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Utilising exhaled nitric oxide information to enhance diagnosis and therapy of respiratory disease – current evidence for clinical practice and proposals to improve the methodology

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**Abstract**

*Introduction:*  
A non-invasive tool to diagnose respiratory diseases and to follow treatment has long been looked-for. Exhaled nitric oxide (NO) is a promising marker of inflammation in asthma but nearly 25-years of research has shown that it works in only certain endotypes of asthma. The modelling of NO dynamics of the lung can give more information than a single F\textsubscript{E}NO value.

*Areas covered:*  
The estimation of the NO production in the conducting airways and in the gas exchange area has given new insight of the NO production in diseases beyond asthma. In this article, we discuss the importance of methodology for NO measurement in the exhaled breath and the indication of applying this technique to detect respiratory disorders. This narrative review is an attempt to examine and discuss the physiological basis underlying exhaled NO measurements and the clinical evidence of the usefulness of this method in asthma and various other respiratory disorders.

*Expert Commentary:*  
Estimation of the NO parameters would aid in our understanding of the NO dynamics of the lung and thereby give more knowledge how to interpret the measured F\textsubscript{E}NO value in clinical practice.
1. Introduction

Nitric oxide (NO) is a ubiquitous gaseous intra- and inter-cellular messenger whose biological role probably preceded the one of oxygen during evolution of all living species [1]. A quarter of century after the first description of measuring NO in humans [2] and with nearly 4,000 papers listed on PubMed [3], including recommendations for standardized procedures for NO measurements, with the latest 2005 [4], the question as to how, and when, to perform this measurement are still a matter of debates within the scientific community. As for all scientific topics, the methodology for NO measurement in the exhaled breath and the indication of applying this technique to detect lung inflammation are based on scientific grounds for the former and clinical evidence for the latter.

This narrative review is an attempt to examine and discuss the physiological basis underlying exhaled NO measurements and the evidence on the usefulness of this method in clinical decision-making. We also aim to provide the reader an insight into what might be the future added value of using mathematical modelling of NO dynamics in disease assessment. As this is a narrative and not a systematic review, a systematic literature search covering all aspects of NO measurements was not conducted.

2. The two-compartment model – theory and physical interpretation

In respiratory medicine, we have developed non-invasive methods to model gas-exchange, energy expenditure and even cardiac output with the use of oxygen and carbon dioxide. To model NO exchange dynamics of the respiratory system seemed therefore a possibility when the NO output was shown to be expiratory flow dependent [5, 6]. Figure 1.

From a system theory perspective, the conductive airways can be seen as a black box adding NO to the exhaled gas of the alveolar region. The NO transfer from bronchial wall to the airway lumen is driven by concentration gradient as described in the Fick’s 1st law of diffusion [7]. Conductive and respiratory parts of the airway tree form the two-compartment model (2CM), which consists of an airway compartment where all airways of the lungs are equally represented and an alveolar or acinar compartment where alveoli as well as respiratory bronchioles give the input to this theoretical model. Table 1 and Figure 2. Alveolar gas contains a low concentration of NO (C\textsubscript{A}NO) and while alveolar gas is expelled through the bronchial tree during exhalation it is conditioned with NO from bronchial mucosa (bronchial NO flux, J\textsubscript{aw}NO). J\textsubscript{aw}NO is dependent on bronchial wall NO concentration (C\textsubscript{aw}NO) and bronchial diffusing capacity of NO (D\textsubscript{aw}NO). The governing equation of the
two-compartment model then describes how $F_{E \text{NO}}$ is dependent on exhalation flow rates ($\dot{V}$) and NO parameters of these two compartments ($J_{\text{aw \text{NO}}}$, $C_A \text{NO}$, $C_{\text{aw \text{NO}}}$, $D_{\text{aw \text{NO}}}$).

The NO parameters can be estimated after measuring $F_{E \text{NO}}$ at different exhalation flows. Several different mathematical approaches to solve the equation have been introduced [8, 9]. In short, one can use the exponential governing equation of the 2CM to calculate $C_A \text{NO}$ and bronchial NO parameters ($J_{\text{aw \text{NO}}}$, $C_{\text{aw \text{NO}}}$, $D_{\text{aw \text{NO}}}$) if $F_{E \text{NO}}$ is measured with at least three exhalation flows including one very low ($\leq 20 \text{ mL/s}$), one intermediate ($\approx 100 \text{ mL/s}$), and one high flow rate ($> 300 \text{ mL/s}$) [10]. Alternatively, one can use a linear approximation of the governing equation if at least three exhalation flows $\geq 100 \text{ mL/s}$ are used. The NO parameters gained from the linear model are $C_A \text{NO}$ and $J_{\text{aw \text{NO}}}$, Figure 3 A, while the non-linear models provide all NO parameters ($C_A \text{NO}$, $J_{\text{aw \text{NO}}}$, $C_{\text{aw \text{NO}}}$ and $D_{\text{aw \text{NO}}}$), Figure 3 B. The quality of the measured data needs to be controlled and exhalation flow should fit the chosen mathematical method. For the linear model to be valid one should be careful not to use too low flow rates ($<100 \text{ mL/s}$) and sufficient linearity of the regression line between flow rate and NO output should be checked ($r>0.95$). A negative $C_A \text{NO}$ should not be interpreted as a consumption of NO from the gas phase of the alveolar region, rather as an indication of inadequacy of the models. In Figure 3 A possible errors in determining $C_A \text{NO}$ are demonstrated. There are two studies presenting values for healthy subjects, one in children [11] and one in adults [12].

The physiological meaning of this 2CM should be put into a clinical context. An inflammatory airway disease, such as atopic asthma has an increased iNOS activity in bronchial mucosa [13] increasing the mucosal NO concentrations ($C_{\text{aw \text{NO}}}$) and thereby NO output from the airway compartment. This increases both $F_{E \text{NO}50}$ and $J_{\text{aw \text{NO}}}$. Also, $D_{\text{aw \text{NO}}}$ can theoretically be increased if the NO producing area of the airway compartment expands or if there are pathophysiological changes in the properties of bronchial mucosa increasing the physical diffusivity of NO. In fact, increased $D_{\text{aw \text{NO}}}$ has been shown in allergic rhinitis and asthma [14]. In COPD both increased and decreased $D_{\text{aw \text{NO}}}$ have been found [15]. Since both $F_{E \text{NO}}$ and $J_{\text{aw \text{NO}}}$ are elevated if either $C_{\text{aw \text{NO}}}$ or $D_{\text{aw \text{NO}}}$ are increased, a more thorough understanding of the disease process is gained by calculating $C_{\text{aw \text{NO}}}$ and $D_{\text{aw \text{NO}}}$ instead of $F_{E \text{NO}}$ or $J_{\text{aw \text{NO}}}$.
\( C_{A}NO \) is determined by the balance between input and output of NO in the alveolar/acinar compartment. The input comes from NO production of the alveolar tissue (either structural or inflammatory cells), by convection of NO from conducting airways during inhalation or by axial back diffusion from conducting airways even during exhalation. The output of NO from acinar compartment is determined by the diffusion of NO from acinar gas to pulmonary capillaries or chemical consumption by e.g. reactive oxygen species. See Figure 2. Increased \( C_{A}NO \) can therefore represent many disease states like parenchymal inflammation and altered diffusivity between alveoli and pulmonary capillaries. In fact, increased \( C_{A}NO \) has been found in e.g. extrinsic allergic alveolitis [16], idiopathic pulmonary fibrosis [17] and chronic obstructive lung disease (COPD) [15, 18], where the cause of increased \( C_{A}NO \) is likely a variable combination of increased NO production in the acinar region and decreased diffusivity of acinar NO to pulmonary capillaries. Also, changes in systemic circulation [19] and hypoxemia in sleep apnoea syndrome have been associated with increased \( C_{A}NO \) [20].

3. Clinical usefulness of \( F_{E}NO \) with a special emphasis on asthma
When increased \( F_{E}NO \) was first discovered in asthma [21, 22] it raised considerable hope as a tool to diagnose and follow-up asthma. However, as many different inflammatory phenotypes of asthma have since been characterised [23], it has become evident that \( F_{E}NO \) is likely not a universal tool to measure inflammatory activity in all types of asthma but its utility may be restricted to certain inflammatory endotypes of asthma. In fact, it has been suggested that \( F_{E}NO \) increases most prominently in eosinophilic asthma with high T2 activity [24, 25, 26, 27].

3.1 Diagnosing asthma
Asthma is defined as a heterogeneous disease with variable and/or reversible airflow limitation that is “usually characterised by chronic airway inflammation” of different types [28]. As \( F_{E}NO \) is not a measure of airflow limitation and as the inflammatory component of asthma may be of such type that it does not increase NO output, \( F_{E}NO \) cannot be used as a diagnostic tool of asthma in every case. In addition, subclinical eosinophilic lower airway inflammation maybe present in subjects with allergic rhinitis without asthma-like airflow limitation, and therefore the diagnostic ability of \( F_{E}NO \) to differentiate between allergic asthma and allergic rhinitis is poor [29]. Hence, increased \( F_{E}NO \) should be considered as a surrogate marker of eosinophilic lower airway inflammation [30] rather than asthma, and whether asthma-like airway hyperresponsiveness or variable airflow limitation are present,
should be evaluated with standard lung function measures. This is supported by the recent Health Technology Assessment where the evidence on the diagnostic ability of FE\textsubscript{NO} was found to be heterogeneous and difficult to interpret based on 27 individual studies [31]. However, that review included all studies where the diagnostic ability of FE\textsubscript{NO} was tested against standard lung function criteria regardless of the inflammatory phenotype of asthma. As FE\textsubscript{NO} is best associated with eosinophilic airway inflammation in asthma (25-28), its diagnostic accuracy might be better if only eosinophilic phenotypes of asthma were considered. In fact, Sato et al. have shown that among subjects with prolonged cough FE\textsubscript{NO} over 39 ppb had sensitivity of 79% and specificity of 91% to differentiate asthma with sputum eosinophilia from other causes of prolonged cough [32].

3.2 Predicting treatment responses to ICS in asthma

Although FE\textsubscript{NO} is not a tool to detect all endotypes of asthma, it might be useful in detecting eosinophilic inflammation susceptible to treatment with ICS, a common “treatable trait” among subjects with airway diseases [33]. It has been shown in both adults and children that among steroid-naive asthmatics higher levels of FE\textsubscript{NO} are associated with better symptom alleviation and improvement of lung function during ICS treatment [34, 35, 36, 37] and the predictive value of exhaled NO in the management of asthma has been lately reviewed [38]. In asthmatics subjects on regular ICS treatment high FE\textsubscript{NO} level predicts increased risk of losing asthma control and experiencing an exacerbation [39, 40, 41], but there is conflicting data whether increasing glucocorticoid treatment in these subjects with high FE\textsubscript{NO} would improve their asthma control and prevent exacerbations [42, 43, 44, 45]. In line with this, those subjects who have low FE\textsubscript{NO} during ICS treatment are at low risk of losing asthma control or experiencing an exacerbation [39, 40, 41] and they do not benefit from augmenting glucocorticoid treatment [38, 42, 43, 44, 45]. Interesting is the finding that FE\textsubscript{NO} and blood eosinophil counts are independently associated with wheeze and asthma events [46].

3.3 Tailoring ICS treatment in asthma based on FE\textsubscript{NO}

As FE\textsubscript{NO} has the ability to predict treatment responses to ICS in asthma, it has been speculated that adjusting ICS dose based on FE\textsubscript{NO} in individual asthmatics on a regular follow-up visit might better focus ICS treatment to those subjects with active eosinophilic inflammation. This hypothesis has been tested in a number of trials in both adults and children and extensively reviewed in recent meta-analyses [31, 45, 47]. According to these reports the evidence is inconclusive although consistent with FE\textsubscript{NO} use resulting in fewer exacerbations,
with a small or zero reduction in ICS use in adults and a possible increase in ICS use in children or patients with more severe asthma. However, there was considerable heterogeneity in the designs of the studies, cut-off levels in FeNO guided management and treatment steps chosen based on FeNO. It is also important to bear in mind that the studies were not focused solely on eosinophilic asthma but included also other phenotypes where NO output may not be related to the activity of asthma. This probably weakens the usefulness of FeNO in treatment tailoring, and in the future these studies should be focused on subjects with high FeNO in the beginning.

3.4 Predicting treatment responses to monoclonal antibodies
As FeNO is increased mainly in association with eosinophilic inflammation [24, 25, 26, 27] and there are new anti-inflammatory therapies introduced in addition to corticosteroids that specifically target eosinophilic or T2-type inflammation, FeNO is likely a useful clinical tool in phenotyping subjects with asthma suitable for these treatments. In fact, high FeNO in subjects with asthma has been associated with better response to treatment with monoclonal antibodies against IgE [48]. In the early studies using anti-interleukin-13 (lebrikizumab) to treat asthma, baseline FeNO50 over 21 ppb was associated with greater improvement in FEV1 [48]. However, in the recent phase III studies lebrikizumab failed to show consistent effect on asthma exacerbations and FeNO was not tested as a predictor of treatment response [49]. Anti-IL-5-antibody mepolizumab has shown slightly higher efficacy in reducing exacerbations in subjects with baseline FeNO50 over 50 ppb than in those with FeNO50 below 50 ppb, but the difference was not significant [50]. Unfortunately this has not been tested in other studies using mepolizumab.

3.5 Using NO modelling in asthma
There are results indicating that the use of extended NO analysis may be useful in monitoring treatment of asthma [51, 52]. Treatment with inhaled corticosteroids will decrease FeNO, but more so CawNO, since DawNO is not affected by this treatment nor by oral steroids [52, 53]. As the effect of ICS on FeNO is probably mediated solely by decreasing CawNO, this might be a more sensitive measure of the anti-inflammatory effect of ICS than FeNO. In a case report it was shown that CawNO decreased into reference levels after one week of ICS with unchanged DawNO, but the level of FeNO was still in the high range due to increased DawNO [52]. DawNO is increased in atopic asthma both in adults [15, 51] and in children [54]. From
the equation in Figure 3 B it can be seen that $F_{E}NO$ is very much dependent on $C_{aw}NO$ and $D_{aw}NO$ (see also Figure 2). Therefore a reference $F_{E}NO$ value for healthy subjects may not be a realistic goal during asthma treatment, as $D_{aw}NO$ is not normalised by inhaled glucocorticoids [52, 53]. A clinical practice guideline suggests that an intermediate $F_{E}NO_{50}$ between 25 ppb and 50 ppb (20–35 ppb in children) should be interpreted cautiously [30]. To gain more information in this situation the $C_{aw}NO$ and $D_{aw}NO$ could be estimated to evaluate whether the intermediate level $F_{E}NO_{50}$ is due to inflammatory activity (increased $C_{aw}NO$ due to iNOS activity) or changes in NO diffusivity (increased $D_{aw}NO$). Increased $C_{aw}NO$ could be an indication for enhancing ICS treatment, but this has not been clinically tested. $C_{A}NO$ has been shown to be unaffected by ICS [55] and seen to be related to nocturnal asthma [56] as well as symptomatic asthma [57]. It might be that in more symptomatic asthma increased $C_{A}NO$ represents small airway inflammation, which may not be adequately treated with conventional inhalation preparations. In fact, in severe asthma oral glucocorticoids have been shown to decrease $C_{A}NO$ even when doubling the dose of ICS was ineffective [58]. Therefore, $C_{A}NO$ might become an important tool in the treatment of individual patients with severe asthma where increased doses of ICS do not improve asthma control.

4. Clinical usefulness of $F_{E}NO$ and its interpretation in other respiratory disorders

There are many association studies on $F_{E}NO$ in different respiratory diseases, but very little predictive studies and hardly any clinical trials focusing on the value of $F_{E}NO$ in clinical decision making. There is a recent review covering the evidence on $F_{E}NO$ in diseases beyond asthma [59].

4.1 Primary ciliary dyskinesia

Primary ciliary dyskinesia (PCD) is a rare, mostly autosomal recessive genetic disorder that causes defects in cilia motility. In PCD there is lower than normal nasal NO production [60], but its possible causal relation to inefficient or unsynchronized cilia motility is not known. Current ERS guideline on PCD recommends the use of nasal NO measurement in the diagnostic algorithm of PCD [61]. Due to heterogeneity of the studies the task force could not recommend a fixed cut-off value, but in different clinical studies nasal NO levels below 30 – 82 nL/min have yielded sensitivity between 90 and 100 % and specificity between 75 % and 97 %. Results on nasal NO in allergic rhinitis and acute and chronic rhinosinusitis have been variable and at the moment there is no evidence on how to use nasal NO in these diseases in clinical practice [62].
4.2 Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is a disease characterized by inflammation of the large and small airways predominantly via the neutrophilic cellular pathway with little effects on the up-regulation of NO. FE\textsubscript{NO} levels vary in patients with chronic obstructive pulmonary diseases based on smoking status [63] and it may also be affected by co-morbidities [64]. It has been proposed to use exhaled NO as a marker of the stability of COPD and related co-morbidities such as heart failure in COPD patients with pulmonary artery hypertension [65].

Inhaled glucocorticoids decrease FE\textsubscript{NO} in subjects with COPD [66] but the ability of FE\textsubscript{NO} to predict treatment responses is sparse and controversial [63, 67]. However, an optimal cut point has been proposed of 19 ppb in predicting sputum eosinophilia in exacerbations [68]. This is much lower than in asthma where 50 ppb may be used to indicate eosinophilic inflammation [30]. In the view of smoking and FE\textsubscript{NO} there is about 23% reduction in ex-smokers and 39% in current smokers [69], which needs to be taken into considerations.

With the progress of the disease there is an involvement in the alveolar region with the formation of emphysema, known to affect gas exchange and V\textsubscript{A}/Q matching [70]. COPD is usually associated with normal airway NO levels and increased alveolar levels [15, 18, 71, 72], while ex-smokers with COPD have slightly higher J\textsubscript{aw}NO levels than currently smoking COPD patients [73]. Treatment with ICS decreases J\textsubscript{aw}NO [66] without affecting C\textsubscript{A}NO [74]. Physical performance measured as 6-Minute Walk Distance (6MWD) correlates negatively with C\textsubscript{A}NO, thus the higher C\textsubscript{A}NO the less distance travelled [75].

As in atopic rhinitis and asthma the D\textsubscript{aw}NO is also elevated in a subgroup of COPD patients [15]. This increase in D\textsubscript{aw}NO in COPD could very well be a sign of tissue changes similar to asthma. Therefore it would be of interest to investigate COPD patients that have an untreated asthma at young age and if they are more likely to have an eosinophilia type of COPD at older age.

4.3 Obstructive sleep apnoea

In obstructive sleep apnoea (OSA) there is a repetitive collapse of the upper airway during sleep resulting in apnoea or hypopneas and subsequent hypoxemia [76]. Obesity is considered to be a major risk factor for OSA [77]. Obesity will also contribute to hypoxemia due to ventilation-perfusion mismatch [78]. Hypoxia increases reactive oxygen species and proinflammatory mediators [79] that can possibly increase the NO production. Therefore
exhaled nitric oxide was applied in numerous studies with divergent results, but summarized to a trend of higher $F_E\text{NO}_{50}$ in individuals with OSA than in healthy individuals [80]. More consistent results have been shown when the extended NO analysis was applied. $C_A\text{NO}$ is increased in OSA and has a positive correlation to the apnoea-hypopnoea index [20], a measure of sleep-disordered breathing. Long-term regular use of continuous positive airway pressure counteracts the hypoxia and proinflammatory mediators [79] therefore normalize $F_E\text{NO}_{50}$ [81, 82].

4.4 Interstitial lung diseases

Interstitial lung diseases (ILD) are a heterogeneous group of diseases affecting the gas-exchanging part of the lung. The intensity and type of inflammation and the extent of fibrosis vary greatly between different disease entities. $C_A\text{NO}$ is increased in subjects with idiopathic interstitial pneumonias such as idiopathic pulmonary fibrosis or non-specific interstitial pneumonia [16, 17]. $C_A\text{NO}$ in these subjects is associated with the degree of lung function impairment and also exercise intolerance. Further, $C_A\text{NO}$ in idiopathic pulmonary fibrosis has been associated with the rate of lung function decline [83] and could therefore be a marker of disease progression in addition to diseases severity.

The use of the extended NO analysis seems favourable and has been applied in patients with ILD associated to systemic sclerosis (SSc), a connective tissue disease characterized by microvascular endothelial damage and fibrosis. In SSc-ILD the estimated value of $C_A\text{NO}$ has been shown to be a marker of ILD, but not serum nitrite and nitrate [84]. Also the severity of the SSc-ILD is related to $C_A\text{NO}$ [85, 86], and increased $C_A\text{NO}$ may even precede radiological changes of ILD [87]. Hence, when ILD and pulmonary hypertension are present the $C_A\text{NO}$ values are increased. Therefore the diagnostic value of $C_A\text{NO}$ to detect interstitial lung disease has been investigated [88]. It was found that there is a relationship between the presence of ILD on lung CT scan, the DLCO impairment, and $C_A\text{NO}$ levels. $C_A\text{NO}$ levels higher than $>5.3$ ppb were associated with early impairment of gas exchange (defined as decreased DLCO).

The incidence of radiation pneumonitis after lung cancer radiation therapy has been reported to be in the range of 9-28% [89]. It has been suggested that it is possible to predict which patients, receiving radiotherapy for lung cancer, will develop radiation induced pneumonitis [90]. In the patients that developed radiation pneumonitis there was a transient elevation in $F_E\text{NO}_{50}$ at the end of radiation therapy. High baseline levels and acute changes of $F_E\text{NO}_{50}$
have also been found to be early markers of development of radiation pneumonitis [91]. In one study the CA\textsubscript{NO} was followed and increased after radiation therapy up to 4 months when it levelled out [92]. To follow CA\textsubscript{NO} would be a more accurate way to estimate parenchymal radiation injury since it reflects the distal part of the lung, Figure 2. However, extended NO analysis is still a new tool to assess ILD and CA\textsubscript{NO} cannot presently replace lung CT or pulmonary function tests, but is a promising new tool in assessing the inflammatory and fibrosing activity in the lung periphery.

4.5 Occupational exposures
A simple, non-invasive test is of urgent need in occupational medicine to determine the reaction of the defence system when healthy subjects get exposed to a variety of substances in their daily work. When the animal lung is exposed to asbestos, the production of free radical increases and causes oxidative stress and inflammation [93]. The asbestos fibres have been shown to activate both macrophages and lung inflammatory cells such as neutrophils [94]. This reaction could possibly explain the increase in F\textsubscript{E}NO\textsubscript{50} that has been observed [95] while others have not seen this increase [96, 97]. In addition, isolated alveolar macrophages from asbestos inhalation in animals have shown increases levels of iNOS mRNA [98]. This could be explained in humans with the extended NO analysis where CA\textsubscript{NO} was increased and J\textsubscript{aw}NO decreased [96] or unchanged [97] in subjects with asbestos induced parenchymal fibrosis. Silica exposure has been shown to induce NO, not detected by F\textsubscript{E}NO\textsubscript{50} but with the use of extended NO analysis. It was seen that CA\textsubscript{NO} was increased with a decrease of J\textsubscript{aw}NO [99]. The lack of increased F\textsubscript{E}NO\textsubscript{50} and J\textsubscript{aw}NO is most likely due to the increase of neutrophils that is known to have a negative correlation to exhaled NO in different lung diseases. Investigation in retired coal miners showed that F\textsubscript{E}NO\textsubscript{50} was unable to distinguish between patients with or without pneumoconiosis [100]. The presence of neutrophils has been shown in coal miners [101]. Hence, the more neutrophils the less increase in NO. It would be interesting to know if CA\textsubscript{NO} could distinguish between patients with or without pneumoconiosis due to mine working.

5. Expert commentary
There is a need to gather more evidence for the use of F\textsubscript{E}NO and NO parameters in disease state, but also to gather reference values for smokers and ex-smokers as well as reference values for the NO parameters in adults and children.
While working with this review it has become clear that in diseases where hypoxemia is intermittent or commonly present the $C_A$NO value has a tendency to increase. In lung fibrosis it is understandable that the diffusion of oxygen is limited but an endogenous up-regulation might improve the diffusion by dilation of the pulmonary capillaries. In severe or nocturnal asthma the hypoxemia might not be considered to happen but could an increased $C_A$NO be sign of that? Can both an increase in $J_{aw}$NO and $C_A$NO be favourable for the improvement in VA/Q distribution in OSA? It is reassuring that research leads to more research but we believe that NO is a biomarker here to stay but the question is what it is a biomarker for. The NO molecule has many faces and therefore possibly different roles in respiratory medicine.

6. Five-year view
It is likely that new inexpensive NO analysers can be used to monitor inflammatory diseases e.g. asthma and COPD by $F_E$NO at the primary care clinic or even at home in severer eosinophilia. Hopefully we will have more studies in asthma children to know the value of $F_E$NO or even the $C_{aw}$NO for treatment decisions. However the modelling of NO dynamics of the lung will still be limited to lung physician or respiratory physiologist due to the interpretation of the results and therapeutic intervention, as well as the cost of the analysers. Chemiluminescence will be the first choice for the estimation of the NO parameters since they are dependent on both a fast analyser and fast flow measurements. For the primary care the electrochemical cell is accurate to measure NO but needs a good flow control to give good repetitive measurements. In a five-years view this is all fulfilled.

7. Conclusion
The extended NO analysis to estimate NO parameters will continue to be a tool for the specialist lung or respiratory physiology clinic. It needs a fast responding analyser and accurate flow measurements to give correct estimations. There are many patient categories that can benefit of these estimations and especially for the alveolar NO. The development of NO analysers with other techniques than chemiluminescence is welcome mostly in the primary care. Exhaled NO is here to stay but it needs to be used in the right patient to give good guidance for diagnosis and treatment evaluation.

8. Key issues
- $F_E$NO$_{50}$ is mostly studied in asthma and currently there is some evidence to use it in detecting bronchial inflammation hence guiding ICS treatment in a large subset of –
but not all – asthmatic patients. Future studies should focus on finding the right asthma phenotype where \( F_{E\text{NO}50} \) could be used.

- \( F_{E\text{NO}50} \) might identify asthmatic patients likely to respond to new monoclonal antibodies against different mediators of T2-type inflammation.
- \( F_{E\text{NO}50} \) has not been shown to be clinically useful in other conditions so far.
- At the moment nasal NO measurement is recommended to be used in the diagnostic work-out of primary ciliary dyskinesia, but its role in other forms of sinonasal disease is not known.
- Modelling of NO dynamics of the lung has been studied much less, but it probably has more clinical applications than \( F_{E\text{NO}50} \). \( C_A\text{NO} \) could be used to assess small airway inflammation in asthma and it may also be useful in assessing different forms of interstitial lung diseases.
- \( C_{aw}\text{NO} \) and \( D_{aw}\text{NO} \) could be more precise measures of inflammatory activity and tissue remodelling, respectively, in asthma and COPD as compared to \( F_{E\text{NO}50} \).

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**Declaration of Interest**

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Figure legends

Figure 1. A schematic drawing representing flow dependence of exhaled NO in an imaginary subject with $C_A\text{NO} \ 3.5 \ \text{ppb}$, $C_{aw}\text{NO} \ 100 \ \text{ppb}$ and $D_{aw}\text{NO} \ 20 \ \text{mL/s}$. At low exhalation flows, like at 50 mL/s, the exhaled NO mostly represents the conducting airways. At high exhalation flow, the time to augment NO from the conducting airways is short and then $F_{E}\text{NO}$ mostly represents the gas exchange area.
Figure 2. The two-compartment model (2CM) consists of the conducting airways where all airways of the lungs are equally represented and a gas exchange area covering alveolar or acinar compartment where alveolar as well as respiratory bronchiole give the input to this theoretical model. The NO parameters of the model are alveolar NO concentration ($C_{A\text{NO}}$), bronchial NO flux ($J_{aw\text{NO}}$), diffusing capacity of NO from the airway wall ($D_{aw\text{NO}}$) and the airway wall NO concentration ($C_{aw\text{NO}}$). The governing equation of the two-compartment model shows how $F_E\text{NO}$ is dependent on the NO parameters and exhalation flow rate ($V$).
Figure 3. Plots of exhaled flow rates and NO output.

A. Linear model with at least 3 flows above 100 mL/s. A regression line is drawn and the slope represents $C_{ANO}$ and the intercept on the Y-axis represents $J_{awNO}$. If three flow rates are used (100, 200 and 300 mL/s) then the $r$-value is 0.99 (grey line) and the $C_{ANO}$ is 1 ppb and $J_{awNO}$ is 0.73 nL/s. When only two flow rates are used the $r$-value is 1.0, but if one of the flow rates is as low as 50 mL/s (grey circle) the slope of the line becomes steeper and the intercept lower; $C_{ANO}$ is 3 ppb and $J_{awNO}$ is 0.59 nL/s. Hence a false high $C_{ANO}$ and low $J_{awNO}$.

B. Non-linear model with three flow rates (<20, 100 and 300 mL/s. The Högman-Meriläinen Algorithm, 3rd order, creates a curve and the fitness to the curve is tested. Another quality control is the calculation of $F_{ENO50}$ from estimated NO parameters and then the comparison with the measured $F_{ENO50}$ (grey circle) can be done.

If the estimation of $C_{ANO}$ is negative then the slope of the regression line is negative, which indicates an inadequacy of either model used.

Adapted from Högman M et al., Extended NO analysis in asthma. *Journal of breath research.* 2007;1:024001 with permission. doi: 10.1088/1752-7155/1/2/024001.
Reference

Papers of special note have been annotated as:
* Of interest
** Of considerable interest

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Table 1. Terminology of exhaled NO and NO parameters from modelling NO dynamics of the lung.

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<td>$F_{\text{ENO}}$</td>
<td>fractional concentration of exhaled NO in the gas phase (ppb). Exhalation flow rate is given as a subscript in mL s$^{-1}$. A flow rate of 50 mL s$^{-1}$ is written $F_{\text{ENO}50}$.</td>
</tr>
<tr>
<td>$C_{\text{A NO}}$</td>
<td>concentration of NO in the gas phase of the alveolar or acinar region (ppb)</td>
</tr>
<tr>
<td>$C_{\text{aw NO}}$</td>
<td>tissue concentration of NO of the airway wall (ppb)</td>
</tr>
<tr>
<td>$D_{\text{aw NO}}$</td>
<td>airway compartment diffusing capacity of NO from the airway wall to the gas stream (mL s$^{-1}$)</td>
</tr>
<tr>
<td>$J_{\text{aw NO}}$</td>
<td>total flux of NO in the conducting airway compartment (nL s$^{-1}$), that takes in to account the value of $C_{\text{A NO}}$ ($J_{\text{aw NO}} = (C_{\text{aw NO}} - C_{\text{A NO}}) \times D_{\text{aw NO}}$).</td>
</tr>
</tbody>
</table>