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## Editorial

### Nitric Oxide in Asthma: What Good Does It Do?

#### Óxido nítrico en el asma ¿para qué sirve?

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Nitric oxide (NO) is a molecule with a simple atomic structure, which, until the end of the eighties, was considered merely as a by-product of gas combustion. In 1987, thanks to studies by Moncada and Ignarro into endothelial cells submitted to oxidative stimuli, NO was recognised as the equivalent to the endothelium-derived relaxing factor (EDRF), due to the similarity in the pharmacological behaviour of both compounds. The possibility of measuring NO indirectly by means of its biological derivatives or directly by chemiluminescence resulting from its reaction with ozone enabled us to find out that this mediator plays an important role in the regulation of many biological functions. Since then, around 70,000 articles are estimated to have been published relating this molecule to basic physiological functions such as vascular relaxation, platelet aggregation, neurotransmission and mitochondrial oxidation.

NO was first associated with respiratory function in 1991, after the study by Gustafsson, who showed it was possible to quantify the amount of this compound in air exhaled by human subjects. Furthermore, it was observed that concentrations were higher in patients with bronchial asthma.<sup>1</sup> These findings provided a breakthrough for an increasing number of papers and lines of investigation into the role of NO in asthma, connected with both its relationship with the pathogenesis of the disease and its use as a diagnostic or prognostic marker for the condition.<sup>2</sup>

NO is synthesized in the endothelial and epithelial cells of the airways and in some inflammatory cells of the airway mucosa such as mastocytes or macrophages. From there, it diffuses as a gas into the airways. Synthesis is produced after a synthase enzyme acts on the amino acid L-arginine forming NO, a highly unstable free radical which reacts with lipid, nitrogenated and aqueous derivatives of the nearby cell structures. Thus, the concentration of NO in exhaled air is the result of the balance between the production of NO and its capacity to react with its surrounding environment. Other factors, such as the diffusion capacity across the bronchial mucosa or the degree of alveolar ventilation and air flow ultimately determine the levels of NO measured in exhaled air (FeNO). The production of NO depends on the activation capacity of the enzymes,

the number of inflammatory cells and the processes which regulate the pH of the airways, and it is in these very areas where its relationship with the intensity and characteristics of the inflammation condition its possible use in asthma. The physiological function of NO in the airways is still to be well-defined, although it is thought to be connected with its capacity as a neurotransmitter of the non-adrenergic non-cholinergic nerves, acting as an endogenous bronchodilator.

Several studies have shown that FeNO values in asthma patients are related to other characteristics of the disease, such as nonspecific bronchial hyperreactivity, symptom intensity or the number of eosinophils in samples from the airways.<sup>3</sup> In this respect, NO has been generically defined as a biological marker of asthma inflammation. Like all the other indicators that have been proposed, it should only be used if it provides some added value to the criteria used up until now, either in the diagnosis or in the therapeutic handling of the disease.

From a diagnostic point of view, the convenience of measuring FeNO in asthma is conditioned not only by the intrinsic properties of the test, but also fundamentally by the setting in which it is applied; in other words, the sensitivity and specificity of the FeNO reference values or cut-off points in the diagnosis of asthma are only useful to us if we take into consideration the prevalence or the prior probability in the individual or population that we aim to analyse. The studies carried out with large groups in healthy non-smoking populations have shown that, once medication is standardised, it is possible to define reference values according to age and height;<sup>4,5</sup> however, with these data it was only possible to explain 11% of the variance due to other factors which influence the FeNO values. These factors, which were not always quantified, include atopy, environmental pollution or nasofacial conditions. In the reference model proposed earlier, the FeNO geometric mean in the healthy population was 16.6 ppb (CI 95: 5-47), this being somewhat higher in atopic individuals and males. Similar values have been described in other studies, such as that performed by Dupont in New Zealand.

At this time, the diagnostic yield of FeNO in asthma is still conditioned by the wide confidence intervals observed in the different papers. This cannot be attributed to the technique, which

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is now standardised, but to the variability of asthma itself. In this respect, rather than use a single cut-off point, most authors have proposed using different values, depending on whether the exclusion or diagnosis of asthma is being tested. Different values have been proposed in the literature. Based on the results in healthy populations, Taylor<sup>6</sup> proposes using a high diagnostic cut-off point of 33 ppb, although using values above 50 ppb has greater specificity.

If we take into consideration Bayesian statistics, we must always consider prior probability when using FeNO measurement as a diagnostic tool. In high probability situations, such as studies in patients hospitalized due to asthma, it is logical that some authors find a higher yield with low cut-off points (13 ppb) in which sensitivity (85-87%) and specificity (85-99%) were very high; on the other hand, in a population tested in primary care clinics,<sup>7</sup> values over 46 ppb are necessary to confirm the diagnosis of asthma (predictive positive value, 80%) while most diagnoses of exclusion are observed when the values are below 12 ppb (negative predictive value: 81%). In the end, what these studies tell us is that how the test is applied is basically a consequence of what we want to obtain from it; if we want to exclude asthma, we use low cut-off points (<12-25 ppb) and if we want to be sure of the diagnosis it is better if these are above 45 ppb. As in any diagnostic test, a previous evaluation of the pre-test probability of asthma must be made and incorporated into the algorithm. This is the only way that we can say that FeNO measurement can be used to diagnose asthma.

The next reason for the interest in FeNO measurement is to find out its value as a criterion of asthma control, either because of its ability to predict exacerbations or in the therapeutic handling of the disease itself. The properties that it must possess are sensitivity to changes in clinical conditions and the ability to do this earlier than other markers. With regard to the FeNO value as a predictor of exacerbations, few papers have been published. It is known that there is an increase in NO in the exhaled air of asthmatic patients during exacerbations. However, comparative analyses with other markers, such as the percentage of eosinophils in sputum, have shown that changes in eosinophils are more sensitive for predicting deteriorations in asthma control. In studies performed with asthma patients whose steroids are withdrawn, there is a high probability that an increase in absolute FeNO values of 10 ppb or 60% above the basal value is associated with an exacerbation (80-90%);<sup>8</sup> however, other criteria such as 4% increase in the number of eosinophils in sputum or an increase of at least one double dose of histamine showed a better predictive capacity.

In longitudinal studies of patients who suffered exacerbations, Gelb et al<sup>9</sup> observed that most (76%) occurred in patients with FeNO values above 28 ppb at the start of the study (PPV: 0.77), while only 33% of the cases suffered crises if the initial values were below this cut-off point (NPV: 0.87).

The benefits of using FeNO as a measure of asthma control compared with simple questionnaires such as the AQT is a cause of ever increasing interest since the questionnaires are very simple. FeNO values above 45 ppb have been shown to correlate with poor asthma control, but only in untreated patients or those with low doses of inhaled steroids, and not in patients taking moderate or high doses. The detailed analysis of the results shows that an increase in FeNO of less than 30% with regard to the initial value or a reduction in FeNO of no more than 40% served to ensure that no changes would be produced in asthma control.

Within this area, one of the most interesting applications is to know if changes in FeNO can be used to guide the reduction or increase in the treatment of asthma – basically, inhaled steroids – because although some studies with antileukotrienes or omalizumab exist, the results are still very limited. The effectiveness of increasing or decreasing steroid use depending on FeNO values has motivated a

recent review by the Cochrane collaboration.<sup>10</sup> The final conclusions were that no final differences were observed in the number of exacerbations, frequency of symptoms or respiratory function depending on whether the treatment was adjusted by FeNO values or by other criteria.

In the adult population, the first randomized trial published<sup>11</sup> compares two steroid increase or reduction strategies in stable asthma patients based on a conventional algorithm or the FeNO value, using a NO value of 30 ppb as a cut-off point to increase or reduce treatment. In this study, a reduction of 40% was reached in the dose of inhaled corticosteroids without compromising asthma control. Completely different results were obtained by Shaw<sup>12</sup> in a similar protocol in which the use of FeNO measurement did not produce a reduction in the number of exacerbations or a lower dose of steroids compared with conventional treatment. Using the same cut-off point for FeNO values to both increase and decrease the dose is probably the cause of the poor results. Another alternative to the studies considered above was performed by Szefer's group.<sup>13</sup> In their paper, the authors do not compare two different strategies but rather analyse if adding FeNO measurement to the usual asthma control results in an improvement in the control. Unfortunately, the results were not satisfactory and, at least in their group of 780 adolescent and young patients with asthma, the use of FeNO meant an increase in the use of steroids, without a significant improvement in symptoms or the number or exacerbations.

Recently, Malerba et al<sup>14</sup> performed a trial with 14 patients with mild to moderate asthma in an unstable clinical situation and compared conventional treatment with a strategy of treatment adjustment and disease control based on the measurement of FeNO or eosinophils in sputum. Based on this criterion, in the patients with values above 20 ppb the dose of steroids was increased, and if they were below 10 ppb the dose was reduced. After one year, the patients treated following this protocol had fewer symptoms and exacerbations than the group of patients given conventional treatment.

If I had to sum up in one line, I would say that despite the many studies performed into the measurement of NO in the exhaled air of asthmatic patients and the interest that has been created due to its simplicity and ease, there are still no definitive conclusions about the value of FeNO in either the diagnosis or control of asthma. As has been done with other tests, it is necessary to study more in depth the characteristics of the setting in which it will be measured and to define its role in all asthma phenotypes.

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