

Editorial

Exhaled NO in asthma: still in touch or definitively out of bounds?

B. Degano

Explorations fonctionnelles – Physiologie, CHU de Besançon, and EA 3920-IFR133 Université de Franche Comté, Cedex, France

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Bruno Degano
Université de Franche Comté – EA 3920 et IFR 133 –
UFR Médecine-Pharmacie
25030 Besançon Cedex
France

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NO is a biologically active free radical synthesized by the oxidative conversion of L-arginine to L-citrulline by the enzymes NO synthases (NOS) in the presence of cofactors. NO is highly reactive, making its direct determination almost impossible in biological tissues. In the gaseous phase, however, NO is relatively stable. Thus, when NO is formed within lung parenchyma adjacent to an airway lumen, a portion escapes by diffusion into the airway gas where it can be detected in the exhaled breath. In the lungs, NO is generated from different cell types including mast cells, macrophages, endothelial cells, epithelial cells, and airway and vascular smooth muscle cells (1). Because many conditions can modify the production and clearance of NO in the respiratory tract, there is considerable inter-subject variability in exhaled breath NO concentration measurements (2). On one hand, levels of exhaled NO concentrations are increased by airway viral infections, by allergic rhinitis, and by intake of a nitrate-rich meals. On the other hand, exercise, tobacco smoking, coffee intake, and laboratory investigations such as spirometry, hyperresponsiveness tests, and sputum induction appear to reduce exhaled NO levels. In addition, a decrease in substrate bioavailability and an increased production of endogenous NOS inhibitors can decrease the amount of NO produced by the lungs. Once produced, NO bioavailability may be decreased when S-nitrosoglutathione reductase activity is increased or when NO reacts with superoxide anion (3).

In patients with asthma, high levels of NO concentration are typically found in exhaled breath (4). This increased NO concentration is attributed to induction of type-2 NOS (NOS-2) transcription in inflamed bronchial

epithelial cells and in inflammatory cells of asthmatic airways. The magnitude of NO concentration in exhaled breath is believed to increase in proportion with bronchial wall inflammation or induced-sputum eosinophilia as well as with airway hyperresponsiveness. The induction of NOS-2 transcription is decreased by corticosteroids (5), and NO in exhaled breath can be reduced following treatment with systemic or inhaled corticosteroids. However, despite extensive investigation, defining the precise role of NO in asthma has proved challenging. NO may activate soluble guanylate cyclase in airway smooth muscle cells and therefore exert a protective function by counteracting the bronchoconstriction mediated by inflammatory mediators. By contrast, NO may have damaging effect when it reacts with superoxide anion to form the highly toxic peroxynitrite anion.

The assessment of asthma control is pivotal to the evaluation of treatment response both in routine clinical practise and in randomized trials. Asthma control is defined as the extent to which the various manifestations of asthma have been reduced or removed by treatment. Evaluation of asthma control includes not only assessment of clinical features such as symptoms and lung function, but also estimation of the patient's risk of future adverse outcomes (6). Inhaled corticosteroids (ICS) are the mainstay of treatment for chronic asthma. International guidelines recommend that doses of ICS should be maintained at the minimum dose that is required to effectively control asthma symptoms to prevent adverse effects. However, the doses necessary to achieve this goal vary considerably between different patients and among individual patients over time, and there is a need for an

easy, effective, and safe method to guide dose titration (6). In this regard, there is considerable interest in the validation of noninvasive methods as an adjunct to clinical assessment of asthma control. Because of logistic and cost issues, measurement of NO concentration in exhaled breath has been considered a promising biomarker in primary care-based asthma studies.

It has been established that exhaled NO should be collected during a vital capacity manoeuvre and that a strong inverse relationship exists between the exhaled concentration of NO and the expiratory flow (7). The plateau of NO concentration measured during exhalation at the constant flow rate of 50 ml/s, referred to as FE_{NO}, has been extensively studied as a potential noninvasive index of asthma control, to titrate the dose of, or predict the response to, corticosteroids. However, results from studies employing FE_{NO} have been inconsistent. Smith et al. demonstrated that adjunct of FE_{NO} measurement permits the same level of asthma control with a significantly lower ICS doses compared to clinical assessment alone (8). Pijnenburg et al. demonstrated a greater improvement in airway responsiveness among patients followed using FE_{NO} compared to matched controls (9). By contrast, Shaw et al. reported that FE_{NO}-managed patients had similar doses of ICS compared to controls, without additional clinical benefit (10). Fritsch et al. (11) and Szeffler et al. (12) reported no difference in asthma symptoms, asthma exacerbations, and results of most pulmonary function tests between control patients and FE_{NO}-managed patients, but in both cases the FE_{NO} groups received higher doses of ICS. Several explanations have been proposed for these seemingly contradictory results. One reason may lie in the differences in study designs and in study populations. Another possible explanation relates to the choice of FE_{NO} itself to evaluate NO metabolism in the lungs. Although FE_{NO} is indicative of NO production, consumption, and release throughout the lungs, the recommended expiratory flow of 50 ml/s is low enough to cause the NO concentration to be predominately of airway origin; hence, the alveolar contribution may be effectively ignored. Under this assumption, researchers have therefore measured exhaled NO at multiple flows in asthmatic patients, allowing

determination of the airway and the alveolar contributions of exhaled NO. In doing so, Lehtimäki et al. reported increased alveolar NO concentration as a measure of inflammation related to small airway disease in asthmatic patients (13). Gelb et al. found that increased alveolar NO concentration was able to predict increased asthma exacerbations (14). These latter studies suffer, however, from several weaknesses, one of which being that the back-diffusion of airway NO that influences alveolar NO concentration was ignored.

In the current issue of *Allergy*, Mahut et al. aimed to overcome some of the limitations of previous studies (15). The authors took into account the back-diffusion of airway NO in the calculation of alveolar NO concentration. In this fashion, they studied whether alveolar NO concentration was associated with asthma control and severity, both at the time of NO measurement and during the subsequent 3 months. The authors questioned whether alveolar NO concentration could reflect subtle levels of control that can be difficult to assess clinically. They therefore deliberately chose to include patients without overt symptoms of loss of asthma control and without recent exacerbation and/or antiasthma treatment modification. They performed a multicentre longitudinal study and demonstrated that alveolar NO concentration was neither associated with asthma control (evaluated by Asthma Control Diary and Questionnaire) nor associated with asthma severity (evaluated by GINA classes) in a large sample of asthma patients (mainly receiving ICS plus long acting β_2 -agonists). The results of their study are useful in daily practice as both the question asked and the study population are those of the 'real life'. Does their negative answer mean that potential interest of alveolar NO measurement as a biomarker of asthma control is definitively ruled out? In treated patients with persistent asthma (i.e., their study population), the answer is very likely to be 'yes'. However, studies have suggested that an inflammatory component is present in distal airways of a subgroup of asthmatic patients, which may indicate a distinct phenotype, and thus, a different clinical course and therapeutic strategy. There might still be a place to study alveolar NO concentration in such a population of asthmatic patients.

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