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Exhaled nitric oxide stability over two years in relation to COPD outcomes

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Keywords: exhaled NO, COPD, GOLD, comorbidity, lung function test, breath test

Supplementary material for this article is available online

Abstract

The non-invasive biological marker exhaled nitric oxide (FE_{NO}) is increasingly used in asthma, but its clinical role in COPD is less established. FE_{NO} has been reported to be both high and low outside the COPD exacerbation period. The study aimed to follow FE_{NO} values over two years during stable conditions in a cohort of COPD subjects participating in the TIE-study (Tools Identifying Exacerbation). The follow-up study included 353 subjects who attended three visits one year apart. The subjects that were ex-smokers ($n = 265$) had higher $FE_{NO,50}$ values (median and IQR) compared with current smokers ($n = 88$), at inclusion 15 (10, 24) versus 9 (7, 15) ppb, after one year 15 (10, 24) versus 10 (7, 18) ppb, and after two years 14 (9, 22) versus 10 (7, 17) ppb, all $p < 0.001$. All subjects were further divided into two FE_{NO} groups: <20 ppb (72%) and ⩾20 ppb (28%). After one year, 81% of the participants remained in the low group and 65% in the high FE_{NO} group. After two years, 71% remained in the low group and 52% in the high FE_{NO} group. The persistent low FE_{NO} group had statistically significantly lower FEV_1%pred and FVC%pred compared to the high FE_{NO} group for all three visits. Among the ex-smokers, the proportion of subjects reporting dyspnoea (mMRC ⩾ 2) was higher in the persistent low FE_{NO} group than in the persistent high FE_{NO} group at all three visits. In conclusion, good consistency in FE_{NO} over two years is promising for monitoring FE_{NO} during stable disease. COPD subjects with persistent low FE_{NO} had poorer lung function and reported more dyspnoea than subjects with persistent high FE_{NO}.

Abbreviations

B-eos	eosinophil blood count
B-neu	neutrophil blood count
BMI	body mass index
CAT	COPD assessment test
CCQ	Clinical COPD questionnaire
COPD	Chronic obstructive pulmonary disease
FE_{NO}	Fraction of exhaled nitric oxide
$FE_{NO,50}$	Fraction of exhaled nitric oxide at a flow of 50 ml s^{-1}
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
GOLD	Global initiative for chronic obstructive lung disease
ICS	Inhaled corticosteroids
mMRC	Modified British Medical Research Council
NO	Nitric oxide
SVC	Slow vital capacity
TIE-study	Tools Identifying Exacerbations in COPD

VC	Vital capacity
V1	First visit at baseline
V2	Second visit after one year
V3	Third visit after two years

1. Introduction

The primary objectives of COPD therapy are to manage symptoms, mitigate inflammation, and maintain lung function. Pharmacological treatment includes bronchodilators and anti-inflammatory drugs, most often ICS, but also oral corticosteroids (OSC) in COPD exacerbations, and biological treatments for severe disease forms. The anti-inflammatory treatments reduce exhaled NO (FE_{NO}). In asthma, FE_{NO} can guide treatment and monitor treatment adherence. Clinical cut-offs are established for asthma, and for example, a high likelihood of response to ICS treatment or support of diagnosis is found at FE_{NO} levels above 50 ppb [1]. In asthma, FE_{NO} levels are typically elevated due to Th2-inflammation, and some COPD patients have concurrent asthma [2–4]. Therefore, FE_{NO} values can be high or low in COPD patients during stable disease outside the exacerbation period [5, 6]. However, we do lack information on relevant cut-off levels for FE_{NO} in COPD, as well as information regarding the stability of FE_{NO} in COPD.

Patients with COPD are usually older than patients with asthma, and FE_{NO} levels increase with age [7, 8]. At exacerbation due to inflammation and upregulation of inflammatory mediators, the FE_{NO} level increases [9–11] and drops soon after treatment. FE_{NO} values in COPD may be low [12] or at the same level as those of healthy control subjects [6] despite ongoing inflammatory changes in the airway. One factor contributing to low values could be tobacco smoking, which is known to inhibit one cofactor (tetrahydrobiopterin) for NO synthase [13]. Another explanation might be that arginase, the enzyme that breaks down L-arginine (the substrate for NO production), is increased in COPD [14]. Smoke and nicotine [15] increase the activity of this enzyme. Yet another factor is the competitive inhibitor of L-arginine, asymmetric dimethylarginine (ADMA), which is elevated in COPD patients, resulting in a reduced availability of L-arginine for conversion [16]. This may account for impaired pulmonary NO production, enhanced pulmonary vasoconstriction, and disease progression [17]. All these factors may lead to cell proliferation, differentiation and collagen formation [18], summarised in a recent review [6].

COPD is a heterogeneous disease with multimorbidity expression, and depending on the subject's status, NO metabolism may vary. Our study aimed to follow FE_{NO} values over two years during stable conditions. Does the FE_{NO} value fluctuate, and what characterises a subject with persistent low FE_{NO} and persistent high FE_{NO}?

2. Material and methods

2.1. Study design and population

Patients from primary and secondary care settings with a diagnosis of COPD (ICD code J44.0, J44.1, J44.8 and J44.9) were recruited for the prospective TIE study from the central Swedish regions of Dalarna, Gävleborg and Uppsala (4). The present analysis is based on data acquired from inclusion visits made between September 2014 and September 2016 (Visit 1, V1) and the follow-up at one year (Visit 2, V2) and two years (Visit 3, V3). The Regional Review Board in Uppsala, Sweden (Dnr 2013/358) approved the study on 25 September 2013. All subjects gave written informed consent.

2.2. Methods

At inclusion, the COPD diagnosis was confirmed with spirometry using the post-bronchodilator (400 μg salbutamol) forced expiratory volume measured in the first second (FEV_1) divided by the highest VC value from a slow (SVC) or forced manoeuvre (FVC) with a ratio of <0.70 (SpiroPerfect spirometer, Welch Allyn, Skaneateles Falls, NY, USA or Jaeger Masterscreen PFT, Erich Jaeger GmbH, Würzburg, Germany). FEV_1 and FVC are expressed as percent predicted using GLI reference values [19]. Height and weight were taken at the visits to calculate the BMI.

Exhaled NO at an exhalation flow of 50 ml s^{-1} ($FE_{NO,50}$) was measured at the different study locations with NIOX VERO (Circassia AB, Solna, Sweden)) and Eco Medics CLD 88 (Eco Medics, Duernten, Switzerland). The measurements were performed following current ATS/ERS 2005 recommendations for NO measurements [20], and a NO questionnaire was filled in for each visit to limit the intra-subject variation. Blood cell counts were assessed (Cell-Dyn 4000, Abbott, Abbott Park, IL, USA and Sysmex XN-10, Sysmex America Inc, Lincolnshire, IL, USA).

Questionnaires, including the CAT, the CCQ and the modified Medical Research Council Scale (mMRC), were used together with questions regarding subject demographics, smoking habits, and medication use. The research nurse reviewed the answers to the questionnaires with the subjects. When a subject did not answer the questions regarding smoking habits in the questionnaire ($n = 2$), exhaled carbon monoxide measurements were used to define their smoking status (Smoke Check, Intramedic AB, Sollentuna, Sweden).

To classify the COPD subjects according to the severity of the disease, the GOLD grade and risk assessment version 2023 A-B-E with symptoms (CAT scale) and the number of exacerbations/hospitalisations were used. An exacerbation was defined as an unscheduled health care visit, a course of oral corticosteroids, or a course of antibiotics due to COPD deterioration. Exacerbations were taken from medical records one year before inclusion, during the study period and one year after the last visit.

2.3. Statistical analysis

To study the consistency of FE_{NO} over two years, the cut-off of 20 ppb was used [5]. A minimal measurable change is suggested as 24% of the FE_{NO} value [21]. Therefore, the variation in $FE_{NO,50}$ was set to be ±5 ppb. Hence, three groups were created: persistently low FE_{NO} (<20 ppb at all three time points), persistently high (⩾20 ppb at all three time points) and variable FE_{NO} (switching between <20 ppb and ⩾20 ppb between visits). The analyses were performed separately for current smokers and ex-smokers. Data in the results are given as *n* (%), mean ± SD and median (interquartile range). For the statistical calculations, Binomial test, Pearson χ^2-test and non-parametric test, i.e. Mann–Whitney U test, Kruskal-Wallis test, related-sample Friedman´s two-way analysis of variance, Spearman's rho and a post hoc test Tukey HSD, (SPSS, v. 24 for Windows, SPSS Inc., Chicago, MI, USA) were used. A *p*-value of $p < 0.05$ was considered significant.

3. Results

3.1. FE_{NO} at inclusion

At inclusion in the TIE-study, our COPD subjects ($n = 572$) had a 94% success rate in the $FE_{NO,50}$ measurements. There was no difference in spirometry values between the subjects who could perform the exhalation for NO measurements and those who could not (data not shown). The median (Q1, Q3) FE_{NO} level at inclusion was 13 (8, 20) ppb. The 5th percentile was 5 ppb, and the 95th percentile was 42 ppb.

3.2. FE_{NO} over the study period

Included in the follow-up of FE_{NO} over two years are subjects who attended all three visits ($n = 353$). Divided by smoking status, GOLD grades (1–4) and GOLD groups (A, B and E) are presented in figure 1. The subjects that were ex-smokers ($n = 265$) had higher FE_{NO} values compared with current smokers ($n = 88$), at inclusion 15 (10, 24) versus 9 (7, 15) ppb, after 1 year 15 (10, 24) versus 10 (7, 18) ppb, and after two years 14 (9, 22) versus 10 (7, 17) ppb, all $p < 0.001$. There was no statistically significant difference in FE_{NO} between the three visits for the ex-smokers or for the smokers.

3.3. FE_{NO} stability

The subjects were further divided into two FE_{NO} groups: <20 ppb (72%) and ⩾20 ppb (28%), as shown in figure 2. After one year, 81% of the participants remained in the low group and 65% in the high FE_{NO} group. After two years, 71% remained in the low group and 52% in the high FE_{NO} group. The intra-class correlation between inclusion and one-year follow-up was 0.76 (0.70, 0.80), and 0.66 (0.58, 0.72) between inclusion and two-year follow-up.

Many subjects had variable FE_{NO} over the two years, as seen in figure 2. Variability was defined as switching sometimes under 20 ppb and sometimes over, with at least a 5 ppb change. Therefore, they were further divided into variable FE_{NO}, which was found in 31% ($n = 114$), consistently low <20 ppb was found in 50% ($n = 184$) over the two years, and consistently high was found in 15% ($n = 55$). Characteristics of these FE_{NO} groups at inclusion (visit 1), after one year (visit 2), and after two years (visit 3) of ex-smokers are presented in table 1, inflammatory markers are presented in table 2, and responses to questionnaires and information from journal records are presented in table 3. The same information is summarised for current smokers in table S1.

3.4. Lung function and FE_{NO}

The low FE_{NO}-group had statistically significantly lower FEV_1%pred and FVC%pred compared to the high FE_{NO}-group for all three visits, $p = 0.003$, $p < 0.001$ and $p = 0.004$ for FEV_1%-pred., and for FVC%-pred., $p = 0.015$, $p = 0.003$, and $p = 0.012$.

There was no statistically significant difference in FEV_1%-pred. or FVC%-pred. in ex-smokers and current smokers at inclusion. In ex-smokers, there was a lung function decline between visit 1 and visit 3, FEV_1%-pred from 61 to 60 ($p = 0.022$) and FVC%-pred. from 86 to 83 ($p < 0.001$). There were no statistically significant differences among current smokers.

The FE_{NO} showed a weak correlation with FEV_1%pred at all visits, rho 0.13, $p = 0.015$, rho 0.10, $p = 0.05$, and rho 0.17, $p = 0.002$, respectively. The correspondent results for FVC%pred are rho 0.12, $p = 0.023$, rho 0.09, $p = 0.10$, and rho 0.17, $p = 0.002$.

3.5. Inflammatory markers

There was a statistically significant difference in B-Lymphocytes, 2.0 (1.5, 2.4) versus 2.1 (1.6, 2.7) 10^9 l^{-1}, $p = 0.013$, B-Leukocytes, 7.2 (6.1, 8.6) versus 7.9 (6.9, 9.4) 10^9 l^{-1}, $p = 0.001$ and B-Neu 4.2 (3.5, 5.3) versus 4.9 (5.0, 6.7) 10^9 l^{-1}, $p = 0.003$, between ex-smokers and current smokers. There were no differences in B-Eos, fibrinogen or CRP. There were no differences in any inflammatory markers between the three FE_{NO} groups (tables 2 and S1). In ex-smokers, a weak correlation was found between FE_{NO} and B-Neu (rho −0.16, $p = 0.009$), B-Eos (rho 0.21, $p < 0.001$), B-Lymphocytes (rho −0.14, $p = 0.022$), and B-Leukocytes (rho −0.16, $p = 0.01$) at inclusion. Further *dividing the group, it was found* that only the constantly high FE_{NO} group over the two years showed a correlation between FE_{NO} and B-Eos (rho 0.37, p = 0.010) and between FE_{NO} and B-Neu rho −0.29, $p = 0.048$. In current smokers,

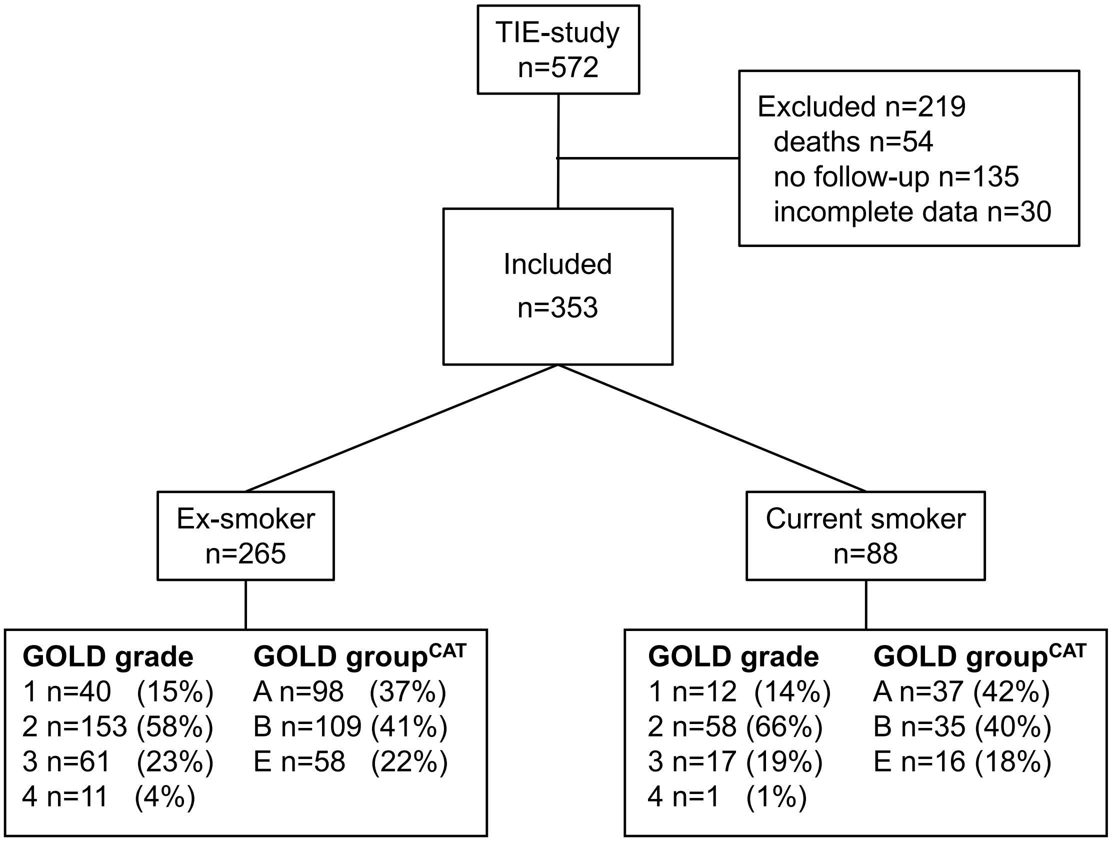

Figure 1. Flow chart of COPD subject excluded, included and grouped according to GOLD 2023.

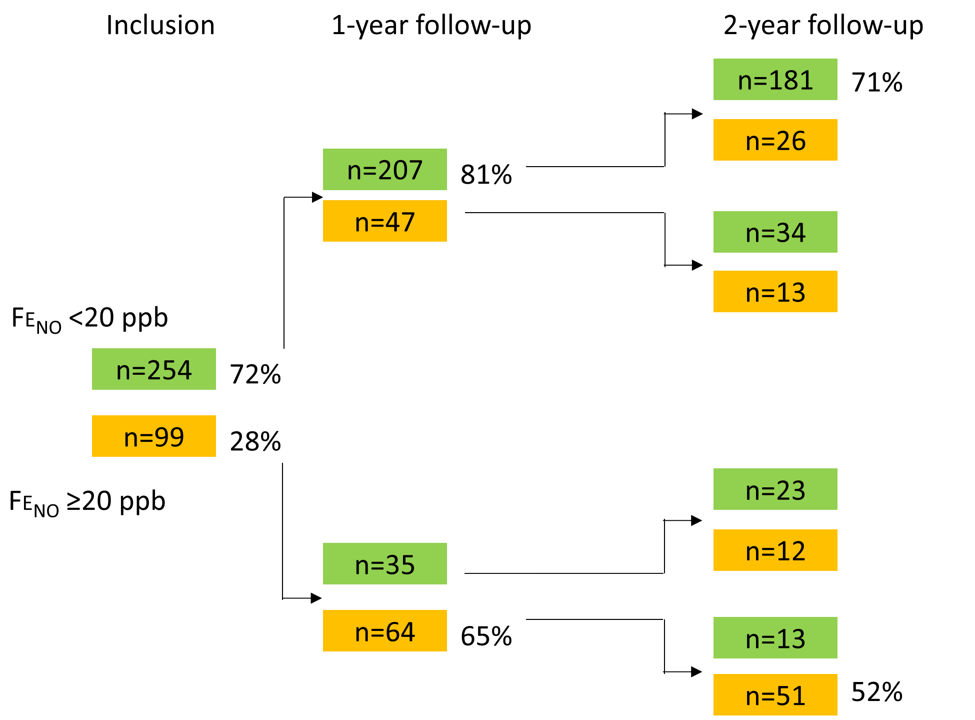

Figure 2. $FE_{NO,50}$ consistency in stable COPD over two years. Green box $FE_{NO,50} < 20$ ppb, yellow box $FE_{NO,50} \geqslant 20$ ppb.

Table 1. Subject characteristics for ex-smokers are divided into groups of $FE_{NO,50}$ at inclusion, inclusion visit (V1), after one year, visit two (V2), and after two years, visit 3 (V3).

		Ex-smokers ($n = 265$)		
$FE_{NO,50}$		Consistent low <20 ppb $n = 121$	Variable $n = 96$	Consistent high ⩾20 ppb $n = 48$
Age yrs		70 ± 7	69 ± 7	67 ± 6
Sex female		58%	52%	40%
BMI		28 ± 5	27 ± 5	28 ± 4
SpO_2		94 (93,96)	94 (93,96)	94 (93,96)
GOLD				
Grade 1		12%	15%	25%
Grade 2		58%	57%	58%
Grade 3		25%	24%	15%
Grade 4		5%	4%	2%
Group A		36%	35%	43%
Group B		46%	38%	38%
Group E		18%	27%	19%
Lung function GLI				
FEV_1-% pred.	V1	59 (47,69)	61 (47,75)	70 (53,79)
	V2	58 (47,69)	60 (47,75)	72 (52,81)
	V3	59 (45,70)	60 (45,74)	69 (53,80)
FVC -% pred.	V1	84 (74,96)	87 (74,95)	92 (79,102)
	V2	82 (71,92)	83 (71,94)	91 (79,100)
	V3	82 (68,93)	82 (68,96)	88 (74,98)
FEV_1/FVC z-score	V1	−2.7 (−3.5,−2.0)	−2.6 (−3.4,−1.9)	−2.4 (−3.3,−1.6)
	V2	−2.6 (−3.3,−1.9)	−2.5 (−3.1,−1.7)	−2.0 (−2.9,−1.5)
	V3	−2.4 (−3.3,−1.8)	−2.4 (−3.2,−1.7)	−2.2 (−3.2,−1.4)

Data are presented as mean ± SD, median (IQR), and percentage (%). Abbreviations: $FE_{NO,50}$ Exhaled nitric oxide at 50 ml s^{-1}; FEV_1, Forced expiratory volume at 1 s; FVC, Forced vital capacity; GOLD, Global initiative for chronic obstructive lung disease.

Table 2. Inflammatory markers in ex-smokers ($n = 265$), divided into groups of $FE_{NO,50}$ at inclusion, visit one (V1), after one year, visit two (V2), and after two years, visit three (V3).

$FE_{NO,50}$		Consistent low <20 ppb $n = 121$	Variable $n = 96$	Consistent high ⩾20 ppb $n = 48$
$FE_{NO,50}$ ppb	V1	11 (8, 13)	18 (14, 25)	32 (27, 50)
	V2	11 (8, 13)	20 (15, 25)	37 (27, 49)
	V3	10 (7, 13)	18 (13, 23)	31 (25, 49)
B-Neu 10^9 l^{-1}	V1	4.4 (3.6, 5.3)	4.2 (3.4,5.2)	4.0 (3.0, 4.6)
	V2	4.3 (3.5,5.4)	4.2 (3.3,5.3)	3.7 (3.0,4.7)
	V3	4.4 (3.7,5.5)	4.1 (3.2,5.1)	3.6 (3.2,4.8)
B-Eos 10^9 l^{-1}	V1	0.19 (0.10,0.22)	0.20 (0.10,0.30)	0.22 (0.10,0.40)
	V2	0.18 (0.10,0.30)	0.20 (0.10,0.30)	0.20 (0,16,0.36)
	V3	0.18 (0.10,0.22)	0.20 (0.10,0.30)	0.20 (0.10,0.30)
B-lymphocytes 10^9 $l^{-1.}$	V1	2.0 (1.6,2.4)	1.9 (1.5,2.4)	2.0 (1.4,2.4)
	V2	1.9 (1.5,2.4)	2.0 (1.5,2.5)	1.9 (1.4,2.4)
	V3	2.0 (1.5,2.4)	2.0 (1.6,2.5)	1.8 (1.3,2.3)
B-fibrinogen g l^{-1}	V1	3.5 (3.0,4.0)	3.5 (3.1,3.8)	3.4 (3.1,3.9)
	V3	3.5 (3.1,4.1)	3.7 (3.1,4.0)	3.4 (3.0,3.9)
CRP mg l^{-1}	V1	2.4 (1.3,4.9)	2.2 (1.0,4.9)	2.6 (1.2,4.4)
	V3	2.5 (1.1,5.2)	2.9 (1.2,4.9)	2.0 (0.8,3.1)

Data are presented as median (IQR). Abbreviations: $FE_{NO,50}$, Exhaled nitric oxide at 50 ml s^{-1}; B-Eos, blood eosinophil count; B-Neu, blood neutrophil count; CRP, C-reactive protein.

Table 3. Information obtained from questionnaires and patient records of ex-smokers ($n = 265$), divided into groups of $FE_{NO,50}$ at inclusion, visit one (V1), after one year, visit two (V2), and after two years, visit three (V3).

$FE_{NO,50}$		Consistent low <20 ppb $n = 121$	Variable $n = 96$	Consistent high ⩾20 ppb $n = 48$
Comorbidities				
Asthma		34%	32%	33%
Chronic bronchitis		33%	26%	27%
Cardiovascular		54%	48%	44%
CAT	V1	12 (6,16)	10 (7.16)	10 (5,15)
	V2	11 (7,18)	10 (6,15)	10 (6,13)
	V3	12 (6,18)	11 (7,16)	8 (6,16)
CAT ⩾ 13	V1	42%	40%	35%
	V2	45%	37%	33%
	V3	50%	39%	33%
mMRC ⩾ 2	V1	54%	43%	25%
	V2	50%	38%	26%
	V3	54%	43%	33%
CCQ total	V1	1.5 (0.8,2.4)	1.3 (0.7,2.4)	1.4 (0.7,2.1)
	V2	1.5 (0.7,2.6)	1.4 (0.7,2.1)	0.9 (0.6,1.7)
	V3	1.6 (0.8,2.6)	1.3 (0.8,2.4)	1.0 (0.5,0.7)
CCQ ⩾ 1.55	V1	48%	47%	46%
	V2	50%	41%	27%
	V3	50%	45%	26%
Exacerbation* and medication				
Episodes				
0–1		68%	62%	56%
2–3		15%	23%	33%
4 and over		17%	15%	11%
ICS** last 6 months	V1	70%	69%	69%
	V2	65%	68%	74%
	V3	65%	69%	69%

*One year before the first visit (V1) at baseline to one year after the last visit (V3) after two years; **ICS alone or in combination with LABA/LAMA. Data are presented as median (IQR) and percentage.
Abbreviations: CAT, COPD Assessment Test; CCQ, Clinical COPD questionnaire; CRP, C-reactive protein; $FE_{NO,50}$, exhaled nitric oxide at 50 ml s^{-1}; ICS, inhaled corticosteroids; mMRC, Modified Medical Research Council dyspnoea scale.

$FE_{NO,50}$ did not correlate with any inflammatory marker.

3.6. Questionnaires

CAT and CCQ showed no difference in smoking status, nor were there differences among the three FE_{NO} groups (tables 3 and S1). The frequency in mMRC ⩾ 2 was higher among ex-smokers at all visits, at V1 $p = 0.004$, V2 $p = 0.007$, and V3 $p = 0.023$. Among the ex-smokers, the low FE_{NO} group had more subjects with mMRC ⩾ 2 than the high FE_{NO} group, as shown in figure 3. The current smokers showed no difference in mMRC ⩾ 2 between the FE_{NO} groups.

The response to the questionnaires about comorbidities showed no difference in the frequency of asthma, chronic bronchitis or cardiovascular disease (atrial fibrillation, myocardial infarction, ischemic heart disease or hypertension) between low, high or variable FE_{NO}. See tables 3 and S1.

3.7. Exacerbations

In stable disease, there is no statistically significant difference in the frequency of exacerbation and the FE_{NO}-value, belonging to the high or low group or belonging to ex-smokers or current smokers. See tables 3 and S1. Subjects with a lower lung function (FEV_1%pred and FVC%pred) were more likely to have an increased frequency of exacerbations over the study period when adjusted for smoking habits ($p < 0.001$).

4. Discussion

This study in COPD subjects during stable disease found good consistency in FE_{NO} over two years. FE_{NO}

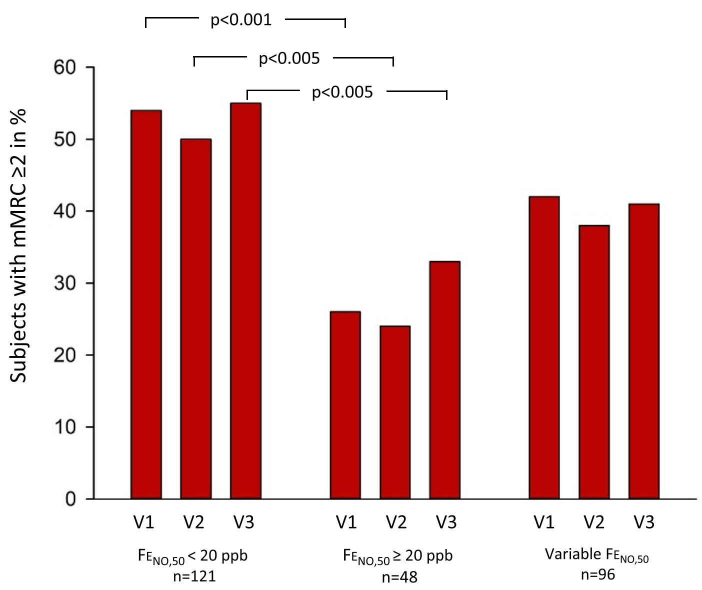

Figure 3. Modified Medical Research Council (mMRC) dyspnoea scale of 2 or above during the 2 years for ex-smokers. Visit one at inclusion (V1), visit two after on year (V2) and visit three after two years (V3).

tended to be lower with lower lung function. In ex-smokers, dyspnoea was less reported in subjects with high FE_{NO}. There was no difference in comorbidities or exacerbation frequency between FE_{NO} groups (low, variable or high) over the two years.

The success rate for our exhaled NO measurements was high, even in patients with respiratory diseases such as COPD. The same person instructed the participants during the study period. COPD subjects with GOLD stage 3–4 can perform FE_{NO} measurements as well as healthy subjects [21]. Another study found poor compliance with the procedure in 9.6% [22]. Bhowmik *et al* reported a success rate of 80% [23], but that was over twenty years ago, and we now have more user-friendly measurement procedures.

In our COPD subjects, there was a clear difference in FE_{NO} between ex-smokers and current smokers. Many studies on COPD do not separate smokers, and in our study, the median value was 13 ppb. This is the same level found in another study, not separated smokers [22]. Our ex-smokers had a FE_{NO} value similar to healthy controls [8, 24–26] and not in line with the meta-analysis [5] as referred to when we set the cut-off level of 20 ppb. It was found that 28% had a value above 20 ppb. Ambrosino *et al* set a cut-off at 25 ppb and found 32% over that cut-off [22].

Interestingly, subjects with persistently high FE_{NO} reported a lower frequency of dyspnoea and exhibited better lung function. Additionally, other researchers report that individuals with high FE_{NO} levels at admission for an exacerbation demonstrated greater improvement in lung function [27], leading the authors to suggest that FE_{NO} may predict treatment response. At a follow-up of this study three years later, they found that the low FE_{NO} group had a higher frequency of exacerbations and hospital admissions [28]. We found that FE_{NO} levels during stable disease were not associated with an increased frequency of exacerbations; however, they were associated with lower lung function. One limitation of our study is the low number of study participants. Only 51 of the 99 participants had consistently high FE_{NO} levels in stable condition over the study period. We found no difference in exacerbation rates or self-reported chronic bronchitis or asthma, which could contribute to high FE_{NO} levels. It is known that FE_{NO} levels rise before an exacerbation [10], remain high upon hospital admission, and decline following treatment [11, 27]. One needs to know the baseline value to recognise a rise in FE_{NO}. When that is set, FE_{NO} could be a marker to monitor closely in primary care and for home monitoring with today's simpler sensor NO devices. In 2006, home monitoring was already suggested for allergen exposure during springtime [29]. It was found that FE_{NO} increased a few days before the worsening of the respiratory symptoms. Such studies have not been performed in COPD yet, but a clear-cut increase appears to be found in exacerbation [10].

A potential role of FE_{NO} monitoring may be in the use of biologics for type 2 inflammation. It has been reported that dupilumab has a clear clinical effect, with a clear-cut decrease in FE_{NO} of a mean value of 10 ppb, even among smokers [30, 31]. This highlights the importance of knowing the patient's 'personal best' FE_{NO} value, as the biologics also affect smokers' FE_{NO}, who are known to have decreased levels. We only had seven currently smoking subjects with high FE_{NO}. It was interesting that they reported a high percentage of asthma. We found a correlation among our ex-smokers between FE_{NO} and B-Eos in the group with consistently high FE_{NO} values only. This favours a non-invasive biomarker such as FE_{NO}, and most speculatively, could it replace B-Eos when evaluating the treatment effects? The complexity of FE_{NO} in COPD can be demonstrated in our study by the subjects with concomitant asthma, who were not related to the FE_{NO} stability patterns, as approximately one-third of ex-smoking subjects had asthma in all three FE_{NO} groups.

In conclusion, good consistency in FE_{NO} over two years is promising for monitoring FE_{NO} during stable disease. Subjects with consistently low FE_{NO} had characteristics of more severe disease than subjects with consistently high FE_{NO}, despite no difference in the reported co-morbidities. Future studies are needed to explore this further and to study specifically suitable cut-offs to predict the effects of anti-inflammatory treatments and the clinical values of longitudinal measurement to identify disease worsening early.

Data availability statement

Swedish personal data protection legislation prohibits the free sharing of study data. However, upon reasonable request and after approval by the Swedish Ethical Review Authority (registrator@etikprovning.se), the corresponding author can make anonymised patient-level data available.

The data that support the findings of this study are available upon reasonable request from the authors.

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Conflict of interest

All authors declare no competing financial or non-financial interests.

Ethics

The Regional Review Board in Uppsala, Sweden (Dnr 2013/358) approved the study on 25 September 2013. All subjects gave written informed consent.

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