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Exhaled nitric oxide from lung periphery is increased in COPD

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ABSTRACT: Single constant flow exhaled nitric oxide (eNO) cannot distinguish between the sources of NO. The present study measured eNO at multiple expired flows (MEFeNO) to partition NO into alveolar ($C_{alv,NO}$) and bronchial ($J_{aw,NO}$) fractions to investigate peripheral lung contribution to eNO in chronic obstructive lung disease (COPD).

MEFeNO were made in 81 subjects including 18 nonsmokers, 16 smokers and 47 COPD patients of different severity by the classification of the Global Initiative for Chronic Obstructive Lung Disease (GOLD): 0 (n=14), 1 (n=7), 2 (n=11), 3 (n=8) and 4 (n=7).

COPD severity was correlated with an increased $C_{alv,NO}$ regardless of the patient's smoking habit or current treatment. The levels of $C_{alv,NO}$ (in ppb) were 1.4±0.09 in nonsmokers, 2.1±0.1 in smokers categorised as GOLD stage 0 (smokers-GOLD0), 3.3±0.18 in GOLD1–2 and 3.4±0.1 in GOLD3–4. $J_{aw,NO}$ levels (pL·s^{-1}) were higher in nonsmokers than smokers-GOLD0 (716.2±33.3 *versus* 464.7±41.8), GOLD3–4 (609.4±71). Diffusion of NO in the airways ($D_{aw,NO}$ pL·ppb^{-1}s^{-1}) was higher ($p<0.05$) in GOLD3–4 than in nonsmokers (15±1.2 *versus* 11±0.5) and smokers-GOLD0 (11.6±0.5). MEFeNO measurements were reproducible, free from day-to-day and diurnal variation and were not affected by bronchodilators.

In conclusion, chronic obstructive pulmonary disease is associated with elevated alveolar nitric oxide. Measurements of nitric oxide at multiple expired flows may be useful in monitoring inflammation and progression of chronic obstructive pulmonary disease, and the response to anti-inflammatory treatment.

KEYWORDS: Chronic obstructive pulmonary disease, exhaled nitric oxide, multiple expiratory flow, small airway inflammation

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Single expiratory flow exhaled nitric oxide (eNO) measurements are simple, highly reproducible [1], have been used to monitor larger airway inflammation in asthma research [2], and are now moving into clinical practice [3]. However, small airways and lung parenchyma are the predominant sites of inflammation in patients with chronic obstructive pulmonary disease (COPD) [4]. Progression of COPD is associated with the accumulation of inflammatory mucous exudates in the lumen and infiltration of the small airway wall by inflammatory cells [4]. There is a high level of expression of inducible NO synthase (iNOS) presence in sputum macrophages [5], alveolar walls, small airway epithelium and vascular smooth muscle of COPD patients [5, 6]. In patients with COPD this may result in an increased production of NO and NO-related species in the lung periphery, which through the function of peroxynitrite may amplify the inflammation and lead to inhaled corticosteroid (ICS) resistance, particularly as the disease becomes more severe [7].

The current single expiratory technique measures predominantly larger airway-derived NO and may only partially reflect peripheral inflammation [8, 9]. eNO is often in the normal range or even reduced in moderate COPD [10], probably due to down-regulation of endothelial NO synthase (eNOS) [11] and iNOS [12] by cigarette smoke.

Recently, methods for measuring eNO at multiple expiratory flows (MEFeNO) [13, 14] have been used to detect elevated levels of alveolar NO in fibrosing alveolitis [13], asthma [15, 16] and COPD [17] leading to the refinement of analytical methods to discriminate exhaled NO sources in the lung [18].

There is agreement that a simple two-compartment model, which is based on MEFeNO, can adequately represent the marked dependence of eNO on exhalation flow [19, 20]. Three flow-independent NO exchange parameters can describe the airway compartment: airway NO diffusing capacity ($D_{aw,NO}$) and either the

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maximum airway wall NO flux, also known as the airway wall NO concentration ($J_{aw,NO}$), and the alveolar region or steady-state alveolar NO concentration ($C_{alv,NO}$) [18]. This model is able, to a certain degree, to partition eNO into an airway source that is reduced by ICS [21] and an alveolar source that is not affected by this treatment [22]. This discriminative analysis may be of particular interest in COPD due to its greater peripheral distribution of inflammation that appears to be relatively resistant to ICS.

The current authors have previously reported that MEFeNO differentiates between bronchial and alveolar inflammation in patients with asthma and COPD, and $C_{alv,NO}$ is increased in COPD patients [23]. The aim of the present study was to validate the MEFeNO measurements in COPD patients of varying severity, and to investigate the effects of smoking history and treatment on these measurements.

METHODS

Subjects

MEFeNO was measured in 81 subjects, comprising 18 non-smokers, 16 smokers, 14 with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage 0 (at risk), seven with stage 1 (mild), 11 with stage 2 (moderate), eight with stage 3 (severe) and seven with stage 4 (very severe) COPD, according to the GOLD classification (table 1).

Study design

All subjects underwent MEFeNO measurements between 09:00–10:00 h, consisting of two exhalations at each flow, which is sufficient to obtain reproducible NO results [1]. In addition, the reproducibility and diurnal variation of MEFeNO measurements were examined in 36 subjects (nine nonsmokers, 12 smokers-GOLD0, 10 with GOLD stage 1–2 and five with GOLD stage 3–4) by measuring their MEFeNO *t.i.d.* between 09:00–10:00, 12:00–13:00 and 15:00–16:00 h. Day-to-day variation, as well as the diurnal variation, of MEFeNO was assessed in eight COPD patients (GOLD2) when their MEFeNO was measured between 09:00–10:00, 12:00–13:00 and 15:00–16:00 h on two consecutive days. MEFeNO levels and forced expiratory volume in one second (FEV_1) were also measured before and 45 min after ipratropium bromide (40 μg) given *via* a spacer in randomly allocated patients to investigate any potential effect of air calibre changes on $C_{alv,NO}$, $J_{aw,NO}$ and $D_{aw,NO}$. All patients refrained from using bronchodilators before the measurements. The effects of smoking and current treatment with ICS were also assessed by comparing values in current *versus* ex-smokers and patients who were treated with and without inhaled steroids. The effect of ambient NO on $C_{alv,NO}$ was studied in five COPD patients (GOLD0–1) whose MEFeNO measurements were made after either one inhalation of NO-free air from the analyser (standard operating

TABLE 1 Subject characteristics

Variables	Nonsmokers	Smokers-GOLD 0	GOLD 1–2	GOLD 3–4
Subjects n	18	30	18	15
Age yrs	45 (37–46)	46 (42–49)	61 (42–72)	62 (55–74)
Sex M:F	8:10	16:14	10:8	9:6
Pack-yrs ex-smokers		3 (32±3)	7 (43±3)	12 (50±7)
Pack-yrs current smokers		27 (35±2)	11 (48±4)	3 (40±5)
FEV_1 L	3.4 (3.2–3.7)	3.5 (3.1–3.7)	2.3 (1.7–2.8)	0.9 (0.7–1.7)
FEV_1 % pred	101 (95–108)	102 (98–107)	73 (63–87)	34 (20–41)
FVC L	4 (3.7–4.4)	4.4 (3.9–4.7)	3.9 (3.2–4.5)	2.8 (2.5–3.2)
FVC % pred	101 (96–107)	106 (101–110)	101 (85–120)	80 (70–96)
FEV_1/FVC % pred	86 (82–90)	80 (78–81)	57 (48–66)	36 (29–40)
PEF % pred		101 (94–106)	74 (61–88)	41 (37–45)
RV % pred		116 (92–140)	140 (122–159)	195 (155–231)
TLC % pred		109 (97–121)	113 (99–127)	125 (110–139)
$T_{L,CO}$% pred		77 (60–94)	65 (54–76)	45 (35–56)
K_{CO} % pred		76 (66–86)	75 (57–93)	59 (42–70)
V_A% pred		105 (93–117)	95 (85–106)	86 (77–96)
MEF 75 % pred		85 (62–108)	50 (32–66)	9.9 (6.8–13)
MEF 50 % pred		61 (31–90)	32 (20–43)	7.3 (5–10)
MEF 25 % pred		45 (14–73)	26 (14–38)	6.7 (3.6–8)
SABA			6	7
LABA			5	10
LABA+ICS			6	12
Theophylline				4

Data are presented as mean±SEM, mean (95% confidence intervals) or n. GOLD: Global Initiative for Chronic Obstructive Lung Disease; M: male; F: female; FEV_1: forced expiratory volume in one second; % pred: per cent predicted; FVC: forced vital capacity; PEF: peak expiratory flow; RV: residual volume; TLC: total lung capacity; $T_{L,CO}$: transfer factor of the lung for carbon monoxide; K_{CO}: carbon monoxide transfer coefficient; V_A: alveolar volume; MEF 75, 50, 25: mean forced expiratory flow during the 75, 50 and 25% of the FVC; SABA: short-acting β_2 agonists; LABA: long-acting β_2 agonists; ICS: inhaled corticosteroids.

procedure for the single exhalation eNO and MEFeNO measurements in the present authors' laboratory) *versus* five consecutive breaths of NO-free air made in two sessions separated by 3 h with recording of ambient NO.

None of the subjects studied had recent (4 weeks prior the study) upper respiratory tract infections, chest infection or COPD exacerbations. All of the subjects were advised not to consume any nitrite-enriched food (*i.e.* spinach) before the study visits. The study was approved by the Ethics Committee of the Royal Brompton Hospital and Harefield NHS Trust (London, UK) and all the patients gave written informed consent.

Lung function

Measurements of FEV1 and FEV1/forced vital capacity (FVC) were made with a dry spirometer (Vitalograph-S; Vitalograph Ltd, Buckingham, UK) which met American Thoracic Society (ATS) standards. Lung volumes and carbon monoxide gas transfer were measured with a Jaeger Master Lab Compact Transfer (Erich Jaeger Ltd, Hoechberg, UK), as described previously [20].

MEFeNO measurements

Standardised single eNO (expiratory flow 50 mL·s^{-1}) was measured by a chemiluminescence analyser (NIOX®; Aerocrine AB, Stockholm, Sweden), as described previously [1]. MEFeNO was measured at expiratory rates 10, 100, 200 and 260 mL·s^{-1} by applying resistors of 10, 100, 200 and 300 cm H_2O mL·s^{-1} to create and maintain the target flow rates. MEFeNO was measured using a vital capacity manoeuvre performed in duplicate [1] to collect plateau NO concentrations. Mean eNO at each expiratory flow was calculated by the analyser during an NO plateau of $\geqslant$3 s with NO variability within 10% of the plateau or $\pm$1 ppb [1]. The patients inhaled NO-free air from the analyser and then exhaled against different linear resistors. The exhalation time was 20 s for 10 mL·s^{-1}, 10 s for 50 and 100 mL·s^{-1}, and 6 s for 200 and 260 mL·s^{-1}. The second manoeuvre (to estimate the effect of ambient NO on $C_{alv,NO}$) involved five breaths of NO-free air from the analyser followed by a standard vital capacity manoeuvre. $J_{aw,NO}$, $C_{alv,NO}$, and $D_{aw,NO}$ were calculated as previously described (Appendix 1) [18, 24].

Statistical methods

The correlation between $C_{alv,NO}$ and FEV1 and other parameters was determined using the Spearman rank correlation test. Intra-class correlation coefficient (ICC; table 2) was used for each flow separately for all the subjects. Nonparametric tests were applied as the distribution of these variables was not known and there were insufficient data for normal distribution analysis. Data were expressed as mean$\pm$SD and/or $\pm$SEM, or mean (95% confidence intervals (CI)). ANOVA was performed using the Kruskall-Wallis test for multiple comparisons. For comparison of two groups, the Mann-Whitney U-test after Bonferroni correction was used. Reproducibility was assessed by the pooled SD and Bland-Altman test. A value of $p<0.05$ was considered statistically significant.

RESULTS

MEFeNO: repeatability, reproducibility and diurnal variation

The mean pooled SD1 of all measurements was 0.32$\pm$0.60 ppb (table 2) and MEFeNO measurements were highly reproducible (fig 1). Pooled SD analysis of all the subjects at the flows 10, 50, 100, 200 and 260 mL·s^{-1} was the highest at the lowest exhalation flow of 10 mL·s^{-1} (table 2). $C_{alv,NO}$ and other measurements (data not shown) made at different visits and time points were highly reproducible and there was no significant day-to-day or diurnal variation (fig. 2).

Effect of ambient NO and NO-free air inhalation on MEFeNO

There was no effect of ambient NO on $C_{alv,NO}$, $J_{aw,NO}$ or $D_{aw,NO}$ (data not shown). There was no difference in $C_{alv,NO}$ measured after one (2.0$\pm$1.13 ppb; 95% CI 0.63–3.43 ppb; ambient NO 72$\pm$27 ppm; 95% CI 38–106 ppm) *versus* five consecutive breaths (2.0$\pm$1.45 ppb; 95% CI 0.20–3.80 ppb; ambient NO 64$\pm$29 ppm; 95% CI 28–100 ppm) of NO-free air.

Effect of bronchodilator

There was no effect of the iptratropium bromide on the $C_{alv,NO}$, $J_{aw,NO}$, $D_{aw,NO}$ and patient's lung function (fig. 2).

Effect of smoking and ICS on $C_{alv,NO}$, $J_{aw,NO}$ and $D_{aw,NO}$

There was no difference in either $C_{alv,NO}$ or $D_{aw,NO}$ between the current and ex-smokers COPD patients (3.2$\pm$0.2 *versus* 3.4$\pm$0.1 ppb and 18.1$\pm$1.4 *versus* 13.5$\pm$0.9 pL·ppb^{-1}s^{-1}, respectively;

TABLE 2 Exhaled NO at multiple expiratory flows measurements at different exhalation flows in the groups of subjects studied

	ICC	Pooled SD_1	Nonsmokers	Smokers-$GOLD_0$	$GOLD_{1–2}$	$GOLD_{3–4}$
Subjects n			18	30	18	15
eNO expiratory rate mL·s^{-1}						
10	0.977**	0.84$\pm$1.5	43.5$\pm$7.3 (39.8–47.1)	28.1$\pm$13.4 (23.1–33.1)	33.2$\pm$22.1 (22.2–42.2)	31.1$\pm$10.8 (25.1–37.1)
50	0.993**	0.30$\pm$0.36	13.5$\pm$2.4 (12.2–14.6)	10.1$\pm$4.3 (8.3–11)	14.6$\pm$7.3 (10.9–18.2)	15.2$\pm$5.7 (12.0–18.4)
100	0.992**	0.18$\pm$0.19	8.0$\pm$1.3 (7.4–8.6)	6.5$\pm$2.4 (5.5–7.4)	9.1$\pm$3.8 (7.2–11.1)	9.2$\pm$2.6 (7.6–10.5)
200	0.989**	0.12$\pm$0.13	5.2$\pm$0.7 (4.8–5.5)	4.7$\pm$1.2 (4.1–5.2)	6.6$\pm$2.1 (5.6–7.7)	6.7$\pm$1.6 (5.8–7.5)
260	0.988**	0.11$\pm$0.13	4.1$\pm$0.6 (3.8–4.4)	3.9$\pm$1.1 (3.4–4.3)	5.6$\pm$1.6 (4.8–6.4)	5.8$\pm$1.3 (5.0–6.4)
Pooled $SD_{1,total}$#		0.32$\pm$0.60				

Data are presented as mean$\pm$SD (95% confidence intervals). ICC: intra-class correlation coefficient on each flow in all subjects (Pearson correlation coefficient); pooled SD_1: pooled SD on each flow in all subjects; GOLD: Global Initiative for Chronic Obstructive Lung Disease; eNO: exhaled nitric oxide; pooled $SD_{1,total}$: pooled SD for total 360 observations (eNO of 72 subjects × 5 exhalation flows=360). **: $p<0.01$ *versus* pooled $SD_{1,total}\pm$SD.#: n=405.

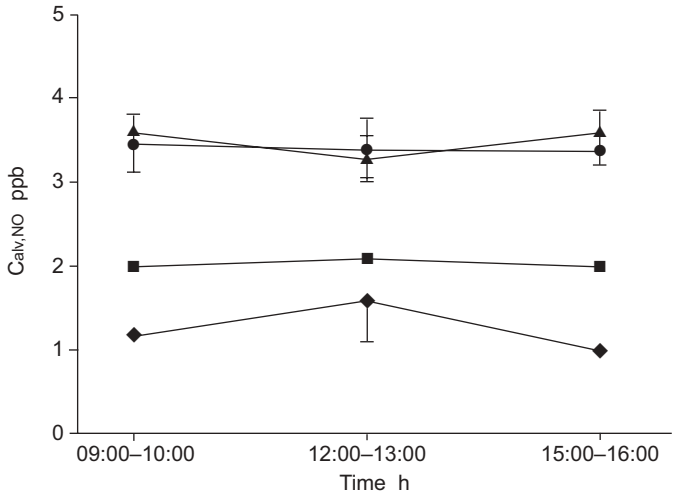

FIGURE 1. Diurnal variation of alveolar nitric oxide concentration ($C_{alv,NO}$) values in 38 subjects measured between 09:00–10:00, 12:00–13:00 and 15:00–16:00 h. ◆: healthy nonsmokers; ■: smokers-Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) stage 0; ▲: GOLD stage 1–2; ●: GOLD stage 3–4.

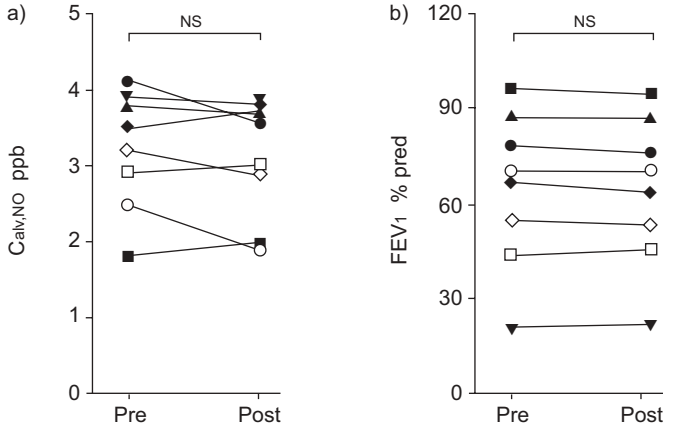

FIGURE 2. Effect of iptratropium bromide (40 μg) on a) alveolar nitric oxide concentration ($C_{alv,NO}$) and b) forced expiratory volume in one second (FEV_1) values in eight chronic obstructive pulmonary disease patients measured after 45 minutes. NS: nonsignificant. Each symbol represents a different subject.

fig. 3). Current COPD smokers, however, had lower $J_{aw,NO}$ (497 ± 88.1 pL·s^{-1}) than ex-smokers (711 ± 81, pL·s^{-1}; $p<0.01$). Although there was a trend towards a lower $J_{aw,NO}$ in COPD patients taking ICS, this was not significant. ICS did not have any effect on $C_{alv,NO}$ (no ICS 3.4 ± 0.1 ppb *versus* treatment with ICS 3.3 ± 0.2; $p>0.05$), nor on $D_{aw,NO}$ (no ICS 14 ± 0.1 *versus* treatment with ICS 17 ± 1.4 pL ppb^{-1}s^{-1}, $p>0.05$; fig. 3).

COPD severity and $C_{alv,NO}$, $J_{aw,NO}$ and $D_{aw,NO}$

There was a significant increase in $C_{alv,NO}$ in COPD patients (fig. 4a; table 3), and significant negative correlation between $C_{alv,NO}$ and FEV_1 ($r=-0.6$, $p<0.0001$), in COPD patients and normal smokers (fig. 5). The levels of $J_{aw,NO}$ (fig. 4b; table 3) were lower in smokers-GOLD0 and COPD GOLD3–4 than in healthy nonsmoking control subjects. The levels of $D_{aw,NO}$

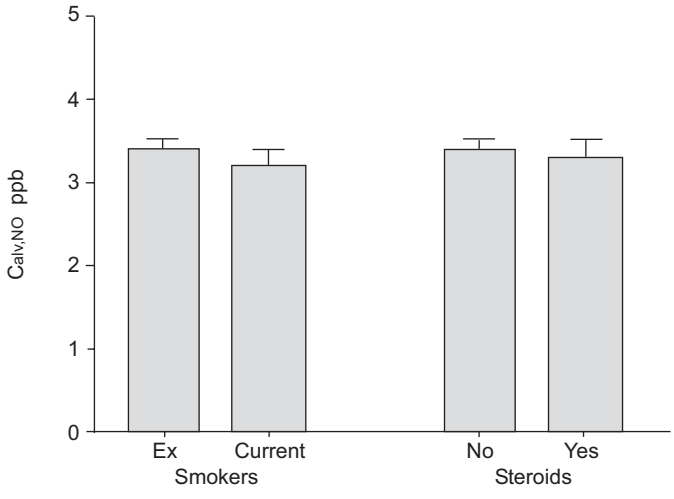

FIGURE 3. Effect of smoking and inhaled corticosteroids on the alveolar nitric oxide concentration ($C_{alv,NO}$) in chronic obstructive pulmonary disease patients.

(fig. 4c; table 3) were increased in COPD patients compared with smokers and healthy controls. A weak negative correlation ($r=-0.4$, $p=0.006$) was found between the $D_{aw,NO}$ and FEV_1 across all COPD and smoking subjects. No correlation was found between $J_{aw,NO}$ and FEV_1 or between $C_{alv,NO}$, $J_{aw,NO}$ and $D_{aw,NO}$ and alveolar volume, transfer of the lung for carbon monoxide or mean forced expiratory flow during the 25% of the FVC (data not shown).

DISCUSSION

The main finding of the present study is that the elevated eNO in COPD patients is derived, predominantly, from the periphery of the lung but is unaffected by smoking, bronchodilators or ICS. The current authors have also shown that MEFeNO measurements are highly reproducible, free of diurnal variation and can be applied in COPD patients of differing severity.

Reproducibility and variability

Identifying the variability of this novel technique is important for the potential clinical utility of the MEFeNO parameters to document longitudinal changes in COPD. The high level of reproducibility of MEFeNO measurements (ICC 0.96) was similar to the high reproducibility of the ATS-standardised eNO measurements (ICC 0.99) [1]. The variability of MEFeNO was not greater in more severe COPD patients, despite the fact that some individuals found it difficult to complete the high exhalation flow manoeuvre (260 mL·s^{-1}) before the required 6 s exhalation time. The most difficult flow to maintain was 10 mL·s^{-1} (pooled SD1 0.8 ± 1.4 ppb), because of the long duration of exhalation (20 s), but this was unrelated to the degree of airway obstruction. The lack of diurnal variability of the MEFeNO in COPD was expected considering the nature of the disease and previously published data demonstrating no diurnal variation of $C_{alv,NO}$ and $J_{aw,NO}$ in either asthmatics or patients with fibrosing alveolitis [13].

Effect of ambient NO

Ambient NO (0–138 ppm) did not influence $C_{alv,NO}$ (the lowest amongst the other NO parameters measured by

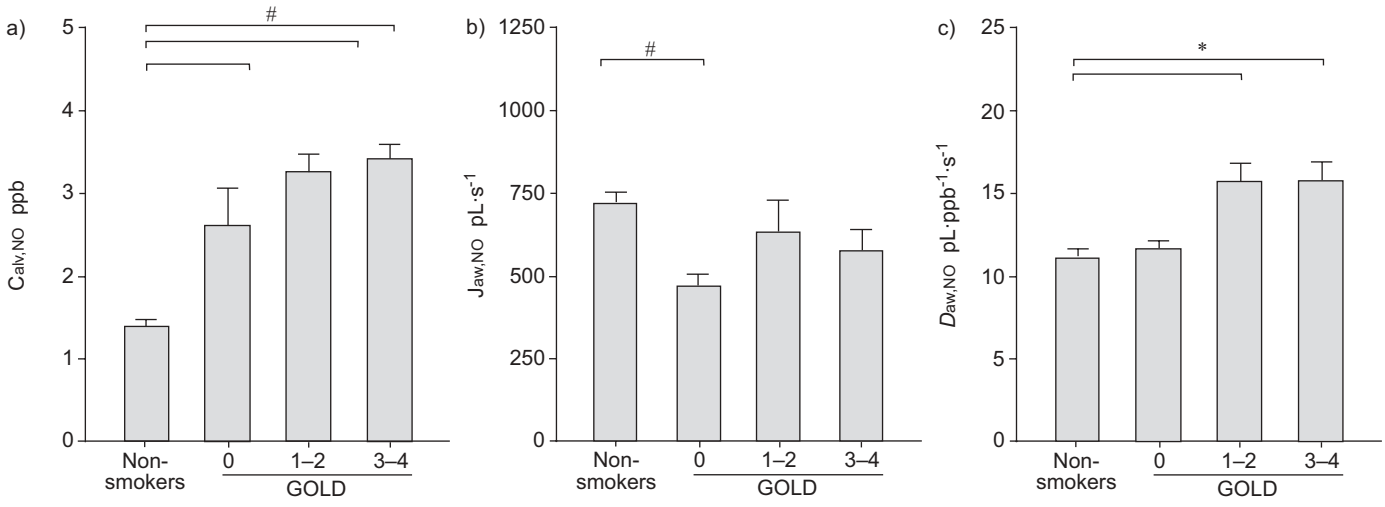

FIGURE 4. a) Alveolar nitric oxide concentration ($C_{alv,NO}$), b) airway wall NO concentration ($J_{aw,NO}$) and c) airway NO diffusing capacity ($D_{aw,NO}$) in nonsmokers, smokers and chronic obstructive pulmonary disease patients of different severity according to the classification of the Global Initiative for Chronic Obstructive Lung Disease (GOLD). *: $p<0.05$; #: $p<0.0001$.

TABLE 3 Alveolar nitric oxide concentration ($C_{alv,NO}$), airway wall NO concentration ($J_{aw,NO}$) and airway NO diffusing capacity ($D_{aw,NO}$) in the studied groups

Variables	Nonsmokers	Smokers-GOLD0	GOLD1–2	GOLD3–4
$J_{aw,NO}$ pL·s^{-1}	716.2±141.7$^{\#,\ \P}$	464.7±229.1^{+}	630.3±417.7	609.4±275.0
	712.5 (645.8–786.7)	385.6 (379–550)	442.9 (422.5–838)	555.5 (457.1–761.7)
$C_{alv,NO}$ ppb	1.4±0.4$^{\#,\ \S,\ \ddagger}$	2.1±0.7$^{\S,\ \ddagger}$	3.3±0.7	3.4±0.6
	1.3 (1.2–1.5)	2.0 (1.8–2.4)	3.2 (2.8–3.6)	3.5 (3.0–3.7)
$D_{aw,NO}$ pL·ppb^{-1}s^{-1}	11.0±2.5$^{\#\#,\ +}$	11.6±3.2$^{\#\#,\ \P}$	15.7±5.1	15.0±5.0
	11.4 (9.8–12.3)	11.2 (10.4–12.8)	14.5 (13.1–18.2)	14.2 (12.2–17.2)

Data are presented as mean±SD and median (95% confidence intervals). GOLD: Global Initiative for Chronic Obstructive Lung Disease. #: $p<0.0001$ *versus* smokers with GOLD stage 0; ¶: $p<0.05$ *versus* smokers with GOLD stage 0; +: $p<0.05$ *versus* patients with GOLD stage 3–4; §: $p<0.0001$ *versus* patients with GOLD stage 1–2; ‡: $p<0.0001$ *versus* patients with GOLD stage 3–4; ##: $p<0.05$ *versus* patients with GOLD stage 1–2.

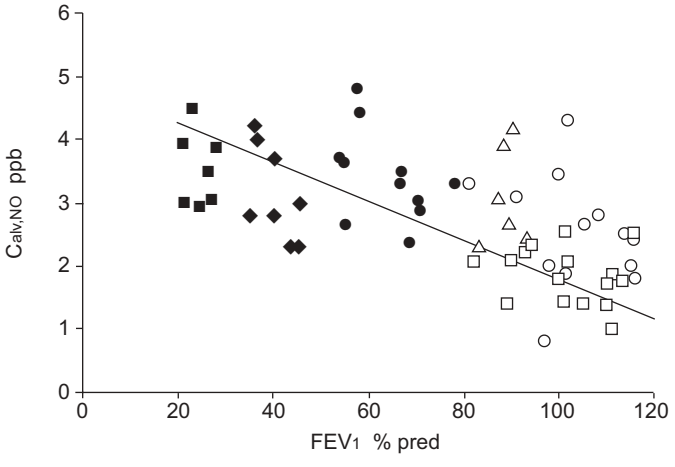

FIGURE 5. Association between the alveolar nitric oxide concentration ($C_{alv,NO}$) and forced expiratory volume in one second (FEV_1) per cent predicted (% pred) for all smokers and chronic obstructive pulmonary disease patients ($r=0.6$; $p<0.0001$). □: smokers; ○: Global Initiative for Chronic Obstructive Lung Disease (GOLD)0; △: GOLD1; ●: GOLD2; ◆: GOLD3; ■: GOLD4.

MEFeNO), and a single inhalation *versus* five inhalations of NO-free air had the same effect on $C_{alv,NO}$. This gives further support for the development of portable NO analysers capable of the MEFeNO measurements, so that $C_{alv,NO}$ might be monitored by COPD patients at home in the future.

Effect of bronchodilators

Although no changes were seen in $C_{alv,NO}$ and other NO parameters or FEV_1 after inhalation of ipratropium bromide that may reduce air trapping, it still too early to say to what extent $C_{alv,NO}$ is related to small airway obstruction and/or to small airway inflammation. Perhaps, both of these factors contribute to elevated levels of $C_{alv,NO}$ in COPD, but their contribution may only be determined once effective anti-inflammatory treatments are developed.

Effect of smoking

There was no difference in $C_{alv,NO}$ between current smokers and ex-smokers, although $J_{aw,NO}$ was reduced, thus, confirming previous studies [8, 13] which show a reduced eNO using the

single exhalation technique. An acute and transient (1–5 min) increase in $J_{aw,NO}$ has been previously reported after smoking a cigarette [13]. This is most likely to be due to release of inhaled NO from haemoglobin and/or nitrosothiols. The reduction in $J_{aw,NO}$ and eNO using the single exhalation technique [8] is likely to be due to down-regulation of both eNOS [11] and iNOS in lung epithelial cells of large airways [12].

In contrast, the increase in $C_{alv,NO}$ in COPD patients (that was almost twice as high as in smokers) is mainly from the peripheral lung and is most likely derived from iNOS in macrophages and alveolar walls that are not directly affected by smoking [6]. The present authors speculate that the elevated $C_{alv,NO}$ in COPD and its relation to the disease severity may be a manifestation of peripheral lung inflammation involving alveolar walls and small airways. Smoking may trigger this inflammatory cascade, but does not have a direct effect on the source of $C_{alv,NO}$.

Effect of corticosteroids

The role of ICS in COPD is under scrutiny, as there is little evidence of their effect on various inflammatory markers in COPD. The current study has shown no effect of ICS on $C_{alv,NO}$ or $D_{aw,NO}$ and a rather small reduction in $J_{aw,NO}$. This is markedly different from asthma, where $D_{aw,NO}$ and $J_{aw,NO}$, but not $C_{alv,NO}$ are significantly reduced by ICS [15, 22, 25]. A short course of oral steroids, but not the inhaled steroids, was able to reduce $C_{alv,NO}$ in patients with moderate asthma [26] suggesting that inhaled steroids may not reach inflammation in peripheral airways. This indicates an important advantage of MEF_{eNO} measurements in COPD for monitoring the inflammatory process that is clearly different from asthma.

COPD severity

The progression of COPD from GOLD stage 0 to GOLD stage 4 is most strongly associated with thickening of the wall of small airways by a repair or remodelling process [4], and with the intensity of the inflammation response in the walls of these airways. The present authors speculate that the severity-related increase of the $C_{alv,NO}$ in COPD may reflect this mechanism of disease progression. Low $J_{aw,NO}$ and high $C_{alv,NO}$ may have different pathophysiological effects and roles, and, therefore, may be pharmacologically corrected using different drugs, including iNOS inhibitors or NOS donors [7]. The present study has shown a significant correlation between $C_{alv,NO}$ and both FEV_1 and FEV_1/FVC ratio, which was not seen in either healthy subjects [27] or in mild asthmatics [15, 26], suggesting that $C_{alv,NO}$ in COPD patients may reflect peripheral inflammation and remodelling resulting in increased peripheral resistance. $C_{alv,NO}$ might be an early and simple marker to diagnose early stages of peripheral inflammation in COPD. Therefore, even COPD and some asthmatic patients may have a similar degree of fixed airway obstruction. The nature of the airway and lung parenchyma inflammation in these diseases is different [28] and elevated $C_{alv,NO}$ may reflect the predominant small airway inflammation in COPD.

The elevated levels of $C_{alv,NO}$ in the present study were similar to the levels reported by HOGMAN *et al*. [17], and may indicate accumulation of NO and NO-related species in the periphery of the lungs, as high iNOS expression has been reported in macrophages [5], alveolar walls, small airway epithelium and vascular smooth muscles of COPD patients [5, 6]. Significantly higher numbers of iNOS-positive cells in alveolar walls in more severe COPD patients [29] may explain the high levels of $C_{alv,NO}$ in GOLD3–4. Interestingly, the $C_{alv,NO}$ was most elevated in patients with GOLD1–2 and GOLD3–4, but there was no significant difference between these two groups. Although the number of patients in the current study with stage GOLD4 is small because of the difficulty in performing the manoeuvre, this may reflect the fact that patients with severe emphysema show a lower percentage of iNOS-positive alveolar macrophages than patients with milder disease [30]. In fact, some of the stage GOLD2 patients had $C_{alv,NO}$ levels similar to those of more severe patients.

Thickening and fibrosis of the airway walls and increased production of mucus might be expected to decrease $D_{aw,NO}$ in COPD patients by increasing the diffusion distance for NO. However, the current authors found higher $D_{aw,NO}$ in COPD patients compared with smokers-GOLD0 and healthy non-smokers. The inflammation may increase the surface area of airways producing NO by stimulating iNOS and increasing the $D_{aw,NO}$. Consumption of NO is also a possibility; *in vivo* NO reacts with several substrates like oxygen, protein thiols (glutathione) and superoxide. In COPD there are an increased number of neutrophils producing toxic radicals, including superoxide, which can react quickly with NO to form peroxynitrite, nitrite and nitrate.

Advantages and limitations

A major advantage of this noninvasive and relatively simple approach of eNO analysis is to monitor eNO, as a marker of inflammation, derived from the lung periphery and to assess the main site of inflammation in COPD. There are, however, potential sources of error in the measurements or interpretation of MEF_{eNO} values that need to be considered. The structural changes in COPD comprise mucoid impaction and atelectasis, often coexisting bronchiectasis, bronchial dilatation and bronchial wall thickening. Bronchoconstriction, small airways obstruction, air trapping, airway hypersecretion and accumulation of inflammatory mucous exudates in the lumen of small airway may result in an overestimation of $C_{alv,NO}$ production and bronchial wall thickening and airway hypersecretion may distort $D_{aw,NO}$ values. Presently, the weakness of current mathematical models, which are used to calculate the NO parameters is that these structural abnormalities are not yet integrated into the analysis. Another factor which may affect MEF_{eNO} in COPD patients of different severity is the surface area of the lung which participates in the exchange process. However, no significant correlation was found between NO exchange parameters and alveolar volume in COPD patients, nor in normal subjects. Finally, the current model that was used in the current authors' calculations assumes that NO is nonreactive. It may be speculated that increased production of superoxide in the airway by neutrophils lowers the NO concentration which may be left unaccounted for in a two-compartment model.

Conclusion

The present authors conclude that measurements of exhaled nitric oxide at multiple expired flows to determine alveolar

nitric oxide concentration reflect inflammation in the peripheral lung of patients with chronic obstructive pulmonary disease; alveolar nitric oxide concentration is not affected by inhaled corticosteroid therapy or smoking and, thus, may provide valuable additional information for assessing the inflammatory process and its response to different therapies.

APPENDIX 1. EQUATIONS FOR CALCULATING EXHALED NITRIC OXIDE CONCENTRATIONS

The equation governing the model [24, 27, 31] predicts the exhaled concentration (C_{exh}, ppb) as a function of the residence time (t_{res}) of each differential bolus of air in the airway compartment, the volume of the airway compartment (V_{air}) and the remaining three parameters ($J_{aw,NO}$, $D_{aw,NO}$, $C_{alv,NO}$).

1. $C_{exh}(t) = (C_{alv,NO} - J_{aw,NO}/D_{aw,NO}) \times e \times (D_{aw,NO}/V_{air} \times t_{res}) + (J_{aw,NO}/D_{aw,NO})$

Mathematical identification of the parameters has been previously described in detail: $C_{alv,NO}$ and $J_{aw,NO}$ can be estimated by using the slope and the intercept of a resulting linear relationship by measuring V_{NO} (elimination rate of nitric oxide (NO) from the breath during exhalation) at multiple constant exhalation flow rates (V_E):

2. $V_{NO} = C_{alv,NO} \times V_E + J_{aw,NO}$

Once $C_{alv,NO}$ and $J_{aw,NO}$ have been calculated, Microsoft Excels' solver tool was used to estimate $D_{aw,NO}$ according to the equation.

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