Bronchial and alveolar components of exhaled nitric oxide and their relationship

To the Editors:

Considerable confusion exists about the clinical use of exhaled nitric oxide measurement in general, and its bronchial and alveolar contributions in particular, for instance in response to treatment. An additional effect needs to be factored in when considering the degree of alveolar nitric oxide abnormality and its response to therapeutic interventions that may or may not be targeted to the lung periphery. Indeed, the alveolar nitric oxide value computed from exhaled nitric oxide measurement at multiple flows with the so-called slope-intercept method [1, 2] can overestimate the true nitric oxide produced by inflammation in the alveolar air spaces. Such overestimation arises when the bronchial nitric oxide back-diffuses into the alveolar air space and thus contaminates the alveolar nitric oxide measurement with nitric oxide that originates from the more proximal airways. Two correction formulas have been published independently [3, 4] proposing to estimate true alveolar nitric oxide by subtracting from the measured alveolar nitric oxide a bronchial nitric oxide-dependent portion corresponding to back-diffusion. However, it has also been shown that airway constriction of peripheral conductive airways may at least partly impair back-diffusion [5]. Thus, in the case of peripheral lung disease, the application of correction formulas that assume unimpaired back-diffusion can erroneously lead to overcorrection and, ultimately, to negative alveolar nitric oxide values. The problem with the real lung is that it is difficult to judge whether and to what extent back-diffusion is impaired, although independent measures of small airway constriction could be envisioned in an attempt to determine this. In the meantime, we advocate here a more pragmatic approach.

One way to inspect uncorrected alveolar nitric oxide concentration ($C_{A,NO}$) for true abnormality is by first plotting it against maximal bronchial nitric oxide production ($J'_{aw,NO}$) as in figure 1a for data retrieved from 30 publications reporting both alveolar nitric oxide and bronchial nitric oxide production in asthma patients; if available from these asthma studies, data on normal control subjects were also retrieved (table 1). Each data point in figure 1a represents the uncorrected values of $J'_{aw,NO}$ and $C_{A,NO}$ corresponding to any given group of asthma patients or normal subjects retrieved from each study. In those papers where exhaled nitric oxide fraction at 50 mL·s^{-1} ($F_{eNO,0.05}$) was reported instead of $J'_{aw,NO}$, the latter was computed using the average multiplicative factor between $J'_{aw,NO}$ and $F_{eNO,0.05}$ obtained from 15 out of the 30 papers where both were reported (mean $\pm$ SD factor 45 ± 4). From figure 1a, we can now assess each ($J'_{aw,NO}$, $C_{A,NO}$) data point with respect to a previously established ''zone of normality'' (dashed lines), which delimits combinations of $C_{A,NO}$ and $J'_{aw,NO}$ for which true alveolar nitric oxide is in fact normal, and any elevated $C_{A,NO}$ value can be attributed entirely to the increased $J'_{aw,NO}$ when full back-diffusion applies [4]. The 95% confidence interval around the

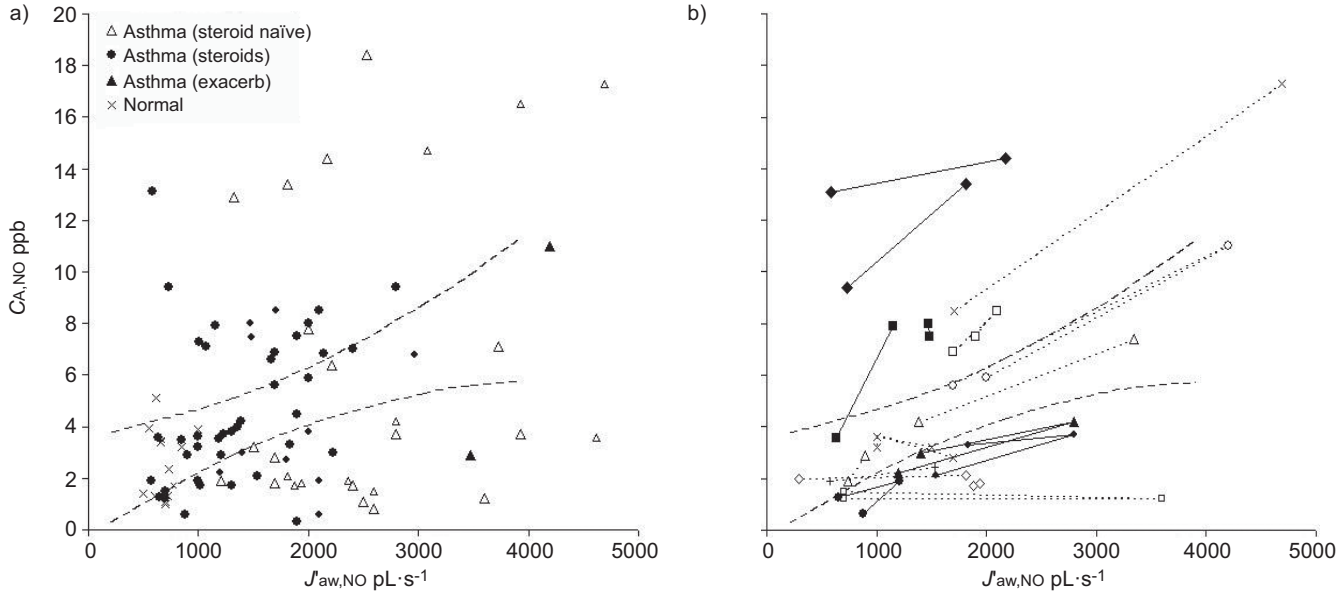

FIGURE 1. a) Uncorrected values of alveolar nitric oxide concentration ($C_{A,NO}$) plotted as a function of bronchial nitric oxide production ($J'_{aw,NO}$) obtained from all studies listed in table 1. Data points corresponding to the average value of $\leqslant$10 subjects are depicted by a slightly smaller symbol. Dashed lines, delimiting the zone of normalcy, indicate the 95% confidence interval around the regression line previously obtained from experimental data on normal subjects and stable asthma patients [4]. b) Uncorrected values of $C_{A,NO}$ plotted as a function of $J'_{aw,NO}$ obtained from interventional asthma studies see table 1 [6–19]; solid lines refer to a subset of interventional studies comparing two treatment arms see table 1 [6–10]. In this panel, different symbol types and sizes are used to identify data originating from different papers. Dashed lines: zone of normality (same representation as in (a)).

TABLE 1 Publication sources from which alveolar nitric oxide concentration and maximal bronchial nitric oxide production values are retrieved and corresponding subject groups under study

First author [ref.]	Subject groups under study
Berry [6]	Asthma/steroids *versus* normal subjects
Fritscher [7]	Asthma/steroids and steroid naïve
Kanazawa [8]	Asthma/steroids and steroid naïve
Nicolini [9]	Asthma/steroids and steroid naïve
Williamson [10]	Asthma/steroids and steroid naïve
Brindicci [11]	Asthma/steroid naïve *versus* normal subjects
Cohen [12]	Asthma/steroids and steroid naïve
Gelb [13]	Asthma/steroids and steroid naïve *versus* normal subjects
Gelb [14]	Asthma/steroids
Gelb [15]	Asthma/steroids and exacerbation *versus* normal subjects
Lehtimäki [16]	Asthma/steroids and steroid naïve *versus* normal subjects
Lehtimäki [17]	Asthma/steroids and steroid naïve *versus* normal subjects
Spears [18]	Asthma/steroids
Van Muylem [19]	Asthma/steroids and steroid naïve
Williamson [20]	Asthma/steroids
Brindicci [21]	Asthma/steroids and steroid naïve and exacerbations *versus* normal subjects
Delclaux [22]	Asthma/steroids *versus* normal subjects
Gelb [23]	Asthma/steroids and steroid naïve *versus* normal subjects
Gelb [24]	Asthma/steroids
Kerckx [25]	Asthma/steroids *versus* normal subjects
Kobayashi [26]	Asthma/steroid naïve *versus* normal subjects
Lehtimäki [27]	Asthma/steroid naïve *versus* normal subjects
Lehtimäki [28]	Asthma/steroid naïve *versus* normal subjects
Lehtimäki [29]	Asthma/steroid naïve *versus* normal subjects
Mahut [30]	Asthma/steroids
Matsumoto [31]	Asthma/steroids
Nihlberg [32]	Asthma/steroid naïve *versus* normal subjects
Van Veen [33]	Asthma/steroids
Verbanck [34]	Asthma/steroids *versus* normal subjects
Williamson [35]	Asthma/steroids *versus* normal subjects

This reference list is limited to those papers where steroid use in asthma study groups or subgroups could be clearly identified.

regression line was previously obtained from experimental data on normal subjects and stable asthma patients [4]. Importantly, the experimental regression line itself (not represented here for clarity) corresponding to an average 1.7-ppb increase in $C_{A,NO}$ for every 1,000 pL·s^{-1} increase in $J'_{aw,NO}$, was almost indistinguishable from that predicted by simulations of convective and diffusive gas transport in a lung model with normal peripheral airways [4].

It can be seen from figure 1a that, despite methodological differences and anthropometric variability in geographical regions from which the different research papers originate, almost all normal data fell within the zone of normality, and more specifically in the lower range of both $J'_{aw,NO}$ (<1,200 pL·s^{-1}) and $C_{A,NO}$ (<5 ppb). At the other end of the spectrum ($J'_{aw,NO}$ >2,000 pL·s^{-1} and outside the zone of normality), we mostly observed data points for groups of asthma patients who were either steroid-naïve (open triangles) or in exacerbation (closed triangles). Two subsets of asthma patients warrant particular attention. On one hand, the patient groups with combinations of $J'_{aw,NO}$ and $C_{A,NO}$ located above the zone of normality are patients with a true increase of nitric oxide originating in the alveolar spaces, *i.e.* an alveolar nitric oxide in excess of what could be expected on basis of their corresponding bronchial nitric oxide production, even in case of full back-diffusion. In these patients, $C_{A,NO}$ values following a full back-diffusion correction would still be abnormal. On the other hand, patient groups with combinations of $J'_{aw,NO}$ and $C_{A,NO}$ below the zone of normality corresponded to patients for whom back-diffusion is hampered by considerable airway constriction, in which case back-diffusion correction would lead to negative alveolar nitric oxide values [19].

Once abnormality of the uncorrected $C_{A,NO}$ is established based on a $J'_{aw,NO}/C_{A,NO}$ plot, the same representation can also help interpret response to treatment. Figure 1b shows data from 15 interventional studies in asthma patients [6–21] including five studies (solid symbols and lines) where two treatment arms were compared, either within the same group of asthma patients or between two comparable patient groups [6–11]. It can be seen that the majority of studies follows the ''normal'' $C_{A,NO}$ decrease with respect to $J'_{aw,NO}$ decrease, in which case the treatment effectively lowers bronchial nitric oxide production but does not really affect alveolar nitric oxide when taking into account the back-diffusion effect. Some studies follow a steeper than normal $C_{A,NO}$ decrease with $J'_{aw,NO}$ decrease, indicating a true alveolar effect, while others show a marked $J'_{aw,NO}$ decrease with no concomitant $C_{A,NO}$ decrease, usually in patients with pre-treatment data points located below the zone of normality. In fact, it has been shown recently that after steroid treatment, such patients may even paradoxically increase their uncorrected $C_{A,NO}$ value, probably because impairment to back-diffusion is lifted [19]. Surely, some interlaboratory methodological issues could influence the absolute value of what constitutes a ''normal'' slope in the relationship between $C_{A,NO}$ and $J'_{aw,NO}$. However, when comparing slopes of $C_{A,NO}$ *versus* $J'_{aw,NO}$ between different treatment arms studied in the same laboratory, a relatively steeper slope should signal a more peripheral effect. In fact, for the five comparative studies (solid lines in fig. 1b), the relatively steeper slope did correspond to the treatment arm with a intended more peripheral therapeutic effect.

In summary, we have taken the opportunity to consider some of the issues with exhaled nitric oxide measurement that have frequently frustrated researchers willing to incorporate this biomarker of inflammation in their study protocol. It would not be the first simple, noninvasive test that has been characterised by an initial outburst of enthusiasm, followed by sound scepticism or discouragement because its interpretation proves to be more complicated than the test itself, at which point it becomes at risk of being all but abandoned. With the comprehensive compilation and interpretation of published alveolar nitric oxide data in asthma to date, we have attempted to reinforce the interest in the exhaled nitric oxide test and, in particular, the components representing alveolar and bronchial nitric oxide. We propose that before readily applying a full back-diffusion correction, individual

values of $C_{A,NO}$ would be plotted *versus* their corresponding value of $J'_{aw,NO}$, and assessed with respect to what has been previously obtained (fig. 1). Besides offering the possibility to diagnose possible equipment-related biases, such an approach could identify patients below the zone of normality for whom the full back-diffusion correction should not be applied. Finally, the proposed $J'_{aw,NO}/C_{A,NO}$ data plots enable a direct comparison of different treatment interventions and identification of a more peripheral effect.

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Efficacy of nebulised liposomal amphotericin B in the attack and maintenance treatment of ABPA

To the Editors:

Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary disorder that results from a hypersensitivity reaction to *Aspergillus* spp. It has been estimated to occur in 1–3% of people with chronic asthma and 2–15% of those with cystic fibrosis [1]. The natural history of ABPA is characterised by exacerbations that can threaten the patient's survival and prognosis [1]. Repetition of such exacerbations is responsible for the development of bronchiectasis, permanent obstructive ventilation defect or fibrotic lung lesions. Prognosis mainly depends on the very early treatment of exacerbations before bronchiectasis sets in. In 2008, the guidelines of the Infectious Diseases Society of America advised combination therapy in ABPA [2]: systemic glucocorticoids to limit the inflammatory component and antifungals to limit mycelium proliferation. However, even though glucocorticoid therapy and antifungals are the treatment of choice for acute-stage ABPA and exacerbations, there are no data to guide the duration of this treatment. Therefore, the current objectives for the management of ABPA are a decrease in the frequency and duration of exacerbations, and a limited solicitation of glucocorticoids. Consequently, the maintenance treatment in the management of ABPA remains a current and progressive problem for pneumologists.

We describe a case of ABPA that was difficult to control using the standard treatment regimen, but which improved dramatically and durably following administration of nebulised liposomal amphotericin B (LAmB).

In May 2010, a 67-yr-old female presented with fever and productive cough, with sputum plugs and a history of epilepsy treated with phenobarbital. In the preceding 3 months, she had presented recurrent chest infections resistant to amoxicillin and ciprofloxacin; glucocorticoids (1 $mg \cdot kg^{-1} \cdot day^{-1}$ prednisolone for 2 weeks) had been started without any clear improvement.

At that time, ABPA was suspected. A thoracic computed tomography (CT) scan showed right upper lobe consolidation with a bronchocele and the patient's absolute eosinophil count was 1,170 $cells \cdot \mu L^{-1}$. Further investigations showed increased total immunoglobulin (Ig)E (1,154 $IU \cdot mL^{-1}$) and specific anti-*Aspergillus fumigatus* IgE levels (12.9 $kU \cdot L^{-1}$), and the presence of specific anti-*A. fumigatus* precipitins in the serum (four lines). Corticosteroids were maintained for 2 weeks (1 $mg \cdot kg^{-1} \cdot day^{-1}$), after which the dose was reduced (5-mg decrease every 2 weeks) and itraconazole was added (200 mg *b.i.d.*).

After 4 weeks of treatment, the patient's clinical status deteriorated and new consolidations appeared on the CT scan (fig. 1a and b); due to phenobarbital interaction, plasma itraconazole concentration was very low, so itraconazole treatment was withdrawn. The patient was therefore treated with the following regimen: prednisolone (0.5 $mg \cdot kg^{-1} \cdot day^{-1}$ for 2 weeks, then reduced by 5 mg every 2 weeks until discontinuation), associated with nebulisations of LAmB (25 mg twice weekly) until steroids were stopped, followed by a maintenance dose of LAmB (25 mg once weekly) to prevent subsequent ABPA exacerbations. As shown on figure 1c and d, the patient improved dramatically and durably, with a significant decrease in eosinophil count, precipitins and total and specific IgE levels over time (values after 6 months of LAmB were 370 $cells \cdot \mu L^{-1}$, two lines, 133 $IU \cdot mL^{-1}$ and 3.05 $kU \cdot L^{-1}$, respectively, and 6 months after discontinuation of LAmB were 310 $cells \cdot \mu L^{-1}$, two lines, 236 $IU \cdot mL^{-1}$ and 3.28 $kU \cdot L^{-1}$, respectively). After 2 months of this regimen, prednisolone was stopped and nebulised LAmB continued (25 mg once weekly) for 6 months as maintenance therapy. In our case, clinical and radiological improvement accompanied that of biological values, without any side-effects.

Systemic glucocorticoids are the treatment of choice for acute ABPA and exacerbations of ABPA. The Cystic Fibrosis Foundation Consensus Conference on ABPA did not propose a specific treatment plan [1]. Two small, uncontrolled clinical trials evaluated glucocorticoids in ABPA with different glucocorticoid regimens that varied in doses and durations (from 2 to 6 months) [2]. Short-term glucocorticoids reduced the number of exacerbations and improved lung function, but caused long-term side-effects (diabetes, dyslipidaemia and osteopenia) and exposed patients to the risk of severe infection (ABPA progression to invasive pulmonary aspergillosis).

Systemic antifungal treatments have been recommended in association with glucocorticoids: the objective being the attenuation or even the eradication of the intrabronchial *Aspergillus* burden, in order to decrease or stop glucocorticoid therapy. Itraconazole is the antifungal agent of choice for this indication, according to the results of two randomised controlled trials