Association of alveolar nitric oxide levels with pulmonary function and its reversibility in stable asthma

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Short title: **Association of alveolar NO with pulmonary function in asthma**

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Key Words: airway reversibility, alveolar NO, asthma, impulse oscillometry system, peripheral airway dysfunction, trumpet model with axial diffusion

Abstract

Background: Inflammation of peripheral airways is implicated in the pathophysiology of severe asthma. However, contributions of peripheral airway inflammation to airway caliber/function in patients with stable asthma, including those with mild to moderate disease, remain to be confirmed.

Objectives: To determine whether peripheral airway inflammation affects airway function in patients with asthma.

Methods: In 70 patients with mild to severe asthma, alveolar nitric oxide ($C_{ANO(TMAD)}$) levels were examined as a noninvasive biomarker of peripheral airway/alveolar inflammation. $C_{ANO(TMAD)}$ and maximal NO flux in the airway compartment, J'awNO, were estimated with a model that incorporated trumpet shaped airways and axial diffusion using exhaled NO output at different flow rates. Measures of pulmonary function were then assessed by spirometry and an impulse oscillometry system, and their bronchodilator reversibility was examined. **Results:** $C_{ANO(TMAD)}$ levels were not correlated with pre- or post-bronchodilator spirometric values, but were significantly associated with pre-bronchodilator reactance at low frequency (Xrs5) (rho=-0.31, p=0.011), integrated area of low-frequency Xrs (AX) (rho=0.35, p=0.003) and negative frequency dependence of resistance (Rrs5-Rrs20) (rho=0.35, p=0.004). Furthermore, $C_{ANO(TMAD)}$ levels were associated with bronchodilator reversibility of FEV_1, $FEF_{25-75\%}$, Xrs5, and AX (rho=0.35, rho=0.31, rho=-0.24, rho=-0.31, respectively; $p \leq 0.05$ for all). No variables were related to J'awNO. **Conclusions**: Elevated $C_{ANO(TMAD)}$, but not J'awNO, partly reflects reversible airway obstruction originating in the peripheral airway. These findings indicate the involvement of peripheral airway inflammation in physiological abnormalities in asthma.

Abbreviations

AX: integrated area of low-frequency reactance

BD: bronchodilator

C_{ANO}: alveolar fraction of exhaled NO

$C_{ANO(TMAD)}$: alveolar fraction of exhaled NO adjusted for trumpet shaped airways and axial diffusion

$FEF_{25-75\%}$: mid forced expiratory flow

FeNO: fraction of exhaled NO

$FeNO_{50}$: FeNO at 50 ml/sec

ICS: inhaled corticosteroids

IOS: impulse oscillometry system

J'_{awNO}: bronchial NO maximal flux adjusted for trumpet shaped airways and axial diffusion

NO: nitric oxide

Rrs: resistance of the total respiratory system

Rrs5: Rrs at 5 Hz

Rrs20: Rrs at 20 Hz

Xrs: reactance of the total respiratory system

Xrs5: Xrs at 5 Hz

Introduction

Asthma is a chronic inflammatory disorder involving proximal airways as well as small peripheral airways [1-4]. Nitric oxide (NO) is a well-established noninvasive biomarker of inflammation in asthmatic airways [5]. Recently, the alveolar fraction of exhaled NO (C_{ANO}) has been introduced to assess inflammation of peripheral airway/alveolar regions [6]. This variable has received renewed interest as a noninvasive tool for both adults [7] and children [8]. Although C_{ANO} is mathematically derived from NO output measured at multiple flow rates, a study by Berry and colleagues has shown a strong correlation between C_{ANO} levels and eosinophil counts in bronchoalveolar lavage fluid [9], suggesting that C_{ANO} may reflect peripheral airway/alveolar inflammation. Moreover, two trumpet models with axial diffusion have recently been developed to correct the effect of axial contamination by small airway NO diffusion on C_{ANO} [10, 11]. This new parameter, $C_{ANO(TMAD)}$, precludes overestimating actual NO production in the alveolar region.

Peripheral airway dysfunction or the degree of air trapping is associated with recurrent exacerbations of severe asthma [12] or with disease severity [13]. Effective prevention of peripheral airway dysfunction requires an understanding of the contribution of peripheral airway inflammation to airway dysfunction. However, such a contribution has been confirmed only for severe refractory asthma [14, 15]. Because peripheral airway inflammation is not limited to patients with severe disease [1], we hypothesized that sustained inflammation in the peripheral airway or alveolar region may be linked to airway dysfunction in the general population of patients with asthma. In this study, we measured $C_{ANO(TMAD)}$, pulmonary function and pulmonary function

reversibility in response to treatment with a bronchodilator (BD) for patients with asthma ranging from mild intermittent to severe persistent. Using an impulse oscillometry system (IOS) [16] [17], we examined negative frequency dependence and low frequency reactance that could be proxies for peripheral airway function.

Methods

Subjects

This cross-sectional study was conducted at the outpatient asthma and cough clinic of Kyoto University Hospital from January through December 2007. We examined NO levels at different flow rates (50, 100, and 200 ml/s) in 75 adults with stable asthma. The inclusion criteria were as follows: 1) a diagnosis of asthma as defined by the American Thoracic Society [18] on the basis of a history of recurrent episodes of wheezing and chest tightness with or without cough; 2) confirmation of airway hyperresponsiveness on past evaluations; 3) regular attendance at our clinic for >3 months; 4) no exacerbation of asthma during the last 4 weeks; 5) no changes in treatment during the last 4 weeks; 6) non-smokers or ex-smokers who had smoked <10 pack-years but had not smoked for >1 year; 7) no evidence of other respiratory diseases on chest X-ray findings. Severity was defined according to the step classification of the 2004 version of the Global Initiative for Asthma guidelines [19]. Three patients with step 2 asthma were treated with leukotriene receptor antagonists because they refused to use inhaled corticosteroids (ICS). To examine patients with persistent airway inflammation, we measured NO levels at higher flows in patients with fraction of exhaled NO (FeNO) at 50 ml/sec ($FeNO_{50}$) values ≥ 25 ppb [5]. The ethics committee of our institution approved the study protocol, and written informed consent was

obtained from each participant.

Measurement of NO and calculation of $C_{ANO(TMAD)}$ and bronchial NO maximal flux

NO levels were measured with a chemiluminescence analyzer (NOA 280, Sievers, Boulder, CO, USA) according to current guidelines, apart from the expiratory flow rate [20]. The analyzer was calibrated daily with gas without NO generated by exposing ambient air to NO scavengers and a standard concentration of 640 ppb NO. The lower detection limit for NO was 2 ppb. The signal output from the NO analyzer was fed to a computer data acquisition program, and concentrations were measured using a data analysis program (NOA Analysis™ Software, Sievers, Boulder, CO, USA). Seated subjects inserted a mouthpiece, inhaled orally to total lung capacity, exhaled immediately against a resistance, and maintained mouth pressure at 20 cmH_2O, displayed on a pressure gauge. The steady-state NO plateau was taken as the FeNO value. By varying expiratory resistances, we measured FeNO levels at three expiratory flows of 50, 100 and 200 ml/s in that order. If an adequate NO plateau was not reached at 200 ml/s, patients were then instructed to exhale at a flow of 150 ml/s instead (n=4).

$C_{ANO(TMAD)}$ was estimated using a model recently developed by Condorelli and colleagues that incorporated trumpet shaped airways and axial diffusion [10] rather than simply assuming that the lung was comprised of two separate regions with a rigid airway compartment and a well mixed expansible compartment [6]. The equation used was the following: $C_{ANO(TMAD)}$ = slope - intercept/740. The slope and intercept of this equation were determined from a linear regression line after plotting NO output (i.e., exhaled NO level times expiratory flow) as a function of expiratory flow. When

$C_{ANO(TMAD)}$ values were < zero, they were assigned a value of zero. Bronchial NO maximal flux (J'_{awNO}), a marker of inflammation in the airway compartment, was also determined by multiplying the intercept by 1.7. Because this trumpet model with axial diffusion was developed based on the assumption that NO flux from the airway tree was a constant and this simplified equation was valid at an expiratory flow rate between 100 ml/s and 250 ml/s, we applied FeNO levels at 100 ml/s and 200 ml/s to obtain a linear regression line between NO output and exhalation flow. Five patients whose results had negative slopes were excluded from further analysis [10].

Pulmonary function tests

After NO measurements, subjects underwent pre- and post-BD (i.e., inhalation of 200 μg salbutamol) pulmonary function testing. Respiratory impedance was measured by IOS followed by spirometric testing. Reversibility of the impedance and spirometric values was calculated by: (post-BD value minus pre-BD value)/pre-BD value.

Spirometry was performed according to the standards of the American Thoracic Society [21] using a ChestGraph HI-701 spirometer (Chest, Tokyo, Japan). FEV_1 and mid forced expiratory flow ($FEF_{25-75\%}$) were determined.

Respiratory impedance was measured using a Jaeger MasterScreen, IOS^{TM} (Erich Jaeger, Hoechberg Germany) that met standard recommendations [22]. The IOS is different from the classical forced oscillation technique by the use of an impulse rather than a pseudorandom noise signal and in data processing. In brief, rectangular mechanical impulses containing a continuous power spectrum ranging from 0 to 100 Hz, generated by a loudspeaker at intervals of 0.2 s, were applied to the respiratory system

through a mouthpiece during tidal breathing. The resulting pressure and flow signals were measured next to the mouthpiece, and were analyzed for amplitude and phase differences using a fast Fourier transform to determine resistance (Rrs) and reactance (Xrs) of the total respiratory system. To reduce loss of energy in the upper airways, the chin and cheeks were supported by the subjects' hands. As proxies for peripheral airway function [16] [17] [23] [24], we used the negative frequency dependence of Rrs between 5 and 20 Hz (Rrs5-Rrs20), Xrs at 5 Hz (Xrs5), and reactance area (AX) that is the integral of Xrs from 5 Hz to the resonant frequency at which Xrs crosses zero. Rrs at 20 Hz (Rrs20) was used as a measure of proximal airway resistance, as oscillatory pressure components at frequencies ≥20 Hz are thought to be damped out before reaching the peripheral airways [16].

Statistical analysis

Data were analyzed using GraphPad Prism 4.00 (GraphPad Software, Inc., La Jolla, CA, USA) and StatView software 5.0 (SAS Institute Inc, Cary, NC, USA). When data were normally distributed, linear regression analysis was used. Otherwise, Spearman's rank-correlation test was used. The correlation coefficient from linear regression analysis is denoted as R, whereas Spearman's correlation coefficient is given as rho. Results are expressed as means ± SDs or medians (ranges). We considered p values of ≤ 0.05 to indicate statistical significance.

Results

Patients' characteristics

The characteristics of the 70 subjects are shown in Table 1. Measures of pulmonary function and $FeNO_{50}$ of the 70 subjects did not differ significantly from those of the 5 patients whose slopes were negative (data not shown).

Correlations between patients' characteristics and $C_{ANO(TMAD)}$ or bronchial flux

Patients with more severe asthma showed a trend for higher J'awNO levels than those with milder disease (rho=0.23, p=0.056), but not for $C_{ANO(TMAD)}$ levels (rho=0.17, p=0.17). The presence of atopy, rhinitis and smoking history were not associated with $C_{ANO(TMAD)}$ or J'awNO (data not shown).

Correlations of $C_{ANO(TMAD)}$ or bronchial flux with pre- and post-BD pulmonary function measures

The associations of $C_{ANO(TMAD)}$ and J'awNO with pulmonary function measures are summarized in Table 2. Correlations of pulmonary function measures with $FeNO_{50}$ that were strongly correlated with J'awNO levels (rho=0.83, p<0.0001) are also shown in this table. There was no association of $C_{ANO(TMAD)}$ with pre-BD $\%FEV_1$, $\%FEF_{25-75\%}$, or Rrs20. $C_{ANO(TMAD)}$ was significantly associated with pre-BD Rrs5-Rrs20, Xrs5, and AX (Fig 1). The association between Xrs5 and $C_{ANO(TMAD)}$ remained significant even when two obvious outliers (one with the highest Xrs5 and another with the lowest Xrs5) were excluded (rho=-0.36, p=0.003 n=68). There was no association between $C_{ANO(TMAD)}$ and any measure of post-BD pulmonary function. J'awNO was not related to any indices of pulmonary function.

Measures of pre-BD Rrs5-Rrs20, Xrs5, and AX were moderately to strongly correlated with each other (|rho| ranged between 0.75 and 0.93, $p<0.0001$). These measures of pre-BD IOS were correlated with pre-BD $\%FEV_1$ (|rho| ranged between 0.33 and 0.40, $p<0.01$).

Correlations between $C_{ANO(TMAD)}$ or bronchial flux and reversibility of pulmonary function measures

Reversibilities of FEV_1 and $FEF_{25-75\%}$, as well as of Xrs5 and AX, were significantly associated with $C_{ANO(TMAD)}$ (Table 2). J'awNO was not related to any indices of reversibility.

Reversibilities of FEV_1, $FEF_{25-75\%}$ Xrs5 and AX were associated with pre-BD levels; reversibility of FEV_1 was correlated with the pre-BD FEV_1 level ($R=-0.56$, $p<0.0001$); for $\%FEF_{25-75\%}$, rho=-0.25, p=0.042; for Xrs5, rho=0.48, $p<0.0001$; for AX, rho=-0.40, p=0.001.

Discussion

To the best of our knowledge, this is the first study to demonstrate that in adult patients with stable asthma, $C_{ANO(TMAD)}$, but not J'awNO, is significantly associated with Rrs5-Rrs20, Xrs5, and AX levels, which are thought to reflect peripheral airway function. Our novel findings are that the increase in $C_{ANO(TMAD)}$ associates with greater reversibility of FEV_1, $FEF_{25-75\%}$, Xrs5 and AX in response to treatment with a BD. These findings suggest that peripheral airway/alveolar inflammation may contribute to reversible airway obstruction originating in the peripheral airway.

Before the introduction of $C_{ANO(TMAD)}$, C_{ANO} had been used as a reasonably well validated marker of inflammation of the peripheral airway/alveolar region [9]. Histological studies have suggested that peripheral airway inflammation has a similar or greater impact on disease severity than does proximal airway inflammation [1, 23, 24]. Studies examining C_{ANO} have drawn similar conclusions; C_{ANO} in refractory asthma is significantly greater than that in mild to moderate asthma [9], and patients with oral steroid-dependent asthma have higher C_{ANO} levels than patients with mild-to-moderate or severe asthma [14] [25]. In this study, J'awNO showed a trend toward increased values in patients with more severe asthma than those with milder disease. However, when $C_{ANO(TMAD)}$ was determined using a trumpet model with axial diffusion that estimated NO concentrations exiting the respiratory bronchioles and alveolar region, $C_{ANO(TMAD)}$ was not correlated with disease severity, which is consistent with a recent finding by Mahut et al [26]. Given that C_{ANO} is not adjusted for NO axial diffusion from conducting airways and could be more contaminated by small airway NO diffusion than $C_{ANO(TMAD)}$, this discrepancy between previous findings with C_{ANO} and those with $C_{ANO(TMAD)}$ in this study may indicate that small airway inflammation

influences asthma severity to a greater extent than the actual alveolar inflammation. Indeed, a histological study of severe asthmatics showed that small airways with perimeters less than 6 mm were the most significantly infiltrated by inflammatory cells across the entire airway tree, from the large airways to the alveolar tissue [23].

Van Veen and colleagues found significant correlations of C_{ANO} with peripheral airway obstruction, as assessed by residual volume, functional residual capacity and closing capacity [14], in patients with severe asthma. However, when patients with mild-to-moderate asthma were included, these correlations disappeared, and only a significant association between C_{ANO} and the slope of the single breath nitrogen washout curve remained. Although C_{ANO} was correlated with $FEF_{25-75\%}$ in children with refractory asthma [15], C_{ANO} was not correlated with $FEF_{50\%}$, $FEF_{25\%}$, or residual volume in adults with asthma [25]. These studies provide little or no evidence of a link between peripheral airway inflammation and dysfunction in the population of adults with asthma.

In the current study, however, $C_{ANO(TMAD)}$ was modestly, but significantly associated with small airway reactance/capacitance (Xrs5, AX) and negative frequency dependence of airway resistance (Rrs5-Rrs20). These variables obtained by IOS, a non-invasive and effort-independent tool, have been proposed as relevant and sensitive indices of peripheral airway function [16] [17] [23] [28] [27] [28]. Previous oscillation studies have demonstrated that as peripheral resistance increases, the resistance at low frequencies increases compared to the higher frequencies, thereby resulting in greater negative frequency dependence of Rrs [16, 29]. Xrs at low frequency is also considered to reflect the capacitative properties of peripheral airways and to detect peripheral airway narrowing [30]. In our recent study of patients with asthma, Rrs5-Rrs20 and AX,

but not Rrs20, are significantly associated with residual volume [23]. Another recent long-term study of controller therapy for pediatric asthma also demonstrates that AX may be sensitive to changes in peripheral airway function [27]. The nature of these indices of IOS may support our premise that Rrs5-Rrs20, Xrs, and, particularly, AX may reflect peripheral airway function. However, as discussed below, the data should be interpreted with caution because IOS indices are indirect measures, and no associations of IOS indices with pathological changes have been demonstrated thus far.

Airway variability is another feature of asthma, implying instability of the disease. Endobronchial and transbronchial biopsy studies of nocturnal asthma have demonstrated a significant contribution of peripheral airway inflammation to nocturnal decrement of pulmonary function as compared with proximal airway inflammation [31]. In studies of pediatric asthma, higher FeNO levels are associated with greater FEV_1 reversibility [32-34]. Tsuburai and colleagues show associations between FeNO levels and FEV_1 reversibility in adult asthmatics with airflow limitation, but not in those without airflow limitation [35]. Our study of adult asthmatics extended the previous findings by showing that smoldering inflammation in the peripheral airway/alveolar compartment is responsible for greater BD response in FEV_1, $FEF_{25-75\%}$, Xrs5, and AX, and that reversibility to a BD depends on the degree of pre-BD obstruction. Given that $C_{ANO(TMAD)}$ was correlated with pre-BD Xrs5 and AX, but not with FEV_1 or $FEF_{25-75\%}$ values, we speculate that peripheral airway/alveolar inflammation may contribute to reversible airway obstruction originating in the peripheral airway.

In sharp contrast to $C_{ANO(TMAD)}$, bronchial NO flux (i.e. J'_{awNO} levels) was not correlated with any indices of pulmonary function and $FeNO_{50}$ was only correlated with reversibility of Xrs5, which was consistent with other studies [7] [36]. This discrepancy

between $C_{ANO(TMAD)}$ and J'awNO could be attributed to differences in their responses to ICS treatment. When patients are treated with an ICS, J'awNO readily decreases, whereas C_{ANO} is resistant to them [37]. Therefore, we speculate that any correlation of J'awNO or $FeNO_{50}$ with pulmonary function might have been modified by ICS treatment. Indeed, in steroid naïve patients, exhaled NO levels are correlated with airway hyperresponsiveness [38], asthma symptom scores [39], or with bronchodilator response [34], whereas such correlations disappear in patients on ICS. Intriguingly, a recent study shows significant associations between exhaled NO levels and FEV_1 or bronchodilator responses in pediatric asthmatics receiving ICS treatments when they are classified according to their ICS doses [40], which may suggest that stratifying patients by ICS doses could provide deeper insights into the roles of exhaled NO in patients on ICS.

The role of $C_{ANO(TMAD)}$ in peripheral airway obstruction should not be overestimated because the associations were modest; elevated $C_{ANO(TMAD)}$ is not a strong indicator for low Xrs5 or increased AX. Also, IOS variables were not compared with closing volume or closing capacity, other proxy measures of peripheral airway obstruction. As mentioned above, IOS measurements have several limitations and should be interpreted cautiously [16] [17] because of issues that include artifact effects of upper airway compliance and leaks at the mouthpiece. Increases in Rrs5-Rrs20 may imply heterogeneity of peripheral airway narrowing in addition to an absolute increase in resistance [41]. Xrs5 is relatively noise-sensitive, whereas AX is less so. Resistance and reactance in the peripheral airway may indicate different properties, given that pre-BD values of Rrs5-Rrs20 were not correlated with reversibility to a BD, whereas pre-BD values of AX and Xrs5 were, although precise mechanisms underlying this discrepancy are unknown.

We may have missed some patients with normal $FeNO_{50}$ and high $C_{ANO(TMAD)}$ by excluding those with $FeNO_{50}$ < 25 ppb. When pediatric patients with asthma are grouped based on their J'awNO and $C_{ANO(TMAD)}$ levels, Puckett et al find that 11% of the patients have normal J'awNO and high $C_{ANO(TMAD)}$ levels [42]. However, when we examined another 21 patients with stable asthma who had $FeNO_{50}$ < 25 ppb, their ratios of $C_{ANO(TMAD)}$ levels to $FeNO_{50}$ levels did not differ from those of the currently studied population (data not shown), suggesting that patients with normal $FeNO_{50}$ and high $C_{ANO(TMAD)}$ are not commonly observed in our patient population. Therefore, we believe that the possibility of missing patients with normal $FeNO_{50}$ and high $C_{ANO(TMAD)}$ may not have severely affected our findings.

Five patients were excluded from the analysis because their results had negative slopes in plots of NO output vs. expiratory flow between 100 ml/s and 200 ml/s. NO axial diffusion from the airway tree increases as bronchial flux increases and its relative impact over NO conductive transport exiting the alveolar region is greater at lower expiratory flows [10]. Therefore, the negative slopes for five patients may indicate that, in these patients, the relative impact of NO axial diffusion on NO output with exhalation flows around 100 ml/s might have remained either because of increased bronchial reflux or because of relatively small NO production in the alveolar region. Measurements with higher expiratory flows would have been desirable, but it was difficult for our patients to obtain a steady-state NO plateau with higher flows, such as at 250 ml/s or 300 ml/s. Despite these limitations, our findings support the superiority of a multiple flow technique to a conventional single flow measurement, as the former provides information on peripheral airway/alveolar inflammation.

In conclusion, inflammation in the peripheral airway/alveolar region as

assessed by $C_{ANO(TMAD)}$ partly contributes to the development of peripheral airway obstruction and to reversibility of pulmonary function in response to a bronchodilator. Monitoring peripheral airway/alveolar inflammation may provide higher-quality information for patients with asthma.

The authors have no conflicts of interest to disclose.

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Figure legend

Figure 1. Relationships between $CA_{NO(TMAD)}$ levels and pre-bronchodilator Xrs5 (left) and Rrs5-Rrs20 (right).

Table 1. Patients' characteristics (n=70)

Gender, M/F	33/37
Age, yr	57.4 ±15.6
Disease duration, yr	12.2 ± 10.4
Asthma severity*, 1/2/3/4	2/35/17/16
Atopy, positive†, %	66
IgE, IU	190 (5, 6800)
Ex smoker, %	33
Sinusitis/rhinitis, %	66
Daily dose of ICS‡, µg	400 (0, 1600)
$\%FEV_1$, %	89.4 ± 16.5
$\%FEF_{25-75\%}$, %	57.4 ± 26.8
Rrs20, $kPa \cdot s \cdot L^{-1}$	0.32 ± 0.11
Rrs5-Rrs20, $kPa \cdot s \cdot L^{-1}$	0.06 (-0.03, 0.43)
Xrs5, $kPa \cdot s \cdot L^{-1}$	-0.13 (-0.56, 0.17)
AX, $kPa \cdot s \cdot L^{-1}$	0.44 (0.01, 5.51)
$FeNO_{50}$, ppb	44.9 (25.0, 224.4)
J'awNO, $pl \cdot s^{-1}$	3638 (0, 15300)
$C_{ANO(TMAD)}$, ppb	3.9 (0, 34.9)

*According to the Global Initiative for Asthma guidelines [19]. †Considered atopic when one or more specific IgE antibodies against house dust mite, cat dander, dog dander, molds, weed, cedar pollen, or grass pollen were positive. ‡Equivalent dose of fluticasone. ICS = inhaled corticosteroids, Rrs20 = resistance of the total respiratory system at 20 Hz, Rrs5 = Rrs at 5 Hz, Xrs5 = reactance at 5 Hz, AX = area of low-frequency reactance, $FeNO_{50}$ = exhaled NO level at flow of 50 ml/s, J'awNO = bronchial NO maximal flux adjusted for trumpet shaped airways and axial diffusion, $C_{ANO(TMAD)}$ = alveolar fraction of exhaled NO adjusted for trumpet shaped airways and axial diffusion

Values are means ± SD or median (range).

Table 2. Correlation coefficients of NO levels with pulmonary function values

	$CA_{NO(TMAD)}$	J'awNO	$FeNO_{50}$
Pre-bronchodilator			
$\%FEV_1$	-0.18	-0.03	-0.12
$\%FEF_{25-75\%}$	-0.09	-0.05	-0.10
Rrs20	0.22	-0.17	-0.05
Rrs5-Rrs20	**0.35**†	-0.09	0.11
Xrs5	**-0.31***	-0.05	-0.19
AX	**0.35**†	-0.03	0.14
Reversibility			
ΔFEV_1	**0.35**†	-0.007	0.09
$\Delta FEF_{25-75\%}$	**0.31***	0.09	0.21
ΔRrs20	-0.23	-0.007	-0.01
Δ(Rrs5-Rrs20)	-0.22	0.05	0.01
ΔXrs5	**-0.24***	-0.16	**-0.25***
ΔAX	**-0.31***	-0.16	-0.10

*: $p \leq 0.05$, †: $p < 0.01$, Correlation coefficients of Spearman's correlation coefficients are shown.

Δ = (post-bronchodilator value minus pre-bronchodilator value) / pre-bronchodilator value, Rrs20 = resistance of the total respiratory system at 20 Hz, Rrs5 = Rrs at 5 Hz Xrs5 = reactance at 5 Hz, AX = integrated area of low-frequency reactance, $CA_{NO(TMAD)}$ = alveolar fraction of exhaled NO adjusted for trumpet shaped airways and axial diffusion, J'awNO = bronchial NO maximal flux adjusted for trumpet shaped airways and axial diffusion, $FeNO_{50}$ = fraction of exhaled NO at 50 ml/sec

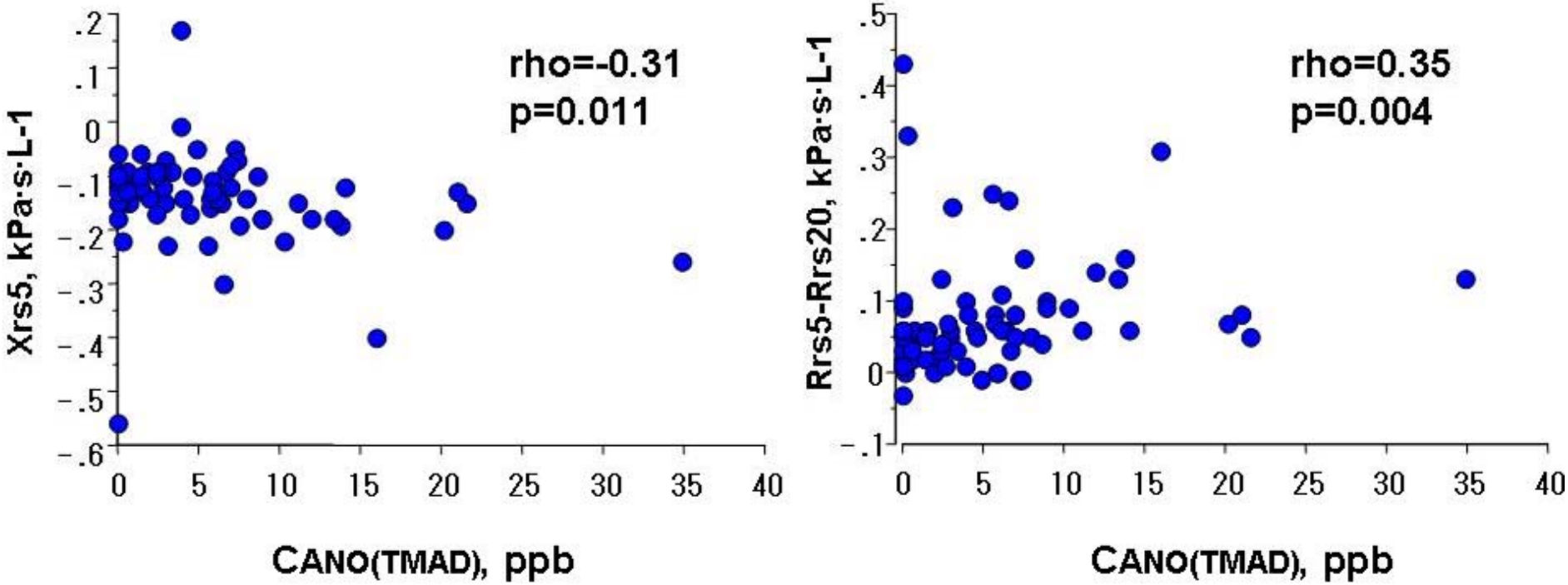
rho=-0.31
p=0.011
Xrs5, kPa·s·L-1
.2
.1
0
-.1
-.2
-.3
-.4
-.5
-.6
0
5
10
15
20
25
30
35
40
CANO(TMAD), ppb
rho=0.35
p=0.004
Rrs5-Rrs20, kPa·s·L-1
.5
.4
.3
.2
.1
0
-.1
0
5
10
15
20
25
30
35
40
CANO(TMAD), ppb

Figure 1