



ORIGINAL ARTICLE

Single breath nitrogen test as predictor of lung function decline and COPD over an 8-year follow-up



F. Pistelli^{a,b,*}, D.L. Sherrill^c, F. Di Pede^{b,d,1}, S. Baldacci^d, M. Simoni^d, S. Maio^d, L. Carrozzi^{a,b}, G. Viegi^d

^a Department of Surgical, Medical and Molecular Pathology and Critical Care Medicine, University of Pisa; Via P. Savi, 10 56126 Pisa (Italy)

^b Pulmonary Unit, Cardiothoracic and Vascular Department, Pisa University Hospital; Via Paradisa, 2 56124 Pisa (Italy)

^c Asthma and Airway Disease Research Center, University of Arizona; 1501 N. Campbell Avenue - 85724 Tucson, AZ (USA)

^d Pulmonary Environmental Epidemiology Unit, CNR Institute of Clinical Physiology, Pisa, Italy, Via Trieste, 41 56126 Pisa (Italy)

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Abstract

Background: The single breath nitrogen (SBN₂) test was proposed for early detection of “small airways disease” in the seventies. Few longitudinal studies have subsequently evaluated the relationships between SBN₂ test measurements and lung function decline or COPD incidence.

Aim: This study evaluates whether SBN₂ test abnormalities may be significant predictors of lung function decline and COPD incidence over an 8-year follow-up.

Study Design and Methods: In this longitudinal study, 907 adults (20+ years old; 56% males) from the prospective Po River Delta epidemiological study underwent SBN₂ test at baseline and spirometry testing at both baseline and follow-up 8-year apart. Multinomial and multiple regression models were used to assess associations of SBN₂ indexes and rates of FEV₁ decline or risk of COPD incidence over time, after adjusting for sex, height and baseline age, FEV₁ and smoking status. COPD was defined according to either GOLD or ATS-ERS criteria.

Results: Among SBN₂ indexes, only the slope of alveolar plateau (N₂-slope) was significantly associated with rates of FEV₁ decline (7.93 mL/year for a one-unit change in N₂-slope, p<0.0001), and with an increased risk of developing COPD as defined by GOLD (RR 1.81, 95%CI 1.29-2.52, mild; RR 2.78, 95%CI 1.70-4.53, moderate or severe obstruction) and ATS-ERS criteria (RR 1.62, 95%CI 1.14-2.29, mild; RR 3.40, 95%CI 1.72-6.73, moderate or severe obstruction).

Conclusion: In this population-based study, N₂-slope from SBN₂ test is a significant predictor of lung function decline and COPD incidence over an 8-year follow-up, confirming the role of the “small airways disease” in the natural history of COPD.

* Corresponding author.

E-mail address: francesco.pistelli@unipi.it (F. Pistelli).

¹ With regard to the manuscript submitted, we inform you that the coauthor Francesco Di Pede is deceased.

Introduction

Small airways disease (SAD) has long pathophysiologically linked to chronic obstructive pulmonary disease (COPD).^{1,2} In the seventies and eighties, early detection of SAD due to cigarette smoking was attempted by using single breath nitrogen (SBN₂) test. Single breath nitrogen test abnormalities were shown to discriminate smokers from non-smokers in selected populations³ and symptomatic from asymptomatic subjects in general population samples.⁴ Conversely, other studies found abnormalities only in small proportions of those with a respiratory disorder not otherwise suspected or diagnosed;⁵ SBN₂ test measurements were less sensitive than those from spirometry.⁶ Few longitudinal studies evaluated the relationships between SBN₂ indexes and lung function decline. In US, an abnormal slope of phase 3 or alveolar plateau (N₂-slope) failed to identify those with an accelerated forced expiratory volume in one second (FEV₁) decline.⁷ In 50-60 year old men from a 7-year population-based Swedish study, an increased baseline N₂-slope was associated with a higher FEV₁ decline.⁸ A higher baseline closing capacity (CC) was associated with subsequent loss of FEV₁ in adults followed-up for 4.7 years.⁹ In two different cohorts, CC/Total Lung Capacity ratio (CC/TLC) was significantly associated with FEV₁ decline.¹⁰ Baseline N₂-slope correlated with FEV₁ and FEV₁/Vital Capacity (VC) ratio at 13-year follow-up, in a group of 56 male smoking steelworkers.¹¹

After 1980s, the prevailing opinion was that SBN₂ test offered no or little advantage over monitoring individuals through spirometry. A renewed interest in SAD has occurred in the new millennium.¹² Small airway pathological findings were related to COPD progression.¹³ Multiple breath wash-out test was shown to be abnormal after only 10 pack-years of smoking.¹⁴ N₂-slope deterioration over 6 years was associated with exhaled nitric oxide measured in both smokers and non-smokers.¹⁵ Further studies were conducted in the last decade. Single breath nitrogen test was shown to be useful in assessing the effects of inhaled medicines on small airways in asthma, by Scichilone et al.,¹⁶ and in COPD, by Pechiari et al.¹⁷ Timmins et al.¹⁸ showed that the severities of COPD and airflow obstruction are independently predicted by the extent of both SAD and emphysema. Khurana et al.¹⁹ found that COPD patients with persistent sputum production had higher closing volume (CV). Boeck et al.²⁰ demonstrated that N₂-slope was increased in COPD patients compared with healthy subjects and associated with FEV₁ predicted. Olofson et al. showed that the cumulative COPD event incidence rate over a 38-year follow-up increased with increasing of N₂-slope,²¹ and it was shown to be higher among heavy smokers with the highest N₂-slope and lowest FEV₁.²²

In this context, the availability of longitudinal data from a population-based study carried out in Italy in 1980-1991 prompted us to evaluate whether SBN₂ test abnormalities are related to lung function decline and COPD incidence over an 8-year follow-up.

Methods

Participants

Data from the Po River Delta prospective study were analyzed. A baseline survey was carried out in 1980-82 on 3284 subjects, of whom 2136 (65%) were followed-up in 1988-91.²³⁻²⁷ This was a population-based study, which included subjects aged 8-64 yrs. Fig. 1 shows the selection of the study subjects, i.e., those aged ≥ 20 yrs, who participated at both baseline and follow-up survey, and had acceptable SBN₂ test measures at baseline along with FEV₁ measures at both baseline and follow-up.

The study protocol was approved by an Internal Revision Board within the Preventive Medicine Targeted Project of the Italian National Research Council.

Measurements

In both surveys, a computerized pneumotachograph (Fleish No. 3) (Pulmonary System 47804/S, Hewlett-Packard, Waltham, Massachusetts, USA) was used.^{27,28} The measurement protocol fulfilled the American Thoracic Society (ATS) recommendations in force at the time of the survey,²⁹ except for the end-point criterion of the forced expirogram.³⁰ Predicted values were computed according to reference equations derived within the baseline survey.^{23,28}

Single breath nitrogen test was performed according to the method of Buist et al.³¹, by using a heated pneumotachograph (Fleisch No. 1) with an attached nitrogen analyzer (Hewlett Packard type 47302A).²³ The pneumotachograph was daily calibrated through a 3 liters syringe. After a slow and complete expiration to residual volume (RV), the valve automatically started the delivery of 100% oxygen, the subject slowly inspired to TLC and then exhaled to RV, maintaining the flow between 0.3 and 0.5 L/s. A maximum of four trials were performed to obtain at least one acceptable tracing. The following indexes were obtained: N₂-slope, CV, CC, CV/VC%, and CC/TLC%. The slope of alveolar plateau was expressed as percent increase of nitrogen concentration per liter of expired volume during phase 3. The first convincing departure from this straight line was detected by the computer algorithm to indicate the beginning of phase 4 and to calculate CV and CC.

Statistical analysis

Chronic obstructive pulmonary disease was defined as presence of airway obstruction according to either Global Initiative for Chronic Obstructive Lung Disease (GOLD)³² or ATS-European Respiratory Society (ERS)³³ criterion. Longitudinal categories of COPD were defined selecting those subjects who had no airway obstruction at baseline and no (0-0), mild (0-1) or moderate/severe (0-2) obstruction at follow-up (Appendix A).

The rate of change in FEV₁ (Δ FEV₁) for each subject was computed as the difference between follow-up and baseline measured (or percentage predicted) values divided by the duration of individual follow-up time in years (mL/year or %pred/year). Predicted values were computed according to Paoletti et al. 1986.²⁸ Associations between Δ FEV₁, as continuous dependent variable, and SBN₂ indexes, as continuous independent variables, were assessed using multiple linear regressions. Each SBN₂ index was fitted independently. Sex, height and baseline age, smoking status (current, ex, never), and FEV₁ were included as potential confounders. Interaction terms between sex and SBN₂ indexes were tested as indicators of gender differences. To verify the results of the multiple linear regression analyses, bootstrapping was applied as method of model validation.

The associations between baseline SBN₂ test indexes and COPD incidence were assessed using multinomial regression models, both for GOLD and ATS-ERS criteria, by adjusting for sex, height and baseline age and smoking status. For each criterion, there were three COPD categories: GOLD (0-0), reference category; GOLD (0-1); GOLD (0-2); ATS-ERS (0-0), reference category; ATS-ERS (0-1); ATS-ERS (0-2). Descriptive statistics are reported as means and proportions; differences between those included and excluded from the analyses were tested using Student's t test for unpaired data and Chi-Square test, respectively. All statistical tests were made assuming a two tailed alternative and alpha = 0.05 significance level.

All statistical analyses were performed in Stata, version 17 (Stata Corporation, College Station, TX).

Results

Characteristics of the study subjects

Descriptive statistics for continuous demographic and lung function values are listed in Table 1 and frequencies for categorical variables are listed in Table 2, stratified by inclusion. There were 907 adult subjects aged ≥ 20 yrs who had acceptable SBN₂ indexes at baseline and FEV₁ measures at both baseline and follow-up, necessary for calculation of Δ FEV₁ (Fig. 1). Of the 907 subjects who completed SBN₂ test, CC was obtained in 761 subjects and CV in 776 subjects. Subjects included in the analyses were significantly younger, slightly taller, had better FEV₁, and lower Δ FEV₁ rates than those excluded. Included participants were on average 37.4 years old (age range 20-63 yrs), 56% males, and 49.8% current smokers at baseline. Among subjects without airway obstruction at baseline, approximately 10% developed COPD at follow-up according to GOLD (8% mild and 2% moderate or severe obstruction), while approximately 5% developed COPD according to ATS-ERS (4% mild and about 1% moderate or severe obstruction) (Table 2).

Out of 769 and 836 subjects without airway obstruction at baseline, according to GOLD and ATS-ERS criteria, there were 75 (9.7%) and 103 (12.3%) subjects, respectively, with abnormal N₂-slope as defined according to Viegi et al. 1988.²³

Single breath nitrogen test as predictor of lung function decline

There was no statistically significant correlation between Δ FEV₁ and CC, CV, CV/VC%, or CC/TLC%, in models fitted

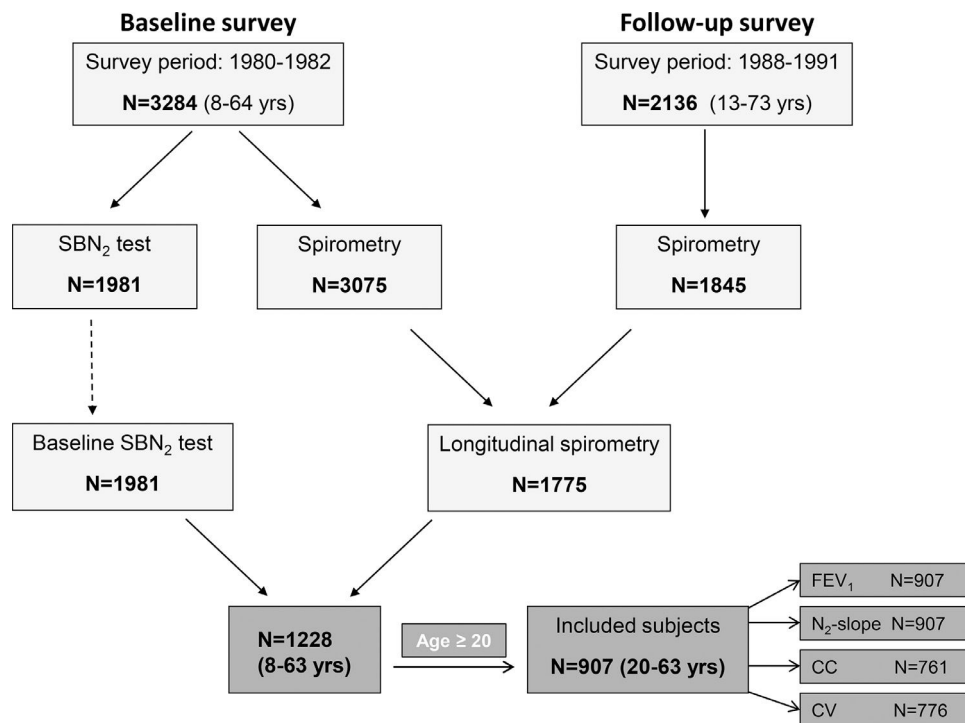


Fig. 1 Flow-chart of the selection of study subjects. FEV₁ = forced expiratory volume in one second; N₂-slope = slope of the single breath nitrogen test phase 3 or alveolar plateau; CC = closing capacity; CV = closing volume.

Table 1 Descriptive statistics: means and standard deviations by inclusion.

Variable	Subjects included in analyses			Subjects excluded from analyses		
	N	Mean	Standard Deviation	N	Mean	Standard Deviation
Age (years)*	907	37.4	11.1	1522	43.4	12.4
FEV ₁ (L)*	907	3.3	0.7	1357	2.9	0.8
FEV ₁ (%pred)*	907	100.4	14.0	1357	96.1	16.0
ΔFEV ₁ (mL/year)*	907	−20.4	30.3	377	−24.2	26.5
ΔFEV ₁ (%pred/year)	907	−0.1	1.2	377	−0.1	1.0
Height (cm)*	907	165.9	9.2	1506	162.7	9.2
Weight (kg)	907	68.9	12.3	1503	69.1	13.1
N ₂ -slope (%N ₂ /L)	907	1.2	0.8	556	1.3	1.0
CC (L)	761	2.3	1.0	447	2.4	1.0
CV (L)	776	0.6	0.3	451	0.6	0.4
CV/VC%*	776	12.7	7.4	449	13.7	8.0
CC/TLC%*	776	35.7	13.5	448	37.4	14.0

Data presented are from baseline survey except for ΔFEV₁. FEV₁ = forced expiratory volume in one second; ΔFEV₁ = rate of change in FEV₁, computed for each subject as difference between follow-up and baseline measured values divided by individual follow-up time in years; FEV₁ (%pred) = FEV₁ percentage predicted value; ΔFEV₁ (%pred/year) = rate of change in FEV₁ %pred, computed for each subject as difference between follow-up and baseline percentage predicted values divided by individual follow-up time in years; N₂-slope = slope of the single breath nitrogen test phase 3 or alveolar plateau; CC = closing capacity; CV = closing volume; CV/VC% = closing volume to vital capacity ratio; CC/TLC% = closing capacity to total lung capacity ratio.

* p < 0.05 for Student t test between those included and excluded.

independently, after adjusting for sex, height and baseline age, smoking status and FEV₁.

Results of multiple regression model to predict the linear association between ΔFEV₁ and N₂-slope are listed in Table 3 and plotted in Figs. 2 and 3. There was a highly significant (p=0.0001) linear association between N₂-slope and ΔFEV₁: for a one unit change in N₂-slope there was an extra 7.93 mL/year in ΔFEV₁. Females had significantly lower ΔFEV₁ than males. With ageing, ΔFEV₁ became steeper by -1.14 mL/year. There was a significant effect of current

smoking (-4.70 mL/year, p=0.041) when subjects of all ages were included. Bootstrap confirmed the validity of the applied model. Fig. 2 illustrates the fitted regression line and its 95% confidence interval. This regression model explained 16.3% of the variability observed in ΔFEV₁. When only subjects over 30 years (n=612) were analyzed, a statistically significant difference (p=0.005) in ΔFEV₁ of -7.4 mL/year was observed between current and never smokers (Fig. 3). In contrast, there was no detectable effect between never and ex-smokers (p=0.418) (Fig. 3).

Table 2 Descriptive statistics: proportions by inclusion.

Variable	Included in Analyses		Excluded from Analyses	
	Total	N (percent)	Total	N (percent)
Sex (Male)*	907	508 (56.0)	1522	654 (43.0)
Smoking Status*				
Never	907	303 (33.4)	1522	659 (43.3)
Ex	907	152 (16.8)	1522	229 (15.0)
Current	907	452 (49.8)	1522	634 (41.7)
GOLD (0-0)	769	693 (90.1)	319	281 (88.1)
GOLD (0-1)	769	63 (8.2)	319	30 (9.4)
GOLD (0-2)	769	13 (1.7)	319	8 (2.5)
ATS-ERS (0-0)	836	796 (95.2)	344	327 (95.1)
ATS-ERS (0-1)	836	34 (4.1)	344	13 (3.8)
ATS-ERS (0-2)	836	6 (0.7)	344	4 (1.2)

Longitudinal categories of Chronic Obstructive Pulmonary Diseases (COPD) according either to Global Initiative for Chronic Obstructive Lung Disease (GOLD) or American Thoracic Society-European Respiratory Society (ATS-ERS) criteria: (0-0) = no airway obstruction at both baseline and follow-up; (0-1) = no airway obstruction at baseline and mild airway obstruction at follow-up; (0-2) = no airway obstruction at baseline and moderate or severe airway obstruction at follow-up.

* p < 0.05 for Chi-square test between those included and excluded.

Table 3 Regression analysis with rate of change in FEV₁ (Δ FEV₁) as the dependent variable.

Variable	Coefficient	Standard Error	p Value
N ₂ -slope (%N ₂ /L)	-7.93	1.27	< 0.0001
Sex (Female)	-17.14	3.08	< 0.0001
Baseline FEV ₁ (mL)	-25.24	2.39	< 0.0001
Age (years)	-1.14	0.11	< 0.0001
Height (cm)	0.54	0.18	0.002
Ex-Smoker	0.11	3.01	0.971
Current Smoker	-4.70	2.30	0.041
Constant	52.28	29.79	0.080

Regression analysis computed on n=907 subjects with Δ FEV₁ and N₂-slope data available. N₂-slope = slope of the single breath nitrogen test phase 3 or alveolar plateau; FEV₁ = forced expiratory volume in one second; Δ FEV₁ = rate of change in FEV₁, computed for each subject as difference between follow-up and baseline values divided by individual follow-up time in years. R² = 16.3%.

Single breath nitrogen test as predictor of incident airway obstruction

There were no statistically significant increased risks detected between CC, CV, CV/VC%, or CC/TLC% and changes in COPD classification, using either GOLD or ATS-ERS criteria.

The results of fitting the multinomial regression models using N₂-slope to predict incidence of COPD at follow-up, after adjusting for sex, height and baseline age and smoking status, are listed in Tables 4, for GOLD and ATS-ERS criteria. The slope of alveolar plateau was associated with an increased risk of developing COPD over the 8-year follow-up, for both criteria. A one unit change in N₂-slope was associated with a 81% increased risk (Relative Ratio (RR)=1.81) of developing COPD GOLD stage I and with a 178% increased risk (RR=2.78) of developing COPD GOLD stage II or higher.

Age was associated with an increased risk for GOLD (0-1) (RR=1.06) and GOLD (0-2) (RR=1.08), indicating a 6 and 8% increased risk for each year, respectively. Sex and current or former smoking were not significant determinants for both GOLD (0-1) and GOLD (0-2). When using the ATS-ERS criterion, the N₂-slope was associated with a 62% increased risk (RR=1.62) of developing ATS-ERS mild airway obstruction and with a 240% increased risk (RR=3.40) of developing ATS-ERS moderate or severe airway obstruction. In this case, age was not significant for ATS-ERS (0-1) nor for ATS-ERS (0-2). As in the GOLD model, sex was not statistically significant for both COPD categories, likewise neither former nor current smoking were statistically significant; in particular, for ex-smoking status there was insufficient number of subjects to estimate the risk for ATS-ERS (0-2). However, smoking status was left in the models because of its known link to COPD.

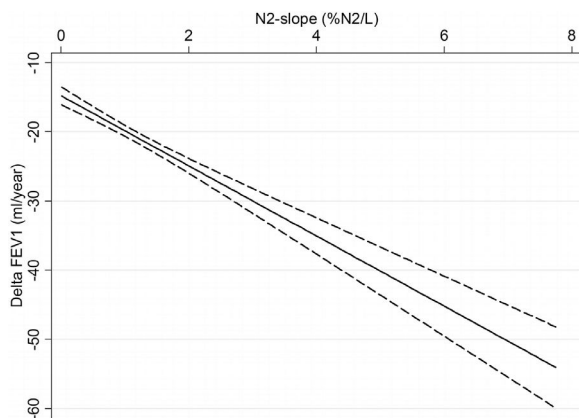


Fig. 2 Linear regression model for rate of change in FEV₁ according to N₂-slope, adjusted for sex, height, baseline age, lung function (FEV₁) and smoking habits, in all study subjects. Solid curve = fitted regression line; dashed curves = 95% confidence interval. Δ FEV₁ = rate of change in FEV₁, computed for each subject as difference between follow-up and baseline values divided by individual follow-up time in years; N₂-slope = slope of the single breath nitrogen test phase 3 or alveolar plateau.

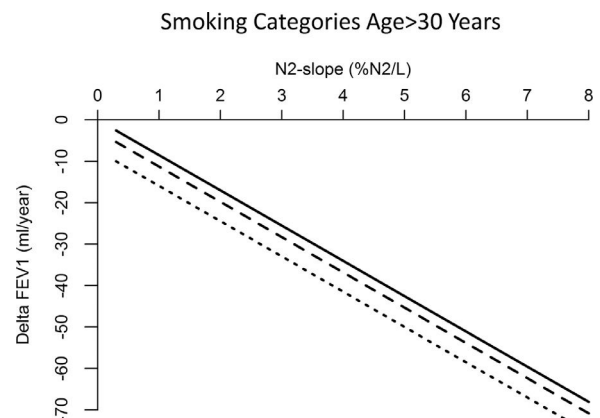


Fig. 3 Linear regression model for rate of change in FEV₁ according to N₂-slope, adjusted for sex, height, baseline age, lung function (FEV₁) and smoking habits, in subjects over 30 years of age by smoking status. Solid curve = never smokers; dashed curve = ex-smokers; dotted curve = current smokers. Δ FEV₁ = rate of change in FEV₁, computed for each subject as difference between follow-up and baseline values divided by individual follow-up time in years; N₂-slope = slope of the single breath nitrogen test phase 3 or alveolar plateau.

Table 4 Results from fitting multinomial regression model predicting changes in airway obstruction based on GOLD and ATS-ERS criteria, with subjects classified as without airway obstruction at both baseline and follow-up as reference group.

Predictive Variable	Relative Risk Ratio (RR)	95% Confidence Interval
GOLD (0-1)		
N ₂ -slope (%N ₂ /L) *	1.81	1.29-2.52
Age (years) *	1.06	1.03-1.09
Sex (Female)	0.73	0.31-1.71
Height (cm)	1.02	0.97-1.07
Ex-smoker	0.58	0.22-1.52
Current smoker	1.20	0.61-2.36
GOLD (0-2)		
N ₂ -slope (%N ₂ /L) *	2.78	1.70-4.53
Age (years) *	1.08	1.02-1.15
Sex (Female)	1.70	0.28-10.57
Height (cm)	1.01	0.92-1.12
Ex-smoker	0.56	0.05-6.37
Current smoker	1.71	0.39-7.59
ATS-ERS (0-1)		
N ₂ -slope (%N ₂ /L) *	1.62	1.14-2.29
Age (years)	0.98	0.95-1.02
Sex (Female)	1.21	0.43-3.45
Height (cm)	0.99	0.94-1.05
Ex-smoker	1.65	0.48-5.66
Current smoker	2.22	0.88-5.63
ATS-ERS (0-2)		
N ₂ -slope (%N ₂ /L) *	3.40	1.72-6.73
Age (years)	1.01	0.91-1.12
Sex (Female)	2.56	0.17-39.45
Height (cm)	0.96	0.81-1.13
Ex-smoker §	–	–
Current smoker †	14.17	0.52-386.09

Longitudinal categories of Chronic Obstructive Pulmonary Diseases (COPD) according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) and American Thoracic Society-European Respiratory Society (ATS-ERS) criteria: (0-1) = no airway obstruction at baseline and mild airway obstruction at follow-up; (0-2) = no airway obstruction at baseline and moderate or severe airway obstruction at follow-up; N₂-slope = slope of the single breath nitrogen test phase 3 or alveolar plateau.

* $p < 0.05$.

§ Insufficient cell sizes to estimate coefficients.

† Low cell sizes affecting estimate of coefficients.

by using the SBN₂ test technique in 460 men aged 50 and 60 years randomly sampled from the general population, Olofsson et al.⁸ found that 1 and 4% N₂/L of baseline N₂-slope produced a decline in FEV₁ of 56 and 82 mL/year, respectively. Beaty et al.⁹ observed a significant negative relationship between baseline CC and Δ FEV₁ in women, and between baseline CC or N₂-slope and Δ FEV₁ in men. Such study on 1912 adults had a shorter follow-up (average of 4.7 years between visits) and used a different SBN₂ technique. By using our same SBN₂ technique, Buist et al.¹⁰ analyzed data from two different cohorts, tested 3-5 times over a mean follow-up of 8.5 and 10.5 years. In both cohorts, Δ FEV₁ rate was significantly associated with CV/VC ($r = -0.16$ in the Portland and -0.7 in the Screening center cohort) and with CC/TLC ($r = -0.33$ and -0.15), but not with N₂-slope ($r = -0.10$ and -0.03). In a preliminary report on 389 subjects from the Tucson Epidemiological Study, Knudson et al.⁷ did not find an abnormal N₂-slope to be a predictor of an accelerated Δ FEV₁ after 4.2 years of follow-up. None of the above longitudinal studies evaluated SBN₂ test indexes as predictors of incident airway obstruction or COPD. Stanescu et al. followed-up an original group of 105 male, long-term smoking, 45-55 years old steelworkers, after 6 (n=85)³⁴ and 13 (n=56) years.¹¹ Baseline N₂-slope was significantly related to FEV₁ decline over 6 years only in obstructed subjects with a baseline FEV₁/VC < 66.6% ($r = 0.59$, $p < 0.01$),³⁴ for whom baseline N₂-slope was significantly related to FEV₁ also at 13-year follow-up ($r^2 = 55\%$), but not to change in FEV₁ over 13 years.¹¹ No other SBN₂ index was found related to FEV₁ decline. Moreover, N₂-slope was related to FEV₁/VC ($r = 0.61$, $p < 0.001$) at baseline but not to change in FEV₁/VC over 13-year follow-up.¹¹ They concluded that middle-aged smokers are at no evident risk of functional deterioration if their FEV₁/VC ratio is normal, even if results of small airways tests are abnormal.¹¹ More recently, using data from a cohort surveyed in 1970s and followed-up for 38 years, Olofsson et al. observed that N₂-slope predicted the cumulative COPD event incidence (i.e. first hospital admission or death for COPD)²¹ and combining the N₂-slope with FEV₁ measurements and smoking history improved the prediction of future COPD events.²²

Overall, previous longitudinal studies have observed links between impairment in small airways as detected by SBN₂ test and subsequent loss in lung function. In our study, N₂-slope appeared to predict lung function decline or airway obstruction. Our result is consistent with those from some previous longitudinal studies,^{8,21,22} but not with others.^{7,9-11} N₂-slope reflects the distribution of ventilation in lung periphery while CV and CC denote the lung volume at which peripheral airway closure begins, and all these indexes may provide an indication of processes associated with parenchymal or small airways dysfunction.² Different sampling procedures, SBN₂ test techniques^{35,36} or statistical approaches of the quoted studies may account for the different findings with our study, along with a younger mean age of our population.

In our study there were 1228/3284 (37.4%) longitudinal subjects who had acceptable baseline SBN₂ test and Δ FEV₁ measures. Among these subjects only adults aged ≥ 20 yrs were selected (n=907), as lung function decline and incidence of COPD over a 8-year follow-up is not expected in subjects aged under 20 yrs. While all our

Discussion

We have prospectively evaluated the association between impairment of small airways and lung function decline or incidence of COPD in a large longitudinal population-based study over 8-year follow-up. We observed that baseline N₂-slope from SBN₂ test is significantly associated with FEV₁ decline and increased risk of incident airway obstruction as defined by GOLD or ATS-ERS criteria.

Our study is comparable to only few other similar studies conducted in the 1970-80s. Over a similar follow-up period,

subjects who completed the SBN₂ test provided a measure of the N₂-slope, only 83.9% and 85.5% provided a measure of CC and CV, respectively. This may be at least in part explained by the difficulty in performing SBN₂ test maneuver among participants in this epidemiological survey.²³ Subjects included in the analyses were significantly younger, slightly taller, and had better FEV₁ with respect to those excluded. The regression analyses to estimate the risk for lung function decline associated with baseline SBN₂ test indexes accounted for these confounders. A significant lower difference was found for Δ FEV₁ between subjects included and excluded from analyses, which may be explained by the smaller number and the younger mean age of those providing acceptable spirometry measurements at both surveys 8-year apart. We observed that baseline N₂-slope was associated with higher risks of lung function decline and airways obstruction incidence in a relatively young population; this result reinforces the importance of SAD in the natural history of COPD, as even higher risks may be expected in an older population possibly exposed to smoking for a longer period.

N₂-slope has been repeatedly found to be the most sensitive SBN₂ index in separating smokers from non-smokers,^{3,22,37} as we previously found.²³ In the present longitudinal study, an association between N₂-slope and decline in FEV₁ was found adjusting for smoking habit, while current or former smoking were not significant determinants for incident airway obstruction. A shorter and lighter history of smoking in current young smokers and the inclusion of occasional among current smokers may explain these effects. Indeed, when only subjects aged over 30 years were analyzed, a statistically significant difference in Δ FEV₁ of -7.4 mL/year was observed between current and never smokers, with no detectable difference between never and ex-smokers. The association between N₂-slope and decline in FEV₁ observed in this young study sample (mean age 37.4±11.1 years) from the general population highlights the relevance of the impairment of small airways in predicting lung function decline even independently from smoking.

Considering the scientific discussion on the spirometry definition of COPD (i.e., FEV₁/FVC < 70% fixed ratio vs. FEV₁/VC < 5th percentile of the predicted value),³⁸ we evaluated SBN₂ test as predictor of COPD incidence defined according to either GOLD³² or ATS-ERS³³ criterion. We found N₂-slope to be a significant predictor of COPD with both criteria. Among subjects without airway obstruction at baseline, approximately 10% vs. 5% developed COPD based on GOLD or ATS-ERS criteria, respectively. This difference may confirm that the use of a non-age-adjusted criterion to define airway obstruction may misclassify the older subjects as obstructed. Indeed, differently from the GOLD model with N₂-slope predicting COPD incidence, in the ATS-ERS model age did not reach the statistical significance. This is consistent with the age-adjustment inherent in the ATS-ERS criterion.

We performed baseline spirometry without using bronchodilator, as at the time of our study there was no suggestion to perform a bronchodilator test when carrying out spirometry in general population samples. A Norwegian group, using a bronchodilator test in a community study,

estimated post-bronchodilator airway obstruction prevalence (defined as FEV₁/FVC < 0.70) to be 27% lower than the pre-bronchodilator values.³⁹ However, the need of using a therapeutic agent in general population studies is still questionable, in view of the widespread lack of awareness of the disease in the general public who, in real life, is unlikely to seek medical advice at the early stage of the disease.⁴⁰

Our study reinforces the usefulness of the SBN₂ test in the early detection of airways obstruction. The reported difficulty in performing SBN₂ test in population-based studies may not apply to the clinical setting. Indeed, high reproducibility for N₂-slope has been shown in different populations of healthy subjects (correlation coefficient for duplicate measurements: $r = 0.96$ ³⁵ or $r = 0.94$ ⁴¹). Other functional or biological tests for exploring small airways obstruction, possibly more patient friendly or easy to apply in clinical practice, are available: e.g., the impulse oscillometry (IOS) or exhaled nitric oxide measurements. Impulse oscillometry parameters have been shown to be reproducible in three measures in healthy children and children with cystic fibrosis (intraclass correlation coefficient = 0.9).⁴² Fractional exhaled nitric oxide (FeNO) measurements have been shown to be reproducible in healthy and asthmatic adults, as well as in children (intraclass correlation coefficient = 0.99).⁴³ While no study has directly compared IOS with the SBN₂ test, strong correlations have been found between the alveolar nitric oxide concentration (CALv) and N₂-slope in patients with severe asthma.⁴⁴ The IOS parameter integrated area of low frequency X has been shown to predict the FEV₁ decline in a group of COPD patients.⁴⁵ There are no prospective data on the association between impairment of small airways, as assessed by IOS, and incidence of COPD. Alveolar nitric oxide concentration has been used to study the small airways in COPD, in addition to asthma,⁴⁶ but longitudinal studies are lacking.

Thus, SBN₂ test might be part of an extended evaluation of respiratory function, in addition to spirometry, in patients with clinically significant risk factors for COPD, e.g., tobacco smoking. Indeed, in the last years, the relevance of targeting treatment to small airways in asthma and COPD has been pointed out,⁴⁷ and SBN₂ test might be used to detect respiratory patients with functionally impaired small airways. In the clinical practice, highlighting abnormal results of the SBN₂ test might also be used to motivate smoking patients to quit; indeed, it is known that diagnosis of airway obstruction motivates smokers to quit smoking⁴⁸ and higher cessation rates are observed in those who are obstructed.⁴⁹

Conclusion

N₂-slope from SBN₂ test is a significant predictor of lung function decline and COPD incidence defined according to either GOLD or ATS-ERS criteria. These results confirm the role of SAD in the natural history of COPD. Large prospective studies are needed to evaluate whether new proposed functional or imaging tests that measure small airways impairment may be useful in the early detection of COPD. Meanwhile, pulmonologists could re-discover an "old" test, which provide important information on their patients at risk for developing COPD.

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Conflict of interest

All the authors have no conflicts of interest to declare in relation to the present manuscript.

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Appendix A. Definition of longitudinal categories of Chronic Obstructive Pulmonary Disease (COPD) according to either Global Initiative for Chronic Obstructive Lung Disease (GOLD)³² or American Thoracic Society-European Respiratory Society (ATS-ERS)³³ criterion

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Longitudinal category of COPD	Lung function at baseline survey	Lung function at follow-up survey
GOLD (0-0)	FEV ₁ /FVC ≥ 0.70	FEV ₁ /FVC ≥ 0.70
GOLD (0-1)	FEV ₁ /FVC ≥ 0.70	FEV ₁ /FVC < 0.70 and FEV ₁ ≥ 80% of predicted value (mild airway obstruction)
GOLD (0-2)	FEV ₁ /FVC ≥ 0.70	FEV ₁ /FVC < 0.70 and FEV ₁ < 80% of predicted value (moderate or severe airway obstruction)
ATS-ERS (0-0)	FEV ₁ /VC ≥ 5th percentile of predicted value	FEV ₁ /VC ≥ 5th percentile of predicted value
ATS-ERS (0-1)	FEV ₁ /VC ≥ 5th percentile of predicted value	FEV ₁ /VC < 5th percentile of predicted value and FEV ₁ ≥ 70% of predicted value (mild airway obstruction)
ATS-ERS (0-2)	FEV ₁ /VC ≥ 5th percentile of predicted value	FEV ₁ /VC < 5th percentile of predicted value and FEV ₁ < 70% of predicted value (moderate or severe airway obstruction)

FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; VC = vital capacity. Predicted values were computed according to Paoletti et al.²⁸

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