmatic subjects and regulation by corticosteroid treatment. Thorax 2001;56:351-7.

90. Visser MJ, Postma DS, Arends LR, de Vries TW, Duiverman EJ, Brand PL. One-year treatment with different dosing schedules of fluticasone propionate in childhood asthma. Effects on hyperresponsiveness, lung function, and height. Am J Respir Crit Care Med 2001;164:2073-7.

91. van Essen-Zandvliet EE. Long-term intervention in childhood asthma: the Dutch study results. Dutch Chronic Nonspecific Lung Disease Study Group. Monaldi Arch Chest Dis 1995;50:201-7.

92. van Essen-Zandvliet EE, Hughes MD, Waalkens HJ, Duiverman EJ, Pocock SJ, Kerrebijn KF. Effects of 22 months of treatment with inhaled corticosteroids and/or beta-2-agonists on lung function, airway responsiveness, and symptoms in children with asthma. The Dutch Chronic Non-specific Lung Disease Study Group. Am Rev Respir Dis 1992;146:547-54.

93. Pijnenburg MW, Bakker EM, Lever S, Hop WC, De Jongste JC. High fractional concentration of nitric oxide in exhaled air despite steroid treatment in asthmatic children. Clin Exp Allergy 2005;35:920-5.

94. Powell H, Gibson PG. High dose versus low dose inhaled corticosteroid as initial starting dose for asthma in adults and children. Cochrane Database Syst Rev 2004:CD004109.

95. Xuan W, Peat JK, Toelle BG, Marks GB, Berry G, Woolcock AJ. Lung function growth and its relation to airway hyperresponsiveness and recent wheeze. Results from a longitudinal population study. Am J Respir Crit Care Med 2000;161:1820-4.

96. Rijcken B, Schouten JP, Xu X, Rosner B, Weiss ST. Airway hyperresponsiveness to histamine associated with accelerated decline in FEV1. Am J Respir Crit Care Med 1995;151:1377-82.

97. Rasmussen F, Taylor DR, Flannery EM, Cowan JO, Greene JM, Herbison GP, Sears MR. Risk factors for airway remodelling in asthma manifested by a low postbronchodilator FEV1/vital capacity ratio: a longitudinal population study from childhood to adulthood. Am J Respir Crit Care Med 2002;165:1480-8.

98. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, Cowan JO, Herbison GP, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med 2003;349:1414-22.

99. Boehmer AL, Merkus PJ. Asthma therapy for children under 5 years of age. Curr Opin Pulm Med 2006;12:34-41.

100. Haby MM, Peat JK, Marks GB, Woolcock AJ, Leeder SR. Asthma in preschool children: prevalence and risk factors. Thorax 2001;56:589-95.

101. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med 1995;332:133-8.

102. Hofhuis W, van der Wiel EC, Nieuwhof EM, Hop WC, Affourtit MJ, Smit FJ, Vaessen-Verberne AA, Versteegh FG, et al. Efficacy of fluticasone propionate on lung function and symptoms in wheezy infants. Am J Respir Crit Care Med 2005;171:328-33.

103. Teper AM, Kofman CD, Szulman GA, Vidaurreta SM, Maffey AF. Fluticasone improves pulmonary function in children under 2 years old with risk factors for asthma. Am J Respir Crit Care Med 2005;171:587-90.

104. Artlich A, Jonsson B, Bhiladvala M, Lonnqvist PA, Gustafsson LE. Single breath analysis of endogenous nitric oxide in the newborn. Biol Neonate 2001;79:21-6.

105. Jöbsis Q, Raatgeep HC, Hop WC, de Jongste JC. Controlled low flow off line sampling of exhaled nitric oxide in children. Thorax 2001;56:285-9.

106. Ratjen F, Kavuk I, Gartig S, Wiesemann HG, Grasemann H. Airway nitric oxide in infants with acute wheezy bronchitis. Pediatr Allergy Immunol 2000;11:230-5.

107. Daniel PF, Klug B, Valerius NH. Measurement of exhaled nitric oxide in young children during tidal breathing through a facemask. Pediatr Allergy Immunol 2005;16:248-53.

108. Silkoff PE, Bates CA, Meiser JB, Bratton DL. Single-breath exhaled nitric oxide in preschool children facilitated by a servo-controlled device maintaining constant flow. Pediatr Pulmonol 2004;37:554-8.

109. Avital A, Uwyyed K, Berkman N, Godfrey S, Bar-Yishay E, Springer C. Exhaled nitric oxide and asthma in young children. Pediatr Pulmonol 2001;32:308-13.

110. Meyts I, Proesmans M, Van Gerven V, Hoppenbrouwers K, De Boeck K. Tidal offline exhaled nitric oxide measurements in a pre-school population. Eur J Pediatr 2003;162:506-10.

111. Franklin PJ, Hall GL, Moeller A, Horak F, Brennan S, Stick SM. Exhaled nitric oxide is not reduced in infants with cystic fibrosis. Eur Respir J 2006;27:350-4.

112. Elphick HE, Demoncheaux EA, Ritson S, Higenbottam TW, Everard ML. Exhaled nitric oxide is reduced in infants with cystic fibrosis. Thorax 2001;56:151-152.

113. Moeller A, Franklin P, Hall GL, Turner S, Straub D, Wildhaber JH, Stick SM. Inhaled fluticasone dipropionate decreases levels of nitric oxide in recurrenty wheezy infants. Pediatr Pulmonol 2004;38:250-5.

114. Straub DA, Moeller A, Minocchieri S, Hamacher J, Sennhauser FH, Hall GL, Wildhaber JH. The effect of montelukast on lung function and exhaled nitric oxide in infants with early childhood asthma. Eur Respir J 2005;25:289-94.

115. Straub DA, Minocchieri S, Moeller A, Hamacher J, Wildhaber JH. The effect of montelukast on exhaled nitric oxide and lung function in asthmatic children 2 to 5 years old. Chest 2005;127:509-14.

116. Delgado-Corcoran C, Kissoon N, Murphy SP, Duckworth LJ. Exhaled nitric oxide reflects asthma severity and asthma control. Pediatr Crit Care Med 2004;5:48-52.

117. Pijnenburg MW, Ghiro L, Baraldi E, De Jongste JC. Exhaled nitric oxide in atopic asthmatic children on inhaled steroids correlates with self-reported compliance. Am J Respir Crit Care Med 2002;165:A796.

118. Baraldi E, Carra S, Dario C, Azzolin N, Ongaro R, Marcer G, Zacchello F. Effect of natural grass pollen exposure on exhaled nitric oxide in asthmatic children. Am J Respir Crit Care Med 1999;159:262-6.

119. de Gouw HW, Grunberg K, Schot R, Kroes AC, Dick EC, Sterk PJ. Relationship between exhaled nitric oxide and airway hyperresponsiveness following experimental rhinovirus infection in asthmatic subjects. Eur Respir J 1998;11:126-32.

120. Kharitonov SA, Yates D, Barnes PJ. Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. Eur Respir J 1995;8:295-7.

121. Gratziou C, Rovina N, Lignos M, Vogiatzis I, Roussos C. Exhaled nitric oxide in seasonal allergic rhinitis: influence of pollen season and therapy. Clin Exp Allergy 2001;31:409-16.

122. de Kluijver J, Evertse CE, Schrumpf JA, van der Veen H, Zwinderman AH, Hiemstra PS, Rabe KF, Sterk PJ. Asymptomatic worsening of airway inflammation during low-dose allergen exposure in asthma: protection by inhaled steroids. Am J Respir Crit Care Med 2002;166:294-300.

123. Baur X, Barbinova L. Latex allergen exposure increases exhaled nitric oxide in symptomatic healthcare workers. Eur Respir J 2005;25:309-16.

124. Murphy AW, Platts-Mills TA, Lobo M, Hayden F. Respiratory nitric oxide levels in experimental human influenza. Chest 1998;114:452-6.

125. Sanders SP, Proud D, Permutt S, Siekierski ES, Yachechko R, Liu MC. Role of nasal nitric oxide in the resolution of experimental rhinovirus infection. J Allergy Clin Immunol 2004;113:697-702.

126. Bush A. Phenotype specific treatment of asthma in childhood. Paediatr Respir Rev 2004;5 Suppl A:S93-101.

127. Bush A. Classification of phenotypes. Pediatr Pulmonol Suppl 2004;26:30-3.

128. Vignola AM, Gagliardo R, Siena A, Chiappara G, Bonsignore MR, Bousquet J, Bonsignore G. Airway remodeling in the pathogenesis of asthma. Curr Allergy Asthma Rep 2001;1:108-15.

129. van den Toorn LM, Prins JB, de Jongste JC, Leman K, Mulder PG, Hoogsteden HC, Overbeek SE. Benefit from anti-inflammatory treatment during clinical remission of atopic asthma. Respir Med 2005;99:779-87.

summary

Airway inflammation is a hallmark of asthma. Since the 1990s there has been considerable interest in nitric oxide (NO) in exhaled breath; NO is produced in the airways and its fractional concentration in exhaled air (F_{ENO}) is elevated in patients with steroid-naive atopic asthma. Higher F_{ENO} separates untreated asthmatics from normals with minimal overlap. In atopic asthmatic adults and children, F_{ENO} correlates with eosinophil counts in induced sputum and with eosinophil infiltration of the airway wall, and this makes F_{ENO} the first noninvasive, valid marker of asthmatic airway inflammation.

This thesis consists of two parts. The first part deals with methodological issues of measuring F_{ENO} in children. The second part of this thesis presents four studies on clinical applications of F_{ENO} measurements in asthmatic children. As asthma is an inflammatory disease, we studied if adjusting anti-inflammatory treatment to F_{ENO} and symptoms could improve childhood asthma outcome.

F_{ENO} measurements in children: methodological issues

In 1999 and 2002 the American Thoracic Society and European Respiratory Society published recommendations for standardized procedures to measure F_{ENO}. Techniques may be online or offline, make use of constant, controlled flow or uncontrolled flow and be performed during a single breath or tidal breathing. Choice of technique is usually determined by the patient's age, the setting (outside or in the lung function laboratory) and the available equipment. Still, these recommendations left several questions unanswered, some of which we addressed in four separate studies.

F_{ENO} strongly depends on exhalation flow; however, children often are unable to perform controlled flow procedures. We developed a device for offline F_{ENO} sampling, in which dynamic flow restriction keeps the exhalation flow constant at 50 ml/s with minimal cooperation of the child needed. Children exhaled into the device and exhaled air was collected in mylar balloons. First we compared F_{ENO} values obtained with this offline technique with values measured online. There was excellent correlation and good agreement between online and offline values. Then, we assessed feasibility of this approach, and obtained normal values for F_{ENO} in children aged 4 – 8 yrs. All but five out of 79 children tested were able to perform the offline procedure. Normal values were obtained in 34 healthy children: geometric mean F_{ENO} was 4.9 (SEM 1.2) parts per billion (ppb) for boys and 7.6 (1.1) ppb for girls. So, in *chapter 2*, we concluded that offline F_{ENO} measurements with dynamic flow restriction are feasible in young children and that obtained values correspond to values obtained using recommended online techniques. This opens the gate for performing F_{ENO} measurements in epidemiological studies or for monitoring of asthma at home.

In the study discussed in *chapter 2*, we collected exhaled air offline in mylar balloons at a primary school and transported them to the clinic for analysis. To examine a possible bias from storage time, humidity and temperature on NO

stability we stored exhaled air samples and calibration gases in mylar balloons at different conditions – i.e. 4, 21 and 37 °C, with or without silica gel, which reduces humidity. The results are reported in *chapter 3*. We found NO to have remained stable for 9 hours at all temperatures, without silica gel. An increase in NO was noted between 9 and 48 hours, but only if initial F_{ENO} was low, and silica gel was found to increase variability. We concluded that mylar balloons are suitable for offline collection of exhaled breath samples, provided these are analyzed within 9 hours. Storage temperature is not critical, and silica gel should not be added. Hence, it is feasible to collect F_{ENO} at remote sites and analyze the samples within 9 hours.

In *chapter 4*, we describe the results of a large international study on normal values for children age 4 to 17 years. More than 400 children participated, including 100 from two schools in Rotterdam. Geometric mean F_{ENO} in all children was 9.7 ppb, and the upper 95% confidence limit was 25.2 ppb. However, F_{ENO} increased significantly with age, and higher F_{ENO} was seen in children with self-reported rhinitis/conjunctivitis or hay fever. The success rate of the single breath online F_{ENO} measurement was age-dependent and ranged from 40% at age 4 years to almost 100% at age 10 years and older.

Spirometry and exercise have been found to reduce F_{ENO} values in adults. However, studies in children report conflicting results. In the study presented in *chapter 5*, we examined if, and to what extent spirometry or exercise could affect F_{ENO} in children. Measurements of F_{ENO} were performed before and 5, 15, 30, 45 and 60 min after spirometry or a 6-min walk test. Slightly lower F_{ENO} was found 5 and 15 min after spirometry. After exercise F_{ENO} values showed larger drops after 5 and 15 min, irrespective of baseline F_{ENO}, and values returned to baseline within 30 min. We conclude that spirometry and especially exercise affect F_{ENO} in asthmatic children and recommend that children should refrain from physical exercise for at least 30 minutes before F_{ENO} measurements and that F_{ENO} measurements can best be performed before spirometric manoeuvres.

F_{ENO} measurements in children: clinical applications

As most methodological issues have now been sorted out, F_{ENO} as a noninvasive marker of airway inflammation might be particularly useful in monitoring asthma in children. We addressed several practical issues related to F_{ENO} measurement in every day paediatric practice.

In *chapter 6*, we studied possible explanations for the elevated F_{ENO} values in almost half of our asthma patients treated with inhaled corticosteroids (ICS). As good inhaler technique is crucial to deliver drugs to the lungs, and a high percentage of children show flawed technique, we hypothesized that optimizing inhalation technique would reduce F_{ENO} in stable atopic asthmatic children who had elevated F_{ENO} while using ICS. However, we failed to substantiate this hypothesis. Several possible explanations present themselves. For one, in view of the high

median ICS doses these children used, inhalation technique may not be that crucial if not absolutely inadequate. Then, in children with persistently elevated FE_{NO} despite optimal inhalation technique, we increased ICS doses in order to decrease FE_{NO} values. Again, increasing ICS from a daily median dose of 800 to 1200 μg budesonide had no significant effect on FE_{NO} suggesting that the maximal effect on FE_{NO} was already attained at the initial dose. Other factors, such as ongoing allergen exposure or high individual baseline values, may be responsible for the persistently elevated FE_{NO} values in this group of patients.

Remission of asthma is common in children. However, it is difficult to determine the best time point at which to reduce or stop ICS. Current practice dictates discontinuation of ICS in asthmatic children who have been symptom-free for at least 6 months on a low dose of ICS. However, some of these children will have a relapse, and there is currently no objective parameter to predict the probability of asthma relapse following steroid withdrawal. In *chapter 7* we studied if FE_{NO} might be a predictor of asthma relapse in children in whom ICS are discontinued. Forty children were included and FE_{NO} was measured before and 2, 4, 12, and 24 weeks after withdrawal of steroids. Nine patients relapsed. Two and 4 weeks after withdrawal of ICS, geometric mean FE_{NO} in children who were about to relapse was higher than in those who did not relapse. FE_{NO} of 49 ppb at 4 weeks after discontinuation of steroids had the best combination of sensitivity (71%) and specificity (93%) for asthma relapse. So, FE_{NO} measured 2 and 4 weeks after discontinuation of steroids in asymptomatic asthmatic children may be an objective predictor of asthma relapse. We accordingly changed our clinical practice and now measure FE_{NO} in asthmatic children at 4 weeks after stopping ICS. Then, if FE_{NO} is above 49 ppb, we start scrupulous monitoring of these children for signs of relapse.

Anti-inflammatory treatment with ICS is the cornerstone of asthma treatment. Decisions to start ICS or to change the dose are now mainly based on symptoms reported by the child or parents. Symptoms, however, are not closely related to the presence and severity of airway inflammation. Therefore, the question we addressed in the study presented in *chapter 8*, is whether asthma treatment in children should be targeted on symptoms or on airway inflammation. The hypothesis was that titrating ICS on both FE_{NO} and symptoms compared to titrating on symptoms only, would result in lower ICS doses and improve childhood asthma management. Allergic asthmatic children using inhaled steroids were randomly allocated to one of two groups. We created a FE_{NO} group (n = 39) for which treatment decisions were made on both FE_{NO} and symptoms, and a symptom group (n = 46) managed on symptoms only. At the end of the study median steroid dose (or change from baseline) did not differ between groups. In the FE_{NO} group, airway hyperresponsiveness improved more than in the symptom group. FE_{NO} increased in the symptom group; change in FE_{NO} from baseline differed between groups. We concluded that ICS dose titration using FE_{NO} improved important objective endpoints in children with moderate to severe allergic asthma.

In our laboratory, FE_{NO} is routinely measured by chemiluminescence analyzers, which are expensive, not easily transportable, require frequent calibration and thus are not suitable for home monitoring. However, a newly developed hand-held NO-analyzer (NIOX MINO™) offers the opportunity to measure FE_{NO} at home. Home monitoring of FE_{NO} has the potential to detect inflammation at an early stage and adapt ICS treatment accordingly. In *chapter 9*, we evaluated the feasibility and variability of FE_{NO} measurements with the NIOX MINO™ in the home situation. We also investigated correlations between symptoms and FE_{NO} over the study period. FE_{NO} was measured twice daily for 2 weeks in 21 stable asthmatic children, with 93% success rate. We found a significant diurnal variation in FE_{NO} with geometric mean morning levels 14% higher than evening levels. Individual children showed marked fluctuation of FE_{NO}, with a mean intrasubject coefficient of variation of 40% for morning and 36% for evening values. FE_{NO} and cumulative symptom scores did not correlate. We conclude that home FE_{NO} measurements are feasible, and offer the possibility to assess airway inflammation on a daily basis.

FE_{NO} measurements provide useful information that may guide the treatment of asthmatic children, in addition to symptoms and lung function tests. They allow for inhaled corticosteroids to be used more rationally and more efficiently. ICS treatment can be adjusted individually, so that patients who really need ICS or a dose increase will indeed get these, and doses may be reduced for those who do not show eosinophilic inflammation. This will prevent under- or over treatment with ICS. We feel that the time has come to incorporate FE_{NO} in the routine assessment of asthmatic children and in treatment guidelines.

samenvatting

Astma is een ziekte die zich kenmerkt door chronische ontsteking van de luchtwegen. De behandeling bestaat dan ook uit ontstekingsremmende medicijnen, meestal corticosteroïden die via inhalatie worden toegediend (ICS).

Sinds het begin van de jaren '90 is er in de medische literatuur veel aandacht voor stikstofmonoxide (NO) in uitademingslucht. Dit gas wordt geproduceerd in de luchtwegen. Bij patiënten met allergisch astma die geen ICS gebruiken wordt een verhoogde concentratie van NO in de uitademingslucht (afgekort als FE_{NO}) gemeten. FE_{NO} correleert goed met eosinofielen (een bepaald type granulocyt, een ontstekingscel die een grote rol speelt bij astma) in sputum en in het slijmvlies van de luchtwegen en daarmee is FE_{NO} een maat voor eosinofiele ontsteking in de luchtwegen.

Het eerste deel van dit proefschrift gaat over de methodologie van het meten van FE_{NO} bij kinderen. Omdat chronische luchtwegontsteking centraal staat bij astma, onderzochten we of we wellicht door het meten van ontsteking met behulp van FE_{NO} de behandeling van kinderen met astma kunnen verbeteren. Onderzoek naar de klinische toepassingen van FE_{NO} bij de behandeling van kinderen met astma, wordt in het tweede deel van dit proefschrift beschreven.

FE_{NO}-metingen bij kinderen: methodologie

Er zijn verschillende methodes om FE_{NO} te meten. Bij de online-meting ademt de patiënt direct uit in de NO-analyzer; bij de offline-meting vangen we de lucht eerst op in een reservoir (bijvoorbeeld een ballonnetje), en meten daarna FE_{NO} in dit reservoir. De uitademingssnelheid kan constant zijn of variëren, en FE_{NO} kan gemeten worden tijdens een enkele uitademing of tijdens rustademhaling. Welke methode gekozen wordt is afhankelijk van o.a. de leeftijd van de patiënt, de locatie waar gemeten wordt (in of buiten het longfunctielaboratorium) en de beschikbare apparatuur. Hoewel er door de Amerikaanse en Europese beroepsverenigingen richtlijnen zijn opgesteld voor het meten van FE_{NO} bij volwassenen en kinderen, blijven er methodologische vragen over. Vier hiervan hebben we getracht te beantwoorden in dit proefschrift.

FE_{NO} is sterk afhankelijk van de snelheid waarmee uitgeademd wordt; in de richtlijnen wordt dan ook een constante snelheid van 50 ml/s geadviseerd. Jonge kinderen zijn echter vaak niet in staat de uitademingssnelheid constant te houden. Daarom hebben we een apparaatje ontwikkeld waarin een 'dynamische flow restrictor' – een soort smoorklep – het veel makkelijker maakt met een snelheid van 50 ml/s uit te ademen. Aan dit apparaatje werd een ballonnetje bevestigd waar de uitgeademde lucht in werd opgevangen. Vervolgens werd de hoeveelheid NO in het ballonnetje gemeten. Allereerst hebben we onderzocht of de FE_{NO}-waarden die met deze offline-techniek verkregen werden overeenkwamen met de waarden verkregen via de gebruikelijke online-methode. Er bleek een goede correlatie en overeenkomst te zijn tussen FE_{NO}-waarden verkregen met beide technieken.

Vervolgens hebben we het nieuwe apparaatje op een basisschool getest bij 79 kinderen van 4 tot 8 jaar. Bij bijna alle kinderen (74 van de 79) lukte het om op deze manier uitademingslucht te verzamelen. Vierendertig van deze kinderen hadden geen astma, allergie, hooikoorts of eczeem, en waren niet verkouden. Bij hen bepaalden we de normaalwaarden. Het geometrisch gemiddelde F_{ENO} bij jongens was 4.9 (SEM 1.2) parts per billion (ppb) en 7.6 (1.1) ppb bij meisjes. De conclusie van dit onderzoek, dat beschreven wordt in *hoofdstuk 2*, was dan ook dat offline F_{ENO}-metingen met dynamische flowrestrictie bij jonge kinderen goed uitvoerbaar zijn en dat de aldus verkregen waarden goed overeenkomen met online verkregen waarden. Dit maakt F_{ENO}-metingen buiten het ziekenhuis voor epidemiologische studies of voor het monitoren van astma in de thuissituatie een reële optie.

Bij het onderzoek dat in *hoofdstuk 2* besproken wordt, werd uitgeademde lucht opgevangen in ballonnetjes en vervolgens werd in het ziekenhuis de hoeveelheid NO hierin bepaald. Omdat we graag wilden weten hoelang NO stabiel blijft in deze ballonnetjes en wat de invloed is van vochtigheid en omgevingstemperatuur, bewaarden we ballonnetjes met uitgeademde lucht en met calibratiegas bij temperaturen van 4, 21 and 37 °C. Aan de helft van deze ballonnetjes werd silicagel toegevoegd om de vochtigheid te verminderen. De resultaten worden beschreven in *hoofdstuk 3*. In de ballonnetjes bleef NO zonder toevoeging van silicagel gedurende 9 uur stabiel, ongeacht de omgevingstemperatuur. Als het uitgangs F_{ENO} normaal of laag was, liep het NO in de ballonnetjes op na die 9 uur; het toevoegen van silicagel verhoogde de variabiliteit. Mits binnen 9 uur geanalyseerd en zonder toevoeging van silicagel zijn deze ballonnetjes dan ook geschikt om uitgeademde lucht in op te vangen voor latere analyse van F_{ENO}.

In *hoofdstuk 4* rapporteren we de resultaten van een grote internationale studie naar normaalwaarden voor F_{ENO} bij kinderen van 4 – 17 jaar. Aan dit onderzoek deden meer dan 400 kinderen mee; waaronder bijna 100 kinderen van twee Rotterdamse scholen. Het geometrisch gemiddeld F_{ENO} (n = 405 kinderen) was 9.7 ppb, met een bovengrens van 25.2 ppb. F_{ENO} bleek toe te nemen met de leeftijd en was hoger bij kinderen met allergische neus- of oogklachten of hooikoorts. Van de kinderen van 4 jaar was 40% in staat de 'single breath online' methode (één enkele uitademing met constante snelheid) succesvol uit te voeren; bij kinderen van 10 jaar en ouder was dit percentage bijna 100%.

Uit onderzoek bij volwassenen is bekend dat longfunctietesten en lichamelijke inspanning vóór het meten van F_{ENO} de gemeten waarden verlagen. Onderzoek hiernaar bij kinderen had nog geen eenduidige conclusies opgeleverd. In *hoofdstuk 5* wordt een onderzoek gepresenteerd naar de effecten van spirometrie (een longfunctieonderzoek) en inspanning op F_{ENO} bij kinderen. F_{ENO} werd gemeten vóór, en 5, 15, 30, 45 en 60 minuten na spirometrie of een 6-minuten looptest. Vijf en 15 minuten na spirometrie daalde F_{ENO} licht. Na inspanning was er een grotere daling merkbaar na 5 en 15 minuten, onafhankelijk van de uitgangswaarde

van FE_{NO}, en na een half uur waren de waarden weer terug op het oude niveau. Spirometrie en vooral lichamelijke inspanning hebben dus invloed op FE_{NO} bij kinderen met astma. De aanbeveling is dan ook dat kinderen zich vanaf 30 minuten voor de FE_{NO} meting niet mogen inspannen en dat FE_{NO} gemeten moet worden vóór spirometrie.

FE_{NO}-metingen bij kinderen: klinische toepassingen

Omdat bij astma chronische ontsteking centraal staat en FE_{NO} een non-invasieve maat voor luchtwegontsteking is, kan FE_{NO} van nut zijn bij het sturen van de behandeling met ICS bij kinderen met astma. In het tweede deel van dit proefschrift worden enkele klinische toepassingen van FE_{NO}-metingen bij kinderen met astma onderzocht.

Ongeveer de helft van de astmapatiënten die onze polikliniek bezoekt, heeft een verhoogd FE_{NO}. In *hoofdstuk 6* worden mogelijke oorzaken onderzocht voor verhoogde FE_{NO}-waarden bij kinderen met astma die ICS gebruiken. Veel kinderen die pufjes gebruiken doen dit niet optimaal, terwijl een juiste inhalatietechniek van het grootste belang is om voldoende medicijnen in de longen te krijgen. Onze hypothese was dan ook dat het verbeteren van de inhalatietechniek bij kinderen met stabiel allergisch astma die ICS gebruikten, maar desondanks een verhoogd FE_{NO} hadden, tot daling van FE_{NO} zou leiden.

Deze hypothese konden we echter niet bevestigen: het optimaliseren van de inhalatietechniek leidde niet tot een daling van FE_{NO}. Een mogelijke verklaring hiervoor is dat deze kinderen vrij hoge doses ICS gebruikten en dat bij deze hoge doseringen de inhalatietechniek minder essentieel is. Immers, ook als deze niet perfect is kan er nog relatief veel medicijn in de longen terecht komen. Bij de kinderen die na het optimaliseren van de inhalatietechniek nog steeds een verhoogd FE_{NO} hadden werd de dosis ICS verhoogd. Het verhogen van de mediane ICS dosis van 800 naar 1200 µg budesonide, had echter geen significant effect op FE_{NO}. Dit wijst er op dat het maximale effect op FE_{NO} al bijna bereikt was met de dosis ICS die de patiënt bij de start van het onderzoek gebruikte. Ook continue blootstelling aan allergenen, slechte therapietrouw of hoge, individuele 'basiswaarden' voor FE_{NO} zijn mogelijke verklaringen voor de verhoogde FE_{NO}-waarden bij deze patiënten.

Sommige kinderen groeien over hun astma heen. In de praktijk is het vaak moeilijk het beste tijdstip te bepalen om de ICS af te bouwen of te stoppen; meestal stoppen we als een kind bij een lage dosis minimaal 6 maanden geen klachten heeft. Een aantal van deze kinderen krijgt vervolgens toch weer astmaklachten en tot nu toe is er geen manier om te voorspellen bij welke kinderen dat het geval zal zijn. In *hoofdstuk 7* werd onderzocht of met FE_{NO} voorspeld kan worden welke kinderen weer last van hun astma krijgen na het stoppen van ICS. Aan deze studie deden 40 kinderen mee, bij wie FE_{NO} werd gemeten vóór het stoppen van de ICS, en 2, 4, 12, en 24 weken erna. Negen

kinderen kregen opnieuw astmaklachten. Bij hen bleek FE_{NO} 2 en 4 weken na stoppen van de ICS hoger te zijn dan bij de kinderen die geen klachten meer kregen. De beste combinatie van sensitiviteit (71%) en specificiteit (93%) werd gevonden voor een waarde van 49 ppb, 4 weken na stoppen van de ICS. We concluderen dat FE_{NO} 2 en 4 weken na stoppen van de ICS dus voorspelt welke kinderen weer astmaklachten krijgen. Voor de dagelijkse praktijk betekent dit dat we kinderen van wie we de medicijnen stoppen, na 4 weken terug laten komen op de polikliniek. Als het FE_{NO} dan hoger is dan 49 ppb ontslaan we de kinderen niet, maar houden ze nog enige tijd onder controle, en beginnen zonodig opnieuw met behandelen.

De beslissing om bij astma te starten met ICS, of de dosering aan te passen, wordt voornamelijk genomen op grond van symptomen. Symptomen van astma komen echter niet goed overeen met de aanwezigheid of de mate van luchtwegontsteking. De vraag is dan ook of de behandeling van astma wel gebaseerd moet zijn op symptomen en of niet beter van luchtwegontsteking kan worden uitgegaan. In *hoofdstuk 8* wordt de hypothese getest dat het aanpassen van de dosis ICS aan de hand van FE_{NO} en symptomen beide, lagere ICS-doses en een betere astmabehandeling mogelijk maakt bij kinderen. Een groep van 85 stabiele allergische astmatische kinderen die behandeld werden met ICS werd random verdeeld in twee groepen. In de FE_{NO} groep (n = 39) werden doseringsbeslissingen om de drie maanden genomen op grond van FE_{NO} en symptomen, in de symptoomgroep (n = 46) alleen op symptomen. Na een jaar was er geen verschil in de mediane ICS-dosering tussen beide groepen. Wel was in de FE_{NO} groep de luchtwegovergevoeligheid (of bronchiale hyperreactiviteit) veel minder dan in de symptoomgroep, d.w.z. de luchtwegen waren minder gevoelig geworden voor prikkels. In de symptoomgroep nam FE_{NO} toe gedurende het onderzoek, ook de verandering in FE_{NO} t.o.v. de uitgangswaarde was voor beide groepen verschillend. De conclusie van dit onderzoek was daarom dat bij kinderen met matig tot ernstig allergisch astma het betrekken van FE_{NO} in beslissingen over de dosis ICS leidt tot een belangrijke verbetering zonder dat daarvoor gemiddeld meer medicijnen nodig zijn.

FE_{NO} wordt meestal gemeten met een chemiluminescentie-analyzer. Dit is een duur apparaat dat nauwelijks te transporteren is, vaak gekalibreerd moet worden, en daarom niet in de thuissituatie gebruikt kan worden. Recent is er een nieuwe, compacte NO-analyzer (NIOX MINO™) ontwikkeld, waarmee thuis FE_{NO} gemeten kan worden. Dit biedt de mogelijkheid ontsteking in een vroeg stadium op te sporen en de behandeling met ICS daarop aan te passen. In *hoofdstuk 9* bestudeerden we of het meten van FE_{NO} met de NIOX MINO™ in de thuissituatie inderdaad haalbaar is en wat de variatie van de metingen is. Ook werd gekeken naar de correlatie tussen symptomen en FE_{NO}. Gedurende twee weken werd FE_{NO} twee keer per dag gemeten bij 21 patiënten met stabiel astma. Dit lukte goed in 93% van de gevallen. Er was variatie van FE_{NO} over de dag genomen, waarbij de ochtendwaarden gemiddeld 14% hoger waren dan de avondwaarden. Ook

individuele patiënten lieten aanmerkelijke variatie van FE_{NO} zien, met een gemiddelde binnenpersoon variatiecoëfficiënt van 40% voor de ochtendwaarden en 36% voor de avondwaarden. Er was geen correlatie tussen symptomen en FE_{NO}. Thuismetingen van FE_{NO} zijn dus haalbaar en maken het mogelijk luchtwegontsteking dagelijks te meten.

Met het meten van FE_{NO} wordt nuttige informatie verkregen over het onderliggend ontstekingsproces bij astma. Deze informatie kan naast symptomen en longfunctieonderzoek gebruikt worden om de behandeling van kinderen met astma te verbeteren. Door het meten van FE_{NO} kunnen ICS rationeel en efficiënter worden toegepast. Bij patiënten zonder eosinofiele luchtwegontsteking (laag FE_{NO}) kan de dosis ICS verlaagd worden, terwijl patiënten die ICS echt nodig hebben (hoog FE_{NO}) hun ICS ook daadwerkelijk krijgen. Onder- of overdosering kan zo voorkomen worden. Wij vinden dan ook dat de tijd rijp is om FE_{NO}-metingen op te nemen in behandelrichtlijnen voor astma bij kinderen.

dankwoord

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publications

1. Pijnenburg MW, Thomasse JE, Odink RJ, Hoekstra HJ. Plantar fibromatosis in infants. Ned Tijdschr Geneeskd 1998;142:2638-40.
2. Pijnenburg MW, Zweens MJ, Bink MT, Reitsma WC, Odink RJ. Hypertensive encephalopathy in a patient with neonatal thyrotoxicosis. Eur J Pediatr 1999;158:789-90.
3. Pijnenburg MW, Cotton MF. Monotherapy in an era of combination therapy: is there a benefit? Experience in HIV-1-infected symptomatic South African children. Ann Trop Paediatr 2000;20:185-92.
4. Pijnenburg MW, Jőbsis Q, de Jongste JC. 'Inflammometry' with nitric oxide in exhaled air: a new test for lung diseases. Ned Tijdschr Geneeskd 2001;145:946-50.
5. Pijnenburg MW, Cotton MF. Necrotising fasciitis in an HIV-1-infected infant. S Afr Med J 2001;91:500-1.
6. Trip J, van Stuijvenberg M, Dikkers FG, Pijnenburg MW. Unilateral CHARGE association. Eur J Pediatr 2002;161:78-80.
7. Pijnenburg MW, Lissenberg ET, Hofhuis W, Ghiro L, Hop WC, Holland WP, de Jongste JC. Exhaled nitric oxide measurements with dynamic flow restriction in children aged 4-8 yrs. Eur Respir J 2002;20:919-24.
8. Bodini A, Pijnenburg MW, Boner AL, de Jongste JC. Exhaled nitric oxide in mylar balloons: influence of storage time, humidity and temperature. Mediators Inflamm 2003;12:47-9.
9. Pijnenburg MW, Cransberg K, Wolff E, Bouquet J, Merkus PJ. Bronchiectasis in children after renal or liver transplantation: a report of five cases. Pediatr Transplant 2004;8:71-4.
10. Van Hest R, De Vries G, Morbano G, Pijnenburg M, Hartwig N, Baars H. Cavitating tuberculosis in an infant: case report and literature review. Pediatr Infect Dis J 2004;23:667-70.
11. Pijnenburg MW, Hofhuis W, Hop WC, De Jongste JC. Exhaled nitric oxide predicts asthma relapse in children with clinical asthma remission. Thorax 2005;60:215-8.
12. Gabriele C, Pijnenburg MW, Monti F, Hop WC, Bakker EM, de Jongste JC. The effect of spirometry and exercise on exhaled nitric oxide in asthmatic children. Pediatr Allergy Immunol 2005;16:243-7.
13. Buchvald F, Baraldi E, Carraro S, Gaston B, De Jongste J, Pijnenburg MW, Silkoff PE, Bisgaard H. Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years. J Allergy Clin Immunol 2005;115:1130-6.
14. Pijnenburg MW, Bakker EM, Hop WC, De Jongste JC. Titrating steroids on exhaled nitric oxide in children with asthma: a randomized controlled trial. Am J Respir Crit Care Med 2005;172:831-6.
15. Pijnenburg MW, Bakker EM, Lever S, Hop WC, De Jongste JC. High fractional concentration of nitric oxide in exhaled air despite steroid treatment in asthmatic children. Clin Exp Allergy 2005;35:920-5.
16. Bogaard R, Huijsmans SH, Pijnenburg MW, Tiddens HA, de Jongste JC, Merkus PJ. Tracheomalacia and bronchomalacia in children: incidence and patient characteristics. Chest 2005;128:3391-7.
17. Pijnenburg MW, Floor SE, Hop WC, De Jongste JC. Daily ambulatory exhaled nitric oxide measurements in asthma. Pediatr Allergy Immunol 2006, in press.
18. Taylor DR, Pijnenburg MW, Smith AD, De Jongste JC. Exhaled nitric oxide measurements: clinical application and interpretation. Thorax 2006, in press.

abbreviations

AHR Airway hyperresponsiveness

AMP Adenosine mono-phosphate

APC Antigen presenting cell

ARDS Acute respiratory distress syndrome

ATS American Thoracic Society

BAL Broncho-alveolar lavage

BALF Broncho-alveolar lavage fluid

BPD Bronchopulmonary dysplasia

BTS British Thoracic Society

CF Cystic fibrosis

CLD Chronic lung disease

cNOS Constitutive nitric oxide synthase

COPD Chronic obstructive pulmonary disease

DFR Dynamic flow restriction

DPI Dry powder inhaler

EBC Exhaled breath condensate

ECP Eosinophil cationic protein

EDN Eosinophil derived neurotoxin

eNOS Endothelial nitric oxide synthase

ENT Ear nose throat

EPO Eosinophil peroxidase

EPX Eosinophil protein X

ERS European Respiratory Society

F_{ENO} Fractional concentration of nitric oxide in exhaled air

FEV_1 Forced expiratory volume in 1 second

GINA Global Initiative for Asthma

GM-CSF Granulocyte macrophages colony stimulating factor

HIV Human immunodeficiency virus

ICS Inhaled corticosteroids

ICAM Intercellular adhesion molecule

IFN Interferon

IgE Immunoglobulin E

IL Interleukin

iNANC Inhibitory nonadrenergic noncholinergic

iNOS Inducible nitric oxide synthase

ISAAC International Study of Asthma and Allergies in Childhood

LABA Long acting β_2-agonist

LTRA Leukotriene receptor antagonist

MBP Major basic protein

MDI Metered dose inhaler

NF-κβ nuclear factor-kappa B

nNO Nasal nitric oxide

nNOS Neuronal nitric oxide synthase

NO Nitric oxide

NO_2^- Nitrate

NO_3^- Nitrite

NOS Nitric oxide synthase

ONOO- Peroxynitrite

PAF Platelet activating factor

PCD Primary ciliary dyskinesia

PEF Peak expiratory flow

RBM Reticular basement membrane

RNS Reactive nitrogen species

ROS Reactive oxygen species

SBOL Single breath online

TGF Transmembrane growth factor

TNF Tumor necrosis factor

VCAM Vascular cell adhesion molecule