Ventilation inhomogeneity in α1-antitrypsin deficient emphysema.

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Short title: New parameter for emphysema progression
Abstract
The slope of phase III of the single-breath nitrogen wash-out test (sbN$_2$-test) measures ventilation inhomogeneity and, in smokers, is strongly associated with small airways pathology. **Aim:** To study ventilation inhomogeneity in emphysema related to type Z alpha-1-antitrypsin and to assess its relationship with indices of parenchymal damage and airways obstruction. **Methods:** Eighteen subjects, ex-smokers, with type Z alpha-1-antitrypsin and emphysema confirmed by CT scan were studied in a cross-sectional design. Post-bronchodilation flow-volume curves and gas transfer parameters were measured; sbN$_2$-test curves were obtained and the slope of phase III was determined. **Results:** The mean value of the slope (4.6±1.3%N$_2$/liter) was higher than reference values +2 standard deviations; it was significantly correlated with TL$_{CO}$ (R = -0.75; p= 0.0001) and K$_{CO}$ (R = -0.58; p= 0.012), but not with airways obstruction. There was no correlation between phase III slope values and cumulative smoking. **Conclusions:** in patients with type Z AAT emphysema, the increased ventilation inhomogeneity reflects parenchymal abnormalities predominantly, demonstrating that measurement of airways obstruction is not sufficient to characterize the disease. Determination of sensitivity of sbN2-test slope in detecting disease progression may give complementary information to spirometry.

**Keywords:** alpha-1-antitrypsin deficiency, cigarette smoking, diffusion capacity, emphysema, single-breath wash-out nitrogen test, small airways.
Introduction

Alpha-1-antitrypsin deficiency (AATD) is a hereditary condition that, in the most severe forms (phenotype Pi ZZ or Null), predisposes to early onset and rapidly progressive COPD, with emphysema as the predominant component. Airways obstruction, measured by the Forced Expiratory Volume in 1 second (FEV₁) and the FEV₁/Forced Vital capacity (FVC) ratio, is the most widely used functional parameter for the monitoring of emphysema in COPD\textsuperscript{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 of more than 200\%\textsuperscript{2}, and mild-to-moderate emphysema can occur without reduction in FEV₁\textsuperscript{3}. Discordance between airways obstruction and gas transfer parameters has been reported in AATD-related emphysema\textsuperscript{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\textsuperscript{7}. Furthermore the use of a single, non-specific, surrogate parameter makes it difficult to assess the effect of specific treatments such as those aiming at reducing or repairing parenchymal damage (e.g. AAT replacement therapy and drugs inducing alveolar repair).

A recent statement of the American Thoracic Society/European Respiratory Society recommended that both airways obstruction and gas exchange should be determined when assessing the overall severity of pulmonary impairment in individuals with AATD\textsuperscript{4}. The gas exchange parameters (TL\textsubscript{CO}:Transfer factor of the lung per carbon monoxide and K\textsubscript{CO}: TL\textsubscript{CO}/Alveolar Volume) measure parenchymal functional damage by assessing the area available for gas diffusion. The measurement of gas
exchange has shown better correlation to the pathological extent of emphysema than spirometric indices⁸ and it presents some potential as tool to monitor emphysema progression. However the method has relatively large longitudinal variability, since TLCO is influenced by changes in alveolar volume⁹ and both TLCO and KCO are influenced by alterations in perfusion over time¹⁰, ¹¹.

The single breath nitrogen 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 parenchyma¹². In non-AATD-related COPD the slope of phase III of the sbN₂-test has been traditionally considered to reflect small airways disease, since it was linked to the pathological score of small airways in smokers with and without airways obstruction¹³. Interestingly, a 13 year follow-up study demonstrated that a high phase III slope associated to a low FEV₁/VC 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 obstruction¹⁴.

Pathologic alterations of the small airways develop in very early stages in non-AATD-related COPD¹⁵. The main known determinant of small airways disease is cigarette smoking, which can induce remodelling of the airway walls; indeed alterations have been reported also in smokers without COPD¹⁵,¹⁶. 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 emphysema¹⁷. Furthermore, in lung tissue from subjects with a panlobular pattern of emphysema, which is characteristic of AATD, small airways showed only minimal pathologic abnormalities¹⁸.
On the basis of this background, we hypothesized that in AATD-related emphysema ventilation inhomogeneity is primarily related to parenchymal destruction, rather than to obstruction due to small airways pathology. To test our hypothesis, we measured ventilation inhomogeneity by the phase III slope of the sbN2-test in patients with type Z AATD-related emphysema, and we 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 part of the Alpha1 International Registry (AIR, nl.air-registry.org) who met inclusion criteria received a written invitation to participate in the study. A group of eighteen subjects entered the study. Inclusion criteria were: a) severe AATD (PiZZ or Pi-Null phenotype), b) emphysema confirmed by CT scan; c) TLco and TLco/VA (Kco) less than 80% of predicted value; d) ex-smokers, who had stopped smoking since at least 6 months, with two negative urine cotinine tests one month apart; e) clinical stability for at least one month prior to the study; f) no changes in inhaled medications and no oral steroid course in the last month prior to the study. The subjects were excluded in case CT scan revealed the presence of giant bullous disease. The study was approved by the Ethics Committee of the Leiden University Medical Centre and patients gave their written informed consent.
**Study design**

The design was cross-sectional, with assessments performed on two consecutive days. At day 1 clinical history was recorded. Smoking history was quantified in pack-years. Quality of life was assessed with the St. George Respiratory Questionnaire and the CRQ-SAS. At day 1 all lung function measurements were performed, except for the sbN\textsubscript{2}-test, which was measured at day 2.

**Lung function**

Lung function tests were performed according to the European Respiratory Society guidelines\textsuperscript{19}. All tests were performed after nebulisation of 5 mg of salbutamol and 500 mcg of ipratropium bromide. The following tests were performed: spirometry with measurement of VC, FE\textsubscript{V}\textsubscript{1}, FVC and FE\textsubscript{V}\textsubscript{1}/FVC; single-breath total lung diffusion capacity (with determination of TLco and Kco) and single-breath nitrogen washout test (sbN\textsubscript{2}-test).

SbN\textsubscript{2}-test was performed using a dry rolling seal spirometer (Morgan Spiroflow) filled with 100% oxygen and equipped with an N\textsubscript{2} meter (Morgan) connected to the mouthpiece, allowing continuous sampling, as previously described\textsuperscript{20,21}. During the test the patients performed a slow full inspiratory and expiratory vital capacity (VC) manoeuvre at a flow rate of 0.5 L/sec. A mechanical flow regulator assured a constant flow. The expiratory N\textsubscript{2} concentration was plotted against volume changes between Total Lung Capacity and Residual Volume, producing the expiratory nitrogen washout curve. The slope of the phase III was calculated by a blinded observer, drawing the best-fit line through phase III of the expiratory volume-concentration curve, and was expressed as % N\textsubscript{2}/L of expired air. This
procedure has been validated in our laboratory, showing good within and between observer repeatability in the slope of phase III, assessed by the intra-class correlation coefficient (Ri = 0.94 within observer and Ri = 0.99 between observer)\(^2\). The measurements were accepted only if the VC during the sbN\(_2\)-test was within 10% of the VC measured by spirometry. All volumes were corrected for body temperature-pressure-saturation with water vapours (BTPS).

**Statistical analysis**

Data analysis was performed using SPSS, version 12.0 (SPSS Inc., Chicago, USA). Values were expressed as mean (standard error of the mean) when normally distributed or as medians (range) when not normally distributed. When the data were not normally distributed, natural logarithmic transformation was performed before data analyses. Parametric correlations were performed to assess the relationship between two parameters at one time, with calculation of Pearson’s coefficient. Results were considered statistically significant at a value of \(p< 0.05\).

**Results**

**Patients’ characteristics**

Demographic and clinical data of the patients are presented in Table 1. All patients were Caucasians, with a mean age of 50 (range: 32-61). Most of the patients had a very early onset the disease, on average before 45 years of age, and the main symptom at onset was dyspnoea on exertion for all the patients but one. All the patients were under treatment with inhaled steroids.
and/or bronchodilators (β2 agonists and/or anti-muscarinic agents) on a regularly basis.

Lung function

As shown in Table 1, AATD patients presented moderate to severe airways obstruction, with preserved forced vital capacity and moderate to severe impairment of gas exchange parameters. At sbN2-test, increased steepness of the slope of phase III was measured in all patients. The mean value of the slope (4.6 ± 1.3 N₂%/L) was higher than the reference values plus 2 standard deviations (upper limits of the reference values: 2.0 N₂%/L for males and 3.0 % N₂% for females) (Figure1)²². The values of the slope were significantly correlated with the gas exchange parameters (TL CO and K CO) but not with airways obstruction, (FEV₁, FEV₁/FVC) (Figure 2). As expected, measures of airways obstruction were negatively associated to smoking history, expressed as pack-years of smoking. On the other hand, neither ventilation inhomogeneity nor gas exchange were linked to smoking history (Figure 3). There was no influence of age on the results of the sbN₂-test (R = 0.114; p < 0.5).

Discussion

We showed 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 our study the abnormalities measured by the sbN₂-test are not associated with cumulative smoking.
This is the first study describing ventilation inhomogeneity in AATD-related emphysema. Increased values of the phase III slope were reported since the late 70's in smokers with and without airways obstruction\textsuperscript{23-26} and have been strongly linked to small airways pathology. A steeper slope reflects a more inhomogeneous lung emptying, which can be caused by heterogeneous airspaces enlargement, airways alterations (remodelling with fibrosis, increased wall thickness, mucus hyper-production) or both. Based on pathology, the smaller, conducting airways have been shown to be the major site of airway obstruction in COPD\textsuperscript{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. On the other hand, in lung tissue from patients with type Z AATD-related emphysema only minimal structural and pathological changes in small airway walls were detected\textsuperscript{17}. These findings, and the good correlation between slope and gas exchange reported in our 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 characteristic even pattern of lobular destruction (panlobular emphysema: PLE) while in non AATD-related emphysema focal lesions are prevalent (centrilobular emphysema: CLE). 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\textsuperscript{28-29} can influence ventilation inhomogeneity by increasing the inhomogeneity of alveolar capacities\textsuperscript{30,31}. Furthermore, loss of elastic recoil
can increase airways resistance during the expiratory phase, by inducing collapse of the small, non-cartilageneous airways. Interestingly, loss of elasticity has been associated with increased airway narrowing in response to bronchoconstrictive stimuli in type Z AAT-related emphysema. The particular anatomical distribution of emphysema in AATD, with prevalent disease at the basal lobes, could also contribute to increase ventilation inhomogeneity. A reduced ventilation in the lower lobes, which are the most ventilated areas of the lung in physiologic conditions, is likely to strongly alter the longitudinal, gravity dependent sequence of lung emptying. The evaluation of the influence of the anatomical distribution of emphysema on ventilation inhomogeneity was not in the aim of our study. Indeed the slope of the phase III has been shown to be influenced by topographical ventilation distribution. Other tests of ventilation inhomogeneity that are more specific for the site of origin, such as the multi-breath nitrogen-test, are required to address this question.

Until now, only gas exchange tests have been investigated as potential tools to monitor alveolar damage in AATD-related emphysema besides spirometry. 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. In addition, 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, it is still 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 with $\text{TL}_{\text{CO}}$ (and $K_{\text{CO}}$) suggests that the parenchymal damage is likely the common pathophysiological denominator of the abnormalities measured by those two tests. However, this does not allow to conclude 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. The $\text{TL}_{\text{CO}}$ 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$^{9-11}$. In contrast to the gas exchange parameters, the results the sbN$_2$-test are hardly influenced by variability in perfusion and ventilation/perfusion mismatch. In the sbN$_2$-test lung volumes and nitrogen values can be calibrated reliably and the slow vital capacity 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 our results we conclude that it is worthwhile to explore the value of the sbN2-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 our study is the fact that the sample size was relatively small. However the range of emphysema severity described in our patients was wide, with values of FEV$_1$ and Kco ranging from mild to very severe, as shown in Table 1. The wide range of emphysema severity and the high correlation between phase III slope and gas exchange values, suggest
that our 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\textsuperscript{36}. However, in our study neither ventilation inhomogeneity nor gas exchange were related to pack-years of smoking history. Even though reporting of smoking history can be affected by recall bias, pack-years is considered to be a valid index in epidemiological studies. Indeed, in line with previous studies\textsuperscript{37}, we found a correlation between smoking history and airways obstruction. Since in our study both ventilation inhomogeneity and gas exchange abnormalities reflect parenchymal damage, our results suggest that determinants other than smoking, such as disease modifier genes or environmental factors\textsuperscript{36,38}, could play a major role alveolar destruction in type Z AATD-related emphysema.

In conclusion, in type Z AATD-related emphysema the increase of ventilation inhomogeneity is likely caused by alveolar destruction rather than by small airways pathology. Our results suggest that the sbN\textsubscript{2}-test can be an interesting surrogate parameter in AATD-related emphysema. Determination of sensitivity of the phase III slope in detecting disease progression may give complementary information to spirometry.

References


17. Tomashefski JF, Crystal RG, Wiedemann HP, Mascha E, Stoller JK; Alpha-1 Antitrypsin Deficiency Registry Study Group. The bronchopulmonary pathology of alpha-1 antitrypsin (AAT) deficiency: findings of the Death Review Committee of the national registry for individuals with Severe Deficiency of Alpha-1 Antitrypsin. *Hum Pathol* 2004: 35: 1452-1461.


Table 1

Clinical and functional patients' characteristics (n=18).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-years</td>
<td>50 (32-61)</td>
</tr>
<tr>
<td>Sex (males/females)</td>
<td>14/4</td>
</tr>
<tr>
<td>Duration of disease-years*</td>
<td>13.5 (6-23)</td>
</tr>
<tr>
<td>Onset of symptoms*</td>
<td>37.5 (24-55)</td>
</tr>
<tr>
<td>Pack-years*</td>
<td>10.2 (3.5-40.5)</td>
</tr>
<tr>
<td>Inhaled bronchodilators / Inhaled corticosteroids #</td>
<td>100 / 11.1</td>
</tr>
<tr>
<td>Symptoms at onset (dyspnoea on exertion/others)</td>
<td>17/1</td>
</tr>
<tr>
<td>FEV₁ % predicted *</td>
<td>39 (29-73)</td>
</tr>
<tr>
<td>FVC % predicted *</td>
<td>103.5 (73-153)</td>
</tr>
<tr>
<td>FEV₁/FVC*</td>
<td>28.7 (19-53)</td>
</tr>
<tr>
<td>TL₂O % predicted*</td>
<td>54.2 (35-82)</td>
</tr>
<tr>
<td>K₂O % predicted*</td>
<td>55.1 (40-68)</td>
</tr>
<tr>
<td>phase III slope (%N₂/liter)§</td>
<td>4.6 ± 1.3</td>
</tr>
<tr>
<td>sat O₂ %§</td>
<td>92.9 (89-98)</td>
</tr>
<tr>
<td>PaO₂§</td>
<td>8.7 (7-11)</td>
</tr>
<tr>
<td>Pa CO₂§</td>
<td>5.1 (4-6)</td>
</tr>
<tr>
<td>SGRQ§</td>
<td>37.7 (13-65)</td>
</tr>
</tbody>
</table>

Data are expressed as § mean (standard deviation), *median (min-max), # percentage of group. SGRQ: St. George Respiratory Questionnaire.
Figure 1

1a

TLC III IV CV

1b

\( dN2 \text{ (%/L)} \)

0 1 2 3 4 5 6 7 8
Figure 2

2a: 
- dN2 (%N2/L) vs. ln TLCO %pred
- r: -0.75
- p: 0.000

2b: 
- dN2 (%N2/L) vs. ln KCO %pred
- r: -0.58
- p: 0.012

2c: 
- dN2 (%N2/L) vs. ln FEV1%pred
- p: > 0.05

2d: 
- dN2 (%N2/L) vs. ln FEV1/FVC
- p: > 0.05
Figure 3

3a: $r = -0.67$, $p = 0.004$

3b: $p > 0.05$

3c: $p > 0.05$

3d: $p > 0.05$
Figure legends

Figure 1

1a. SbN₂-test curve
On the x-axis lung volumes from total lung capacity (TLC) to residual volume (RV). On the y-axis expired nitrogen concentrations.
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: closing volume.

1b. Distribution of the values of the phase III slope
Mean value of the phase III slope (continuous line) was higher than the reference values ± 2 standard deviations (upper limits of normal range: 2.0%N₂/liter for males and 3.0%N₂/liter for females (dotted lines).

Figure 2
Correlation between phase III slope and TL_{CO} (2a), KCO (2b), FEV₁ (2c) and FEV₁/FVC (2d).

Figure 3
Correlation between pack-years and FEV₁ (3a), phase III slope (3b), TLco (3c) and Kco (3d).