



Ventilation inhomogeneity in α_1 -antitrypsin-deficient emphysema

L. Fregonese, H.P.A.A. van Veen, P.J. Sterk and J. Stolk

ABSTRACT: The slope of phase III of the single-breath nitrogen wash-out test (sbN₂-test) measures ventilation inhomogeneity and, in smokers, is strongly associated with small airways pathology. The present authors aimed to study ventilation inhomogeneity in emphysema related to type Z α_1 -antitrypsin (AAT) and to assess its relationship with indices of parenchymal damage and airways obstruction.

A total of 18 subjects, consisting of ex-smokers with type Z AAT and emphysema (confirmed by computed tomography scan), were studied in a cross-sectional design. Post-bronchodilation flow–volume curves and gas transfer parameters were measured; sbN₂-test curves were obtained and the slope of phase III was determined.

The mean value of the slope ($4.6 \pm 1.3\% \text{ N}_2 \cdot \text{L}^{-1}$) was higher than reference values +2SD; it was significantly correlated with the transfer factor of the lung for carbon monoxide ($R = -0.75$) and the transfer coefficient of the lung for carbon monoxide ($R = -0.58$), but not with airways obstruction. There was no correlation between phase III slope values and cumulative smoking.

In patients with type Z α_1 -antitrypsin emphysema, the increased ventilation inhomogeneity predominantly reflects parenchymal abnormalities, demonstrating that measurement of airways obstruction is not sufficient to characterise the disease. Determination of the sensitivity of the single-breath nitrogen wash-out test slope in detecting disease progression may give complementary information to spirometry.

KEYWORDS: α_1 -Antitrypsin deficiency, cigarette smoking, diffusion capacity, emphysema, single-breath nitrogen wash-out test, small airways

Alpha-1-antitrypsin deficiency (AATD) is a hereditary condition that, in the most severe forms (phenotype PiZZ or null), predisposes to early onset and rapidly progressive chronic obstructive pulmonary disease (COPD), with emphysema as the predominant component. Airways obstruction, measured by the forced expiratory volume in one second (FEV₁) and the FEV₁/forced vital capacity (FVC) ratio, is the most widely used functional parameter for monitoring emphysema in COPD [1, 2]. However, the sole use of FEV₁ is not sufficient to characterise emphysema and its progression. Different definitions of airway obstruction may produce prevalence estimates of COPD that vary by >200% [2], and mild-to-moderate emphysema can occur without reduction in FEV₁ [3]. Discordance between airways obstruction and gas transfer parameters has been reported in AATD-related emphysema [4–7], and assessing only FEV₁ can lead to overestimation of severity in patients with predominantly basal emphysema, such as the majority of the subjects with AATD [7]. Furthermore, the use of a single, nonspecific, surrogate parameter makes

it difficult to assess the effect of specific treatments, such as those aiming at reducing or repairing parenchymal damage (*e.g.* α_1 -antitrypsin (AAT) replacement therapy and drugs inducing alveolar repair).

A recent statement of the American Thoracic Society/European Respiratory Society (ERS) recommended that both airways obstruction and gas exchange should be determined when assessing the overall severity of pulmonary impairment in individuals with AATD [4]. The gas exchange parameters (transfer factor of the lung for carbon monoxide (TL_{CO}), and TL_{CO} /alveolar volume (VA) *i.e.* KCO) measure parenchymal functional damage by assessing the area available for gas diffusion. Diffusion capacity has shown better correlation to the pathological extent of emphysema than spirometric indices [8] and it presents some potential as a tool to monitor emphysema progression. However, the method has relatively large longitudinal variability, since TL_{CO} is influenced by changes in alveolar volume [9] and both TL_{CO} and KCO are influenced by alterations in perfusion over time [10, 11].

AFFILIATIONS

Lung Function Laboratory, Dept of Pulmonology, Leiden University Medical Centre, Leiden, The Netherlands.

CORRESPONDENCE

L. Fregonese
Dept of Pulmonology (C3-P)
Leiden University Medical Centre
P.O. Box 9600
2300 RC Leiden
The Netherlands
Fax: 31 715266927
E-mail: l.fregonese@lumc.nl

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The single-breath nitrogen wash-out test (sbN₂-test) offers a way to measure inhomogeneous ventilation as a reflection of abnormalities in the peripheral lung at the level of small airways and/or parenchyma [12]. In non-AATD-related COPD, the slope of phase III of the sbN₂-test curve has traditionally been considered to reflect small airways disease, since it was linked to the pathological score of small airways in smokers with and without airways obstruction [13]. Interestingly, a 13-yr follow-up study demonstrated that a high phase III slope of the sbN₂-test curve associated with a low FEV₁/FVC ratio identifies a subset of smokers at high risk of developing COPD, thus showing some predictive value of the sbN₂-test toward the development of airways obstruction [14].

Pathological alterations of the small airways develop in very early stages in non-AATD-related COPD [15]. The main known determinant of small airways disease is cigarette smoking, which can induce remodelling of the airway walls. Indeed, alterations have also been reported in smokers without COPD [15, 16]. In contrast, in a recent report, only minimal abnormalities of small airways were demonstrated in lung specimens from patients with end-stage type Z AATD-related emphysema [17]. Furthermore, in lung tissue from subjects with a panlobular pattern of emphysema, which is characteristic of AATD, small airways showed only minimal pathological abnormalities [18].

On the basis of this background, the current authors hypothesised that ventilation inhomogeneity is primarily related to parenchymal destruction in AATD-related emphysema, rather than to obstruction due to small airways pathology. To test this hypothesis, the present authors measured ventilation inhomogeneity by the phase III slope of the sbN₂-test curve in patients with type Z AATD-related emphysema, and evaluated the relationship of the slope with the indices of parenchymal damage and airways obstruction.

METHODS

Subjects

A total of 25 patients recorded in the Dutch section of the Alpha-1 International Registry who met inclusion criteria received a written invitation to participate in the study. A group of 18 subjects entered the study. Inclusion criteria were: 1) severe AATD (PiZZ or Pi null phenotype); 2) emphysema confirmed by computed tomography (CT) scan; 3) $T_{L,CO}$ and $T_{L,CO}/VA$ (KCO) <80% of the predicted value; 4) ex-smokers, who had stopped smoking for ≥ 6 months, with two negative urine cotinine tests 1 month apart; 5) clinical stability for ≥ 1 month prior to the study; and 6) no changes in inhaled medications and no course of oral steroids in the last month prior to the study. Subjects were excluded in cases where the CT scan revealed the presence of giant bullous disease. The study was approved by the Ethics Committee of the Leiden University Medical Centre (Leiden, the Netherlands) and patients gave written informed consent.

Study design

The design was cross-sectional, with assessments performed on 2 consecutive days. On day 1, clinical history was recorded and smoking history was quantified in pack-yrs. Quality of life was assessed with the St George's Respiratory Questionnaire

and the Chronic Respiratory Disease Questionnaire. On day 1, all lung function measurements were performed, except for the sbN₂-test, which was measured on day 2.

Lung function

Lung function tests were performed according to the ERS guidelines [19]. All tests were performed after nebulisation of 5 mg of salbutamol and 500 μ g of ipratropium bromide. The following tests were performed: 1) spirometry with measurement of vital capacity (VC), FEV₁, FVC and FEV₁/FVC; 2) single-breath total lung diffusion capacity (with determination of $T_{L,CO}$ and KCO) and sbN₂-test.

SbN₂-test was performed using a dry rolling seal spirometer (Morgan Spiroflow; P.K. Morgan Ltd, Kent, UK) filled with 100% oxygen and equipped with an N₂ meter (P.K. Morgan Ltd) connected to the mouthpiece, allowing continuous sampling, as previously described [20, 21]. During the test the patients performed a slow full inspiratory and expiratory VC manoeuvre at a flow rate of 0.5 L·s⁻¹. A mechanical flow regulator assured a constant flow. The expiratory N₂ concentration was plotted against volume changes between total lung capacity and residual volume, producing the expiratory nitrogen wash-out curve. The slope of phase III of the sbN₂-test curve was calculated by a blinded observer, drawing the line of best-fit through phase III of the expiratory volume-concentration curve, and was expressed as % N₂·L⁻¹ of expired air [22]. This procedure was validated in the laboratory of the current authors, showing good intra- and inter-observer repeatability in the slope of phase III, assessed by the intra-class correlation coefficient (Ri=0.94 and 0.99 for intra-observer and inter-observer repeatability, respectively) [21]. The measurements were only accepted if the VC during the sbN₂-test was within 10% of the VC measured by spirometry. All volumes were corrected for body temperature and ambient pressure, saturated with water vapour.

Statistical analysis

Data are presented as mean \pm SEM when normally distributed, or as median (minimum–maximum) when not normally distributed. When the data were not normally distributed, natural logarithmic transformation was performed before data analysis. Parametric correlations were performed to assess the relationship between two parameters and Pearson's coefficient was calculated. Results were considered statistically significant at a p-value <0.05.

RESULTS

Patient characteristics

Demographic and clinical data of the patients are presented in table 1. All patients were Caucasian, with a mean age of 50 yrs, and were ex-smokers. Most of the patients had very early onset of the disease, on average before the age of 45 yrs, and the main symptom at onset was dyspnoea on exertion for all but one of the patients. All patients were being treated with inhaled steroids and/or bronchodilators (β_2 -agonists and/or anti-muscarinic agents) on a regular basis.

Lung function

As shown in table 1, AATD patients presented moderate-to-severe airways obstruction, with preserved FVC and moderate-to-severe impairment of gas exchange parameters.

TABLE 1 Clinical and functional patient characteristics

Characteristic	
Subjects n	18
Age yrs	50 ± 7.6
Sex M/F	14/4
Duration of disease yrs	13.5 (6–23)
Onset of symptoms	37.5 (24–55)
Pack-yrs	10.2 (3.5–40.5)
Inhaled bronchodilators/ICS#	100/11.1
Symptoms at onset: dyspnoea on exertion/ others n	17/1
FEV ₁ % pred	39 (29–73)
FVC % pred	103.5 (73–153)
FEV ₁ /FVC	28.7 (19–53)
TL,CO % pred	54.2 (35–82)
KCO % pred	55.1 (40–68)
Phase III slope % N ₂ ·L ⁻¹	4.6 ± 1.3
Sa _a O ₂ %	92.9 ± 2.3
Pa _a O ₂	8.7 ± 0.9
Pa _a CO ₂	5.1 ± 0.4
SGRQ	37.7 ± 15

Data are presented as mean ± SD or median (minimum–maximum), unless otherwise stated. M: male; F: female; ICS: inhaled corticosteroids; FEV₁: forced expiratory volume in one second; % pred: per cent predicted; FVC: forced vital capacity; TL,CO: transfer factor of the lung for carbon monoxide; KCO: transfer coefficient of the lung for carbon monoxide; Phase III slope: the slope of phase III of the single-breath nitrogen wash-out test curve; Sa_aO₂: arterial oxygen saturation; Pa_aO₂: arterial oxygen tension; Pa_aCO₂: arterial carbon dioxide tension; SGRQ: St George's Respiratory Questionnaire. #: percentage of the group.

In the sbN₂-test curve, increased steepness of the slope of phase III was measured in all patients. The mean value of the slope ($4.6 \pm 1.3\% \text{ N}_2 \cdot \text{L}^{-1}$) was higher than the reference values +2SD (upper limits of the reference values: $2.0\% \text{ N}_2 \cdot \text{L}^{-1}$ for males and $3.0\% \text{ N}_2$ for females; fig. 1) [20]. The values of the slope were significantly correlated with the gas exchange parameters (TL,CO and KCO) but not with airways obstruction (FEV₁, FEV₁/FVC; fig. 2). As expected, measures of airways obstruction were negatively associated to smoking history, expressed as pack-yrs of smoking. In contrast, neither ventilation inhomogeneity nor gas exchange was linked to smoking history (fig. 3). There was no influence of age on the results of the sbN₂-test ($r=0.114$; $p<0.5$).

DISCUSSION

The present authors have shown that ventilation inhomogeneity, measured by the sbN₂-test, is increased in AATD-related emphysema, and that increased values of the phase III slope are linked to indices of parenchymal impairment but not to airways obstruction. Furthermore, in the present study, the abnormalities measured by the sbN₂-test are not associated with cumulative smoking.

The current study is the first study describing ventilation inhomogeneity in AATD-related emphysema. Increased values of the phase III slope have been reported since the late 1970s in smokers with and without airways obstruction [23–26] and

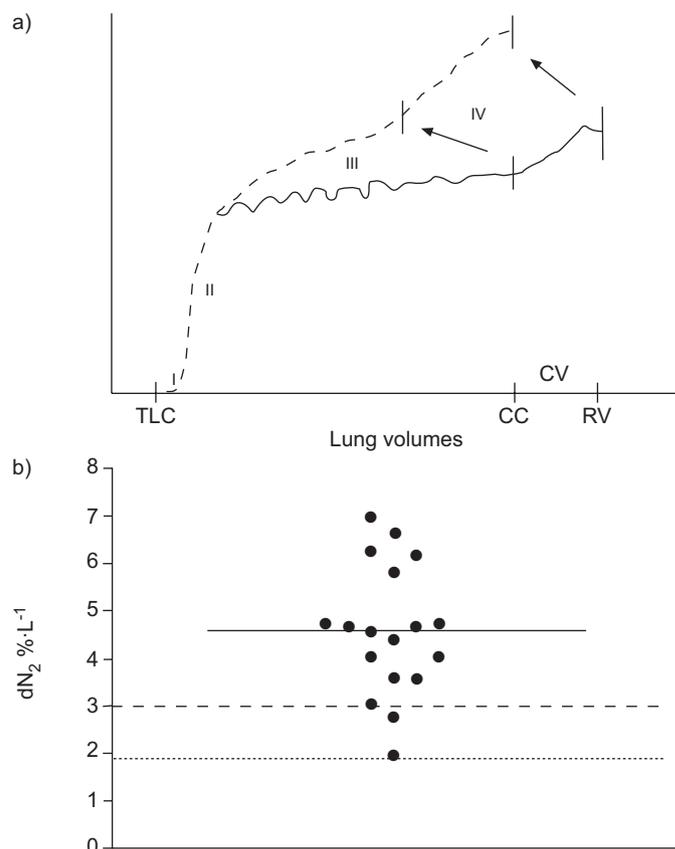


FIGURE 1. a) Single-breath nitrogen wash-out test curve. Lung volumes, including total lung capacity (TLC), closing capacity (CC), closing volume (CV) and residual volume (RV) are shown on the x-axis. Expired nitrogen concentrations are shown on the y-axis. Phase I: gas from the dead space; phase II: mixture of dead space and alveolar gas; phase III: slope (alveolar plateau); junction between phase III and phase IV: CV (left-to-right arrow). b) Distribution of the values of the phase III slope. Mean value of the phase III slope (—) was higher than the reference values ±2SD (upper limits of normal range: $2.0\% \text{ N}_2 \cdot \text{L}^{-1}$ for males and $3.0\% \text{ N}_2 \cdot \text{L}^{-1}$ for females).

have been strongly linked to small airways pathology. A steeper slope reflects more inhomogeneous lung emptying, which can be caused by heterogeneous airspaces enlargement, airways alterations (remodelling with fibrosis, increased wall thickness, mucus hyperproduction) or both. Based on pathology, the smaller, conducting airways have been shown to be the major site of airway obstruction in COPD [27]. Therefore, increased airways resistance due to remodelling at the level of small airways is likely to be a major cause of increase in ventilation inhomogeneity in non-AATD-related COPD. In contrast, in lung tissue from patients with type Z AATD-related emphysema only minimal structural and pathological changes in small airway walls were detected [17]. These findings, and the good correlation between slope and gas exchange reported in the current study, suggest that parenchymal destruction, rather than airways remodelling, is the main determinant of the increased ventilation inhomogeneity in AATD-related emphysema.

By which mechanisms can parenchymal damage increase ventilation inhomogeneity? Emphysema in AATD presents a

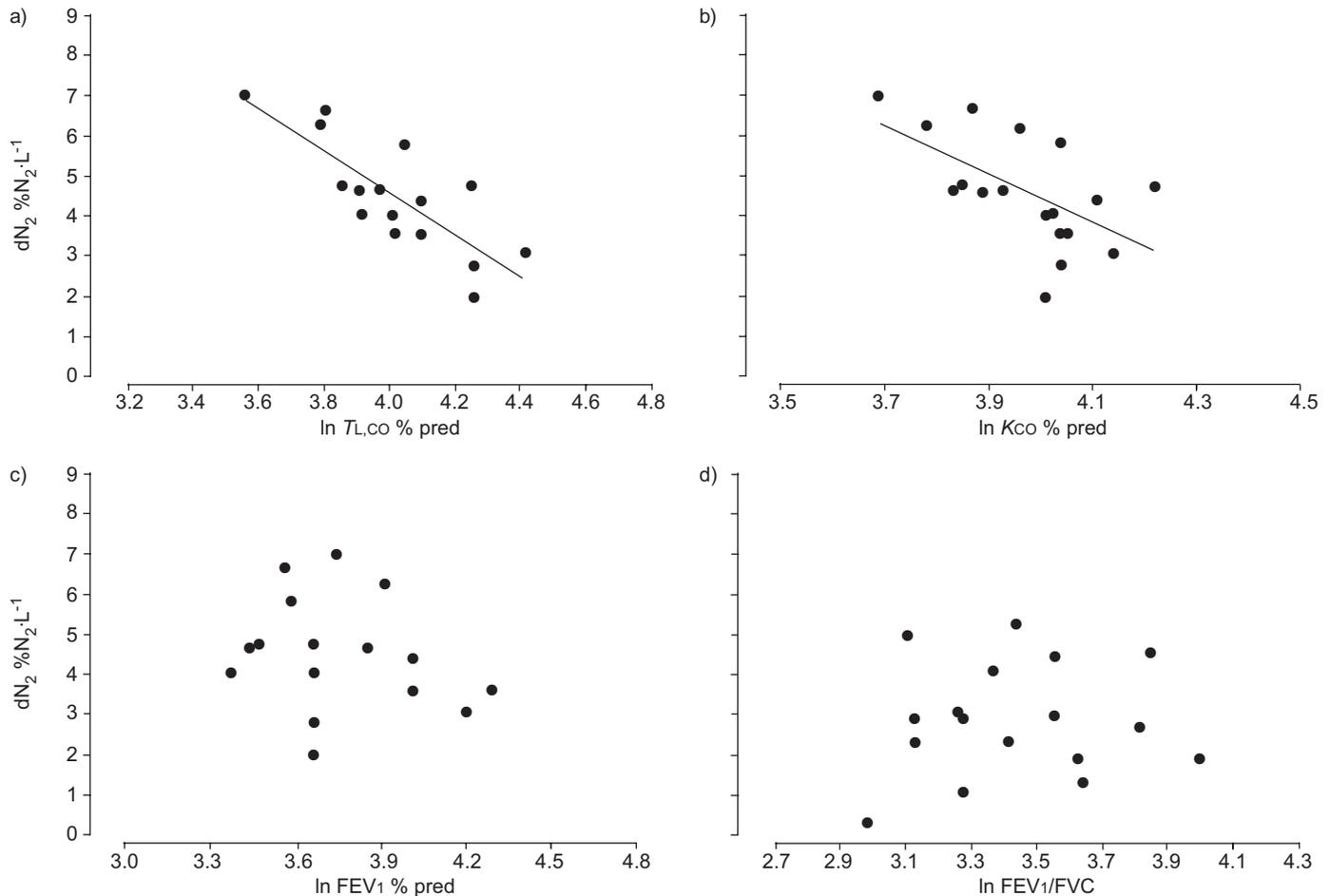


FIGURE 2. Correlation between phase III slope of the single-breath nitrogen wash-out curve (dN_2) and a) transfer factor of the lung for carbon monoxide ($T_{L,CO}$; $r = -0.75$; $p = 0.000$), b) transfer coefficient of the lung for carbon monoxide (K_{CO} ; $r = -0.58$; $p = 0.012$), c) forced expiratory volume in one second (FEV_1 ; $p > 0.05$) and d) FEV_1 /forced vital capacity (FVC ; $p > 0.05$). % pred: per cent predicted.

characteristic even pattern of lobular destruction (panlobular emphysema (PLE)), while in non-AATD-related emphysema, focal lesions are prevalent (centrilobular emphysema). These patterns result in different mechanical properties of the lung even in the presence of a very mild degree of emphysema. In particular, the very pronounced loss of elastic recoil and the increased compliance reported in PLE [28–29] can influence ventilation inhomogeneity by increasing the inhomogeneity of alveolar capacities [30, 31]. Furthermore, loss of elastic recoil can increase airways resistance during the expiratory phase by inducing collapse of the small, noncartilagenous airways. Interestingly, loss of elasticity has been associated with increased airway narrowing in response to bronchoconstrictive stimuli in type Z AAT-related emphysema [32].

The particular anatomical distribution of emphysema in AATD, with prevalent disease at the basal lobes, could also contribute to increase ventilation inhomogeneity. Reduced ventilation in the lower lobes, which are the most ventilated areas of the lung in physiological conditions, is likely to strongly alter the longitudinal, gravity dependent sequence of lung emptying [33]. The evaluation of the influence of the anatomical distribution of emphysema on ventilation inhomogeneity was not one of the aims of the current study. Indeed

the slope of phase III of the sbN_2 -test curve has been shown to be influenced by topographical ventilation distribution [34]. Other tests of ventilation inhomogeneity that are more specific for the site of origin, such as the multi-breath nitrogen test [25], are required to address this question.

Until now, besides spirometry, only gas exchange tests have been investigated as potential tools to monitor alveolar damage in AATD-related emphysema. However, gas exchange measurements can be subject to high variation in longitudinal studies, because of the difficulty in standardising gas transfer calibration, and for changes in lung perfusion over time [35]. Furthermore, the breath-hold manoeuvre at repeatable lung volume might be difficult to perform by severely affected patients. For these reasons, and for the fact that airway obstruction and gas exchange are not always well correlated in emphysema [3–7], it is still a matter of debate which lung function measurement is the most representative in AATD, and the most useful in monitoring the progression of the disease and the response to treatment.

The correlation of the phase III slope of the sbN_2 -test curve with $T_{L,CO}$ (and K_{CO}) suggests that the parenchymal damage is probably the common pathophysiological denominator of the

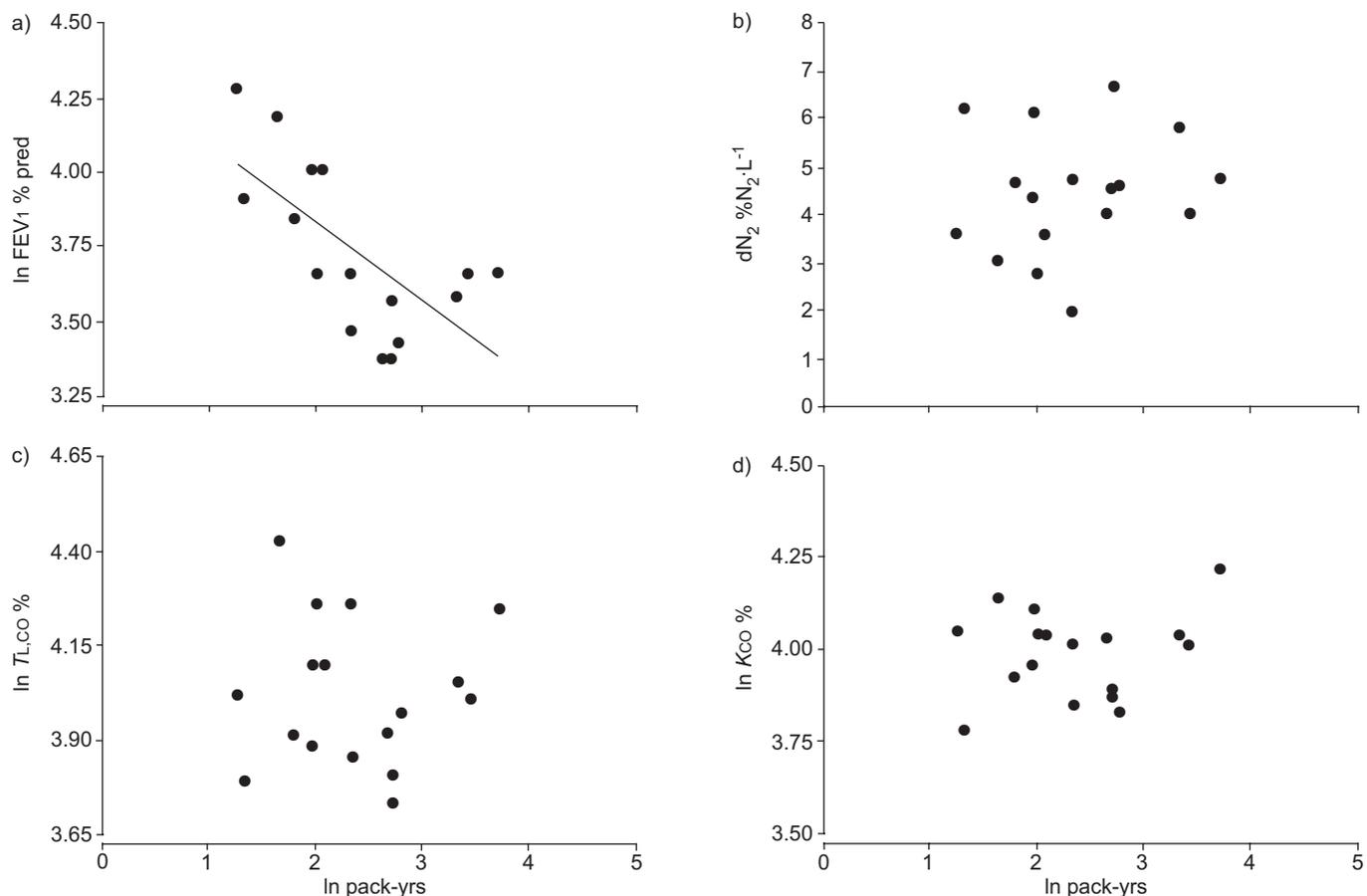


FIGURE 3. Correlation between pack-yrs and a) forced expiratory volume in one second (FEV₁; $r = -0.67$; $p = 0.004$), b) phase III slope of the single-breath nitrogen wash-out curve (dN_2 ; $p > 0.05$), c) transfer factor of the lung for carbon monoxide (TL_{CO} ; $p > 0.05$) and d) transfer coefficient of the lung for carbon monoxide (KCO ; $p > 0.05$). % pred: per cent predicted.

abnormalities measured by these two tests. However, this does not allow the conclusion that the two tests are interchangeable but only that they provide a different view over the disease. The sbN_2 -test informs about the degree of inhomogeneous ventilation, which is determined by the absolute levels of ventilation and its distribution within the lungs. TL_{CO} is a measure of gas transfer across the alveolar–capillary interface, thus it is determined by the absolute levels of ventilation and perfusion, their distribution with respect to each other, and the diffusion characteristics of the alveolar–capillary membrane [9–11, 36]. In contrast to the gas exchange parameters, the results the sbN_2 -test are hardly influenced by variability in perfusion and ventilation/perfusion mismatch. Furthermore, in the sbN_2 -test, lung volumes and nitrogen values can be calibrated reliably and the slow VC manoeuvre is easier for the patient to perform. Longitudinal studies to identify which are the most effective parameters to monitor the progression of the different types of emphysema should be encouraged. In particular, on the basis of the results of the present study, the authors conclude that it is worthwhile to explore the value of the sbN_2 -test in longitudinal studies in AATD-related emphysema and probably also in non-AATD-related-emphysema, as the slope change over time may be “less noisy” than the change in gas exchange parameters over time.

One possible limitation in the current study is that the sample size was relatively small. However, the range of emphysema severity described in the presented patients was wide, with values of FEV₁ and KCO ranging from mild to very severe, as shown in table 1. The wide range of emphysema severity and the high correlation between the phase III slope and gas exchange values, suggest that the present author’s observation is likely a representative sample of the general population with type Z AAT-related emphysema.

As for non-AATD-related COPD, cigarette smoking is considered the most important risk factor for the development of emphysema in type Z AATD [36]. However, in the present study, neither ventilation inhomogeneity nor gas exchange was related to smoking history measured in pack-yrs. Even though reporting of smoking history can be affected by recall bias, pack-yrs is considered to be a valid index in epidemiological studies. Indeed, in line with previous studies [37], the current authors found a correlation between smoking history and airways obstruction. Since ventilation inhomogeneity and gas exchange abnormalities both reflect parenchymal damage in the present study, the current results suggest that determinants other than smoking, such as disease modifier genes or environmental factors [36, 38], could play a major role in alveolar destruction in type Z AATD-related emphysema.

In conclusion, in type Z α_1 -antitrypsin deficiency-related emphysema the increase of ventilation inhomogeneity is probably caused by alveolar destruction rather than by small airways pathology. The present results suggest that the single-breath nitrogen wash-out test can be an interesting surrogate parameter in α_1 -antitrypsin deficiency-related emphysema. Determination of the sensitivity of the phase III slope of the single-breath nitrogen wash-out test curve in detecting disease progression may give complementary information to spirometry.

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