Nitric oxide is an important regulatory mediator throughout the body. Its measurement in exhaled air (FeNO) is useful in disease monitoring in asthma, and in the diagnosis of congenital ciliary dyskinesias. Recommendations on the emerging application of nitric oxide measurement in pulmonary and cardiovascular disease are currently impossible.

**Physiological and pathophysiological significance of NO**

The vasodilator effect of the purely descriptively termed "endothelium-derived relaxing factor" (EDRF) was found experimentally in the 1980s. In the early 1990s it became clear that this factor was in fact nitric oxide (NO). Thereafter its genesis, metabolism and other functions were discovered. Endogenous NO is formed from L-arginine by the enzyme NO synthase, which exists in 3 isoforms.

NO is a gaseous molecule with an unpaired electron. It is highly lipophilic and diffuses readily. Its unpaired electron is responsible for its free radical character, extreme reactivity and biologic effects. It reacts with haem proteins and iron-sulphur centres. This leads to modulation of the activity of important intracellular enzymes. NO diffuses from vascular endothelial cells to adjacent vascular wall smooth muscle cells, where it reacts with the iron molecule of the haem complex of the soluble cytosolic guanylate cyclase, which it activates via conformational change. The activated guanylate cyclase produces cyclic guanosine-3’5’-monophosphate (cGMP), which causes muscle relaxation. NO also reacts with protein-bound iron molecules in the mitochondrial respiratory chain, and with enzymes in microorganisms responsible for synthesizing nuclear DNA, inhibiting enzyme activity with bactericidal effect. This mechanism appears important for the paranasal sinuses, in particular.

NOS isoforms also exist in the human pulmonary vasculature, in the bronchial tree and in the parenchyma. NOS is expressed in the following cell types: arterial and venous endothelial cells, epithelial cells, macrophages, mast cells, neutrophils, eosinophils, non-adrenergic-non-cholinergic nerves, fibroblasts, smooth muscle cells and platelets. The rise in FeNO which is measurable in asthma in particular is attributable to its increased production in bronchial epithelial cells. It plays an important role in the recruiting and activation of eosinophilic granulocytes, albeit the pathophysiological significance of NO has not yet been elucidated in detail (1).
Among the physiological roles of NO are the inhibition of platelet activation and aggregation, leucocyte adhesion and defence against specific microorganisms, modulation of bronchial muscle tone, and vasodilatation. NO is a weak bronchodilator, but a potent vasodilator. Excessive NO production, which occurs for example in septic shock, contributes to hypotension and disturbances in vascular reactivity. Inhaled NO prevents hypoxemia-induced pulmonary vasoconstriction. NOS inhibitors, conversely, lead to pulmonary vasoconstriction. A number of studies have addressed the subject of FeNO measurement, and are summarized here. The American Thoracic Society and the European Respiratory Society recently published an updated guideline on standardized measurement, technical requirements and reference values. (2).

**Origin of exhaled NO**

Gustafsson et al. detected NO in exhaled air in 1991. Most exhaled NO arises from NOS in the epithelial cells of the paranasal sinuses and bronchial tree. The paranasal sinuses have the highest concentrations, at 1 000 to 30 000 ppb. The NO concentration in the healthy individual’s bronchial tree is around 10 ppb using standard measuring techniques, but around 3 to 5 ppb in the alveolar area, where diffusion out of the surrounding lung tissue and especially into the blood occurs. Exhaled NO concentrations are affected by high NO concentrations in inhaled air. For this reason the subject is given NO-free air during the measurement process.

**FeNO measurement technique**

Exhaled NO is normally determined by chemiluminescence analysis, which depends on the reaction of NO with ozone. The ozone is produced in the measuring machinery from atmospheric oxygen. The resulting nitrogen dioxide emits light quanta, which are quantified as follows:

\[ 3\text{NO} + \text{O}_3 \rightarrow 3\text{NO}_2^* \]
\[ \text{NO}_2^* \rightarrow \text{NO}_2 + h\nu \]

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Chemiluminescence analysis is highly sensitive, as the light produced can be measured photometrically and is directly proportional to the concentration of NO. A number of devices are now available, some of them portable.

The NO concentration from the bronchoalveolar space is measured by means of a slow exhalation via a mouthpiece with the closed velum to prevent contamination with air from the upper respiratory tract. The velum is closed via exhalation against a pressure of around 10 cm H₂O. The current pressure is shown on a display and the patient is asked to keep it as accurately as possible within the desired region of 10 cm H₂O, if necessary by varying air flow (diagram 2). First, NO-free air is inhaled to capacity via a mouthpiece. Then the patient exhales to close to the residual volume at an even flow rate (ideally 50ml/s for routine measurement). The exhaled NO concentration rapidly plateaus after an initial rise (diagram 2). This manoeuvre is repeated twice, (as recommended by the American Thoracic Society, ATS, and the European Respiratory Society, ERS [2]). Higher or lower flow rates are useful to determine other parameters or results. This measurement can be carried out from age 4 to 5 upwards. It is important to keep the breath flow constant and steady, as the exhaled NO plateau reflects a constant value for transport from the bronchi, and is therefore not an “alveolar plateau.” The faster the exhalation, the shorter the time for which the air remains in the bronchi, and therefore the lower the concentration of exhaled NO. It is also possible to measure NO after collection into a Mylar bag (2). It should be noted that the inhalation of NO-free air is important here, too, and that storage is limited and liable to contamination by external NO entering, among other things.

FeNO does not show a diurnal rhythm, and does not vary significantly between the sexes. The normal range for adults is between 5 and 20 ppb, and for children between 4 and 10 ppb. Smokers have lower values of between 2 and 6 ppb, depending on levels of cigarette consumption.
FeNO changes in respiratory disease

In recent years, changes in FeNO in association with diseases of the respiratory tract as well as in systemic illness have been described (table). Of particular note is the marked elevation in allergic asthma, which is consistent across studies. FeNO monitoring has been shown to be more helpful than regular lung function testing in the prediction of acute exacerbations in severe asthma (3, 4). Some centers are already using this method for screening (4, 5, 6).

The value of FeNO measurement in non-allergic asthma remains uncertain and contentious, however. NO measurement has an established place in pediatric screening for primary ciliary dyskinesias, where bronchial NO is largely absent (7, 8). Numerous other diseases are associated with less marked variations in NO levels, as shown in the table. There are significant overlaps between groups, including the reference values. What becomes clear is that NO measurement is more useful for intra-individual investigation and prognosis than for inter-group comparisons.

The mild FeNO elevation found in healthy atopic individuals, who show a type I hypersensitivity reaction to environmental allergens, is noteworthy (9). Patients with allergic asthma and allergic rhinitis have significantly elevated basal FeNO levels and react to allergenic challenge with a significant rise in FeNO (10, 11) (diagram 3). Smoking leads to a reduction in FeNO, possibly due to the high NO levels in cigarette smoke. In addition, it is thought that both endogenously produced and exogenously introduced oxidants react with NO, thus reducing FeNO levels. Both allergic smokers and allergic non-smokers show a significant increase in FeNO following exposure to allergen (10).

FeNO is useful in the identification of relapse following a reduction or discontinuation of corticosteroid treatment (3, 4, 13). A multicentre randomized controlled trial of 144 children with asthma showed a significant correlation between reduction of FeNO and improvement in respiratory status both clinically and in pulmonary function tests (peak expiratory flow [PEF], forced expiratory volume in one second [FEV1]/forced vital capacity [FVC]), and response to bronchodilator (13).

A recent single blind study by Smith et al. (4) investigated the effect of FeNO monitoring in 97 adult asthmatic patients on steroid treatment. After an initial dose-finding phase, one subgroup received steroid doses as indicated by the clinical parameters specified in the GINA guideline, the other group according to current FeNO levels. Over one year’s follow-up the FeNO group showed a significant 45% reduction in steroid requirement relative to the control group, with at least equivalent symptom control in respect of individual outcomes.

Pijnenborg et al. (14, 15) investigated children older than one year with allergic asthma, with steroid dosage adjusted according to clinical symptoms, or to FeNO levels. No difference was found between the two groups in cumulative steroid dose. However, the FeNO group showed a significant reduction in bronchial hyper-reactivity and a trend towards a reduction on severe exacerbations, as well as an improvement in FEV1. FeNO was also reduced in the treatment arm at the end of follow-up, relative to the control arm. These results support the use of FeNO in following up inflammatory parameters and optimizing treatment, with the effect of reducing bronchial inflammation.

Discussion and future perspectives

FeNO in respiratory inflammation

Exhaled air can be cooled to produce an exhaled breath condensate (EBC) which contains dozens of different airway-derived components. pH measurement has been shown to be relatively reliable. The pH value is reduced in inflammation, asthma, COPD and cystic fibrosis. Other interesting parameters include H2O2, nitric and nitrous oxide, adenosine, leukotrienes, arachidonic acid metabolites and 8-isoprostane. However, these parameters remain to be validated for the most part (16).

There is considerable evidence that sputum induction via the inhalation of hypertonic saline solution is helpful, particularly in asthma. However, this is significantly more labor and time intensive than FeNO measurement. A recent study by Lemière et al. (17) demonstrated increased expression of the leukotriene receptors CysLT1 and BLT1 on neutrophils in the sputum of asthmatics exposed to inhalational isocyanate, as well as an increase in the leukotrienes LTB4 and IL-8. These findings require confirmation, in particular in other diseases.

In a multicentre prospective study by Girard et al. of 49 patients with suspected occupational asthma (18), 23 subjects were found to have a positive allergen provocation
test after a two week work phase (allergen provocation) and a two week break. The authors performed PEF measurement, and quantified the eosinophil levels in induced sputum. The sputum examination increased the specificity of the PEF monitoring by 18% (with elevated eosinophil levels by 2%).

In a large epidemiological study, Hendriksen et al. (19) studied FeNO singly and in combination with bronchial hyperreactivity (FEV₁ reduction of 20% following inhalation of ≤ 2 mg methacholine) as a potential test for asthma symptoms. FeNO (threshold level: 8 ppb; flow rate 250 ml/s) showed a sensitivity of 52% but a high specificity of 80%. The hyperreactivity testing had a sensitivity of 74% and a specificity of 75%. The combination of both tests yielded a specificity of 82% and a high negative predictive value of 92.5%.

Smith et al. (5) showed that raised FeNO levels (cut off: 20 ppb) had a significantly higher sensitivity (88%) and a higher negative predictive value than reduced FEV₁ or FEV₁/FVC values, in asthma. (sensitivity 29% and 35%; negative predictive value 71% and 73%). Only the eosinophil levels in sputum (threshold level: 3%) had a comparable diagnostic potential (sensitivity 86%; negative predictive value 92%). A high negative predictive value indicates a high diagnostic accuracy of FeNO for exclusion of asthma and is extremely helpful both in screening for asthma and for prospective studies, and can often save unnecessary investigation. According to Barreto et al. (9), FeNO correlates significantly with the type I sensitization (positive skin prick test) associated with eosinophilia, but also with respiratory symptoms, in particular wheeze.
Overall, FeNO could be said to have a comparable or higher sensitivity and specificity level than the more resource-intensive sputum analysis, lung function tests or bronchial hyperreactivity measurement.

**New developments in FeNO analysis**

FeNO is an integrative parameter. It remains unclear what role each part of the respiratory tract plays, and whether there are differences between the various pulmonary diseases. In order to understand the relationship between NO production in the lung and expiratory NO, Silkoff et al. (20) developed a conceptual model to describe the negative correlation between the respiratory flow rate and FeNO. On this basis the authors developed a mathematical model including the 3 parameters independent of flow rate – bronchial wall NO concentration, alveolar NO concentration and the NO diffusion coefficient (21). These calculations have recently been used increasingly as part of differential diagnosis (21, 22). This might be particularly useful in interstitial lung diseases.

**Nasal NO**

The measurement of nasal NO has so far had only limited use in clinical practice due to the lack of standardization. Its concentration is about 100 fold that of FeNO. Its use may be indicated in the following conditions: allergic rhinitis, the diagnosis of primary ciliary dyskinesias, cystic fibrosis and paranasal sinusitis.

**Areas for further research**

While the clinical value of FeNO measurement in the monitoring of poorly controlled asthma and in the diagnosis of primary ciliary dyskinesias is uncontroversial, more research is needed in respect of the considerable overlapping of the remaining diseases and disease constellations, as shown in the table. Attempts are currently being made to glean differential diagnostic information via the determination of bronchial NO diffusion and separation of the bronchial and alveolar fractions. However, significant methodological questions remain unresolved. The as yet sporadic practice of using NO therapeutically also needs

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**DIAGRAM 3**

FeNO levels before and 22 h after exposure to latex allergen in hospital employees most of whom showed signs of latex allergy (31 of 45 subjects). The untreated latex-exposed subjects have a higher basal FeNO level and a significantly greater increase in FeNO 22 h after exposure, compared with the treated latex sensitive group and the latex non-sensitized group.
further research in order to establish appropriate indications, dosages and its use in cardiovascular and pulmonary disease. Inhalational NO given at a concentration of 1 to 100 ppm can improve gas exchange in intrapulmonary shunt situations, especially in acute non-obstructive pulmonary failure (ALI, ARDS), alone or in combination with other interventions by improving the ventilation/perfusion ratio, and can improve survival in severe chronic obstructive pulmonary disease (COPD) in long term use (23). In addition, benefit has been found in pulmonary hypertension, bronchopulmonary dysplasia in the neonate, and sickling crises (23, 24). Recent studies have focused on synthetic vascular implants which release NO, with promising results (25).

Recommendations for practice
Recent publications justify the use of FeNO measurement in primary diagnosis and in disease monitoring of severe asthma, whether in association with severe allergic reaction or bronchial hyperreactivity, or poor patient compliance with treatment (6). FeNO monitoring allows the extent of inflammation to be measured and the steroid dose to be tailored to it. Its most significant role is in the early detection of exacerbations.

Its high sensitivity (88%) and high negative predictive value (92%) mean in practice that a fall in FeNO suggests clinical improvement with around 90% certainty. If on the other hand the FeNO levels remain high, this means that the inflammatory process has not improved, i.e. that the exacerbation is ongoing. Only in one in ten cases are both findings in error. The predictive value is higher than any other parameter, such as sputum analysis or pulmonary function tests. Another important area of application is the non-invasive screening of children for primary ciliary dyskinesias.

Its use in other pulmonary diseases, in particular COPD, is not clearly established. It should be noted that FeNO measurement is not yet standard practice, and is not yet reimbursable under German health insurance funds. The purchase price is between 20,000 and 30,000 Euros. The consumables cost around 5 Euros per case for the stationary apparatus, around 10 per case for the portable devices.

Conflict of interest statement
The authors declare that no conflict of interest exists according to the Guidelines of the International Committee of Medical Journal Editors.

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