ORIGINAL ARTICLE

Comparison of 2 Methods to Correct for Peripheral Nitric Oxide Exchange in the Lungs

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Abstract

Background and objective: Two methods have been developed to account for the impact of airway nitric oxide (NO) production on alveolar NO (CA_{NO}) concentration. In the first (Condorelli method), CA_{NO} is corrected using bronchial NO flux ($J'aw_{NO}$) values, whereas in the second (Kerckx method), knowledge of the exhaled NO concentration at a flow of 50 ml/s (FE_{NO50}) is required. The aim of the present study was to determine the influence of each correction method on CA_{NO} values.
Methods: Ninety-one adults (27 asthmatics, 46 patients with allergic rhinitis, and 18 healthy volunteers) were studied. FE_{NO50} was obtained according to a standardized method and exhaled NO was measured at multiple flows (100, 200, and 300 ml/s). CA_{NO} was adjusted for the trumpet shape of the airway tree and axial diffusion from central to peripheral airways using both the Condorelli and Kerckx methods.
Results: The mean (95% CI) CA_{NO} value obtained with the Condorelli method was 1.27 ppb (0.93-1.60), compared to 0.87 ppb (0.55-1.19, $P<.001$) with the Kerckx method. Differences in CA_{NO} values obtained with each method were identified in individuals with high FE_{NO50} values (n=55) and in those with normal FE_{NO50} values (n=36), but were significantly greater in the first group ($P=.01$).
Conclusions: Our findings suggest that CA_{NO} values obtained with the 2 methods reported to adjust for the trumpet shape of the airway tree and axial diffusion from central to peripheral airways are not equivalent and cannot be used interchangeably.

Key words: Nitric oxide. Alveolar nitric oxide. Bronchial nitric oxide. Asthma. Allergic rhinitis.

Resumen

Antecedentes y objetivo: Para cuantificar el impacto de la producción de óxido nítrico (NO) bronquial sobre el NO alveolar (CA_{NO}) se han desarrollado dos métodos. En el primero (método de Condorelli), la CA_{NO} se corrige utilizando el valor de NO bronquial (J'awNO), mientras que el segundo (método de Kerckx) requiere la cuantificación de la concentración de NO exhalado a un flujo de 50 ml/s (FE_{NO50}). El objetivo del presente estudio fue determinar la influencia del método utilizado para realizar la corrección sobre los valores de CA_{NO}.
Métodos: Se estudiaron 91 adultos (27 asmáticos, 46 pacientes con rinitis alérgica y 18 individuos sanos). Los valores de FE_{NO50} se obtuvieron mediante un método estandarizado. Además se identificaron las concentraciones de NO exhalado a flujos múltiples (100, 200 y 300 ml/s). La CA_{NO} se ajustó para tener en cuenta la morfología en trompeta del árbol bronquial y la difusión axial desde las vías aéreas centrales hacia las periféricas, utilizando tanto el método de Condorelli como el de Kerckx.
Resultados: Los valores medios (95% CI) para la CA_{NO} obtenidos mediante el método de Condorelli eran de 1.27 ppb (0.93-1.60), comparado con valores de 0.87 ppb (0.55-1.19, $P<0.001$) mediante el método de Kerckx. Estas diferencias entre los valores obtenidos con cada método se identificaban tanto en los individuos con FE_{NO50} elevado (n=55) como en los que presentaban valores de FE_{NO50} normales (n=36), pero eran significativamente mayores en el grupo con valores de FE_{NO50} altos ($P=0.01$).
Conclusiones: Nuestros hallazgos sugieren que los valores de CA_{NO} obtenidos mediante los dos métodos propuestos para ajustar el efecto trompeta del árbol bronquial y la difusión axial desde las vías centrales hacia las periféricas no son equivalentes y no pueden comparase.

Palabras clave: Óxido nítrico exhalado. Óxido nítrico alveolar. Óxido nítrico bronquial. Asma. Rinitis alérgica.

Introduction

Exhaled nitric oxide (NO) is thought to reflect a balance between production and catabolism within the respiratory tract [1] and can be easily and noninvasively measured. NO exchange in the lungs exhibits 2 characteristic features, namely the bronchial and alveolar origin of exhaled NO and the flow-dependence of exhaled NO [2]. Therefore, a method measuring exhaled NO at different flow rates has been proposed to estimate exhaled NO sources in the lung. It is based on a simple 2-compartment model of the lungs [3] that separates the lung into a rigid trumpet-shaped airway region characterized by constant NO production (or flux of NO from the airway wall surface) and an expansile small airway/alveolar region characterized by a steady-state NO concentration. Although different approaches have been used, the most common method is a series of single exhalation maneuvers in which different exhalation flows that are held constant are used [3,4]. This model is able, to a certain degree, to partition exhaled NO into an airway source ($J'aw_{NO}$) and a small airway/alveolar source (CA_{NO}). These initial 2-compartment models were, however, extremely simple because they neglected potentially important physiological phenomena [5-7]. Inappropriate use of the slope-intercept model with inclusion of exhaled NO data obtained from measurements at excessively low flow rates could theoretically lead to falsely elevated CA_{NO} values and decreased J'awNO values [8]. Furthermore, NO from the central airways can contaminate the alveolar region, leading to an overestimation of alveolar NO concentration [6,9]. Similarly, a trumpet-shaped geometry for the airways must be incorporated instead of a constant diameter tube [10].

Two methods have been developed and experimentally validated to account for the impact of airway NO production on alveolar concentration [9,10]. In the method described by Condorelli et al [10] (referred to hereafter as the Condorelli method), CA_{NO} is corrected using $J'aw_{NO}$, whereas the approach reported by Kerckx et al [9] (the Kerckx method) involves a simple correction that requires knowledge of the exhaled concentration at a flow of 50 mL/s. Both methods have been found to lead to similar estimations of CA_{NO} in individuals with asthma and in healthy volunteers [9]. While Kerckx et al used exhalation flows of 50, 175, and 300 mL/s, Condorelli et al used flow ranges more commonly employed by different research groups (100, 150, 200 and 250 mL/s). In the present study we aimed to determine the influence of the correction method on CA_{NO} values obtained using identical exhalation flow rates. Some of the results have been previously reported in the form of an abstract [11].

Table 1. Characteristics of Study Individualsa

Characteristics	Data
Age, y	42 (39-45)
Male/female, No	37/54
Ex-smokers, No.	16
Asthma, diagnosis, No.	27
Allergic rhinitis, diagnosis, No.	46
Healthy volunteers, diagnosis, No.	18
FEV_1, % predicted	103.4 (100.5-106.3)
FVC, % predicted	113.9 (111.2-116.7)
FEV_1/FVC, %	76.4 (74.8-78.0)
FE_{NO50}, ppb	43.2 (35.2-51.2)

Abbreviations: FEV_1, forced expiratory volume in the first second; FE_{NO50}, fractional exhaled nitric oxide at 50 mL/s; FVC, forced vital capacity.
[a]Data are presented as mean (95% CI) unless otherwise indicated.

Individuals and Methods

Individuals

Ninety-one individuals volunteered for this study (Table 1). Forty-six patients with allergic rhinitis and 27 patients with intermittent asthma treated with inhaled ß$_2$-agonists on demand were recruited from the allergy clinic of our institution. We also recruited 18 healthy volunteers in the laboratory and among students. Individuals aged 18 to 70 years were eligible for the study. They were all nonsmokers, and none had a history of chronic bronchitis, emphysema, or respiratory tract infections in the 4 weeks before the study. Pregnant women and individuals with significant renal, hepatic, or cardiovascular disease were excluded. Asthmatic patients abstained from short-acting bronchodilators for 6 hours. The study protocol was approved by the Dr Peset University Hospital ethics committee, and informed consent was obtained from all participants.

Table 2. Small Airway/Alveolar Nitric Oxide Concentration (CA_{NO}) in ppb Computed Using the Methods of Condorelli et al [10] and Kerckx et al [9][a]

	No.	Condorelli	Kerckx	*P* Value
All individuals	91	1.27 (0.93-1.60)	0.87 (0.55-1.19)	<.001
Group with FE_{NO50} >25 ppb	55	0.92 (0.63-1.22)	0.40 (0.18-0.61)	<.0001
Group with FE_{NO50} ≤25 ppb	36	1.79 (1.09-2.49)	1.59 (0.89-2.29)	.03
Asthma	27	1.43 (0.70-2.17)	0.85 (0.12-1.59)	.0002
Allergic rhinitis	46	1.16 (0.69-1.63)	0.82 (0.38-1.26)	.001
Healthy volunteers	18	1.28 (0.65-1.91)	1.02 (0.41-1.63)	.0003

Abbreviations: FEV_1, forced expiratory volume in the first second; FE_{NO50}, fractional exhaled nitric oxide at 50 mL/s; FVC, forced vital capacity.
[a]Values are presented as mean (95% CI).

Lung Function

Lung function (flow-volume curves) was measured using a calibrated pneumotachograph (Jaeger MasterScope, Erich Jaeger GmbH) according to standardized guidelines [12]. Reference values were those of the European Community for Coal and Steel [13].

Nitric Oxide Measurements

Exhaled NO measurements were performed using a chemiluminescence analyzer (NiOx, Aerocrine). In this study exhaled NO at 50 mL/s was referred to as FE_{NO50} and was obtained according to a standardized method [14], as described elsewhere [15,16]. Exhaled NO was also measured at expiratory flow rates of 100, 200, and 300 mL/s. Dynamic flow resistors (Aerocrine) were used to enable the individuals to achieve the target flow rate. The technique of Tsoukias and George [3] was used to calculate $J'aw_{NO}$ in pl/s (intercept with the y-axis) and CA_{NO} in ppb (slope) by applying linear least squares to a plot of the output of NO (exhaled NO values times the expired flow) vs flow.

CA_{NO} was adjusted for the trumpet shape of the airway tree (increasing cross-sectional area with distance to the lungs) and axial diffusion from the central to the peripheral airways (CA_{NOcor}) using the Condorelli method, ie, CA_{NOcor} (ppb) = CA_{NO} (ppb) – $J'aw_{NO}$ (pl/s)/860, and the Kerckx method, ie, $CA_{NOcor} = (CA_{NO}$ – 0.08 x $FE_{NO50})/0.92$, where CA_{NO} and J'awNO are small airway/alveolar and large airway NO concentrations, respectively, computed by the method of Tsoukias and George [3], and FE_{NO50} is fractional exhaled nitric oxide at 50 mL/s.

Statistical Analysis

All numerical variables are reported as arithmetic means with 95% CIs. Negative CA_{NO} values were recorded using the Condorelli method in 16 individuals and the Kerckx method in 15. These values were replaced with 0. The Kolmogorov-Smirnov test was used to evaluate normality of distribution and a *P* value of greater than .05 was obtained. The difference in CA_{NOcor} between the 2 methods was assessed using the *t* test. *P* values are 2-sided, and values of less than .05 were considered statistically significant. In addition, CA_{NOcor} measurements with the 2 methods were shown graphically by plotting the difference against the mean as recommended by Bland and Altman [17]. Data were analyzed with a statistical software package (Prism 5, GraphPad Software).

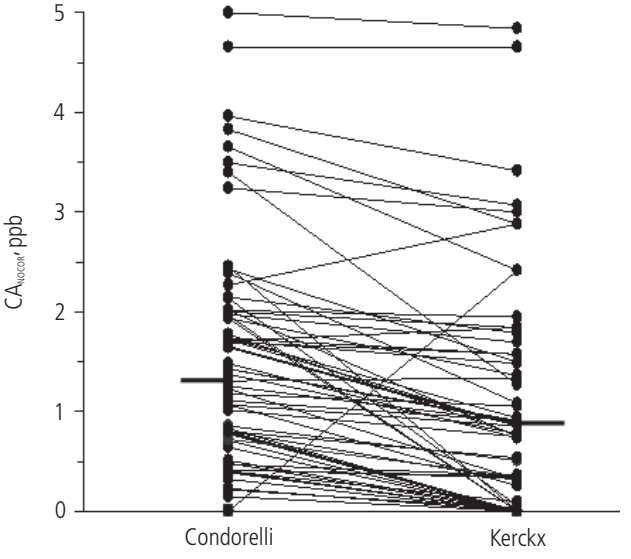

Figure 1. Individual values for small airway/alveolar nitric oxide (CA_{NO}) computed by the correction methods of Condorelli et al [10] and Kerckx et al [9] in 91 individuals. Horizontal lines are means.

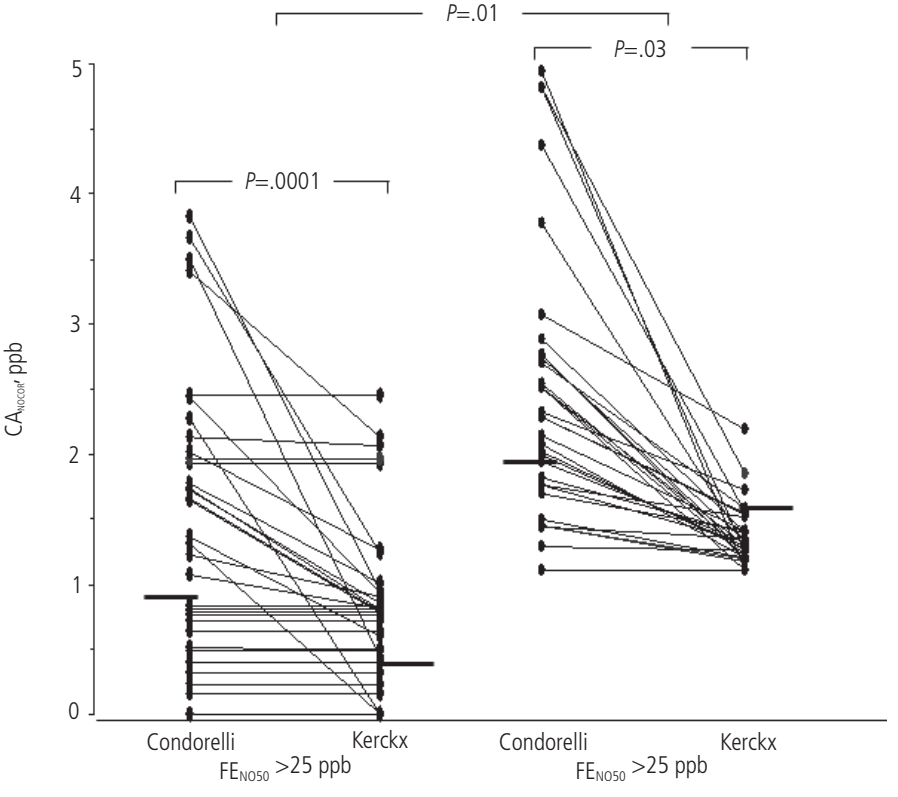

Figure 2. Individual values for small airway/alveolar nitric oxide (CA_{NO}) computed with each method in individuals with high (>25 ppb) FE_{NO50} values and with normal (≤25 ppb) FE_{NO50} values. Horizontal lines are means. FE_{NO50} indicates fractional exhaled nitric oxide at 50 mL/s.

Results

CA_{NOcor} values computed using the Condorelli method were significantly higher than those obtained using the Kerckx method. These differences were observed in the population as a whole (Table 2 and Figure 1), as well as in asthmatics, individuals with allergic rhinitis, and healthy volunteers (Table 2). Although significant differences in CA_{NOcor} values computed with each method were detected in both individuals with high FE_{NO50} values (>25 ppb) and those with normal FE_{NO50} levels (≤25 ppb), they were significantly higher in individuals from the first group (Table 2 and Figure 2); the mean difference was 0.34 ppb (95% CI, 0.08-0.59 ppb; *P*=.01). Similar findings were obtained using a cutoff point of 20 ppb for FE_{NO50} (data not shown).

Figure 3 shows the differences between CA_{NOcor} values obtained with each approach plotted against the mean according to Bland and Altman [17]. The mean difference in CA_{NOcor} values obtained with each method was 0.40 ppb (95% CI, 0.27-0.53 ppb), but differences were more evident in individuals with mean CA_{NO} values of over 1 ppb. Furthermore, differences between the 2 methods were not absolutely consistent because CA_{NO} values obtained with the Kerckx method were higher than those obtained with the Condorelli method in 4 individuals [10].

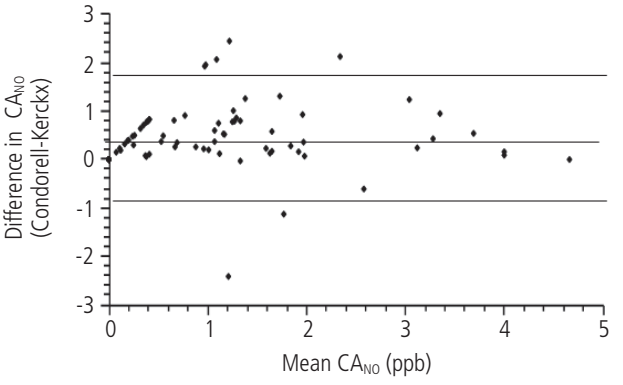

Figure 3. Difference between the small airway/alveolar nitric oxide (CA_{NO}) values obtained using the methods of Condorelli et al [10] and Kerckx et al [9] (Condorelli – Kerckx) plotted against the mean CA_{NO} values. The continuous line represents mean difference and the dashed lines represent ±2 SDs for the differences.

Discussion

This study demonstrates significant differences in CA_{NO}values obtained using the Condorelli and the Kerckx methods to adjust for the trumpet shape of the airway tree and axial diffusion from central to peripheral airways. Another important finding is that differences in CA_{NO} values obtained with each method were significantly greater in individuals with high FE_{NO50} values than in those with normal FE_{NO50} levels. These results suggest that CA_{NO} values obtained with each approach are not equivalent and cannot be used interchangeably.

Although a multicompartment model was recently proposed to account for regional heterogeneity in ventilation and NO production [18,19], current theoretical models of NO dynamics in the lungs utilize a single-path trumpet shape that partitions the exhaled concentration into one airway and one alveolar contribution [3,18,19]. NO has sources from both the airway and alveolar regions, which has been determined by implementing 2-compartment mathematical models [3,20,21]. These initial models were, however, extremely simple, and consisted of separating the lung into a rigid trumpet-shaped airway region characterized by constant NO production (or flux of NO from the airway wall surface) and an expansile distal airway/alveolar region characterized by a steady-state NO concentration. In all these models, the slope and intercept reflected alveolar and bronchial NO, respectively. Although these models explained the strong flow dependence of exhaled NO, they neglected potentially important physical and physiological phenomena such as axial gas phase diffusion. Because of the concentration gradient between bronchial and alveolar regions, some NO molecules produced in the airways diffuse toward the alveoli (back diffusion) without being expired [5,6]. One consequence of the presence of NO back diffusion is that models assuming 2 independent compartments lend to an underestimation of actual bronchial NO production [5,6]. Another consequence of NO back diffusion is that it constitutes an additional NO source for the alveolar compartment and, hence, contributes to an increase in alveolar NO concentration.

At present, 2 methods have been validated to account for the impact of airway NO production on alveolar concentration. In the first method, introduced by Condorelli and colleagues [10], alveolar NO is corrected using bronchial NO values. The second method, proposed by Kerckx et al [9] involves a simple correction that requires knowledge of the exhaled concentration at a flow of 50 mL/s. The results of the present study indicate that the correction introduced by the Condorelli method provides significantly higher CA_{NO} values than those obtained using the Kerckx method. In addition, these differences were observed both in the population as a whole and in each group of individuals studied (asthmatics, individuals with allergic rhinitis, and healthy volunteers). This appears to contradict the results of 2 previous investigations [9,22] reporting that the 2 methods provided similar values for alveolar NO. There are several possible explanations for this apparent contradiction. In one of these previous studies [9], the expiratory flows were 50, 175, and 300 mL/s. It has been demonstrated that when flows of under 100 mL/s are used, the relationship between exhalation flows and NO output is nonlinear and it has been recommended that only data points corresponding to a flow of 100 mL/s or higher should be used to calculate alveolar and bronchial NO [1]. Therefore, the differences between the results of the present study and those reported by Kerckx et al [9] might be attributable to methodological differences. Furthermore, a significant proportion of individuals with asthma in the 2 previous studies [9,22] were treated with inhaled corticosteroids, whereas our asthmatic patients were treated only with short-acting inhaled $ß_2$-agonists on demand.

This is important because some studies have reported that treatment with inhaled corticosteroids is associated with significant reductions in both FE_{NO50} [23] and $J'aw_{NO}$[24]; the effect on CA_{NO}, by contrast, has been less consistent [24,25]. Therefore, it may be speculated that, at least in the group with asthma, the differences between our results and those previously reported [9,22] might be explained by the effect of inhaled corticosteroids on bronchial NO. The greater differences between the CA_{NO} values obtained by each method in the group with increased FE_{NO50} values are in line with the explanation that the steroid-induced decrease in bronchial NO may explain, at least in part, differences between the results of the present study and those reported in previous studies [9,22]. Finally, differences between the results of the present study and those reported by other investigators [9,22] might be due to differences in exhalation flow rates employed for NO measurement, because it has been demonstrated that studies using different flow rates yield different absolute CA_{NO} values [26].

On the other hand, adjusting for the trumpet shape of the airway tree and axial diffusion produces an alveolar NO concentration close to zero. This result is consistent with previous observations [8,10]. In addition, differences in CA_{NO} values between the 2 methods reported to adjust for the trumpet shape and axial diffusion are not absolutely consistent and are more evident in individuals with mean alveolar NO values of over 1 ppb (Figure 3). Although these aspects must be studied further, our findings indicate that the alveolar NO values obtained using the Condorelli and the Kerckx method are not comparable.

In summary, our results demonstrate that alveolar NO values obtained using the Condorelli method are significantly higher than those calculated using the Kerckx method. Although the differences are more evident in individuals with increased exhaled NO, they were also detected in individuals with normal NO values. These results suggest that alveolar NO values obtained with each approach are not equivalent and cannot be used interchangeably.

Acknowledgments

The authors wish to thank Diater Laboratorios (Madrid, Spain) for their help and collaboration and Rocio Rojas and Ana Lucas for their technical assistance.

Funding

The authors confirm that no funding was received for the present study.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Previous Presentation

The results of this study were previously reported at the European Academy of Allergy and Clinical Immunology Congress 2011, Istambul, Turkey.

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Manuscript received October 29, 2012; accepted for publication, February 13, 2013.

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