Chapter 9

The nitric oxide transfer factor as a tool for the early diagnosis of emphysema

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ABSTRACT

The transfer factor for nitric oxide (Tlno) is independent of pulmonary capillary blood volume due to the very high affinity of nitric oxide (NO) to hemoglobin, in contrast to the transfer factor for carbon monoxide (Tlco). Therefore the sensitivity for the detection of alveolar destruction is supposed to be higher.

We measured flow volumes curves, Tlno, Tlco, the transfer coefficients Kno and Kco and performed high-resolution computed tomography (HRCT) in 263 randomly selected heavy smokers. Participants with areas ≥1% of the total lung volume showing an attenuation <-950 Hounsfield Units were considered to have emphysema. In 36 participants emphysema was diagnosed with HRCT, an abnormal Kno was present in 94 participants, and in 95 participants a FEV_1/FVC ratio <70% was seen. The area under the ROC curve for the Kno was 0.894 and for the Kco 0.822. The Kno therefore showed a slightly higher sensitivity to detect emphysema, compared to the Kco. The positive predictive value of K_{NO} however was low (34.7%), while the negative predictive value of Kno was very high (98.2%), indicating an exclusion test. The Tlno/Tlco ratio is significantly higher compared to normal participants, indicating a strong influence of a decreased microvascularity.

INTRODUCTION

The prevalence of chronic obstructive pulmonary disease (COPD) is expected to increase in the next decades, leading to a decreased quality of life in older participants, as well as an increased financial burden to society [1]. Estimations of the percentage of smokers developing COPD vary from 0.3 to 8.5%, depending on diagnostic criteria [2-4].

Emphysema is a component of COPD and is defined as an abnormal enlargement of the terminal bronchioles and alveoli [5]. Gevenois et al [6] demonstrated that the amount of abnormally low attenuation areas on high resolution computed tomography (HRCT) scans correlated well with the amount of emphysema present in lung specimens. Several investigators showed that the transfer factor (Tlco) and transfer coefficient (Kco) for carbon monoxide (CO) showed a stronger correlation with HRCT emphysema indices than the FEV_1 and FEV_1/FVC ratio [7-9]. HRCT as an instrument for mass screening for COPD has the disadvantage of high costs. The Tlco and Kco are simple and cheap parameters, available in any pulmonary function laboratory, and may be used as a tool for early detection of emphysema.

The Tlco is dependent on the thickness and surface area of the alveolocapillary membrane, its solubility in water and binding to hemoglobin. This has been formulated by Roughton and Forster [10] in 1959: $1/Tlco=1/Dmco + 1/\theta co*Vcap$, where Dmco is the membrane diffusing capacity for CO, θco the CO uptake by erythrocytes and Vcap the pulmonary capillary blood volume. Since 15 years studies have been performed with nitric oxide (NO) instead of CO [11;12]. NO has a much stronger affinity for hemoglobin, so θ_{NO} is very high, leading to a negligible value for $1/\theta_{NO}*Vcap$. Therefore, the transfer factor for NO (Tlno) is supposed to represent the true membrane diffusing capacity [13]. A predominantly vascular disease will lower the Tlco, but not the Tlno, as it is not influenced by erythrocyte NO uptake (=decreased Vcap). The Tlno/Tlco will tend to increase when predominantly vascular disease is present [14]. On the other hand [15], a predominantly membranous disturbance will affect both Tlco and Tlno, and the alleged high sensitivity of the Tlno for membranous disturbances will tend to sharply decrease Tlno. The Tlco, being partly dependent on membranous damage will not change that sharply: the ratio will tend to decrease.

The aim of this study was to determine whether the Tlno is a better screening tool for the detection of emphysema than the Tlco, and if the Tlno/Tlco ratio differs from healthy participants.

METHODS

PARTICIPANTS

All participants of the current study were participating in the NELSON-project, a Dutch-Belgian multi-center lung cancer screening trial. The participants were all male, 50 to 75 years of age with a smoking history of at least 16 cigarettes/day for 25 years or 11 cigarettes/day for 30 years. Exclusion criteria were current or past melanoma, renal breast or lung cancer diagnosed <5 years before recruitment, a chest CT scan <1 year before recruitment, a body weight ≥140 kilogram and quitting smoking >10 years before start of the trial.

This study was performed in the University Medical Center Utrecht, one of the participating hospitals in the NELSON study. The NELSON-project was approved by the Dutch ministry of health and by the ethics committee of the participating hospital; informed consent was obtained from all participants. From three thousand participants who underwent the screening in our hospital, randomly one out of three screened participants was selected for pulmonary function testing on the same day.

PULMONARY FUNCTION TESTING

Spirometry and flow-volume curves measurements were obtained via pneumotachography, according to ERS guidelines [16]. No reversibility testing was done. In line with the GOLD classification [17], a FEV1/FVC ratio <70% was labeled as abnormal. A simultaneous single breath Tlno and Tlco test was performed directly after spirometry, as described earlier [18]. At least two measurements per participant were obtained and a difference <10% in Tlco was acceptable. All pulmonary function values are given as a mean with standard deviation (SD), and as a percentage of predicted values [16;19]. For Tlno we used references equations from an earlier study. Values <-1.64 times the standard deviation were considered abnormal. The Tlno/Tlco and FEV1/FVC ratio's are expressed as absolute values.

CT SCANNING AND EMPHYSEMA QUANTIFICATION

CTs were performed on a 16 detector-row scanner (Mx8000 IDT or Brilliance 16P, Philips Medical Systems, Cleveland, OH), scan time was 12 seconds, in spiral mode with 16 x 0.75mm collimation, 1.0 mm reconstruction thickness, without contrast-injection. After automatic lung segmentation connecting all areas below -500 Hounsfield units (HU) starting in the trachea and excluding the main bronchi, quantification of emphysema was done highlighting low attenuated areas [20] using in-house developed software (ImageXplorer, Image Sciences Institute, Utrecht, The Netherlands), with a threshold of -950 HU. Emphysema scores (ES) were calculated as the volume with an attenuation <-950 HU indexed to total lung volume. Participants with an ES≥1% were considered to suffer from emphysema [7;21;22].

STATISTICS

Parameters are depicted as means with standard deviations (SD). Spearman correlation coefficients were used to assess significant relationships; the area under the curve (AUC) of the receiver operator curves (ROC) were used to assess the capability of the pulmonary function parameters to signal the absence or the presence of emphysema. One-way ANOVA was used to compare differences between groups. All statistics were calculated with SPSS for Windows release 13 (SPSS Inc, Chicago, Ill.).

RESULTS

Between October 2004 and April 2005 263 male participants were included in the study. Several characteristics of the study population are given in Table 1. Thirty-six (13.6%) participants were shown to suffer from emphysema (ES≥1).

Significant negative correlations were observed between all diffusion capacity parameters, the FEV_1/FVC ratio and the ES (Table 2). The mean Tlno/Tlco ratio was 4.9, which is higher than the 4.3 value reported by Borland et al [11] in healthy participants and also significantly higher (p<0.001) than the 4.6 value in 65 males or the 4.4 value in men and women pooled, derived from the previous data.

The AUC of the ROC curves showed the highest values for Kno (as percentage of predicted) to detect emphysema, with Kco (also as percentage predicted) and FEV_1/FVC in second respectively in third highest position (Table 3). The differences between the AUC ROC for the Kno and Kco were small and clinically irrelevant.

The sensitivity and specificity of all parameters to detect an ES≥1% are given in Table 4, as well as the positive (PPV) and negative (NPV) predictive values. The NPV is especially high indicating that a normal Kco, Kno or FEV1/FVC virtually excludes an ES≥1%. The low PPV values indicate that an abnormal Kco, Kno or FEV1/FVC only points at an ES≥1% in a minority of the cases.

Participants with low Kno values, participants with emphysema on HRCT and participants with a FEV1/FVC ratio lower than 70% form partially overlapping groups, which can be illustrated with a Venn diagram (Figure 1).

	Mean	Range	SD
Age, years	60.3	52.3-76.9	5.4
Height, m	1.78	1.61-2.00	0.07
VC, %pred	105.5	61.1-147.5	13.2
FEV_1/FVC ratio, %pred	93.6	42.3-113.9	11.7
FEV_1, %pred	97.7	43.0-140.8	16.8
FEV_1/FVC ratio	71.5	32.4-86.9	9.0
Tlco, %pred	87.4	49.8-140.4	16.1
K_{CO}, %pred	84.4	46.6-140.2	15.9
Tlno, %pred	87.5	45.3-121.3	13.5
K_{NO}, %pred	90.4	53.7-121.6	12.4
Tlno/Tlco ratio	4.9	3.8-6.4	0.4
emphysema score	0.6	0.0-14.7	1.5

Table 1

Characteristics of the study population, n = 263

	Emphysema score
FEV_1/FVC ratio	-0.43*
Tlco, %predicted	-0.26*
K_{CO}, %predicted	-0.38*
Tlno, %predicted	-0.29*
K_{NO}, %predicted	-0.50*

* = $p < 0.01$

Table 2

Correlation coefficients of the emphysema score versus pulmonary function data

	AUC	p=	95%CI
FEV_1, %pred	0.656	<0.003	0.551-0.761
FEV_1/FVC, %pred	0.795	<0.001	0.710-0.880
Tlco, %pred	0.727	<0.001	0.622-0.833
K_{CO}, %pred	0.822	<0.001	0.757-0.887
Tlno, %pred	0.711	<0.001	0.608-0.815
K_{NO}, %pred	0.894	<0.001	0.850-0.938

Table 3

Area under the curve (AUC) with 95% confidence intervals (95% CI) of the receiver operator curves (ROC).

	Sensitivity	Specificity	PPV	NPV
Tlco	58.3%	81.5%	33.3%	92.5%
Tlno	50.0%	81.9%	30.5%	91.2%
Kco	88.9%	57.3%	24.8%	97.0%
Kno	91.7%	72.7%	34.7%	98.2%
FEV_1/FVC	77.8%	70.1%	29.5%	95.2%

Table 4

Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of the measured parameters to detect emphysema.
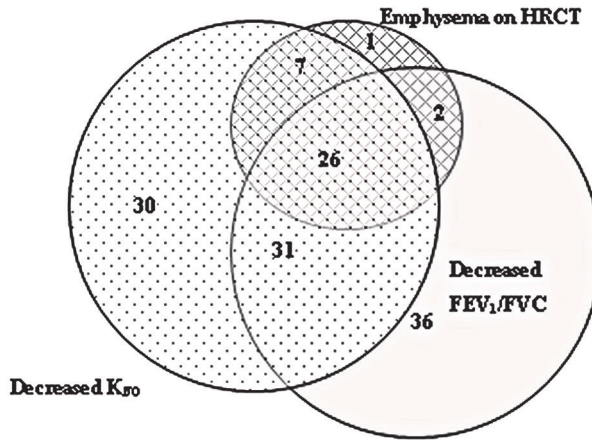

Figure 1

Venn diagram: the presence of emphysema on HRCT (n=36), a decreased Kno (n=94) and a decreased FEV_1/FVC ratio (n=95) are partially overlapping entities.

DISCUSSION

We measured the single breath transfer factor and transfer coefficient for CO and NO in a large sample of current and former heavy smokers. The hypothesis was that the Tlno would better detect emphysema on HRCT than the Tlco, based on its alleged higher sensitivity for alveolar membrane destruction. However, this proved not to be the case.

The Tlno/Tlco ratio in these smokers is significantly higher than in healthy participants, and reaches values seen in participants with pulmonary arterial hypertension [14;15], suggesting that in this group of smokers vascular damage is an important phenomenon and strongly influences the diffusion capacity. In the data from Borland et al a Tlno/Tlco ratio of 5.0 in participants with PAH versus 4.5 in healthy participants was reported [23].

The study outcome indicates that lung function is not very sensitive to detect emphysema, even if the parameter used is maximally influenced by a loss of alveolar membranes, like the Tlno and the Kno. Apparently the early presence or a low degree of emphysema does not influence the lung function significantly and other processes, not directly linked to emphysema, are more important for the changes in lung function. We conclude that, at least in the beginning, the status of the non-emphysematous parts of the lung influences lung function parameters more than the emphysematous parts. One must acknowledge that the lung function is influenced by the status of the entire lungs and that emphysematous parts are only subdivisions of that total, so small amounts of emphysema will easy go undetected. The CT-scan can however detect such small amounts of emphysema more easily.

The reason why the Tlno and Kno are only marginally better in detecting emphysema compared to the Tlco and KCO is probably due to the fact that in our study population the majority of the tested participants showed either no or only small amounts of emphysema. These small amounts of loss of alveolar tissue and the higher sensitivity of the Tlno and Kno is apparently not sufficient to overcome the problem of the low emphysema expression in lung function. When the non-emphysematous parts of the lungs dictate the decrease in function strongly the Tlno and Kno will lose their theoretical advantage.

As a result the correlation between CT and lung function apparently can not be strong. In older reports discrepancies between the pulmonary function testing and the presence of CT-based emphysema have been reported in participants with COPD [20] and in candidates for lung resection or transplantation [7]. This confirms our conclusion.

In this population based study in heavy (former) smokers the correlation between pulmonary function tests and CT based assessment of emphysema is even weaker than reported earlier [20]. This is not unexpected, because our participants were not selected on the presence of COPD via lung function testing. In studies in which such work-up bias was present, one might expect stronger correlations, due the

presence of more severe disease. As a consequence, the correlation between for example the FEV_1/FVC ratio and the emphysema score is low: 8 out of 36 participants with emphysema on HRCT had a FEV_1/fvc ratio above 70%.

Similar arguments can be used to explain why the spirometric parameters (FEV1 and FEV_1/FVC) also show rather low AUC's of the ROC curve values. As with the diffusion parameters: they are less useful to diagnose emphysema. Again we must conclude that the presence or the degree of emphysema does not influence spirometry significantly.

As mentioned before in the introduction, a high Tlno/Tlco ratio points at a significant influence of diffusion parameters on vascular damage. The Tlno is not influenced to the same extent by vascular damage as the Tlco: the ratio therefore will increase if such damage is significant. Now we calculated this ratio for the entire sample, and most participants had no emphysema detectable on CT. The reduction of the diffusion capacity parameters therefore seems to be caused to a large extent by damage of the alveolar vascular compartment by smoking. This functional damage may be renamed as vascular malfunctioning. The fact that the high Tlno/Tlco ratio also characterizes the non-emphysematous participants points at the fact that functional alveolar vascular damage precedes the loss of alveolar tissue measurable via CT-scanning. This is not an illogical approach: overt alveolar tissue loss will be small in early disease. It is conceivable that alveolar vessel function already is impaired in such an early stage of the disease, followed by the more overt loss of tissue later. The loss of alveolar vascular function will of course coincide with a loss of recoil because it is hard to conceive that only the vessels in the alveolar membrane will suffer from smoking.

Spirometry, gas transfer and CT-scans are complementary in the detection of emphysema and partially overlap (Figure 1). In clinical practice this can be used in excluding emphysema: only when combining all measures a complete description of the pathophysiology of the lung will be obtained.

The commonly used GOLD criteria [17] are mainly based on the presence of airflow limitation. The FEV_1 has been chosen as the major determinant because abundant data are available to correlate the FEV_1 with symptoms, prognosis and mortality. Such data are not available for HRCT-detected emphysema, and are scarce for diffusion parameters. Emphysema, however, is not always accompanied by significant airflow limitation. Therefore, one must realize that the COPD is more than just airflow limitation. In the GOLD criteria stage 0 is defined as "at risk": this compasses a FEV_1/FVC ratio >70% combined with complaints of coughing and increased phlegm production. By strictly using the FEV_1/FVC cut off point of 70% and ignoring the transfer factor (or tissue destruction on HRCT), selection bias is introduced.

LIMITATIONS

Although it was recommended that the participants refrained from smoking 24 hours prior to testing, the question is always open whether this advice was

followed. This could have led to a small decrease in the Tlco based on higher CO-backpressure. In our believe this could not alter the main results, because the smoking effect is very small: the Tlco decreases by 1% for each percent COHb present. So when the presence of COHb is 7%, the Tlco decrease by approximately 7% [25]. Such high levels of COHb are seldom.

The Tlno references equations are based on a previous study in 124 participants, of which were 65 males with a mean age of 40.1 years. The mean age in this study was higher. However, the Tlco values in that previous study match exactly with the ECCS vales 19, so gross deviations in estimating age, and height effects are unlikely. The extrapolation to older participants is therefore possible.

The cut off value for the amount of emphysema on HRCT scan suffers from a lack of consensus: we defined a cut-off point of 1% at -950 HU based 1 mm slice techniques, based on the study of Kinsella et al [22].

Spirometry is very important for the diagnosis and classification of emphysema, but only measures airway obstruction. The Kno is a sensitive measure for the detection of emphysema on HRCT. Pathophysiological changes in emphysema also compass microvascular changes and parenchymal loss. It is not unlikely that microvascular disease precedes extensive parenchymal loss eventually leading to airway obstruction. Longitudinal studies are needed to further explore this concept.

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