# Nitric oxide and carbon monoxide

# diffusing capacity of the lung

***Diffusiecapaciteit van de long voor***
***stikstofmonoxide en koolmonoxide***

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

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Voor mijn ouders

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## Contents

Chapter 1: General Introduction ..... 1
History ..... 1
The carbon monoxide diffusing capacity of the lung ($DL_{CO}$) ..... 3
Clinical use of the $DL_{CO}$ ..... 5
The dependency of the $DL_{CO}$ on the alveolar volume ($V_A$) ..... 6
The value of the measurement of Dm/Vcap ..... 8
The carbon monoxide diffusing capacity of the lung ($DL_{NO}$) ..... 10
Interpretation of diffusion impairment ..... 13
Conclusion ..... 14
Outline of this thesis ..... 16
References ..... 17
Part 1: The carbon monoxide diffusion capacity of the lung
Chapter 2: Pattern of diffusion disturbance related to clinical diagnosis: the $K_{CO}$ has no diagnostic value next to the $DL_{CO}$ ..... 23
Abstract ..... 24
Introduction ..... 25
Methods ..... 26
Results ..... 30
Discussion ..... 32
References ..... 39
Chapter 3: Alveolar volume determined by single-breath helium dilution correlates with the high-resolution computed tomography-derived nonemphysematous lung volume ..... 43
Abstract ..... 44
Introduction ..... 45
Methods ..... 46
Results ..... 47
Discussion ..... 50
References ..... 53
Chapter 4: Early diagnosis of emphysema: computed tomography versus pulmonary function testing ..... 55
Abstract ..... 56
Introduction ..... 57
Methods ..... 58
Results ..... 60
Discussion ..... 65
References ..... 68

Part 2: The nitric oxide diffusion capacity of the lung
Chapter 5: Diffusing capacity for nitric oxide: reference values and dependence on alveolar volume ..... 73
Abstract ..... 74
Introduction ..... 75
Methods ..... 76
Results ..... 78
Discussion ..... 82
References ..... 85
Chapter 6: The effect of red cell transfusion on nitric oxide diffusing capacity ..... 87
Abstract ..... 88
Introduction ..... 89
Methods ..... 90
Results ..... 93
Discussion ..... 95
References ..... 97
Chapter 7: Diffusing capacity for nitric oxide and carbon monoxide in patients with diffuse parenchymal lung disease and pulmonary arterial hypertension ..... 99
Abstract ..... 100
Introduction ..... 101
Methods ..... 102
Results ..... 104
Discussion ..... 107
References ..... 111
Chapter 8: The nitric oxide transfer factor as a tool for the early diagnosis of emphysema ..... 115
Abstract ..... 116
Introduction ..... 117
Methods ..... 118
Results ..... 119
Discussion ..... 122
References ..... 126
Chapter 9: Summary, conclusions and view ..... 129
Samenvatting ..... 137
Dankwoord ..... 149
Curriculum vitae ..... 152

# Chapter 1

# General Introduction

## History

Simple measurements of vital capacity were first performed in the middle of the 17th century by Borelli (1679). Hutchinson (1846) designed a spirometer to assess vital capacity and performed some studies. In that second half of the 19th century, rapid progression was noted in the field of lung mechanics and from the beginning of the 20th century pulmonary gas exchange became a research topic in physiology. Great differences in views were present: J.S. Haldane (1860-1936) and Christian Bohr (1855-1911) supported the concept of "oxygen secretion" as the major function of the lung. In this concept the oxygen uptake by the lung was seen as an active process. August Krogh (1874-1949) on the contrary supported the concept of a passive diffusion of oxygen from the alveolar air to the pulmonary capillaries. Marie Krogh (1874-1943), August's wife, was the first to develop the basis underlying the measurement of the diffusing capacity of the lung [1]. She used carbon monoxide in a single breath inspiration method and up to now this method has not changed very much. After the development of the fast infrared carbon monoxide meter in the Second World War the measurement of the diffusing capacity of the lung was standardized and it became a routine method in many lung function laboratories [2]. Roughton and Forster [3] revitalized the diffusing capacity measurement by distinguishing two major components: the passage of the (test)gas through the alveolocapillary/red blood cell membrane and the uptake of gas by hemoglobin in the red blood cell. They developed a method which could be used in a lung function laboratory with a double measurement of the diffusing capacity for carbon monoxide with a low and a high oxygen concentration, which results in a value of the diffusing capacity of the alveolocapillary membrane (Dm) and a value for the pulmonary capillary blood volume (Vcap) (see later). This method is still in practice today in some pulmonary function laboratories.

## The carbon monoxide diffusing capacity of the lung ($DL_{CO}$)

The main function of the lung is gas exchange, which can be assessed in several ways. Spirometry measures the flow and volumes of inspired and expired air, and does not provide information about gas exchange *per se*. An arterial blood gas sample is the most simple way to assess pulmonary gas exchange, although it has the disadvantage that abnormalities are only seen when substantial changes in lung function are present. Arterial blood gas sampling during exercise, preferable combined with oxygen uptake and carbon dioxide emission measurements, is a very good and sensitive way to assess gas exchange abnormalities. Unfortunately this is a time consuming method with substantial discomfort for the patient. The measurement of the carbon monoxide diffusing capacity of the lung ($DL_{CO}$) is a fast and reproducible method to assess the pulmonary gas exchange. There are three methods available: 1] a single breath method, 2] a steady state method and 3] a rebreathing method. The single breath method is the most frequently used method: easy to perform and widely available. This review will be confined to the single breath method.

The method is simple: after exhaling to residual volume, the test subject inhales a mixture of carbon monoxide, helium and air to the level of total lung capacity. After a breath holding period of 10 seconds the subject exhales as fast as possible. The first 750 ml of the expiratory air is discarded and the following sample of air is considered to represent alveolar air. A pneumotachometer measures air volumes and the concentrations of inspiratory and expiratory carbon monoxide and helium are measured. To compensate for dilution, the alveolar inspiratory carbon monoxide concentration is multiplied by the ratio of the expiratory/ inspiratory helium concentrations.

$$DL_{CO} = \frac{V_A}{t} \times \log \frac{FA,_{CO}\,(t=0)}{FA,_{CO}\,(t=t)} \qquad \textbf{Equation 1}$$

***Equation 1***. *Calculation of $DL_{CO}$. $V_A$ is the effective alveolar volume, t is the breath holding time, $F_{A,CO}$ (t=0) is the alveolar CO concentration at t=0, and $F_{A,CO}$ (t=t) is the alveolar CO concentration at t=t.*

Some investigators prefer the term transfer factor of the lung instead of diffusion capacity, because *diffusion* is not the only physical process that is measured. The term $DL_{CO}$ compasses two entities: first, the diffusion of the test gas through the alveolocapillary membrane, the plasma and the intra-erythrocytic compartment, and second, the binding of the test gas to hemoglobin. The first process is determined by the solubility of the gas, the molecular weight, the surface and the thickness of the membrane, and the pressure gradient. The second process is limited by the reaction rate of the test gas binding to hemoglobin. An important factor is that the two compartments (the alveolar air and the hemoglobin in the red blood cells) are not homogeneously distributed.

The determination of the Dm and Vcap is based on the determination of the components of the single breath $DL_{CO}$, as defined by Roughton and Forster in Equation 2. This equation contains two unknown figures and therefore can not be solved. $\Theta_{CO}$ depends on the alveolar oxygen concentration and is known from experiments. Equation 2 can be solved via two measurements at high and low alveolar oxygen concentrations.

$$\frac{1}{DL_{CO}} = \frac{1}{Dm_{CO}} + \frac{1}{\Theta_{CO} \times [Hb] \times Vcap} \quad \textbf{Equation 2}$$

***Equation 2.*** *Roughton and Forster equation for the $DL_{CO}$. $Dm_{CO}$ is the membrane diffusing capacity for carbon monoxide, Vcap the pulmonary capillary blood volume, $\Theta_{CO}$ the reaction rate of CO to hemoglobin at a hemoglobin concentration of 9.0 mmol/l, and [Hb] the actual hemoglobin concentration.*

The values of $Dm_{CO}$ and of Vcap can be used to determine whether the diffusion impairment is located at the alveolocapillary membrane or in the vascular compartment. The $DL_{CO}$ is strongly associated with the level of exercise of a subject and therefore a standardized $DL_{CO}$ measurement is performed after 10 minutes of rest. At increasing exercise levels, the $DL_{CO}$ increases linearly [4]. The reason for this phenomenon can be recruitment of alveoli (increasing $Dm_{CO}$), recruitment of pulmonary capillaries (increasing Vcap), better matching of the perfusion and ventilation or a combination of these

factors. Since the $Dm_{CO}$ and the Vcap are not independent variables (in order to measure $Dm_{CO}$ capillary blood flow is a prerequisite), the measurement of the subdivisions of the carbon monoxide diffusing capacity can not clearly distinguish between these options. It can point to the relative contribution of the membrane and vascular compartment for the whole lung. In other words, the lung is observed as a mono-alveolar object, and not as many parallel coupled alveoli.
Subjects have to refrain from smoking at least 24 hours before testing [5,6] because the accumulation of COHb causes an anemia effect and smoking decreases the $DL_{CO}$ and the Vcap, possible due to pulmonary vasoconstriction [7].

## Clinical use of the $DL_{CO}$

The $DL_{CO}$ is determined by sex, height and age, and reference equations have been calculated including these parameters [6;8]. In patients with chronic obstructive pulmonary disease (COPD) the $DL_{CO}$ is an independent prognostic factor [9], next to the $FEV_1$. The $DL_{CO}$ has been shown to predict desaturation during exercise in patients with COPD, when using a threshold of 55% of the predicted value [10], and in this respect the $DL_{CO}$ performs better than the $FEV_1$. Other authors found a cut-off point of 62% of the predicted $DL_{CO}$ in 8017 patients (most of them with airway obstruction, but restrictive pulmonary diseases were also included), with 75% sensitivity and specificity for desaturation during exercise [11]. In 217 COPD patients with long term oxygen therapy, the $DL_{CO}/V_A$ appeared to be a very strong predictor for mortality [12]. In a study comparing the sensitivity of the $DL_{CO}$ and the pressure-volume curves as determined by transpulmonary pressure measurements with an esophageal balloon to detect pathologically assessed emphysema in resected lung specimens, the $DL_{CO}$ appeared to be superior [13].
There are several pathological conditions [14] associated with an increased $DL_{CO}$: asthma, obesity, polycythemia, hemoptysis, and left-to-right shunt. The latter three are due to increased pulmonary capillary blood volume, or free alveolar red blood cells. The fact that ventilation inhomogeneity in asthma does not lead to lowering of the $DL_{CO}$ is remarkable. The most likely explanation is that in asthma more perfusion is present at the apices of the lung [15], leading to a higher $DL_{CO}$. It is not inconceivable that this is closely associated with

sequential filling of the lungs, because this phenomenon has a major impact on the diffusion capacity, and probably plays an important role especially in obstructive pulmonary diseases [16].

The $DL_{CO}$ is an important tool in the diagnosis and prognosis of patients with diffuse parenchymal lung disease (DPLD) [17], and is used to assess the response to therapy. In patients with systemic sclerosis and interstitial lung disease, a significant correlation between the $DL_{CO}$ and the amount of lymphocytes in bronchial alveolar lavage fluid (as a quantitative marker for inflammation) has been observed [18]. In subjects with DPLD the reason for the exercise limitation lies solely in the diffusion limitation, because an increase in capillary blood flow can not compensate the decrease in oxygen uptake [19]. Furthermore, in DPLD-patients the transfer factor is lowered during exercise. The reason for this phenomenon is that the mean transit time of an erythrocyte in the alveolar capillaries is about one second at rest and during the first 0.3 second complete oxygen saturation is achieved in healthy subjects. During exercise the transit time is shorter, which means that complete saturation will not be achieved when a diffusing disturbance is present due to thickened alveolocapillary membranes. This will cause hypoxemia during exercise.

In pulmonary arterial hypertension (PAH) the $DL_{CO}$ is often decreased. Sun et al. [20] found a decreased $DL_{CO}$ in 75% of 79 patients with primary pulmonary hypertension and the $DL_{CO}$ correlated better with the decrease in peak oxygen uptake than spirometric values. In subjects with the CREST syndrome, who are prone for the development of PAH, a decrease in $DL_{CO}$ can precede the clinical assessment of PAH [21].

In assessing subjects who are candidates for lung resection therapy and even thoracotomy alone the $DL_{CO}$ is an indispensable test, next to spirometry [22].

## The dependency of the $DL_{CO}$ on the alveolar volume ($V_A$)

The dependence of the $DL_{CO}$ on the $V_A$ is known for a long time, and is cumbersome because all reference values are valid only at maximum TLC levels. In subjects with lung disorders and a decrease of TLC, the $DL_{CO}$ has to be lower because of the lower TLC level. Johnson [23] measured the change in $DL_{CO}$ and $K_{CO}$ in 24 healthy subjects, and formulated reference equations to adjust the predicted $DL_{CO}$ and $K_{CO}$

for $V_A$. He evaluated these reference equations in 2313 patients with various obstructive and restrictive pulmonary diseases, and he advised to use $V_A$-adjusted reference equations for patients with pulmonary diseases for a better assessment of lung function. A similar advise is given by Stam [24], based on thorough research with 55 healthy subjects [25], in whom he found a strong dependency of $DL_{CO}/V_A$ on $V_A$: the $K_{CO}$ rises if the $V_A$ decreases. This phenomenon is also present in patients with restrictive pulmonary diseases [24]. Stam advised to use reference equations at corresponding lower TLC-levels in patients with restrictive lung diseases [24], excluding the restrictive factor as the cause of the diffusing disturbance. Frans et al. [26] also observed that the $DL_{CO}$ and $DL_{CO}/V_A$ strongly depend on $V_A$. They measured $DL_{CO}$ and $DL_{CO}/V_A$ in 23 healthy subjects at four different inspiratory levels, in patients with high $V_A$, in patients with COPD and in patients with DPLD. They concluded that the use of correction formulas on the theoretical values in restrictive pulmonary diseases should be promoted, or simple to "consider that in patients with restrictive lung disease, $DL_{CO}$ is underestimated and $DL_{CO}/V_A$ is overestimated" [26]. Chinn et al. [27] proposed the use of a linear model in order to replace $K_{CO}$. Their model included the term of $V_A$*height$^{-2}$ next to the already used components of sex, height and age. They claim that reference equations composed with this model improve the accuracy of normal values for the $DL_{CO}$, especially in patients with disturbed $V_A$. As far as we know neither of these or other models [28] are used on a broad scale in pulmonary medicine today. There are some possible explanations for this, at first some of these models are very complex, and hard to fathom for clinicians who are not familiar to such a degree with gas exchange physiology. A second problem is that the models are based on healthy subjects who performed $DL_{CO}$ measurements at different inspiratory levels. The question remains whether these results can be extrapolated to subjects with diseased lungs. Concerning the use of the $K_{CO}$, experts in the field of physiology have different opinions, some think that that $K_{CO}$ is a very useful tool [29], some have a different opinion [30].

## The value of the measurement of Dm/Vcap

### *Clinical studies*

For a good understanding of the underlying pathophysiology in subjects with impaired gas exchange, it is important to know at which anatomical localizations alterations can occur. In patients with DPLD a different disease mechanism will lead to impaired gas transfer as compared to subjects with COPD. There are a several possibilities: 1] the lung volume can be decreased, 2] the alveolocapillary membranes can be thickened, 3] a decreased perfusion of ventilated alveoli is present, or 4] a combination of these three pathological entities. It seems logical to use the Dm and Vcap measurements to obtain insight in the localization of the defect causing the diffusion disturbance. Therefore, many studies using Dm and Vcap measurements have been conducted. Saumon et al. [31] analyzed 77 patients with sarcoidosis by measurement of single breath $DL_{CO}$ at different inspiratory oxygen concentrations. They found a decrease in $DL_{CO}$ from stage I (only lymph node enlargement on the chest X-ray) to stage III (DPLD without lymph node enlargement on the chest X-ray). The Vcap was decreased in the stage III group, but not in the groups with stage I and II (DPLD with lymph node enlargement on the chest X-ray), whereas the lowering of the Dm was similar to that of the $DL_{CO}$ from group I to III. He also investigated a group of 20 patients with other types of DPLD and all had very low $DL_{CO}$ values with both diminished Dm and Vcap values. Lamberto et al. [32] found reduced $DL_{CO}$ and Dm in 24 patients with sarcoidosis, and very slightly reduced Vcap values. The $DL_{CO}$ and Dm were the strongest predictors for gas exchange abnormalities during exercise.

In 1960, Bates et al. [33] published a stimulating paper describing the clinical use of steady state $DL_{CO}$ with its components. They investigated healthy subjects and a variety of patients with pulmonary diseases, in which this method yielded valuable clinical information. In subjects with DPLD the Dm was diminished but the Vcap not or only slightly.

Steenhuis et al. [34] measured the $DL_{CO}$ and its components in 19 patients with primary pulmonary hypertension (PPH) and in 8 patients with CTEPH (chronic thromboembolic pulmonary hypertension). Although it is well known that pulmonary hypertension can lead to a decreased diffusion capacity [35], the expectation was that in patients

with CTEPH the Dm and Vcap could differentiate between CTEPH and PPH. Unfortunately this was not the case. The $DL_{CO}$, the Vcap and the Dm were lower in both groups to a similar degree, which forced the authors to appoint the possibility of functional impairment of the alveolocapillary membrane. Bernstein et al. found similar results [36].
The Dm and Vcap cannot discriminate between subjects with DPLD with PAH and subjects with DPLD without PAH [37]. The use of the Dm and Vcap measurement in pulmonary embolism did not have additional value next to the $DL_{CO}$ measurement [38;39].
Subjects with chronic heart failure have decreased $DL_{CO}$ and Dm [40], which does not improve after heart transplantation [41], probably due to irreversible changes to the alveolocapillary membrane. The lowered $DL_{CO}$ in patients with chronic heart failure is strongly correlated with exercise limitation [42].

### *Dependency on exercise*

The increase of the $DL_{CO}$ as measured during exercise is due to an increase in the Vcap, whereas the $Dm_{CO}$ remains unchanged [33]. During exercise also a strong linear relation has been observed between the $DL_{CO}$, the $Dm_{CO}$, the Vcap and the Qc (the pulmonary capillary blood flow) [43].

### *Dependency on $V_A$*

The decrease of the $DL_{CO}$ when measured at 50% of TLC is mainly due to a decrease of the $Dm_{CO}$, and is not based on a change in the Vcap [44]. The reason for the dependency of the $DL_{CO}$ on $V_A$ is not evident, but is probably due to a recruitment of capillaries, because at full TLC level the negative intrathoracic pressure will lead to accumulation of blood in the thorax. Another explanation could be the red blood cell orientation in the pulmonary capillaries: when the red blood cells are positioned with longitudinal axis parallel to the alveolar surface, which is achieved at high inflation pressure (full inspiration), this will lead to a shorter diffusion distance which can increases gas diffusion significantly [45].

***Dependency on posture***

The $DL_{CO}$ dependency on posture was shown by several investigators. An increase in $DL_{CO}$ from sitting to supine position has been observed [46], with a relative greater increase in Vcap than in Dm [47]. The single breath $DL_{CO}$ also differs between a prone and a supine position. In 14 healthy subjects, the single breath $DL_{CO}$ with its subdivisions was acquired with the high/low oxygen method and the $DL_{CO}$ was 8% lower in the prone than in the supine position [48]. Dm and Vcap were slightly but not significantly lower in the prone position. The authors interpreted the results as a consequence of the position of the heart in the thorax [49]. These investigations led to the recommendation that the $DL_{CO}$ measurement has to be performed in a sitting or standing position [5,6].

## The carbon monoxide diffusing capacity of the lung ($DL_{NO}$)

In search for a more specific method to measure the membrane diffusing capacity than the $DL_{CO}$, the $DL_{NO}$ has been developed. The binding of nitric oxide (NO) to hemoglobin is about 280 times faster than that of CO [50].

$$\frac{1}{DL_{NO}} = \frac{1}{Dm_{NO}} + \frac{1}{\Theta_{NO} \times [Hb] \times Vcap} \quad \textbf{Equation 3}$$

In the Roughton and Forster equation for the $DL_{NO}$ (Equation 3), $\Theta_{NO}$ is very high, and $1/ \Theta_{NO}$*Vcap will be negligible. Therefore $DL_{NO}$ equals the $Dm_{NO}$, and $DL_{NO}$ only represents the membrane diffusing capacity. The relationship between the $DL_{NO}$ and the $Dm_{CO}$ can be calculated from the molecular weights (MW) and the solubility factors ($\alpha$) of NO and CO (Equation 4).

$$\frac{DL_{NO}}{Dm_{CO}} = \frac{\alpha NO}{\alpha CO} x \sqrt{\frac{MW_{CO}}{MW_{NO}}} = \frac{0.0364 mL^{-1} atm^{-1}}{0.0183 mL^{-1} atm^{-1}} x \sqrt{\frac{28}{30}} = 1.93 \quad \textbf{Equation 4}$$

Most investigators used the combination of $DL_{NO}$ and $DL_{CO}$ measurements to calculate the Dm and Vcap. The $Dm_{CO}$ is calculated by dividing the $DL_{NO}$ by 1.93 (Equation 4), the Vcap can be calculated from Equation 2. In this way, one measurement is sufficient for the

calculation of the two components of the diffusing capacity. The advantage above duplicate measurements is obvious, because changes in the distribution of test gas can affect the measurement. Furthermore, the time for the measurement procedure is reduced by half. Another approach is to use the $DL_{NO}/DL_{CO}$ ratio to assess the location of the diffusion impairment. Changes in the Dm/Vcap ratio will also be expressed in the $DL_{NO}/DL_{CO}$ ratio. An advantage of the $DL_{NO}/DL_{CO}$ ratio as compared to the Dm and Vcap is that the $\Theta_{CO}$ value, necessary for the calculation of the Vcap, is not exactly known. The investigators who published results concerning the $Dm_{CO}$ used different values for $\Theta_{CO}$, therefore the results are difficult to compare.
In 1989 Borland et al. [51] measured the combined single breath $DL_{NO}/DL_{CO}$ in 13 volunteers, with a mean ratio of 4.3. There was no evident interaction between CO and NO, because $DL_{CO}$ and $DL_{NO}$ measured together or separately were identical. The $DL_{NO}$ did not change when alveolar oxygen concentration increased from 18 to 68% in five subjects, whereas the $DL_{CO}$ was reduced with 54%. The $DL_{NO}$ responded stronger to a fall in alveolar volume than the $DL_{CO}$ in 5 subjects.

Guenard et al. [52] calculated the Dm and Vcap from the combined single breath $DL_{NO}/DL_{CO}$ measurements in 14 healthy subjects. Using a very short breath holding time (NO-analyzers were not very sensitive yet) of three seconds they found a mean $DL_{NO}/DL_{CO}$ ratio of 5.3, and values for Dm and Vcap comparable with earlier reported values obtained with the high/low oxygen method. In patients with COPD the $DL_{CO}$ and $DL_{NO}$ values were underestimated due to such a short breath holding time that sufficient gas mixing in the lungs could not take place [53].
Manier et al. [54] used the combined single breath $DL_{NO}/DL_{CO}$ to investigate post-exercise changes in Vcap and Dm. They found that the $DL_{CO}$ normalized 30 minutes post-exercise, but $DL_{NO}$ and subsequently the derived Dm was slightly but significantly lower. Vcap, which was elevated directly post-exercise, reached normal pre-exercise values 30 minutes post-exercise. The authors could not give a valid explanation for these results. Moinard et al. [55] used the combined $DL_{NO}/DL_{CO}$ measurement to determine the Dm and Vcap in patients with chronic renal failure who were treated with hemodialysis. After hemoglobin correction they found normal Vcap values with decreased

Dm, which was related to the time of the hemodialysis, and they assumed that a change in the alveolocapillary membrane occurred during the hemodialysis.
Phansalkar et al. [56] measured the combined $DL_{NO}/DL_{CO}$ at rest and during exercise in 18 healthy subjects and in 25 patients with stage II-III sarcoidosis with a rebreathing technique at two alveolar oxygen levels, which enabled them to calculate the Dm and Vcap by the classical Roughton and Forster method [3] and by the NO-CO method. They found excellent agreement between the two methods. At rest $Dm_{CO}$ and Vcap were significantly lower in the patients with sarcoidosis as compared to the normal subjects. During increasing exercise the Dm hardly increased, whereas the Vcap increased to a similar degree as measured in the healthy subjects. The authors concluded that the membrane barrier is mainly responsible for the impaired gas transfer.
Tamhane et al. [50] also measured the combined $DL_{NO}/DL_{CO}$ with a rebreathing technique at rest and during exercise in 12 healthy volunteers, on high and low oxygen concentration which allowed the calculation of Dm and Vcap by the classical Roughton and Forster method and by the NO-CO method. The alveolar oxygen concentration did not effect the $DL_{NO}$ measurement, in agreement with the earlier published results of Borland et al. [57]. The mean $DL_{NO}/DL_{CO}$ ratio was 3.98 and did not change with increasing exercise and there was good agreement between Vcap and Dm calculated with the two methods. The $DL_{NO}/Dm_{CO}$ ratio was 2.49, which is considerable higher than the expected 1.93, based on the theoretical relationship between membrane diffusing capacity of NO and CO (Equation 4).
Zavorsky et al. [58] measured combined single breath $DL_{NO}$ and $DL_{CO}$ and found a ratio of 4.52 in 8 healthy subjects, which did not change during various exercise intensities.
Recently Harris et al. [59] measured the single breath $DL_{NO}$ and $DL_{CO}$ in mechanically ventilated sheep, before and after pulmonary artery occlusion and autologous clot embolism. After occlusion the $DL_{NO}/DL_{CO}$ ratio increased from 4.8 to 6.4, after clot embolism the ratio increased from 7.6 to 11.6. This phenomenon was independent of the fraction of inspired oxygen. The reason for a greater disturbance of the $DL_{CO}$ than the $DL_{NO}$ is that CO accumulates in stagnant arteries, because of the much lower concentration of NO and the much greater affinity of Hb for NO, $DL_{NO}$ is not or hardly changed, leading to

higher ratios. The authors conclude that the $DL_{NO}/DL_{CO}$ ratio is a function of the recruited pulmonary capillary bed.

## Interpretation of diffusion impairment

Measurement of the diffusion capacity is a frequently used method to determine the diagnosis, the prognosis and the therapy of a variety of pulmonary diseases. Most emphasis is put on the diagnostic quality of the $DL_{CO}$, but prospective investigations are lacking, and all recommendations are based on cross sectional analysis of small groups. Several pathophysiological mechanisms are responsible for impaired diffusion and therefore the interpretation of the diffusion capacity is not simple. Agreement exists that combining the $DL_{CO}$, the $K_{CO}$ and the $V_A$ with parameters obtained with spirometry and whole body plethysmography is to be recommended to obtain a better understanding in impairment of diffusion. The factors influencing the single-breath $DL_{CO}$ are 1] alveolar-capillary membrane factors, i.e. total surface area and thickness of the alveolocapillary membrane, 2] hemodynamic factors like hemoglobin concentration, pulmonary capillary blood volume, and ventilation-perfusion inhomogeneity and 3] technical factors, i.e. CO-backpressure and inspiration time [29;60-62]. An impaired $DL_{CO}$ therefore can be caused by several mechanisms, and medical history, physical examination and other diagnostic tools are necessary for an exact understanding of the mechanisms responsible for this impairment. For instance, one should be cautious to exclude impaired gas transfer solely based on a normal $DL_{CO}$ since exercise testing can reveal diffusion abnormalities also when spirometric and diffusion measurements at rest are normal. The sole use of the $K_{CO}$ to exclude impaired gas transfer is even more risky, because of the strong dependency of the $K_{CO}$ on the $V_A$, and patients with small $V_A$ and lowered $DL_{CO}$ values may have a normal $K_{CO}$.
Nonetheless, on theoretical bases the $DL_{CO}$ combined with the $V_A$ can differentiate between different pathophysiological conditions [60;62], although this concept has not been formally proven valid in clinical practice. In Table 1 this is shown, based on several publications [60;61].

| | $DL_{CO}$ | $V_A$ | $K_{CO}$ | Mechanism, explanation |
|---|---|---|---|---|
| Restriction | ↓ | ↓ | n | Decreased functional units, normal function |
| Emphysema | ↓↓ | n/↓ | ↓↓ | Decreased surface area, decreased Vcap |
| DPLD | ↓↓ | ↓ | ↓ | Increased membrane thickness, loss of functional units |
| Heart failure | ↓↓ | ↓ | ↓ | Increased membrane thickness, decreased $V_A$ due to increased heart volume |
| Asthma | n/↑ | n | n/↑ | Increased $DL_{CO}$ due to increased upper zone blood flow |
| Obesity | ↑ | n/↓ | n/↑ | Increased Vcap due to increased cardiac output, higher ventilation/perfusion ratio |
| Left-to-right shunt | ↑↑ | n | ↑ | Increased Vcap |
| Chronic bronchitis | n | n | n | Normal gas transfer |
| PAH | ↓ | n | ↓ | Decreased perfusion of ventilated alveoli |
| Bullous emphysema or bullae | ↓ | ↓ | n | Areas inaccessible to test gas, so $K_{CO}$ is normal |
| Anemia | ↓↓ | n | ↓↓ | Decreased binding sites for CO |

**Table 1.** *The effects of different diseases on $DL_{CO}$, $V_A$, and $K_{CO}$.*

## Conclusion

The single breath $DL_{CO}$ is a cheap and easy to perform method, available in most pulmonary function laboratories. It can provide important information on the diagnosis and the prognosis of several pulmonary diseases, and sometimes can be used to guide therapy. The measurement of the subdivisions of the $DL_{CO}$, the $Dm_{CO}$ and the Vcap, may give additional information, but is time-consuming and is more cumbersome for the patient. Moreover, the true significance of these

last two parameters remains unknown. The single breath $DL_{NO}$, especially when combined with the $DL_{CO}$ in one breath holding period, probably is a better measure of the membrane diffusing capacity than the $Dm_{CO}$. The $DL_{NO}/DL_{CO}$ ratio can give information about the localization of the diffusion impairment, i.e. the vascular compartment or the alveolocapillary membrane.

## Outline of this thesis

The aim of this thesis was to explore the diagnostic quality and the diagnostic possibilities and impossibilities of the diffusion measurement. In Part 1, aspects of the carbon monoxide diffusion capacity are studied. We investigated the diagnostic quality of the $DL_{CO}$ and the $K_{CO}$ in a broad spectrum of pulmonary diseases, as presented to our outpatient clinic. The results are described in Chapter 2. In Chapter 3 the value of the single breath alveolar helium dilution ($V_A$) as used for the calculation of the diffusing capacity is studied in patients with COPD. The $V_A$ is very sensitive to ventilatory disturbances, a phenomenon frequently encountered in patients with COPD. We compared $V_A$ with the lung volumes with low attenuation on high resolution computed tomography (HRCT) scans, which has shown good correlation with pathological extent of emphysema [63]. In Chapter 4 we studied in heavy smokers the value of spirometric parameters and the $DL_{CO}$ and $K_{CO}$ to diagnose emphysema in comparison with low attenuation areas on HRCT scans as gold standard.
Part 2 of this thesis compasses investigations concerning the nitric oxide diffusion capacity. At first, we created reference values by measuring healthy individuals. A subset of healthy individuals was used to study the dependence of the $DL_{CO}$ and $DL_{NO}$ as well as the $K_{CO}$ and $K_{NO}$ on alveolar volume. These two studies are described in Chapter 5. On theoretical basis $DL_{NO}$ should be independent on hemoglobin, which is tested in the study described in Chapter 6. For this purpose we performed measurements in patients who were admitted for red cell transfusion. The $DL_{NO}$ was measured before and shortly after the transfusion. The clinical value of the $DL_{NO}$, with special attention on the $DL_{NO}/DL_{CO}$ ratio, was tested in subjects with PAH and DPLD, as described in Chapter 7. The value of the $DL_{NO}$ and the $K_{NO}$ for the early diagnosis and the assessment of the severity of COPD was studied in Chapter 8, using low attenuation areas on CT scan as gold standard. In Chapter 9 a summary with the main results obtained in this thesis together with recommendations for further research is given.

## References

1. Krogh M. The diffusion of gases through the lungs of man. J Physiol Lond 1915; 49:271-296.
2. Blakemore WS, Forster RE, Morton JW et al. A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. J Clin Invest 1957; 36(1 Part 1):1-17.
3. Roughton FJ, Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J Appl Physiol 1957; 11(2):290-302.
4. Hsia CC. Recruitment of lung diffusing capacity: update of concept and application. Chest 2002; 122(5):1774-1783.
5. American Thoracic Society. Single-breath carbon monoxide diffusing capacity (transfer factor). Recommendations for a standard technique--1995 update. Am J Respir Crit Care Med 1995; 152(6 Pt 1):2185-2198.
6. Cotes JE, Chinn DJ, Quanjer PH et al. Standardization of the measurement of transfer factor (diffusing capacity). Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:41-52.
7. Sansores RH, Pare PD, Abboud RT. Acute effect of cigarette smoking on the carbon monoxide diffusing capacity of the lung. Am Rev Respir Dis 1992; 146(4):951-958.
8. Paoletti P, Viegi G, Pistelli G et al. Reference equations for the single-breath diffusing capacity. A cross-sectional analysis and effect of body size and age. Am Rev Respir Dis 1985; 132(4):806-813.
9. Traver GA, Cline MG, Burrows B. Predictors of mortality in chronic obstructive pulmonary disease. A 15-year follow-up study. Am Rev Respir Dis 1979; 119(6):895-902.
10. Owens GR, Rogers RM, Pennock BE et al. The diffusing capacity as a predictor of arterial oxygen desaturation during exercise in patients with chronic obstructive pulmonary disease. N Engl J Med 1984; 310(19):1218-1221.

11. Hadeli KO, Siegel EM, Sherrill DL et al. Predictors of oxygen desaturation during submaximal exercise in 8,000 patients. Chest 2001; 120(1):88-92.
12. Dubois P, Machiels J, Smeets F et al. CO transfer capacity as a determining factor of survival for severe hypoxaemic COPD patients under long-term oxygen therapy. Eur Respir J 1990; 3(9):1042-1047.
13. Morrison NJ, Abboud RT, Ramadan F et al. Comparison of single breath carbon monoxide diffusing capacity and pressure-volume curves in detecting emphysema. Am Rev Respir Dis 1989; 139(5):1179-1187.
14. Saydain G, Beck KC, Decker PA et al. Clinical significance of elevated diffusing capacity. Chest 2004; 125(2):446-452.
15. Weitzman RH, Wilson AF. Diffusing capacity and over-all ventilation:perfusion in asthma. Am J Med 1974; 57(5):767-774.
16. Tsoukias NM, Wilson AF, George SC. Effect of alveolar volume and sequential filling on the diffusing capacity of the lungs: I. theory. Respir Physiol 2000; 120(3):231-249.
17. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med 2000; 161(2 Pt 1):646-664.
18. Domagala-Kulawik J, Hoser G, Doboszynska A et al. Interstitial lung disease in systemic sclerosis: comparison of BALF lymphocyte phenotype and DLCO impairment. Respir Med 1998; 92(11):1295-1301.
19. Hughes JM, Lockwood DN, Jones HA et al. DLCO/Q and diffusion limitation at rest and on exercise in patients with interstitial fibrosis. Respir Physiol 1991; 83(2):155-166.
20. Sun XG, Hansen JE, Oudiz RJ et al. Pulmonary function in primary pulmonary hypertension. J Am Coll Cardiol 2003; 41(6):1028-1035.
21. Stupi AM, Steen VD, Owens GR et al. Pulmonary hypertension in the CREST syndrome variant of systemic sclerosis. Arthritis Rheum 1986; 29(4):515-524.
22. Beckles MA, Spiro SG, Colice GL et al. The physiologic evaluation of patients with lung cancer being considered for resectional surgery. Chest 2003; 123(1 Suppl):105S-114S.

23. Johnson DC. Importance of adjusting carbon monoxide diffusing capacity (DLCO) and carbon monoxide transfer coefficient (KCO) for alveolar volume. Respir Med 2000; 94(1):28-37.
24. Stam H, Splinter TA, Versprille A. Evaluation of diffusing capacity in patients with a restrictive lung disease. Chest 2000; 117(3):752-757.
25. Stam H, Hrachovina V, Stijnen T et al. Diffusing capacity dependent on lung volume and age in normal subjects. J Appl Physiol 1994; 76(6):2356-2363.
26. Frans A, Nemery B, Veriter C et al. Effect of alveolar volume on the interpretation of single breath DLCO. Respir Med 1997; 91(5):263-273.
27. Chinn DJ, Cotes JE, Flowers R et al. Transfer factor (diffusing capacity) standardized for alveolar volume: validation, reference values and applications of a new linear model to replace KCO (TL/VA). Eur Respir J 1996; 9(6):1269-1277.
28. Gonzalez MN, Aviles Ingles MJ, Peces-Barba G et al. A simple method of correcting diffusing capacity for alveolar volume reduction in restrictive lung diseases. Respiration 1987; 52(3):163-170.
29. Hughes JM, Pride NB. In defence of the carbon monoxide transfer coefficient Kco (TL/VA). Eur Respir J 2001; 17(2):168-174.
30. Cotes JE. Carbon monoxide transfer coefficient KCO (TL/VA): a flawed index. Eur Respir J 2001; 18(5):893-894.
31. Saumon G, Georges R, Loiseau A et al. Membrane diffusing capacity and pulmonary capillary blood volume in pulmonary sarcoidosis. Ann N Y Acad Sci 1976; 278:284-291.
32. Lamberto C, Nunes H, Le Toumelin P et al. Membrane and capillary blood components of diffusion capacity of the lung for carbon monoxide in pulmonary sarcoidosis: relation to exercise gas exchange. Chest 2004; 125(6):2061-2068.
33. Bates DV, Varvis CJ, Donevan RE et al. Variations in the pulmonary capillary blood volume and membrane diffusion component in health and disease. J Clin Invest 1960; 39:1401-1412.
34. Steenhuis LH, Groen HJ, Koeter GH et al. Diffusion capacity and haemodynamics in primary and chronic thromboembolic pulmonary hypertension. Eur Respir J 2000; 16(2):276-281.
35. Rubin LJ. Primary pulmonary hypertension. N Engl J Med 1997; 336(2):111-117.

36. Bernstein RJ, Ford RL, Clausen JL et al. Membrane diffusion and capillary blood volume in chronic thromboembolic pulmonary hypertension. Chest 1996; 110(6):1430-1436.
37. Bonay M, Bancal C, De Zuttere D et al. Normal pulmonary capillary blood volume in patients with chronic infiltrative lung disease and high pulmonary artery pressure. Chest 2004; 126(5):1460-1466.
38. Sharma GV, Burleson VA, Sasahara AA. Effect of thrombolytic therapy on pulmonary-capillary blood volume in patients with pulmonary embolism. N Engl J Med 1980; 303(15):842-845.
39. Fennerty AG, Gunawardena KA, Smith AP. The transfer factor and its subdivisions in patients with pulmonary emboli. Eur Respir J 1988; 1(2):98-101.
40. Ohar J, Osterloh J, Ahmed N et al. Diffusing capacity decreases after heart transplantation. Chest 1993; 103(3):857-861.
41. Mettauer B, Lampert E, Charloux A et al. Lung membrane diffusing capacity, heart failure, and heart transplantation. Am J Cardiol 1999; 83(1):62-67.
42. Al Rawas OA, Carter R, Stevenson RD et al. Exercise intolerance following heart transplantation: the role of pulmonary diffusing capacity impairment. Chest 2000; 118(6):1661-1670.
43. Johnson RL, Jr., Spicer WS, Bishop JM et al. Pulmonary capillary blood volume, flow and diffusing capacity during exercise. J Appl Physiol 1960; 15:893-902.
44. Stam H, Kreuzer FJ, Versprille A. Effect of lung volume and positional changes on pulmonary diffusing capacity and its components. J Appl Physiol 1991; 71(4):1477-1488.
45. Nabors LK, Baumgartner WA, Jr., Janke SJ et al. Red blood cell orientation in pulmonary capillaries and its effect on gas diffusion. J Appl Physiol 2003; 94(4):1634-1640.
46. Huang YC, Helms MJ, MacIntyre NR. Normal values for single exhalation diffusing capacity and pulmonary capillary blood flow in sitting, supine positions, and during mild exercise. Chest 1994; 105(2):501-508.
47. Chang SC, Chang HI, Liu SY et al. Effects of body position and age on membrane diffusing capacity and pulmonary capillary blood volume. Chest 1992; 102(1):139-142.
48. Peces-Barba G, Rodriguez-Nieto MJ, Verbanck S et al. Lower pulmonary diffusing capacity in the prone vs. supine posture. J Appl Physiol 2004; 96(5):1937-1942.

49. West JB, Wagner PD. Ventilation-perfusion relationships. In: Crystal RG, editor. The lung: scientific foundations. Philadelphia: Lippincott-Raven Publishers, 1997: 1693-1710.
50. Tamhane RM, Johnson RL, Jr., Hsia CC. Pulmonary membrane diffusing capacity and capillary blood volume measured during exercise from nitric oxide uptake. Chest 2001; 120(6):1850-1856.
51. Borland CD, Higenbottam TW. A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. Eur Respir J 1989; 2(1):56-63.
52. Guenard H, Varene N, Vaida P. Determination of lung capillary blood volume and membrane diffusing capacity in man by the measurements of NO and CO transfer. Respir Physiol 1987; 70(1):113-120.
53. Moinard J, Guenard H. Determination of lung capillary blood volume and membrane diffusing capacity in patients with COLD using the NO-CO method. Eur Respir J 1990; 3(3):318-322.
54. Manier G, Moinard J, Stoicheff H. Pulmonary diffusing capacity after maximal exercise. J Appl Physiol 1993; 75(6):2580-2585.
55. Moinard J, Guenard H. Membrane diffusion of the lungs in patients with chronic renal failure. Eur Respir J 1993; 6(2):225-230.
56. Phansalkar AR, Hanson CM, Shakir AR et al. Nitric oxide diffusing capacity and alveolar microvascular recruitment in sarcoidosis. Am J Respir Crit Care Med 2004; 169(9):1034-1040.
57. Borland CD, Cox Y. Effect of varying alveolar oxygen partial pressure on diffusing capacity for nitric oxide and carbon monoxide, membrane diffusing capacity and lung capillary blood volume. Clin Sci (Lond) 1991; 81(6):759-765.
58. Zavorsky GS, Quiron KB, Massarelli PS et al. The relationship between single-breath diffusion capacity of the lung for nitric oxide and carbon monoxide during various exercise intensities. Chest 2004; 125(3):1019-1027.
59. Harris RS, Hadian M, Hess DR et al. Pulmonary artery occlusion increases the ratio of diffusing capacity for nitric oxide to carbon monoxide in prone sheep. Chest 2004; 126(2):559-565.
60. Ayers LN, Ginsberg ML, Fein J et al. Diffusing capacity, specific diffusing capacity and interpretation of diffusion defects. West J Med 1975; 123(4):255-264.

61. Weinberger SE, Johnson TS, Weiss ST. Clinical significance of pulmonary function tests. Use and interpretation of the single-breath diffusing capacity. Chest 1980; 78(3):483-488.
62. Miller WF, Scacci R., Gast L.R. Diffusion. Laboratory Evaluation of Pulmonary Function. J.B. Lippincott Company Philadelphia, 1987: 398-440.
63. Gevenois PA, de Maertelaere V, De Vuyst P et al. Comparison of computed density and macroscopic morphometry in pulmonary emphysema. Am J Respir Crit Care Med 1995; 152(2):653-657.

# Chapter 2

# Pattern of diffusion disturbance related to clinical diagnosis: the $K_{CO}$ has no diagnostic value next to the $DL_{CO}$

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## Abstract

The diffusion capacity of the lung for carbon monoxide ($DL_{CO}$) is an important tool in the diagnosis and follow-up of patients with pulmonary diseases. In case of a decreased $DL_{CO}$ the $K_{CO}$, defined as $DL_{CO}/V_A$ ($V_A$ is alveolar volume), can differentiate between normal alveolocapillary membrane (normal $K_{CO}$) and abnormal alveolocapillary membrane (low $K_{CO}$). The latter category consists of decreased surface of the membrane, increased thickness or decreased perfusion of ventilated alveoli. The $V_A$/TLC (TLC is total lung capacity determined by whole body plethysmography) can partially differentiate between these categories. The aim of this study was to investigate the diagnostic value of the specific diffusion disturbances, which can be constructed by combining the $DL_{CO}$, $K_{CO}$ and $V_A$/TLC.
In 460 patients the diagnosis made by clinicians were fitted into five diagnostic categories: asthma, COPD (chronic obstructive pulmonary disease), treatment effects of haematological malignancies, heart failure and diffuse parenchymal lung diseases (DPLD). These categories were linked to the pattern of diffusion disturbance.
Almost all patients with asthma have a normal $DL_{CO}$, most patients in the other groups do not have the expected pattern of diffusion disturbance, especially in the group with DPLD a bad match is observed.
In this study the pattern of diffusion disturbance is of limited use in establishing a diagnosis. The use of the $K_{CO}$ next to the $DL_{CO}$ has no additional diagnostic value. Regional ventilation-perfusion inequality probably forms an important underlying mechanism of decreased $DL_{CO}$.

## Introduction

The diffusion capacity of the lung for carbon monoxide ($DL_{CO}$) is a standard test in the pulmonary function laboratory. The $DL_{CO}$ is used in the assessment of restrictive as well as obstructive pulmonary diseases, and is an indicator of disease severity. In chronic obstructive pulmonary disease (COPD) and in diffuse parenchymal lung diseases (DPLD) the $DL_{CO}$ is a strong predictor for desaturation during exercise [1;2]. Furthermore, the $DL_{CO}$ is an important parameter in the assessment of response to therapy in idiopathic pulmonary fibrosis [3] and other DPLD.
The $K_{CO}$ is defined as the $DL_{CO}/V_A$, where $V_A$ is the alveolar volume: the $K_{CO}$ is often referred to as "$DL_{CO}$ corrected for $V_A$", or the diffusion capacity per litre lung volume. $V_A$ is measured by a single breath helium dilution technique and is sensitive to ventilatory disturbances. When $V_A$ is less than 85% of TLC, as measured by whole body plethysmography, ventilation inhomogeneity is considered to be present [4]. The discriminative properties of the $V_A$/TLC ratio only accounts for TLC measured with whole body plethysmography, because then all air containing parts of the thorax are measured (using multiple breath helium dilution inaccessible parts of the lungs are still not included).

In several publications [5-7] a method of interpretation of diffusion disturbances has been proposed, based on the $DL_{CO,}$ the $K_{CO}$ and the $V_A$/TLC ratio. When the $DL_{CO}$ is decreased, the $K_{CO}$ locates the diffusion abnormality at the level of the alveolocapillary membrane or not. A low $K_{CO}$ indicates a situation where the $DL_{CO}$ is decreased solely or where it is decreased more than a lowered $V_A$. Both phenomena point to pathology at the level of the alveolocapillary membrane. The cause can be a decreased surface with ventilation inhomogeneity (e.g. emphysema), an increased thickness (fibrosis) or a decreased perfusion of ventilated alveoli. Using the $V_A$/TLC ratio, emphysema can be detected, due to the presence of ventilation inhomogeneity, which leads to a low $V_A$/TLC ratio. In fibrotic disorders such ventilatory disturbances are not present and therefore the $V_A$/TLC ratio will be normal [4].
In case of a low $DL_{CO}$ and a normal $K_{CO}$, the decreased diffusion is due to a volume effect (to a so called small lung, as in

lobectomy/pneumectomy, chest cage restriction) or to the presence of non-communicating air as in bullous emphysema. The $V_A$/TLC ratio again can discriminate between these possibilities: a low $V_A$/TLC ratio (<85%) indicates the presence of ventilation inhomogeneity (bullous emphysema), a normal $V_A$/TLC ratio indicates a small lung syndrome or chest cage restriction.
Most authors [5] present algorithms as shown in Figure 1.

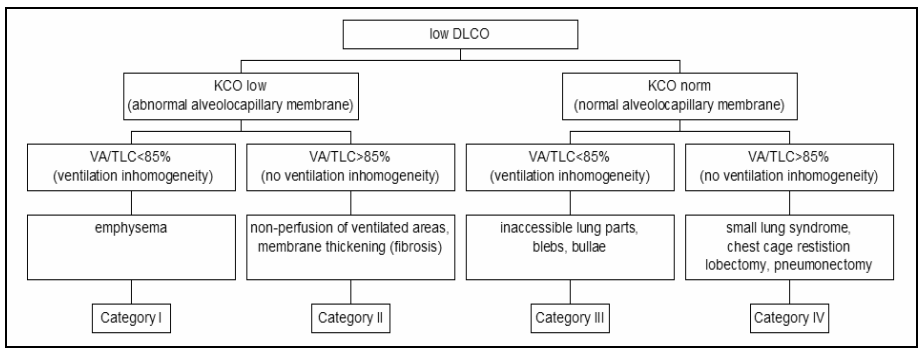


***Figure 1.*** *Commonly used algorithm in interpreting diffusing abnormalities. $DL_{CO}$ is corrected for anemia, $K_{CO}$ is $DL_{CO}/V_A$, $V_A$/TLC is single breath helium dilution alveolar volume divided by total lung capacity determined by whole body plethysmography.*

Although these algorithms are meant to give insight in the underlying pathology, and are easy to understand, they have never been tested in clinical practice, as far as we know. The aim of this study is to test the clinical relevance of this scheme. We investigated whether the clinical diagnosis and the diffusion patterns as sketched above would match sufficiently with emphasis on the discrimination between COPD and DPLD.

## Methods

All new consecutive patients referred to the pulmonary function laboratory between July 1999 and November 2002 were assessed. Only patients who performed $DL_{CO}$, spirometry and whole body plethysmography on the same day were included in this study. From patients who underwent PFT more than once, only the entry test was used.

### *Pulmonary function data*

Plethysmography, spirometry and the diffusion tests were carried out according to ERS guidelines [8;9] using a bodyplethysmograph (Jaeger Masterlab) and a Jaeger Masterscreen FRC system by qualified lung function technicians. Upon arrival, patients routinely rest for 15 minutes before any lung function test was determined, while whole body plethysmography was always performed immediately before spirometry. Diffusing measurements were made after spirometry, but before reversibility testing. The $DL_{CO}$ measurement was performed based on ATS recommendations [10], which include refraining from smoking for 24 hours before the test (to minimize CO backpressure), and all $DL_{CO}$ values are corrected to standard haemoglobin of 14.6 g/dl for men and 13.4 g/dl for women (to rule out anaemia effects). For reversibility testing, all patients received salbutamol 200 µg pMDI via Volumatic and after 15 minutes spirometry was repeated.
Measured variables were single breath $DL_{CO}$, $V_A$, $DL_{CO}/V_A$, TLC, residual volume, forced expiratory volume in one second ($FEV_1$), (forced) vital capacity ((F)VC), peak flow, maximum expiratory flow at 25, 50 and 75% of expiration.
In the pulmonary function laboratory daily quality control procedures were performed as recommended by the European Respiratory Society [8;9].

### *Patterns of diffusion disturbance*

For the $DL_{CO}$ and $K_{CO}$ the actual measured value was expressed as a percentage of the predicted value: a value outside the 95% confidence interval was labelled as abnormal [9]. The $V_A$/TLC is defined as the single breath helium dilution $V_A$, as determined with the $DL_{CO}$ measurement, divided by the TLC determined by plethysmography. We used the 85% cut off point proposed by Cotes [4] to separate normal from diseased.
We defined five categories (Figure 1): category 0 is no diffusion disturbance (not shown in figure), meaning a normal $DL_{CO;}$ category I is a low $DL_{CO}$, a low $K_{CO}$ and $V_A$/TLC <0.85; category II is a low $DL_{CO}$, a low $K_{CO}$ and $V_A$/TLC > 0.85; category III is a low $DL_{CO}$, normal $K_{CO}$ and $V_A$/TLC <0.85 and category IV is a low $DL_{CO}$, normal $K_{CO}$ and $V_A$/TLC > 0.85.

### *Diagnostic categories*

All patients were seen by experienced pulmonary physicians, who took a case history, performed a physical examination, reviewed a chest X-ray and assessed all pulmonary function data including flow-volume curves.
We defined the following diagnostic categories: asthma, COPD, treatment effects of haematological malignancies, heart failure and DPLD (Table 1) and defined the corresponding diffusion patterns.

| Group | Description | Expected diffusion pattern |
|---|---|---|
| Asthma | According to ATS guidelines [11] | No diffusion abnormality |
| COPD | According to ATS guidelines [11] | Abnormal ventilation |
| Treatment effects of hematological malignancies | Malignant lymphoma, all leukemia's, multiple myeloma, including treatment effects | Abnormal alveolocapillary membrane, normal ventilation |
| Heart failure | Diagnosed by experienced cardiologists | Abnormal alveolocapillary membrane, normal ventilation |
| DPLD | Diffuse parenchymal lung diseases (DPLD) as defined by ATS/ERS criteria [12] | Abnormal alveolocapillary membrane, normal ventilation |

***Table 1.*** *Description of the diagnostic groups*.

The diagnosis of asthma was based on clinical assessment with typical symptoms, PFT including reversibility testing in all patients and bronchial provocation testing with histamine in most patients, the measurement of eosinophilic leucocytes, total and specific IgE antibodies [11]. The expected diffusion pattern is *category 0*.
The diagnosis of COPD was mainly based on clinical assessment including smoking history, radiology and spirometry, and the exclusion of other obstructive pulmonary diseases [11;13]. The expected diffusion pattern of the COPD group is *category I and III*.
The group of treatment effects of haematological malignancies includes different haematological malignancies as described in Table 1 and their various treatments. Chemotherapy often leads to lowering of the diffusion capacity, whereas thoracic radiotherapy leads to restriction [14]. Therefore the expected diffusion pattern is *category II*, and *category IV* (if the lowering of the $DL_{CO}$ is only due to restriction).

Heart failure patients suffer from irreversible diseased alveolocapillary membranes [15] and reduction of lung size, due to the enlarged heart, which will direct the subjects to a *category II*.
In all patients with DPLD [12] the diagnosis was made after intensive clinical assessment, radiological investigations including high resolution computed tomography (HRCT) scanning in all patients, PFT including exercise testing, bronchoscopy with bronchoalveolar lavage in most patients, transbronchial or thoracoscopic lung biopsy whenever indicated, and consultation of any other medical specialist (for example cardiologists and rheumatologists) when indicated. The expected diffusion pattern in this group is *category II.*

We reviewed the charts of all patients in order to confirm the accuracy of the diagnosis, and excluded patients with diagnostic dilemmas. Exclusion criteria were as follows: patients in which the clinician could not differentiate between two diagnosis (i.e. asthma or COPD), patients with more than one diagnosis from the categories and patients who could not be fitted into any of the categories.

## Results

Initially 639 patients were reviewed, after the exclusion of patients that did not fit the inclusion criteria 460 patients remained: 208 females and 252 males. Most of the excluded patients had more than one diagnosis from the list of categories. Mean age was 51 years, ranging from 18 to 88. A selection of the PFT data is displayed in Table 2.

| | # | Age | $FEV_1$ %pred | TLC %pred | $FEV_1/FVC$ % | $DL_{CO}$ %pred | $K_{CO}$ %pred | $V_A/TLC$ |
|---|---|---|---|---|---|---|---|---|
| Asthma | 188 | 45 (15) | 89 (18) | 104 (13) | 76 (10) | 84 (17) | 90 (14) | 91 (8) |
| COPD | 143 | 62 (11) | 64 (17) | 114 (15) | 57 (11) | 56 (21) | 62 (22) | 82 (10) |
| hematological malignancies | 49 | 43 (12) | 74 (19) | 78 (16) | 83 (7) | 59 (22) | 81 (21) | 92 (8) |
| Heart failure | 21 | 50 (16) | 72 (20) | 82 (17) | 84 (10) | 54 (17) | 74 (15) | 89 (10) |
| DPLD | 59 | 50 (15) | 71 (18) | 74 (14) | 80 (11) | 51 (18) | 79 (23) | 89 (11) |
| Total | 460 | 51 (16) | 77 (21) | 100 (20) | 72 (15) | 67 (24) | 78 (22) | 88 (10) |

***Table 2.*** *Pulmonary Function Tests Results. Clinical diagnosis versus number of patients (#), mean age (standard deviation (SD)), mean $FEV_1$ as percentage predicted (SD), mean TLC determined by plethysmography as percentage predicted (SD), mean $FEV_1/FVC$ ratio as percentage (SD), mean $DL_{CO}$ corrected for hemoglobin as percentage predicted (SD), mean $K_{CO}$ as percentage predicted (SD), mean $V_A/TLC$ as percentage (SD).*

The overall mean $DL_{CO}$ was 67% of the predicted value: patients with asthma had the highest $DL_{CO}$. The lowest mean $K_{CO}$ was seen in the COPD group, followed by the heart failure and DPLD groups. The group of asthma patients had highest $FEV_1$, as expected. The diagnosis of COPD was made in 143 patients, 132 of these had a $FEV_1/FVC$ ratio <70%. In 7 out of 59 patients from the DPLD group a $FEV_1/FVC$ ratio <70% was observed. The relation of the clinical diagnosis to the patterns of diffusion disturbance is shown in Table 3: the majority of the patients with asthma had a normal $DL_{CO}$, and of those with a lowered $DL_{CO}$ most had a normal $K_{CO}$.

| | Category 0 | Category I | Category II | Category III | Category IV | Total |
|---|---|---|---|---|---|---|
| Asthma | 126 | 1 | 4 | 21 | 36 | 188 |
| COPD | 34 | 57 | 30 | 20 | 2 | 143 |
| haematological malignancies | 12 | 3 | 12 | 6 | 16 | 49 |
| Heart failure | 2 | 2 | 7 | 3 | 7 | 21 |
| DPLD | 10 | 8 | 12 | 12 | 17 | 59 |
| Total | 184 | 71 | 65 | 62 | 78 | 460 |

***Table 3.*** *Diagnostic groups versus pattern of diffusion disturbance.*

Within the COPD subjects a minority (18%) of those with a lowered $DL_{CO}$ had a normal $K_{CO}$ and were hence labeled as *category III*: most showed a ventilation inhomogeneity. Within the lowered $K_{CO}$ group 34% showed a $V_A$/TLC ratio >85% and were placed in *category II*. An emphysematous diffusion pattern (combining *category I* and *III*) is present in 54% of the COPD cases, in those with a lowered $DL_{CO}$ (n= 109) 71% of the COPD patients show a "correct" pattern. On the other hand, of the 142 subjects with an emphysema pattern only 77 were true emphysema subjects: in 54% the diffusion pattern correctly denotes emphysema.
Of the subjects with DPLD with a lowered $DL_{CO}$, 59% had a normal $K_{CO}$ and hence a large minority a lowered one. In the latter group 40% of cases showed a lowered $V_A$/TLC ratio and therefore were incorrectly labelled with emphysema. In the end, only 12 out of 59 DPLD subjects showed 'the correct' fibrotic diffusion pattern (*category II*).
Both heart failure and haematological malignancy subjects showed highly variable patterns with a preference for *category II* and *IV*.
The group of subjects with DPLD is very heterogeneous: we retrieved all diagnosis from the patients' charts and related it to the diffusion patterns (Table 4).

| | Cat 0 | Cat I | Cat II | Cat III | Cat IV | Total |
|---|---|---|---|---|---|---|
| IPF | 0 | 3 | 0 | 2 | 1 | 6 |
| Sarcoidosis | 8 | 1 | 3 | 4 | 6 | 22 |
| COP | 0 | 2 | 0 | 1 | 1 | 4 |
| Col-vasc disease | 0 | 0 | 5 | 2 | 7 | 14 |
| Fibrosis n.c. | 2 | 2 | 4 | 3 | 2 | 13 |
| Total | 10 | 8 | 12 | 12 | 17 | 59 |

***Table 4.*** *Categories (Cat) of diffusion disturbance in patients with DPLD. DPLD is diffuse parenchymal lung diseases, IPF is idiopathic pulmonary fibrosis, COP is cryptogenic organizing pneumonia, col-vasc disease is DPLD associated with collagen vascular disease, fibrosis n.c. means non-classified.*

Again, a bad match is observed between the specific fibrotic disorders and the diffusion pattern. Idiopathic pulmonary fibrosis (IPF) is mostly seen as an archetype of fibrotic lung disease, but none of the six subjects with IPF has the expected fibrotic diffusion pattern (*category II*).

## Discussion

We investigated whether an interpretation scheme of diffusion patterns would indeed render a reliable clinical diagnosis. The match between the suggested patterns and the clinical diagnosis can not considered as sufficiently close for the diagnostic scheme to be used satisfactorily in daily practice.
There are several possible explanations for this observation. The first one is that our disease categories were not well defined. However, patients with more than one diagnosis were excluded: concomitant pulmonary disease is hence very improbable, which eliminates much overlap and maximises the inclusion of classical disease patterns. Cardiologists of course made the initial diagnosis of heart failure after echocardiography: all heart failure patients were referred to the lung function laboratory as part of the standard cardiological work up. The exclusion of pre-existing severe pulmonary disease renders the possibility of isolated right heart failure low. All patients with

haematological disorders were seen and referred by haematologists, because of possible pulmonary effects of the treatment and disease itself.

There is no absolute dividing line between the diagnosis asthma and COPD. Almost all (92%) patients with COPD have a $FEV_1/FVC<70\%$, hence we feel that not many diagnostic mistakes were made in that group. Some asthmatics may truly be COPD-patients, which could account for the 12% (22 out of 188) of emphysematous diffusion patterns (*category I* and *III*) in this group.

Of course, the possibility that technical mistakes with pulmonary function testing were made must also be kept in mind. If that was to be the sole cause of the noted discrepancies, at least 30% of the measurements must have been flawed. We feel that is too far fetched, taking into account the daily quality control procedures and the vast experience of the technicians.

We observed that in subjects with DPLD apparently a lowered $DL_{CO}$ and a normal $K_{CO}$ is quite standard and this must mean that the lowering of $V_A$ and $DL_{CO}$ were of similar magnitude. Taking this argument one step further: a lowered $K_{CO}$ is not so common. Some may be tempted to judge the pre/absence of disease by just assessing the $K_{CO}$. According to this study this approach is incorrect: most patients will be missed.

Another finding is that $V_A/TLC$ ratios <85% are quite common in DPLD. The most probable explanation for this phenomenon is the existence of ventilation abnormalities in some of the patients in the DPLD group, not assumed to be present and easily obscured by the fibrotic process. DPLD is a very heterogeneous group of lung diseases and sometimes smoking related, for example IPF. So, when someone is diagnosed with IPF it is possible that some co-existing and indistinct obstructive disease is present which leads to the low $V_A/TLC$ ratio. The obstruction is not detectable via spirometry and/or body plethysmography. It is well known that cryptogenic organizing pneumonia (COP) as well as sarcoidosis can lead to airway obstruction, so it would be incorrect to label these patients with a second diagnosis of COPD. From this group only 7 patients (12%) have $FEV_1/FVC<70\%$, 3 patients with sarcoidosis, 2 with COP and 2 with fibrosis of unknown cause.

In the COPD group a normal $K_{CO}$ is much less common than in the DPLD group. Again, a normal $K_{CO}$ does not exclude disease, because most of these subjects showed a significant degree of ventilation inhomogeneity, labelling these subjects with bullous emphysema. Unfortunately, in 30 of the 87 COPD subjects with a lowered $DL_{CO}$ and $K_{CO}$, the $V_A$/TLC is >85% and hence the pattern suggests fibrosis. We examined the characteristics of these 30 subjects: 11 male and 19 female, mean age 56, mean $FEV_1$ is 74% of predicted, mean TLC 110% predicted, mean $FEV_1$/FVC is 61% of predicted. It appeared that 26 of them have $FEV_1$/FVC<70% and when reviewing the charts none of these patients had diagnostic controversies. In these 30 subjects the possibility exists that the pathophysiological phenomenon responsible for the decreased $K_{CO}$ is thickening of the membranes, but we think that is very unlikely: in none of the patients the radiological studies are compatible with fibrosis. Therefore, it is likely that non-perfusion of ventilated alveoli (ventilation-perfusion inequality) is the cause of the lowered $K_{CO}$. With the available data we cannot further investigate this hypothesis, because invasive investigations are necessary, but regional ventilation-perfusion inequality is an important cause of diffusion limitation as earlier investigators described [16]. Of course this pattern could also be found with pulmonary embolism [17], but after reviewing the patients' charts in none of the cases strong arguments for the existence of pulmonary embolism were present, although it was not formally excluded using ventilation-perfusion scintigraphy.

In this study almost all included patients with heart failure show a decreased $DL_{CO}$, which has been described earlier [18]. These patients did not show a specific diffusion pattern and hence may be categorised variably. Of course, subtle pulmonary abnormalities in these groups can explain some of these differences, bearing in mind that many patients with heart failure are former smokers.

The category of treatment effects of haematological malignancies is heterogeneous. All patients in this category received chemotherapy, which is known to cause a reduction of the diffusing capacity [14;19]. Moreover, patients with haematologic malignancies treated with high dose chemotherapy can often develop serious pulmonary diseases, which can give different pulmonary syndromes (e.g. bronchiolitis obliterans and graft-versus-host disease can lead to a modest restriction

and lowering of the $DL_{CO}$ [20]). Some patients received concomitant radiotherapy, which can have additive effects on pulmonary function, mostly leading to restriction [14]. Furthermore, subtle smoking induced abnormalities can lead to a lowered $V_A$/TLC, which can obscure other treatment effects.

### *Accuracy of model*

A possible explanation for the weak correlation between the disease categories and the diffusion patterns is that the model is incorrect. It is possible that the pathophysiology underlying diffusion abnormalities is much more complicated than this rather simple model. The distinction between $DL_{CO}$ and $K_{CO}$ plays an important role in this model. In the calculation of $K_{CO}$ ($DL_{CO}/V_A$, Figure 2) the term $V_A$ is used in the numerator and in the denominator. In the numerator $V_A$ is used to transform gas volume into moles of gas.

$$K_{CO} = (DL_{CO})/V_A = \left( \frac{V_A}{t * (P_B - P_{H_2O})} * \ln \frac{F_{0,CO}}{F_{t,CO}} \right) / V_A = \frac{c}{t * (P_B - P_{H_2O})} * \ln \frac{F_{0,CO}}{F_{t,CO}}$$

***Figure 2.*** *The equation of the single breath KCO, in which t is breath hold time, $P_B$ is barometric pressure, $P_{H2O}$ is water vapour pressure, $F_{0,CO}$ is fractional alveolar [CO] at time 0, $F_{t,CO}$ fractional alveolar [CO] at time t, $V_A$ is alveolar volume. $K_{CO}$ can be expressed in two ways: as $DL_{CO}/V_A$ and as the exponential decay in alveolar [CO] multiplied by constant c.*

In the denominator the $V_A$ is expressed in litres of gas volume, so there are two ways of looking at the value of $K_{CO}$. The first and most used way is to see the $K_{CO}$ as $DL_{CO}$ corrected for $V_A$, the second way as the rate constant of exponential decay of alveolar CO concentration, because the $V_A$ in the numerator and in the denominator cancel each other out [21]. The fact that the $K_{CO}$ is not simply a parameter that describes the diffusion per unit lung volume obscures this model, i.e. it makes the model more difficult to understand. Others have observed the lack of clinical relevance of the $K_{CO}$ next to the $DL_{CO}$ in the recent past [22].

Another important factor is that the TLC as percentage predicted is not incorporated in this model. Pulmonologists know that the TLC is an important parameter in the assessment of especially restrictive pulmonary diseases. From this study can be derived that the $V_A$/TLC ratio cannot replace the TLC parameter.
Another possible weakness in this model is that the group of subjects with low $DL_{CO}$, low $K_{CO}$ and normal $V_A$/TLC ratio is heterogeneous, and contains thickening of the alveolocapillary membrane and non-perfusion of ventilated areas. A distinction between these two cannot be made with this scheme. Ventilation-perfusion relationships have a major effect on gas-exchange [23]. The fact that postural changes [24;25] and gravitational changes [26] affects the $DL_{CO}$ is based on alterations in the regional ventilation-perfusion relationship. In subjects with obstructive as well as restrictive pulmonary diseases the regional ventilation-perfusion relationship contributes to impaired diffusion [16]. Therefore the measurement of the $DL_{CO}$ is closely related to the relationship of pulmonary ventilation and perfusion. As Roughton and Forster showed in 1957 [27], the $DL_{CO}$ is composed of the alveolocapillary membrane conductance (Dm) and of the product of the pulmonary capillary blood volume (Vcap) and $\theta_{CO}$ (the rate of carbon monoxide uptake by whole blood). Until now it still is unclear which proportions of Dm and Vcap are determining the $DL_{CO}$ measurement [28] in various pulmonary diseases. Of course it is possible to calculate a value for Dm and Vcap with the high/low oxygen method [27] or with the NO (nitric oxide)-CO method [29;30] in subjects with pulmonary diseases. However, the Roughton and Forster model is a mono-alveolar model, therefore the distinction between thickening of the alveolocapillary membranes on the one hand and ventilation-perfusion inequality on the other hand cannot be made with the division of the $DL_{CO}$ in Dm and Vcap.

***Reference values***

This model is strongly dependent on accurate reference values for the $DL_{CO}$ and $K_{CO}$. Concerning the $K_{CO}$ an ongoing debate exists whether the reference values are of much use [31;32]. The main problem lies in the fact that the regression equations for $K_{CO}$ simply do not exist: it is common practice to divide the reference value for $DL_{CO}$ by the reference value of the TLC [9]. These two reference values are obtained

from different populations, which of course is not an ideal situation. Furthermore, $K_{CO}$ is calculated as $DL_{CO}$ divided by $V_A$, instead of $DL_{CO}$ divided by TLC. The reason for this is merely historical, and used overall in Europe and North America. Because $V_A$ is the single breath helium dilution measurement, $V_A$ is lower than TLC, especially in obstructive pulmonary diseases, were the $V_A$/TLC ratio can drop to far below 85%. Therefore, in obstructive pulmonary diseases, the use of $V_A$ instead of TLC leads to higher values of $K_{CO}$, and therefore a higher percentage of patients in the category "normal $K_{CO}$". Another issue concerning the reference values compasses the fact of the dependence of $K_{CO}$ on $V_A$. In healthy patients, the $K_{CO}$ rises if the $V_A$ decreases, as Stam et al. [33] determined some years ago. Indications are present that this phenomenon is also present in patients with restrictive pulmonary diseases [34]. If true, this means that for patients with a restriction, the reference values for the $K_{CO}$ should be higher. In that case the flow chart leading to the fibrotic diffusion pattern (*category II*) will be more complicated. Although some investigators found ways of recalculating the $K_{CO}$ by using adjusted $V_A$ values [32;34-36], this has never been adopted by clinicians on a broad scale.
The cut-off value for $V_A$/TLC ratio we used was 85%. This rather arbitrary value is based on recommendations of Cotes [4], who on his turn based it on earlier publications, which are hard to trace back. The value of 85% is assuming an exact dividing line between normal and disturbed ventilation, which of course is not the case, in fact there is a continuous scale between normal and disturbed ventilation. This could explain the percentage of 39% (56 out of 143) patients with COPD and normal $V_A$/TLC ratio. The finding of decreased $DL_{CO}$ with relative undisturbed ventilation in patients with COPD has been described earlier [37].

In conclusion, the measurement of the $DL_{CO}$ is of utmost importance in the assessment of the gravity of different pulmonary diseases, and it has an important value in assessing the response to treatment in DPLD. However, the simple diagnostic flow chart used in some publications has limited value in establishing a diagnosis. The use of the $K_{CO}$ next to the $DL_{CO}$ has no additional diagnostic value, and therefore a normal $K_{CO}$ can never rule out major pulmonary pathology. The $DL_{CO}$ should be used as a tool in the diagnosis, assessment and follow-up of

patients, but a diagnosis can never be based on diffusion disturbance only.

## References

1. Hadeli KO, Siegel EM, Sherrill DL et al. Predictors of oxygen desaturation during submaximal exercise in 8,000 patients. Chest 2001; 120(1):88-92.
2. Owens GR, Rogers RM, Pennock BE et al. The diffusing capacity as a predictor of arterial oxygen desaturation during exercise in patients with chronic obstructive pulmonary disease. N Engl J Med 1984; 310(19):1218-1221.
3. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med 2000; 161(2 Pt 1):646-664.
4. Cotes JE. Assessment of distribution of ventilation and of blood flow through the lung. In: Cotes JE, editor. Lung function. Assessment and application in medicine. Oxford: Blackwell Scientific publications, 1993: 213-262.
5. Miller WF, Scacci R., Gast L.R. Diffusion. Laboratory Evaluation of Pulmonary Function. J.B. Lippincott Company Philadelphia, 1987: 398-440.
6. Hughes JM, Pride NB. In defence of the carbon monoxide transfer coefficient Kco (TL/VA). Eur Respir J 2001; 17(2):168-174.
7. Ayers LN, Ginsberg ML, Fein J et al. Diffusing capacity, specific diffusing capacity and interpretation of diffusion defects. West J Med 1975; 123(4):255-264.
8. Quanjer PH, Tammeling GJ, Cotes JE et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:5-40.
9. Cotes JE, Chinn DJ, Quanjer PH et al. Standardization of the measurement of transfer factor (diffusing capacity). Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:41-52.
10. American Thoracic Society. Single-breath carbon monoxide diffusing capacity (transfer factor). Recommendations for a standard

technique--1995 update. Am J Respir Crit Care Med 1995; 152(6 Pt 1):2185-2198.
11. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. Am Rev Respir Dis 1987; 136(1):225-244.
12. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. Am J Respir Crit Care Med 2002; 165(2):277-304.
13. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. American Thoracic Society. Am J Respir Crit Care Med 1995; 152(5 Pt 2):S77-121.
14. Hirsch A, Vander Els N, Straus DJ et al. Effect of ABVD chemotherapy with and without mantle or mediastinal irradiation on pulmonary function and symptoms in early-stage Hodgkin's disease. J Clin Oncol 1996; 14(4):1297-1305.
15. Mettauer B, Lampert E, Charloux A et al. Lung membrane diffusing capacity, heart failure, and heart transplantation. Am J Cardiol 1999; 83(1):62-67.
16. Yamaguchi K, Mori M, Kawai A et al. Inhomogeneities of ventilation and the diffusing capacity to perfusion in various chronic lung diseases. Am J Respir Crit Care Med 1997; 156(1):86-93.
17. Fennerty AG, Gunawardena KA, Smith AP. The transfer factor and its subdivisions in patients with pulmonary emboli. Eur Respir J 1988; 1(2):98-101.
18. Puri S, Baker BL, Dutka DP et al. Reduced alveolar-capillary membrane diffusing capacity in chronic heart failure. Its pathophysiological relevance and relationship to exercise performance. Circulation 1995; 91(11):2769-2774.
19. Van Barneveld PW, Sleijfer DT, van der Mark TW et al. Natural course of bleomycin-induced pneumonitis. A follow-up study. Am Rev Respir Dis 1987; 135(1):48-51.
20. Chiou TJ, Tung SL, Wang WS et al. Pulmonary function changes in long-term survivors of chronic myelogenous leukemia after

allogeneic bone marrow transplantation: a Taiwan experience. Cancer Invest 2002; 20(7-8):880-888.
21. Hughes JM. The single breath transfer factor (Tl,co) and the transfer coefficient (Kco): a window onto the pulmonary microcirculation. Clin Physiol Funct Imaging 2003; 23(2):63-71.
22. Kanengiser LC, Rapoport DM, Epstein H et al. Volume adjustment of mechanics and diffusion in interstitial lung disease. Lack of clinical relevance. Chest 1989; 96(5):1036-1042.
23. West JB, Wagner PD. Ventilation-perfusion relationships. In: Crystal RG, editor. The lung: scientific foundations. Philadelphia: Lippincott-Raven Publishers, 1997: 1693-1710.
24. Chang SC, Chang HI, Liu SY et al. Effects of body position and age on membrane diffusing capacity and pulmonary capillary blood volume. Chest 1992; 102(1):139-142.
25. Peces-Barba G, Rodriguez-Nieto MJ, Verbanck S et al. Lower pulmonary diffusing capacity in the prone vs. supine posture. J Appl Physiol 2004; 96(5):1937-1942.
26. Rohdin M, Linnarsson D. Differential changes of lung diffusing capacity and tissue volume in hypergravity. J Appl Physiol 2002; 93(3):931-935.
27. Roughton FJ, Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J Appl Physiol 1957; 11(2):290-302.
28. Klocke RA. Diffusion, chemical reactions involving respiratory gases, and diffusing capacity. In: Fishman AP, editor. Fishman's Pulmonary diseases and disorders. New York: Mc Graw-Hill, 1998: 193-202.
29. Borland CD, Higenbottam TW. A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. Eur Respir J 1989; 2(1):56-63.
30. Guenard H, Varene N, Vaida P. Determination of lung capillary blood volume and membrane diffusing capacity in man by the measurements of NO and CO transfer. Respir Physiol 1987; 70(1):113-120.
31. Hughes JM, Pride NB. Carbon monoxide transfer coefficient (transfer factor/alveolar volume) in females versus males. Eur Respir J 2003; 22(1):186-187.

32. Chinn DJ, Cotes JE, Flowers R et al. Transfer factor (diffusing capacity) standardized for alveolar volume: validation, reference values and applications of a new linear model to replace KCO (TL/VA). Eur Respir J 1996; 9(6):1269-1277.
33. Stam H, Hrachovina V, Stijnen T et al. Diffusing capacity dependent on lung volume and age in normal subjects. J Appl Physiol 1994; 76(6):2356-2363.
34. Stam H, Splinter TA, Versprille A. Evaluation of diffusing capacity in patients with a restrictive lung disease. Chest 2000; 117(3):752-757.
35. Frans A, Nemery B, Veriter C et al. Effect of alveolar volume on the interpretation of single breath DLCO. Respir Med 1997; 91(5):263-273.
36. Johnson DC. Importance of adjusting carbon monoxide diffusing capacity (DLCO) and carbon monoxide transfer coefficient (KCO) for alveolar volume. Respir Med 2000; 94(1):28-37.
37. Clark KD, Wardrobe-Wong N, Elliott JJ et al. Patterns of lung disease in a "normal" smoking population: are emphysema and airflow obstruction found together? Chest 2001; 120(3):743-747.

# Chapter 3

# Alveolar volume determined by single-breath helium dilution correlates with the high-resolution computed tomography-derived nonemphysematous lung volume

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Respiration, Sept 30 2006

## Abstract

The alveolar volume ($V_A$), determined by single-breath helium dilution, is a measure for the total lung capacity (TLC) that is very sensitive to ventilatory disturbances. In chronic obstructive pulmonary disease (COPD), the emphysematous lung parts are less accessible to test gas; therefore, the $V_A$ is smaller than TLC measured by multiple-breath helium dilution ($TLC_{He}$). The aim of this study was to investigate whether the $V_A$ represents the nonemphysematous lung parts.

We measured $V_A$ as part of the diffusing capacity for carbon monoxide ($DL_{CO}$), $TLC_{He}$ and spirometry in 50 patients with COPD. High-resolution computed tomography (HRCT) scans of all subjects were analyzed with the density mask method, where parts with an attenuation of less than –950 Hounsfield units were considered as emphysematous.

A strong correlation was observed between the $V_A$ (mean 5.2 liters) and nonemphysematous HRCT lung volume (mean 5.2 liters, $r^2 = 0.9$) and between the $TLC_{He}$ (mean 6.6 liters) and total HRCT lung volume (mean 6.4 liters, $r^2 = 0.9$). Bland-Altman plots showed considerable disagreement between the $V_A$ and the nonemphysematous HRCT lung volume. A weak correlation between the forced expiratory volume in 1 s (mean 46% predicted) and $DL_{CO}$ (mean 46% predicted) versus the HRCT emphysema ratio (nonemphysematous/total HRCT lung volume) was observed ($r^2 = 0.3$ and 0.3, respectively).

We concluded that the $V_A$ correlates with the nonemphysematous HRCT lung volume, although the two measurements are not equivalent, possibly due to technical factors.

# Introduction

The alveolar volume ($V_A$) is a measure for lung size, and is mostly determined during the measurement of the carbon monoxide diffusion capacity ($DL_{CO}$) via a single-breath helium dilution technique. Due to that single-breath approach, the measurement is sensitive to ventilatory disturbances. In healthy subjects the $V_A$ equals the total lung capacity (TLC) determined by multiple-breath helium dilution ($TLC_{He}$). In subjects with ventilatory impairment the VA often is much lower than the $TLC_{He}$, due to the insufficient mixing of gas [1]. The $V_A/TLC_{He}$ ratio can be used as an estimator for ventilation disturbances; Cotes [2] proposed an 85% cutoff point of the $V_A/TLC_{He}$ ratio as a boundary between healthy and diseased subjects.

Emphysema, which is the major component of chronic obstructive pulmonary disease (COPD), can be visualized on high-resolution computed tomography (HRCT) scans as areas with abnormally low attenuation [3]. These areas with decreased attenuation can be assessed with semiautomated computer software, which quantifies the amount of lung volume below a certain attenuation threshold. In this way the total emphysematous lung volume can be measured and expressed as percentage of the total HRCT lung volume. In the recent past a strong correlation between the percentage of low attenuation areas on HRCT scan and pathological grading of emphysema was found [4-6]. Furthermore, a good correlation between the amount of low attenuation areas on HRCT scan and pulmonary function testing (PFT) has been assessed in multiple studies [7-9].

Classical lung physiology theories predict that inspired gas will show almost a zero flow at the entrance of the alveoli and the alveolar membrane is reached by diffusion [10;11]. In emphysema this diffusion pathway is significantly lengthened and we hypothesized that the emphysematous degenerated parts of the lung are not or less accessible to test gas and hence the nonemphysematous lung size equals that found with the $V_A$ measurement.

In this study we compared the nonemphysematous lung volume calculated by means of HRCT with that of the $V_A$ determined by the single-breath helium dilution technique.

# Methods

From April 2001 until February 2003, a search was performed to identify subjects diagnosed with COPD based on the American Thoracic Society criteria [12], who underwent an HRCT scan of the lungs. Only patients with a $V_A$ measurement as part of the $DL_{CO}$ measurement and a $TLC_{He}$ measurement less than 3 months before or after the performance of the HRCT were included in this study.

### *Pulmonary Function Testing*

Multiple-breath $TLC_{He}$ and forced expiratory volume in 1 s ($FEV_1$) were determined on a MasterScreenFRC (Erich Jaeger, Würzburg, Germany). The $V_A$ determined by single-breath helium dilution was measured as part of the determination of the $DL_{CO}$ (MasterLab Pro, Erich Jaeger). All PFT data were expressed as absolute values or as percentages of predicted [13].

### *HRCT Scans and Evaluation*

The scans were made using Philips CT Secura system (Philips Medical Systems, Best, the Netherlands), tube current 120 kV, and 120 mA. Subjects were supine, in end-inspiratory state. The slice thickness was 1 mm, there was a 10-mm interval between the slices, and scanning time was 1,000 ms. All images were smoothed slice-by-slice with a Gaussian filter (SD = 3 pixels) to reduce noise. The lungs were segmented by selecting the pixels with a value below -500 Hounsfield units (HU), the trachea and main bronchi were removed semiautomatically. Per voxel the amount of pixels with attenuation between –500 and –1,000 HU was multiplied by voxel volume, from which the HRCT-determined total lung volume ($TLC_{HRCT}$) was calculated by adding up all voxel volumes in all slices. The nonemphysematous HRCT lung volume was defined as $TLC_{HRCT}$ minus the total volume of attenuation less than –950 HU [4;14]. We defined the HRCT emphysema ratio as the nonemphysematous HRCT lung volume divided by $TLC_{HRCT}$. This ratio provides information about the emphysema fraction: normal is a ratio of 1.0, which means no evident emphysema; a lower ratio points to a significant amount of emphysema.

### *Statistical Analysis*

The pulmonary function tests and the HRCT emphysema ratio were compared via Pearson correlation coefficients (r). The HRCT-derived lung volumes and the helium dilution-derived lung volumes were compared with Pearson correlation coefficients and their mutual relation was graphically depicted by Bland-Altman plots [15]. Statistical significance was defined by $p < 0.05$.

## Results

A total of 50 patients (21 female and 29 male) with COPD were reviewed. All subjects were current or former smokers. Physiological characteristics of the subjects are displayed in Table 1.

| | *Mean ± SD* | *Range* |
|---|---|---|
| Age, years | 59.5±11.7 | 29–83 |
| $FEV_1$, l | 1.417±0.9 | 0.4–4.7 |
| $FEV_1$, % predicted | 46.4±24.1 | 13.4–117.5 |
| $FEV_1$/FVC, % | 39.2±15.6 | 16–86 |
| $TLC_{He}$, l | 6.6±1.6 | 4.1–10.8 |
| $TLC_{He}$, % predicted | 105.4±17.0 | 68.6–150.7 |
| $V_A$, l | 5.2±1.2 | 3.3–9.0 |
| $DL_{CO}$, % predicted | 45.9±17.0 | 14.9–95.8 |
| $K_{CO}$, % predicted | 55.8±20.8 | 17.5–117.8 |
| $TLC_{HRCT}$, l | 6.4±1.5 | 2.9–10.9 |
| Nonemphysematous HRCT lung volume, l | 5.2±1.1 | 2.9–7.4 |
| HRCT emphysema ratio | 0.8±0.1 | 0.6–1.0 |
| $V_A/TLC_{He}$, % | 79.8±11.5 | 55.0–102.0 |

***Table 1.*** *Characteristics of the 50 patients with COPD (21 female, 29 male).*

A weak correlation was found between the emphysema fraction and the $DL_{CO}$ as percentage predicted ($r^2 = 0.3$) and the $FEV_1$ percentage predicted ($r^2 = 0.3$; Figure 1). The mean $TLC_{He}$ (6.6 liters) and mean $TLC_{HRCT}$ (6.4 liters) have a strong correlation ($r^2 = 0.9$; Figure 2); the mean $V_A$ (5.2 liters) and the mean nonemphysematous HRCT lung volume (5.2 liters) also have a strong correlation ($r^2 = 0.9$; Figure 3). In order to compare the HRCT-derived volumes with the $V_A$ and $TLC_{He}$ we constructed Bland-Altman plots (Figure 4, 5), which showed that there are considerable differences between the two methods.

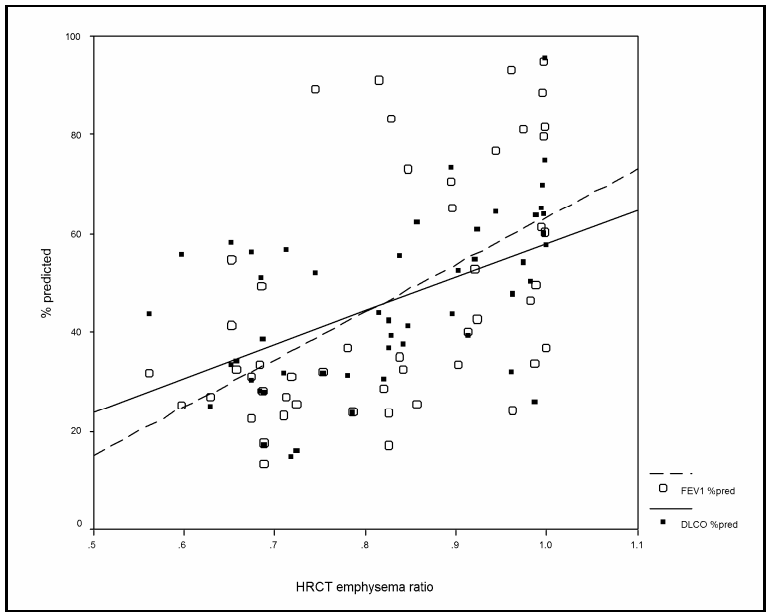


***Figure 1.*** *Scatter diagram of HRCT emphysema ratio versus $FEV_1$ (% predicted) and versus $DL_{CO}$ (% predicted).*

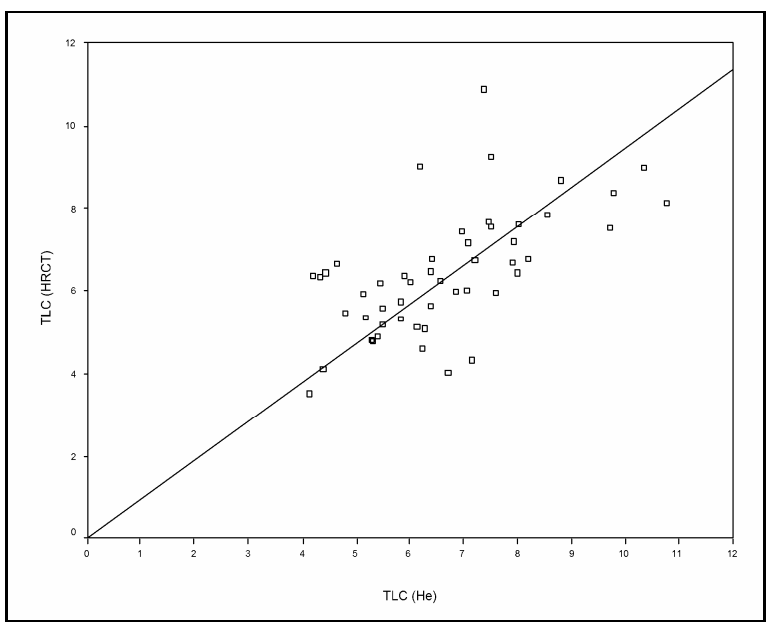


***Figure 2.*** *Scatter diagram of $TLC_{He}$ and $TLC_{HRCT}$.*

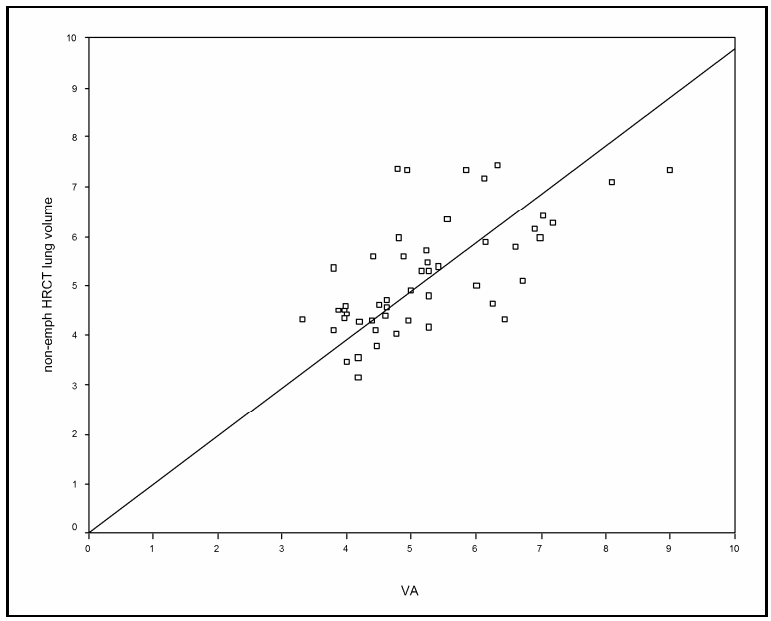


***Figure 3.*** *Scatter diagram of $V_A$ and nonemphysematous HRCT lung volume.*

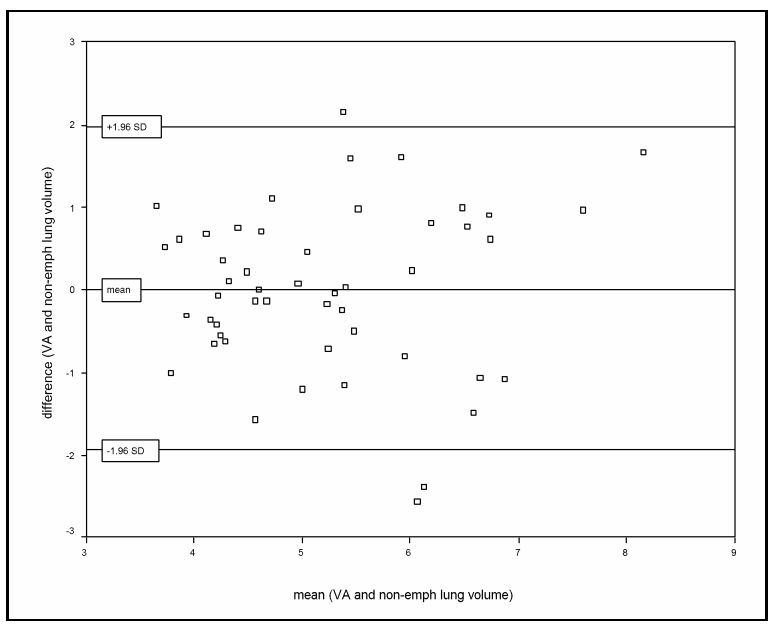


***Figure 4.*** *Bland-Altman plot of $V_A$ and nonemphysematous HRCT lung volume.*

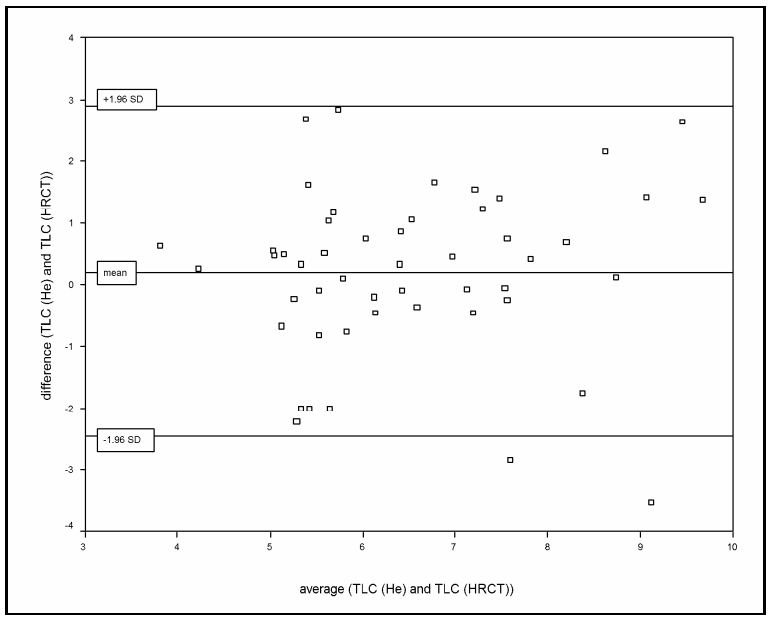


***Figure 5.*** *Bland-Altman plot of $TLC_{He}$ and $TLC_{HRCT}$.*

## Discussion

We found a strong correlation between nonemphysematous HRCT lung volume and $V_A$, although the agreement between the two measurements based on Bland-Altman plots is weak. The difference between the two measurements is up to 2 liters, which is clinically very relevant; therefore, these two measurements cannot be used interchangeably. Remarkable is the fact that the difference between the $TLC_{HRCT}$ and the $TLC_{He}$ shows the same amount of disagreement. A probable explanation is that PFT is performed in the sitting position, and the patients are encouraged by experienced pulmonary function technicians to do their best. The HRCT scans are performed in the supine position, and although patients are told to maximally inhale, this is not checked by experienced personnel or with the aid of spirometry. The only way to avoid this discrepancy between these two methods would be to use spirometric-controlled HRCT scans in a prospective study, and to correlate this with the $V_A$ measurement performed in the supine position. Another advantage of this approach

would be that a better distinction could be made between areas with extreme hyperinflation and emphysematous areas, because in our study the density mask method cannot discriminate between these two phenomena.
The $V_A$ gives a functional assessment of the total ventilation inhomogeneity, based on the dilution of helium. Air-containing parts of the lung, which are not accessible to test gas or inspired air, do not contribute to the $V_A$. In normal subjects (or in patients with a restrictive pulmonary impairment) $V_A$ is expected to equal the $TLC_{He}$. In subjects with emphysema the $TLC_{He}$ is higher than the $V_A$, providing that enough time is taken to let the helium wash in the less accessible areas. Areas inaccessible to test gas, for example bullae, are not measured with multiple-breath helium dilution, but are measured with whole body plethysmography, which measures the entire air-containing lung space.
As far as we know this is the first study that correlates the size of $V_A$ to the size of the nonemphysematous HRCT lung volume. This notion leads to an interesting hypothesis: in measuring the diffusion capacity the test gas used is a mixture of helium and CO which is distributed over the lung in exactly the same way. CO hence also does not enter the emphysematous lung parts and it can be assumed that these inaccessible parts do not contribute significantly to the diffusion capacity. Still in emphysema the diffusion capacity is often lowered and so this lowering reflects the integrity of the accessible parts of the lungs. It reflects the functional status of the nonemphysematous lung parts and it might be expected that there is a correlation between the extent of emphysema and the damage to the rest of the lung. That damage is not (yet) visible, however, on HRCT scans as plain emphysema; therefore, HRCT scanning and diffusion capacity are complementary approaches.
Recently others measured ventilated lung volumes using a hyperpolarized $^3$He MRI scan, which showed a good correlation with nonemphysematous lung volume as measured by CT scan [16]. The investigators used it to determine split-lung volumes in single-lung transplant recipients, where standard pulmonary function tests are insufficient to assess the left-right distribution. Of course, the $V_A$ measurement is much more uncomplicated and cheaper than an MRI scan, and in a subject without single-lung transplant there is no need to determine split-lung volumes.

We have not been the only ones to find a rather weak correlation of $DL_{CO}$ and $FEV_1$ with the HRCT emphysema ratio: Gelb et al. [5] also found a weak correlation and concluded that emphysema does not appear to be primarily responsible for the expiratory airflow limitation in COPD. A recent publication describes a strong association between small-airway obstruction and the progression of COPD [17]. Both investigations (among others) focus on small airway disease as the cause of the persistent expiratory airflow limitation in COPD, and not on the existence of emphysema.
In conclusion, the measurement of $V_A$ by single-breath helium dilution has a good correlation with the measurement of the nonemphysematous HRCT lung volume, although the differences between the two measurements can increase to 2 liters, which of course in a clinical situation is very relevant. Spirometry-controlled CT scanning related to $V_A$ measurement will be needed before we can state without reservation that the $V_A$ represents the easy-accessible air parts of the lungs.

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## References

1. Roberts CM, MacRae KD, Seed WA. Multi-breath and single breath helium dilution lung volumes as a test of airway obstruction. Eur Respir J 1990; 3(5):515-520.
2. Cotes JE. Assessment of distribution of ventilation and of blood flow through the lung. In: Cotes JE, editor. Lung function. Assessment and application in medicine. Oxford: Blackwell Scientific publications, 1993: 213-262.
3. Webb WR, Stein MG, Finkbeiner WE et al. Normal and diseased isolated lungs: high-resolution CT. Radiology 1988; 166(1 Pt 1):81-87.
4. Gevenois PA, De Vuyst P, de Maertelaer V et al. Comparison of computed density and microscopic morphometry in pulmonary emphysema. Am J Respir Crit Care Med 1996; 154(1):187-192.
5. Gelb AF, Hogg JC, Muller NL et al. Contribution of emphysema and small airways in COPD. Chest 1996; 109(2):353-359.
6. Muller NL, Staples CA, Miller RR et al. "Density mask". An objective method to quantitate emphysema using computed tomography. Chest 1988; 94(4):782-787.
7. Park KJ, Bergin CJ, Clausen JL. Quantitation of emphysema with three-dimensional CT densitometry: comparison with two-dimensional analysis, visual emphysema scores, and pulmonary function test results. Radiology 1999; 211(2):541-547.
8. Sakai N, Mishima M, Nishimura K et al. An automated method to assess the distribution of low attenuation areas on chest CT scans in chronic pulmonary emphysema patients. Chest 1994; 106(5):1319-1325.
9. Verschakelen JA, Scheinbaum K, Bogaert J et al. Expiratory CT in cigarette smokers: correlation between areas of decreased lung attenuation, pulmonary function tests and smoking history. Eur Radiol 1998; 8(8):1391-1399.
10. Miller WF, Scacci R., Gast L.R. Diffusion. Laboratory Evaluation of Pulmonary Function. J.B. Lippincott Company Philadelphia, 1987: 398-440.
11. Scheid P, Piiper J. Diffusion. In: Crystal RG, West JB, Weibel ER et al, editors. The lung: scientific foundations. Philadelphia: Lippincott-Raven Publishers, 1997: 1681-1692.

12. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. American Thoracic Society. Am J Respir Crit Care Med 1995; 152(5 Pt 2):S77-121.
13. Quanjer PH, Tammeling GJ, Cotes JE et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:5-40.
14. Gevenois PA, de M, V, De Vuyst P et al. Comparison of computed density and macroscopic morphometry in pulmonary emphysema. Am J Respir Crit Care Med 1995; 152(2):653-657.
15. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; 1(8476):307-310.
16. Zaporozhan J, Ley S, Gast KK et al. Functional analysis in single-lung transplant recipients: a comparative study of high-resolution CT, 3He-MRI, and pulmonary function tests. Chest 2004; 125(1):173-181.
17. Hogg JC, Chu F, Utokaparch S et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med 2004; 350(26):2645-2653.

# Chapter 4

# Early diagnosis of emphysema: computed tomography versus pulmonary function testing

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## Abstract

To determine the capability of pulmonary function tests (PFT) to detect emphysema demonstrated by the density mask method on computed tomography (CT).
We studied current and former heavy smokers participating in a population based randomized lung cancer screening trial, screened between April 2004 and March 2005 in one of the participating centers with low-dose baseline CT (16x0.75 mm slice collimation). Only participants who also performed PFT on the same day were included. Emphysema on CT was determined as lung volume with attenuation below -950 HU relative to total lung volume using the density mask method and expressed as emphysema score (ES). Subjects with ES>1 were considered having emphysema. Positive (PPV) and negative predictive values (NPV) for PFT were calculated, using CT as gold standard.
We included 545 men (51-74 y, mean 62 y) for analysis. Seventy-five cases (14%) showed emphysema on CT. $DL_{CO}/V_A$ was the most accurate parameter, missing only 13 (17%) cases with emphysema. All subjects with ES>5 showed abnormal $DL_{CO}/V_A$ (<70% of predicted). However, 149 of 211 subjects (71%) with abnormal $DL_{CO}/V_A$ did not show emphysema on CT. NPV of $DL_{CO}/V_A$ was 96%, while PPV was only 23%. $FEV_1/VC$ showed a NPV of 93% and a PPV of 31%, while $FEV_1$ showed a NPV of 89% and a PPV of 35%.
Normal PFT results are useful in excluding emphysema. However, pulmonary function tests lack discriminatory power to distinguish emphysema from other pulmonary diseases.

# Introduction

Chronic obstructive pulmonary disease (COPD) is the most frequent chronic disease in developed countries and will the third cause of death in 2020 [1]. A major problem is that the diagnosis of COPD is often made late in the course of the disease. Early detection of COPD by screening could identify subjects in an earlier and milder stage and would enable initiation of treatment before the occurrence of exacerbations [2]. Since the GOLD guidelines [3] require spirometry for diagnosis, COPD mass screening has been performed with pulmonary function testing [4-9] and has used pulmonary function parameters as surrogate markers for emphysema.

Emphysema is defined anatomically as an abnormal permanent enlargement of the airspace distal to the terminal bronchioles without fibrosis [10]. Therefore, histology is required for diagnosis of emphysema. Several groups showed a good correlation between emphysema in histological specimens and low attenuation areas detected on computed tomography (CT), enabling non-invasive diagnosis [11-15]. These areas with abnormally low attenuation on CT, due to disappearance of lung tissue, can be highlighted by the density mask method [11-15], first described by Müller and co-workers [16] and validated for high resolution CT against pathology by Gevenois and co-workers [15].

Several investigators correlated the extent of emphysema determined on CT to pulmonary function parameters and reported that $FEV_1/VC$ and $DL_{CO}/V_A$ were the best correlating parameters ($r= -0.44$ and $r= -0.71$, respectively) [17;18]. These moderate to good correlations suggest that pulmonary function tests and CT partly overlap, but may also detect different entities of smoking-related pulmonary pathology. Secondly, these correlations were calculated for patients with a prior diagnosis of emphysema, which can bias the results.

The aim of this study was to determine the predictive values of pulmonary function parameters for the presence or absence of emphysema as detected with high resolution volume CT and the correlation between both techniques in a large group of heavy current and former smokers, without a prior diagnosis of emphysema.

# Methods

### *Subjects*

The NELSON-project is a population based randomized Dutch-Belgian multi-center lung cancer screening trial, studying male, and to a lesser extent female, current and former heavy smokers. The trial was approved by the Dutch ministry of health and by the ethics committee of each participating hospital. Selection of participants for the trial was performed by sending a questionnaire about among others smoking history to people between 50 and 75 years old and living in the areas around the participating centers. Subjects meeting the inclusion criteria of a minimum of 16 cigarettes/day for 25 years or 11 cigarettes/day for 30 years, who gave informed consent were equally randomized to either the screening arm or the control arm. Before inviting eligible subjects, persons with a moderate or bad self-reported health status who were unable to climb two flights of stairs were excluded. Persons with current or past renal cancer, melanoma, breast cancer or with lung cancer diagnosed less than 5 years before recruitment were excluded as well as subjects who had a chest CT scan less than one year before they filled in the first NELSON questionnaire and persons with a body weight greater of equal to 140 kilogram. Subjects in the screening arm receive baseline CT and at least two follow-up CTs during a period of four years. Three thousand participants underwent baseline CT in our hospital and randomly one in three screened subjects was referred for pulmonary function testing.
We included participants who were screened between April 2004 and February 2005 in our hospital and who performed pulmonary function tests on the same day. Since the trial started with screening men, only male participants were included in the present study.

### *CT scanning and calculation of emphysema scores*

CT scanning was performed by a 16 detector-row scanner (Mx8000 IDT or Brilliance 16P, Philips Medical Systems, Cleveland, OH) with 16x0.75 mm collimation. A caudo-cranial scan direction was applied and the entire chest was scanned in approximately 10 seconds. No intravenous contrast injection was used. Exposure settings were 30 mAs at 120 kVp for patients weighing ≤80 kg and 30 mAs at 140 kVp

for those weighing >80 kg. We reconstructed axial images of 1.0 mm thickness at 0.7 mm increment, using the smallest field of view (FOV) to include the outer rib margins at the widest dimension of the thorax. All scans were reconstructed with a soft kernel (Philips "B") at 512x512 matrix.

### *Emphysema quantification*

Extent of low-attenuation areas was determined with a density mask method, using in-house developed software (imageXplorer (iX), Image Sciences Institute, Utrecht, The Netherlands) and expressed relative to total lung volume as emphysema score (ES).
Total lung volume was calculated using the following steps. Segmentation of trachea, left and right lung was performed by a fully automated region growing program starting in the trachea, which included all connected areas below -500 HU. In a second step, trachea and main bronchi were excluded from the lungs. The algorithm is similar to the one described by Hu and co-workers [19]. The number of voxels within the segmented area was multiplied by the size of a voxel to calculate total lung volume. Finally, segmented lungs were filtered for image noise with a median filter [20]. ES was calculated as percentage of total lung volume with an attenuation below -950 HU as recommended by Gevenois [15] and Parr [21].
Subjects were divided in two groups based on the presence or absence of areas with an attenuation below -950 HU, which is usually used for subjective scoring [22;23] and mentioned by Kinsella and co-workers, who used the density mask method [24]: no emphysema or non-significant emphysema (ES=0-1) or significant emphysema (ES≥1).

### *Pulmonary Function Tests*

Pulmonary function tests (PFT) included spirometry and flow-volume curves measurements with a pneumotachograph and assessment of diffusion capacity, according to ERS guidelines [25]. Upon arrival, subjects rested for 15 minutes after which non-forced spirometry was performed, immediately followed by recording flow-volume curves. No reversibility testing was done.
Diffusing capacity measurements were performed after spirometry. The inhalation mixture contained 0.3% CO and 10% He with balance air. A breath holding period of 10 seconds was used. Participants were

asked to refrain from smoking, but the $DL_{CO}$ was not corrected for Hb, because in a normal population such correction is not useful [26]. Abnormal pulmonary function parameters were defined as values ≤-1.64 standard deviations below reference values [25]. Subjects were staged according to updated GOLD guidelines [27].

### *Statistics*

We calculated means, standard deviations and 95% confidence intervals for normal distributed parameters and medians and 25%/75% quartiles for non-normal distributed parameters. Spearman's correlation coefficients were used to assess a relationship between lung function parameters and emphysema scores. We performed Kruskal-Wallis tests to detect differences between GOLD-stages in both pack years (one pack of cigarettes a day during one year) and median emphysema scores. To compare our results to studies including only patients with a prior diagnosis of emphysema, we simulated the effect of selection by performing the calculations in the entire sample and in a subpopulation of patients with GOLD stage II, III and IV.
Based on presence or absence of emphysema on CT as defined above and lowered pulmonary function parameters, we calculated positive and negative predictive values of lung function parameters using CT as gold standard.
All statistics were calculated with SPSS statistical software package version 12 (SPSS, Chicago, USA). P-values <0.05 were considered significant.

## Results

### *Subjects*

In total 1386 male subjects received baseline screening between April 2004 and February 2005. Five hundred forty-five of them (50-74y, mean 62y), 185 smokers and 360 ex-smokers, also performed pulmonary function tests on the same day. All data were eligible for analysis. Characteristics of the study subjects are shown in Table 1. No subjects fulfilled the criteria for GOLD stage 4. Age differences between groups were significant (p=0.03), while pack year differences were not (p=0.06).

***Pulmonary Function Parameters compared to computed tomography***

In Figure 1 the extent of emphysema is displayed, showing 75 subjects (14%) with emphysema and 470 without emphysema. Median ES was 0.16 (±3.3), ranging from 0 to 31. Median emphysema scores according to GOLD stage are shown in Table 2.
In Figure 2, we show percentages of abnormal $FEV_1$, $FEV_1/VC$ and $DL_{CO}/V_A$ ratios as function of ES. Comparison between emphysema detected on CT and PFT showed significant but low correlations: r =-0.156 for $FEV_1$, r= -0.484 for $FEV_1/VC$ and r= -0.467 for $DL_{CO}/V_A$ ($p<0.001$). The effect of selection bias on correlation coefficients becomes clear in Table 3. The total sample has been compared to a subgroup with only those subjects who are likely to seek medical help due to more severe disease and would have been included in a hospital based study. Especially for $FEV_1$ and $DL_{CO}$, the correlations coefficients increase considerably, while for $FEV_1/VC$ and $DL_{CO}/V_A$ smaller increases become clear.

***Positive and negative predictive values***

$FEV_1$ was lowered in 60 (11%) subjects, but only 21 (35%) of them showed emphysema on CT. $FEV_1/VC$ was lowered in 143 (26%) subjects, but only in 44 (31%) of them emphysema was detected on CT. Abnormal $DL_{CO}/V_A$ was demonstrated in 211 subjects, while only 62 (29%) of them showed emphysema on CT. In the group of 75 subjects demonstrating emphysema on CT, 53 (71%) subjects had a normal $FEV_1$, 30 (40%) subjects showed normal $FEV_1/VC$ values, while $DL_{CO}/V_A$ was normal in only 13 (17%) subjects.
The prior probability to detect the absence of emphysema on CT was 86%, since this number of subjects did not show emphysema. $FEV_1$ showed a negative predictive value (NPV) of 89%, while NPV for $FEV_1/VC$ was 93% and for $DL_{CO}/V_A$ even 96% (Table 4). These results show that these parameters are useful to exclude emphysema on CT. The positive predictive values of $FEV_1$, $FEV_1/VC$ and $DL_{CO}/V_A$ were 35%, 31% and 29% respectively. These results show that an abnormal result for pulmonary function parameters represent the presence of emphysema in only a minority of cases, while abnormal results can also be caused by diseases other than emphysema, which could not be detected on computed tomography with the density mask method.

| GOLD stage | age (ys) | VC | $FEV_1$ | $FEV_1$/VC | $DL_{CO}$ | $DL_{CO}/V_A$ | Pack years |
|---|---|---|---|---|---|---|---|
| All | 59.8 (5.5) | 105.1 (13.6) | 97.3 (17.9) | 90.6 (12.1) | 83.7 (17.8) | 90.0 (24.5) | 37.8 (27.3-48.3) |
| at risk (n=339) | 59.3 (5.4) | 106.2 (13.1) | 104.9 (13.9) | 97.8 (6.0) | 87.1 (14.6) | 94.2 (15.7) | 37.8 (27.3–48.3) |
| mild (n=135) | 60.5 (5.4) | 112.4 (11.3) | 94.3 (9.1) | 83.3 (5.9) | 80.4 (17.3) | 81.3 (16.1) | 42.6 (33.3–48.3) |
| moderate (n=62) | 61.2 (6.4) | 94.7 (11.5) | 69.5 (7.2) | 73.1 (8.3) | 75.0 (18.6) | 83.3 (19.7) | 44.8 (33.3–58.8) |
| severe (n=9) | 60.7 (2.4) | 90.5 (10.8) | 44.1 (2.2) | 49.0 (6.8) | 47.4 (14.3) | 50.8 (13.8) | 37.8 (37.8–57.0) |
| p-value for group differences | 0.029 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.058 |
| p-value for linear trend | 0.376 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | - |

***Table 1.*** *Descriptive statistics shown as mean values (±SD) according to GOLD stage. All lung function parameters are expressed as percentage of the predicted value. Pack years are depicted by a median and 25/75th percentile values and evaluated by a Kruskal-Wallis test.*

| Gold stage | Median ES |
|---|---|
| all | 0.2 |
| at risk | 0.1 |
| mild | 0.3 |
| moderate | 0.4 |
| severe | 4.2 |
| p-value for group difference | <0.001 |

***Table 2.*** *Median tissue destruction (percentage of total lung volume), according to GOLD stage. Evaluation by Kruskal-Wallis test.*

| GOLD stage | VC | $FEV_1$ | $FEV_1$/VC | $DL_{CO}$ | $DL_{CO}/V_A$ |
|---|---|---|---|---|---|
| all | $0.231^{**}$ | $-0.156^{**}$ | $-0.484^{**}$ | $-0.284^{**}$ | $-0.467^{**}$ |
| ≥2 only | 0.081 | $-0.346^{*}$ | $-0.442^{**}$ | $-0.484^{**}$ | $-0.491^{**}$ |

***Table 3.*** *Non-parametric correlation coefficients between the degree of tissue destruction and pulmonary function parameters, expressed as percentage of predicted results, in the total sample and in a subgroup with GOLD stage ≥2 (** p<0.001;* p<0.01).*

| Parameter | Positive predictive value | Negative predictive value |
|---|---|---|
| VC | 25.0 (10.4 – 48.8) | 86.8 (84.2 – 89.0) |
| $FEV_1$ | 35.0 (24.2-47.6) | 89.1 (86.0 – 91.5) |
| $FEV_1/VC$ | 30.8 (23.8 – 38.8) | 92.5 (89.5 – 94.7) |
| $DL_{CO}$ | 29.9 (24.5 – 36.1) | 93.6 (91.2 – 95.4) |
| $DL_{CO}/V_A$ | 29.4 (23.7 – 35.9) | 96.1 (93.5 – 97.7) |

***Table 4.*** *Positive predictive and negative predictive values of lung function parameter values to detect or exclude the presence of emphysema on computed tomography.*

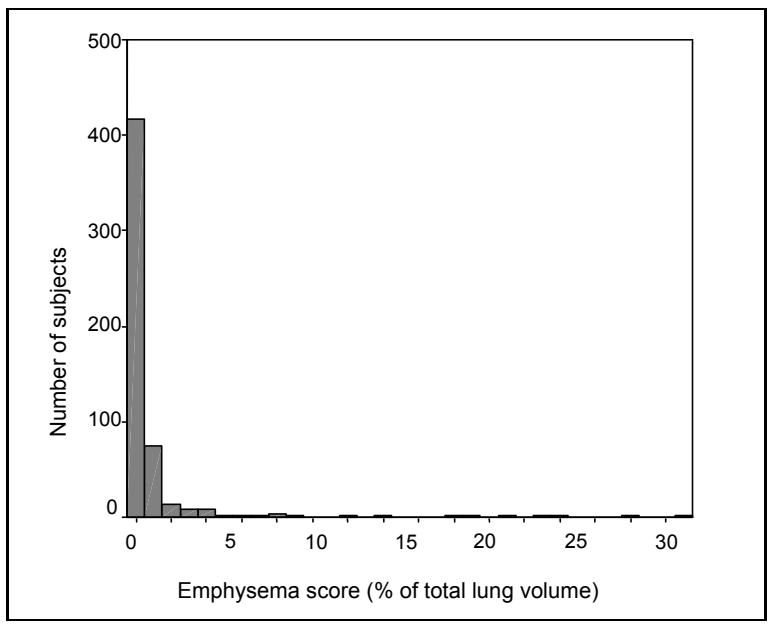
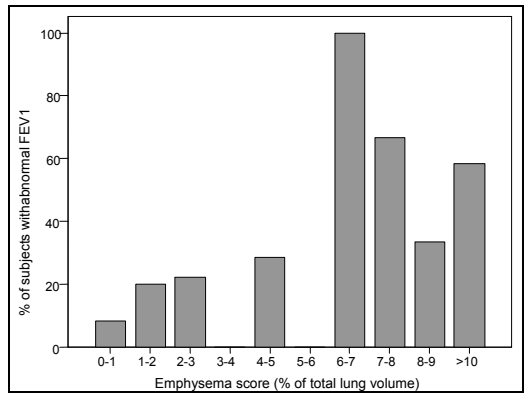
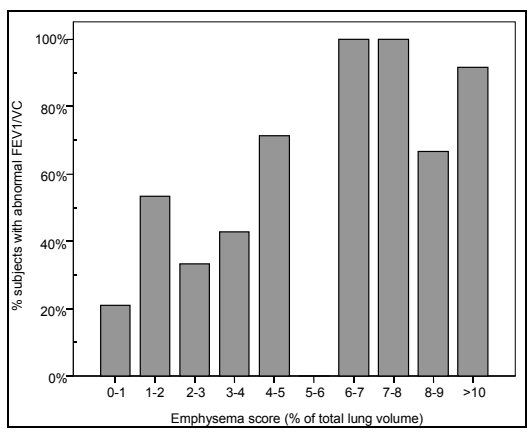



***Figure 1.*** *Emphysema scores plotted against the number of subjects showing these emphysema scores. The majority of subjects show an emphysema score of zero, representing no emphysema on computed tomography.*



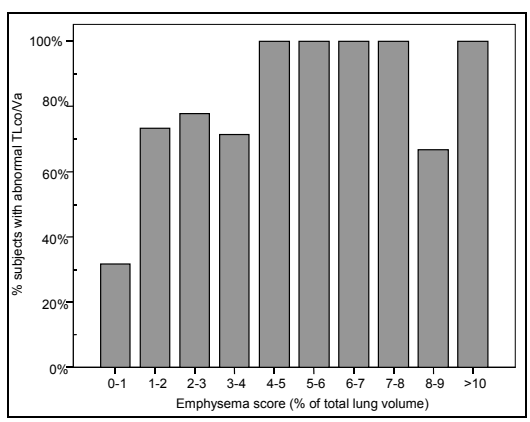




***Figure 2.*** *Emphysema scores plotted against percentages of subjects with lowered $FEV_1$, $FEV_1/VC$ and $DL_{CO}/V_A$.*

## Discussion

In this study we demonstrated that a minority (14%) of current and former heavy smokers suffer from emphysema detected on CT, defined as areas with attenuation below -950 HU. Normal pulmonary function testing parameters appear to be useful to exclude emphysema on CT, but function testing also frequently showed abnormal parameters, even when no emphysema was detected on CT.

The low positive predictive values for lowered pulmonary function parameters to be caused by emphysema shown on CT, suggest the ability of PFT to detect smoking-related abnormalities other than emphysema. Smoking has been shown not only to be associated to emphysema, but also to small airways and parenchymal diseases, such as respiratory bronchiolitis [28]. These abnormalities have been demonstrated to be also present in asymptomatic smokers by Remy-Jardin and co-workers [22;29]. About 30% of their smoking study-population showed areas with ground-glass attenuation and/or micronodules on CT. And those subjects with areas of ground-glass attenuation showed a more rapid decline in $FEV_1$ than subjects without these areas. Diffusion tests were not performed during their study. We performed visual inspection of CTs of subjects with lowered $K_{CO}$ and without emphysema on CT and determined that some of these CTs showed areas with ground-glass attenuation. We did not further evaluate these cases, since that was beyond the scope of this study, but more emphasis on the non-emphysematous tissue could elicit the influence of smoking-related diseases other than emphysema on spirometry and diffusion test results.

$FEV_1$ and (F)VC are mechanical parameters, and sensitive to mucosal thickening or loss of elasticity resulting from airway inflammation and remodeling. These phenomena can lead to airway obstruction without lung destruction, resulting in $FEV_1$ and (F)VC impairments, but will not be detected by the density mask method, which is only able to detect areas with loss of tissue.

The hypothesis that pulmonary function parameters were influenced by these pathophysiological processes can explain the low correlations between PFT and emphysema present on CT. This assumption is supported by comparisons to several other studies [17;18], showing higher correlations. We hypothesized that differences between these studies

and ours were due to selection bias: in these studies COPD was diagnosed with pulmonary function tests and only in these subjects CT-scanning was performed. These subjects form a subgroup with more advanced disease. We recalculated correlations in a subgroup of our sample, i.e. subjects with GOLD stage 2 and higher and we could confirm that correlations coefficients were higher compared to the total group. We can confirm the results reported by Gurney, who found similar increases of correlations in a relatively small group of emphysema patients with lung function disturbances compared to those without emphysema [30]. Selection bias seems to inflate correlation coefficients and may therefore explain to a large extent the differences in outcome.

Our results can also be compared to the study performed by Kinsella and co-workers [24]. They included 85 patients with suspected lung malignancy in their study and correlated results of the density mask method to pulmonary function testing. They reported moderate to good correlation coefficients: -0.56 for $FEV_1$, -0.53 for $DL_{CO}/V_A$ and -0.72 for $FEV_1$/FVC. However, they excluded patients with evidence of interstitial lung diseases, used a cut off level of -910 HU, and performed CTs at 10 mm collimation and after injection of intravenous contrast medium. We hypothesized that the differences between their results and the results we reported in this study can be explained by their exclusion of patients with interstitial diseases and difference in scanning and evaluation protocols.

For the diagnosis of emphysema, histology is required [10]. No histology was available in the present study, but computed tomography has shown to be able to detect lung tissue destruction, based on a good correlation with histology, rendering CT-scanning a reliable surrogate marker for pathology [11-15;31]. We detected lung destruction by the density mask method, which is an extensively described method to determine emphysema by highlighting the low density areas [14;15;17;18;21;24;32-37]. The density mask method was validated against pathology for area measurements on 2D images [15;16] but measurements on 2D and 3D CT-images have been demonstrated to show good correlations, indicating that the method is also reliable in a volume setting [18].

The main disadvantages of CT are the costs and radiation burden [38;39]. Introduction of low-dose protocols has reduced the radiation risk [39;40], but the increase of image noise on low-dose scans can influence results

of the density mask. Schilham showed that emphysema scores performed on filtered low-dose scans revealed comparable results to ES performed on standard-dose scans realized in the same session [41]. Shaker showed that emphysema scoring realized on low-dose CT was reliable, using 5.0 mm collimation, as long as more than 8 mAs is delivered [36].

In conclusion, we demonstrated in a large group of current and former heavy smokers that normal pulmonary function tests can exclude the presence of emphysema on CT. However, pulmonary function test have limited value in distinguishing emphysema from other pulmonary diseases.

## References

1. Feenstra TL, van Genugten ML, Hoogenveen RT et al. The impact of aging and smoking on the future burden of chronic obstructive pulmonary disease: a model analysis in the Netherlands. Am J Respir Crit Care Med 2001; 164(4):590-596.
2. Calverley PMA. COPD : Early Detection and Intervention. Chest 2000; 117(90052):365S-371.
3. Pauwels RA, Buist AS, Calverley PMA et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmo-nary Disease . NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop Summary. Am J Respir Crit Care Med 2001; 163(5):1256-1276.
4. Zielinski J, Bednarek M. Early detection of COPD in a high-risk population using spirometric screening. Chest 2001; 119(3):731-736.
5. Tzanakis N, Anagnostopoulou U, Filaditaki V et al. Prevalence of COPD in Greece. Chest 2004; 125(3):892-900.
6. Stratelis G, Jakobsson P, Molstad S et al. Early detection of COPD in primary care: screening by invitation of smokers aged 40 to 55 years. Br J Gen Pract 2004; 54(500):201-206.
7. Shin C, Lee S, Abbott RD et al. Respiratory Symptoms and Undiagnosed Airflow Obstruction in Middle-Aged Adults: The Korean Health and Genome Study. Chest 2004; 126(4):1234-1240.
8. Gorecka D, Bednarek M, Nowinski A et al. Diagnosis of Airflow Limitation Combined With Smoking Cessation Advice Increases Stop-Smoking Rate. Chest 2003; 123(6):1916-1923.
9. Buffels J, Degryse J, Heyrman J et al. Office Spirometry Significantly Im-proves Early Detection of COPD in General Practice: The DIDASCO Study. Chest 2004; 125(4):1394-1399.
10. The definition of emphysema. Report of a National Heart, Lung, and Blood Institute, Division of Lung Diseases workshop. Am Rev Respir Dis 1985; 132(1):182-185.
11. Coxson HO, Rogers RM, Whittall KP et al. A quantification of the lung sur-face area in emphysema using computed tomography. Am J Respir Crit Care Med 1999; 159(3):851-856.
12. Madani A, Keyzer C, Gevenois PA. Quantitative computed tomography as-sessment of lung structure and function in pulmonary emphysema. Eur Respir J 2001; 18(4):720-730.

13. Bergin C, Muller N, Nichols DM et al. The diagnosis of emphysema. A com-puted tomographic-pathologic correlation. Am Rev Respir Dis 1986; 133(4):541-546.
14. Newell JD, Jr., Hogg JC, Snider GL. Report of a workshop: quantitative computed tomography scanning in longitudinal studies of emphysema. Eur Respir J 2004; 23(5):769-775.
15. Gevenois PA, De Maertelaer V, De Vuyst P et al. Comparison of computed density and macroscopic morphometry in pulmonary emphysema. Am J Respir Crit Care Med 1995; 152(2):653-657.
16. Muller NL, Staples CA, Miller RR et al. "Density mask". An objective method to quantitate emphysema using computed tomography. Chest 1988; 94(4):782-787.
17. Arakawa A, Yamashita Y, Nakayama Y et al. Assessment of lung volumes in pulmonary emphysema using multidetector helical CT: comparison with pulmonary function tests. Comput Med Imaging Graph 2001; 25(5):399-404.
18. Park KJ, Bergin CJ, Clausen JL. Quantitation of emphysema with three-dimensional CT densitometry: comparison with two-dimensional analysis, visual emphysema scores, and pulmonary function test results. Radiology 1999; 211(2):541-547.
19. Hu S, Hoffman EA, Reinhardt JM. Automatic lung segmentation for accurate quantitation of volumetric X-ray CT images. IEEE Trans Med Imaging 2001; 20(6):490-498.
20. Hilts M, Duzenli C. Image filtering for improved dose resolution in CT polymer gel dosimetry. Med Phys 2004; 31(1):39-49.
21. Parr DG, Stoel BC, Stolk J et al. Influence of calibration on densitometric studies of emphysema progression using computed tomography. Am J Respir Crit Care Med 2004; 170(8):883-890.
22. Remy-Jardin M, Edme JL, Boulenguez C et al. Longitudinal Follow-up Study of Smoker's Lung with Thin-Section CT in Correlation with Pulmonary Func-tion Tests. Radiology 2002; 222(1):261-270.
23. Vikgren J, Friman O, Borga M et al. Detection of mild emphysema by com-puted tomography density measurements. Acta Radiol 2005; 46(3):237-245.
24. Kinsella M, Muller NL, Abboud RT et al. Quantitation of emphysema by computed tomography using a "density mask" program and correlation with pulmonary function tests. Chest 1990; 97(2):315-321.

25. Quanjer PH, Tammeling GJ, Cotes JE et al. Lung volumes and forced ventila-tory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:5-40.
26. Stam H, Hrachovina V, Stijnen T et al. Diffusing capacity dependent on lung volume and age in normal subjects. J Appl Physiol 1994; 76(6):2356-2363.
27. Pauwels RA, Buist AS, Calverley PM et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. Am J Respir Crit Care Med 2001; 163(5):1256-1276.
28. Heyneman LE, Ward S, Lynch DA et al. Respiratory bronchiolitis, respiratory bronchiolitis-associated interstitial lung disease, and desquamative inter-stitial pneumonia: different entities or part of the spectrum of the same dis-ease process? AJR Am J Roentgenol 1999; 173(6):1617-1622.
29. Remy-Jardin M, Remy J, Boulenguez C et al. Morphologic effects of ciga-rette smoking on airways and pulmonary parenchyma in healthy adult volunteers: CT evaluation and correlation with pulmonary function tests. Radiol-ogy 1993; 186(1):107-115.
30. Gurney JW, Jones KK, Robbins RA et al. Regional distribution of emphy-sema: correlation of high-resolution CT with pulmonary function tests in un-selected smokers. Radiology 1992; 183(2):457-463.
31. Coddington R, Mera SL, Goddard PR et al. Pathological evaluation of com-puted tomography images of lungs. J Clin Pathol 1982; 35(5):536-540.
32. Kemerink GJ, Kruize HH, Lamers RJ et al. CT lung densitometry: depend-ence of CT number histograms on sample volume and consequences for scan protocol comparability. J Comput Assist Tomogr 1997; 21(6):948-954.
33. Kalender WA, Rienmuller R, Seissler W et al. Measurement of pulmonary parenchymal attenuation: use of spirometric gating with quantitative CT. Ra-diology 1990; 175(1):265-268.
34. Lamers RJ, Thelissen GR, Kessels AG et al. Chronic obstructive pulmonary disease: evaluation with spirometrically controlled CT lung densitometry. Radiology 1994; 193(1):109-113.

35. Stoel BC, Stolk J. Optimization and standardization of lung densitometry in the assessment of pulmonary emphysema. Invest Radiol 2004; 39(11):681-688.
36. Shaker SB, Dirksen A, Laursen LC et al. Short-term reproducibility of computed tomography-based lung density measurements in alpha-1 antitrypsin deficiency and smokers with emphysema. Acta Radiol 2004; 45(4):424-430.
37. Shaker SB, Dirksen A, Laursen LC et al. Volume adjustment of lung density by computed tomography scans in patients with emphysema. Acta Radiol 2004; 45(4):417-423.
38. Haaga JR. Radiation dose management: weighing risk versus benefit. AJR Am J Roentgenol 2001; 177(2):289-291.
39. Brenner DJ, Doll R, Goodhead DT et al. Cancer risks attributable to low doses of ionizing radiation: assessing what we really know. Proc Natl Acad Sci USA 2003; 100(24):13761-13766.
40. Brenner DJ. Radiation risks potentially associated with low-dose CT screening of adult smokers for lung cancer. Radiology 2004; 231(2):440-445.
41. Schilham A, Van Ginneken B, Gietema H et al. Local Noise Weighted Filter-ing for Emphysema Scoring of Low-dose CT Images. IEEE Transactions on Medical Imaging 2006;accepted for publication.

# Chapter 5

# Diffusing capacity for nitric oxide: reference values and dependence on alveolar volume

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submitted

## Abstract

Nitric oxide (NO) has a much stronger affinity for hemoglobin than carbon monoxide (CO), therefore the $DL_{NO}$ (diffusing capacity for NO) is less influenced by changes in capillary blood volume than the $DL_{CO}$ (diffusing capacity for CO), and represents the true membrane diffusing capacity.
We measured the combined single breath $DL_{NO}/DL_{CO}$ in 124 healthy subjects, and generated reference equations for the $DL_{NO}$ and $K_{NO}$. In a subset of 21 subjects the measurements were performed on different inspiratory levels.
The reference equation for $DL_{NO}$ in females is 53.47*H (height)-0.077*A (age)-48.28 (RSD 5.22) and for males 59.84*H-0.25*A-44.20 (RSD 6.39). Reference equations for $K_{NO}$ in females is -2.03*H-0.025*A+11.52 (RSD 0.48) and for males -0.15*H-0.045*A +9.47 (RSD 0.65). The $K_{CO}$ ($DL_{CO}/V_A$) increases when $V_A$ (alveolar volume) decreases, probably due to an increase of blood volume per unit lung volume.
The $DL_{NO}$ was much stronger related to the $V_A$, the $K_{NO}$ was almost independent on $V_A$. Because of the relative independence of the $K_{NO}$ on $V_A$, the $K_{NO}$ appears to be a much better index for the diffusion capacity per unit lung volume (transfer coefficient) than the $K_{CO}$.

## Introduction

The carbon monoxide (CO) diffusing capacity of the lung ($DL_{CO}$), also known as the transfer factor, is a commonly used measure for lung gas uptake. The single breath approach is mostly used, because it is a fast method with good reproducibility. The CO lung uptake is influenced by two factors: 1) the diffusion limited passage of CO through the alveolocapillary membrane and 2) the passage of the CO through the plasma, the intra-erythrocytic compartment and the chemical binding to hemoglobin. The latter process leads to a decreased $DL_{CO}$ in case of anemia and/or reduced capillary blood volume. Roughton and Forster constructed the well-known equation: $1/DL_{CO}=1/Dm_{CO} + 1/\theta*Vcap$, where $Dm_{CO}$ is the membrane diffusing capacity for CO, $\theta_{CO}$ the CO uptake by erythrocytes and Vcap the pulmonary capillary blood volume. For the correct interpretation of the $DL_{CO}$ clinicians need to be aware that the alveolocapillary membrane resistance approximately accounts for half of the total resistance [1]. Another important issue is lung size: larger lungs show a stronger CO uptake and therefore the $DL_{CO}$ is dependent on alveolar volume ($V_A$). In search for an index independent of lung size, clinicians often use the $K_{CO}$, or transfer coefficient, defined as the $DL_{CO}$ divided by $V_A$. Unfortunately, the $K_{CO}$ increases when $V_A$ is (voluntary) decreased [2;3], probably due to a relative increase in blood volume per unit lung volume [2]. This phenomenon makes the $K_{CO}$ hard to interpret [4] in subjects with a small total lung volume, as is frequently seen in interstitial lung disease. Many authors proposed methods for correction of the $K_{CO}$ when $V_A$ is lower than its normal high value [3;5-7], but these methods have never been adopted on a wide scale.

Due to the high affinity of nitric oxide (NO) to hemoglobin, the nitric oxide diffusing capacity ($DL_{NO}$) reflects the properties of the alveolocapillary membrane much better than the $DL_{CO}$ [8]. The strong binding of NO to hemoglobin leads to a very high value for $\theta_{NO}$, thus the last term in the Roughton and Forster equation equals zero ($1/DL_{NO}=1/Dm_{NO}$). Therefore $DL_{NO}$ can be defined as the true alveolocapillary membrane diffusing capacity [9]. The $DL_{NO}$ only has been investigated by a small group of researchers, and is still not implemented in clinical routine. As far as we know references values for the single breath $DL_{NO}$ test are not available yet, because in

previous studies [8;10] too few subjects were tested to generate useful reference equations.
Because the $DL_{NO}$ is not influenced by pulmonary capillary blood volume we hypothesized that the $K_{NO}$ will not react to the relative increase in lung blood volume at decreasing $V_A$. If the membrane itself will not change (i.e. will not become thicker) during decreasing $V_A$, the $K_{NO}$ will be independent of $V_A$, and can be considered as the true membrane diffusing coefficient.
This study was performed to generate clinical useful reference values for the single breath $DL_{NO}$, and to investigate the dependence of the $DL_{NO}$ and the $K_{NO}$ on the alveolar volume.

## Methods

### *$DL_{NO}$ measurement*

A standard $DL_{CO}$ apparatus (MasterLab Pro, Erich Jaeger GmbH, Wurzburg, Germany) was changed drastically. An electronic switchboard was added which followed the processing of the apparatus, thereby controlling the addition of a small amount of NO in $N_2$ (750 ppm, Hoekloos Medical, the Netherlands) to the standard test gas containing CO 0.25%, helium 9% with balance air. The NO was added shortly before the measurement, the concentration in the inhalation mixture was 7-9 ppm. In the exhaled air a small amount of the sample was lead via a side arm to an NO-chemoluminescence analyzer with rapid reaction time (CLD 77 AM, Eco Physics, Zurich, Switzerland). The analyzer was calibrated with 5 ppm NO in nitrogen and NO-free air. The output from the NO-analyzer was transferred to a personal computer and later offline combined with the data from the $DL_{CO}$-apparatus. The single breath procedure was performed according to ATS recommendations [11] with an effective breath-holding period of 10 seconds, discard volume 750 ml, sample volume 750 ml. The $DL_{NO}$ and $DL_{CO}$ measurements were performed simultaneously. A minimum of two measurements were performed, in which a change of 10% or less of the $DL_{CO}$ and the $V_A$ was acceptable. The $DL_{NO}$ is calculated according the equation: $DL_{NO}=V_A/t*\ln(Fi_{NO}/Fa_{NO})*1/P_b-P_{H2O}$ ($V_A$ is alveolar volume BTPS corrected, t is effective breath holding time, $Fi_{NO}$ is inspiratory alveolar NO concentration, $Fa_{NO}$ is expired alveolar

NO concentration, Pb is atmospheric pressure and $P_{H2O}$ is the vapour pressure of water at 37 ºC, which is 6.3 kPa) [8].

### *Reference equations study*

The combined single breath $DL_{NO}$ and $DL_{CO}$ was measured in healthy volunteers who were recruited from local hospital personnel. Anemia was an exclusion criterion. The $DL_{CO}$ was not corrected for hemoglobin concentration, because in subjects with normal hemoglobin levels the correction only leads to very small changes [2]. In all subjects whole body plethysmography was performed (6200 Autobox DL, SensorMedics Cooperation, Yorba Linda, California, USA) with the determination of static and dynamic lung volumes. Inclusion criteria consisted of never smokers without pulmonary complaints and without medication. Exclusion criteria consisted of serious chronic illnesses, a history of asthma or other pulmonary diseases and diabetes mellitus. This study had been approved by our local ethics committee and informed consent was obtained from each participant.

### *$V_A$ dependency study*

A subset of the subjects included in the reference equation study performed combined $DL_{NO}/DL_{CO}$ measurements when inspiring to 50, 70% and 100% of TLC. The $DL_{CO}$, $DL_{NO}$, $K_{CO}$, $K_{NO}$ and $V_A$ were expressed as fraction of the value at TLC [3].

### *Statistics*

Linear regression analysis (SPSS for Windows version 11.0, SPSS Inc, Chicago, USA) was used to calculate the relation between the $DL_{CO}$, the $DL_{NO}$, the $K_{CO}$ and the $K_{NO}$ versus $V_A$. For generation of the reference equations a correlation matrix was produced in search of factors dependent of the $DL_{NO}$, $K_{NO}$ and $V_A$. Linear regression analysis was used calculate the regression equations. Data are presented as means ± standard deviation (SD), significance was defined as $p<0.01$.

## Results

### *Reference equations study*

A total of 124 healthy subjects were enrolled, 59 females and 65 males. All had normal flow-volume loops, and normal values for $DL_{CO}$ [12] and TLC [13]. The characteristics of the study population are shown in Table 1: we observed no statistically significant differences in the pulmonary function parameters between the male and female subjects, when expressed as percent predicted values. The mean values for the diffusion parameters are displayed in Table 2. The mean $DL_{CO}$ was 100.1% of the predicted value in women and 104.1% of the predicted value in men. The $K_{CO}$ as percentage of predicted differed slightly but significantly between males and females. We constructed reference equations for all diffusion parameters (Table 3). The references equations we constructed for the $DL_{CO}$ were comparable with the ERS references equations [12], for men as well as women (Table 3). The ERS reference equations for the $K_{CO}$ are defined as the $DL_{CO}$-reference divided by the TLC-reference. The question remains whether this is a valid method to calculate reference equations [14], nonetheless we used the ERS equations to evaluate the $K_{CO}$ values we found, although our equations are hard to compare with the ERS-equations. We found that the $DL_{NO}$ is about 4.5 times higher than the $DL_{CO}$, in concordance with the findings of others [8]. In this study the $DL_{NO}$ strongly depends on height, for female as well as male subjects [9].

### *$V_A$ dependency study*

In the $V_A$ dependency study 21 subjects were included, 15 female and 6 male, mean age 39 years. All subjects had normal $DL_{CO}$ and $V_A$ [12]. We observed a decrease of the $DL_{NO}$ and to a lesser extent the $DL_{CO}$ with a lowering of $V_A$. A strong negative relation was seen between the $K_{CO}$ and the $V_A$ (Figure 1): the $K_{CO}$ increases when $V_A$ decreases. The $K_{NO}$ only slightly increases when $V_A$ decreases (Figure 2). Because of the different response of the $DL_{NO}$ and $DL_{CO}$ on inspiratory level, the $DL_{NO}/DL_{CO}$ ratio decreases when the $V_A$ diminishes (Figure 3). We constructed equations for all diffusion parameters as percentage of the value at maximal TLC (Table 4).

| | Females (59) | Males (65) |
|---|---|---|
| Age (years) | 37.9 ± 11.8 [ns] | 40.1 ± 12.6 |
| Height (m) | 1.69 ± 0.07 [*] | 1.82 ± 0.07 |
| Weight (kg) | 77.0 ± 10.1 [*] | 80.4 ± 10.2 |
| TLC (L) | 5.9 ± 0.9 [*] | 7.9 ± 1.0 |
| $FEV_1$/FVC | 0.81 ± 0.06 [ns] | 0.81 ± 0.05 |
| $FEV_1$ %pred | 107.3 ± 14.0 [ns] | 112.7 ± 15.7 |
| FVC %pred | 114.5 ± 16.4 [ns] | 113.1 ± 14.4 |
| TLC %pred | 110.0 ± 12.4 [ns] | 105.7 ± 12.4 |
| $V_A$ (L) | 5.5 ± 0.9 [*] | 7.4 ± 1.0 |
| $V_A$/TLC | 0.94 ± 0.06 [ns] | 0.93 ± 0.06 |

***Table 1.*** *Characteristics of the study population. Depicted are means ± standard deviation (SD) (* $p<0.01$, ns not significant).*

| | Females (59) | Males (65) |
|---|---|---|
| $V_A$ (L) | 5.5 ± 0.9 [*] | 7.4 ± 1.0 |
| $DL_{CO}$ (mmol/min/kPa) | 9.2 ± 1.6 [*] | 12.0 ± 2.2 |
| $DL_{CO}$ %pred [12] | 100.1 ± 14.9 [ns] | 104.1 ± 15.1 |
| $DL_{NO}$ (mmol/min/kPa) | 39.1 ± 6.3 [*] | 54.3 ± 8.7 |
| $DL_{NO}/DL_{CO}$ | 4.3 ± 0.4 [*] | 4.6 ± 0.5 |
| $K_{CO}$ (mmol/min/kPa/L) | 1.7 ± 0.2 [ns] | 1.6 ± 0.3 |
| $K_{CO}$ %pred [12] | 98.2 ± 11.7 [*] | 105.8 ± 14.1 |
| $K_{NO}$ (mmol/min/kPa/L) | 7.1 ± 0.6 [ns] | 7.4 ± 0.9 |

***Table 2.*** *Summary of diffusion parameters. Depicted are means ± standard deviation (SD)([*] $p<0.01$, [ns] not significant).*

| sex | Regression equation | RSD |
|---|---|---|
| $DL_{NO}$, females | 53.47*H-0.077*A-48.28 | 5.22 |
| $DL_{NO}$, males | 59.84*H-0.25*A-44.20 | 6.39 |
| $DL_{CO}$, females | 10.51*H-0.030*A-7.43 | 1.37 |
| $DL_{CO}$, males | 12.02*H-0.074*A-6.88 | 1.74 |
| $K_{CO}$, females | -0.88*H-0.0083*A+3.48 | 0.20 |
| $K_{CO}$, males | -0.14*H-0.012*A+2.37 | 0.22 |
| $K_{NO}$, females | -2.03*H-0.025*A+11.52 | 0.48 |
| $K_{NO}$, males | -0.15*H-0.045*A+9.47 | 0.65 |
| *$DL_{CO}$, females, ERS* | *8.18*H-0.049*A-2.74* | *1.17* |
| *$DL_{CO}$, males, ERS* | *11.11*H –0.066*A-6.03* | *1.41* |

***Table 3.*** *Regression equations for the diffusion parameters in female and male subjects. For comparison the ERS [12] reference equations are displayed in the two lower rows. H is height in m, A is age in years, RSD is residual standard deviation.*

| Parameter | Equation |
|---|---|
| $DL_{CO}$ (mmol/min/kPa) | $DL_{CO}/DL_{CO,TLC}=0.42+0.58*V_A/V_{A,TLC}$ |
| $DL_{NO}$ (mmol/min/kPa) | $DL_{NO}/DL_{NO,TLC}=0.13+0.88*V_A/V_{A,TLC}$ |
| $K_{CO}$ (mmol/min/kPa/L) | $K_{CO}/K_{CO,TLC}=1.72-0.73*V_A/V_{A,TLC}$ |
| $K_{NO}$ (mmol/min/kPa/L) | $K_{NO}/K_{NO,TLC}=1.20-0.21*V_A/V_{A,TLC}$ |
| $DL_{NO}/DL_{CO}$ | $DL_{NO}/DL_{CO} =2.50+2.06* V_A/V_{A,TLC}$ |

***Table 4.*** *Equations for the relation between diffusion parameters and alveolar volume ($V_A$).*

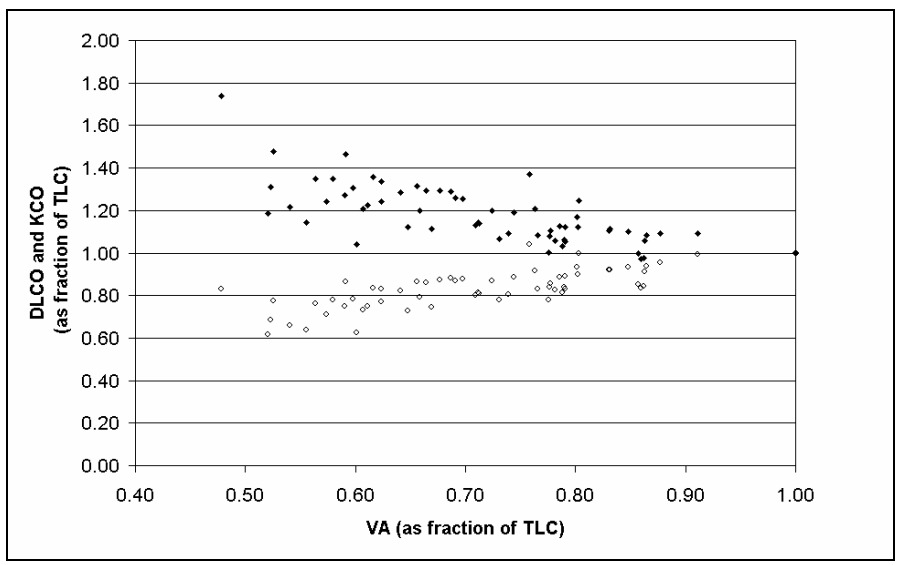


***Figure 1.*** *$DL_{CO}$ (open circles) as fraction of the value at TLC, $K_{CO}$ (closed diamonds) as fraction of the value at TLC, versus $V_A$ depicted as the fraction of the value at TLC.*

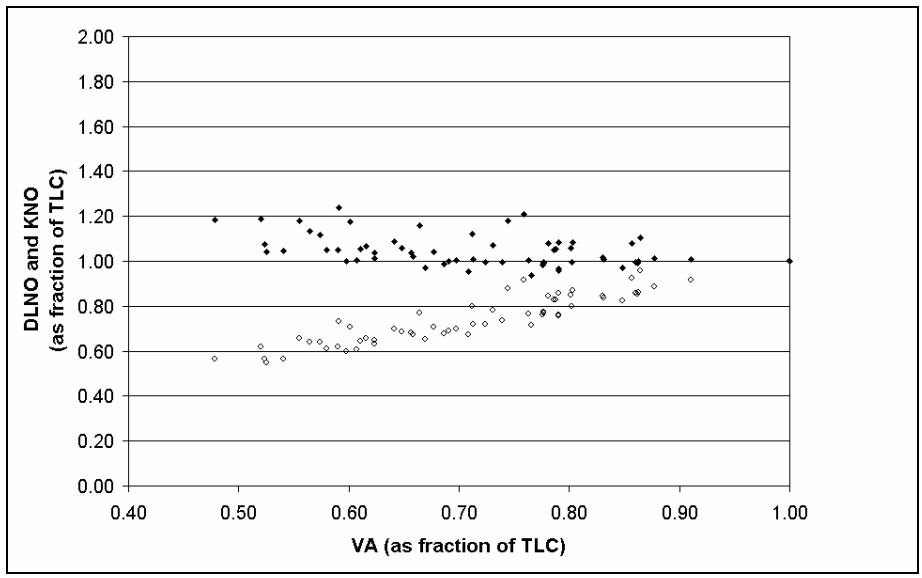


***Figure 2.*** *$DL_{NO}$ (open circles) as fraction of the value at TLC, $K_{NO}$ (closed diamonds) as fraction of the value at TLC, versus $V_A$ depicted as the fraction of the value at TLC.*

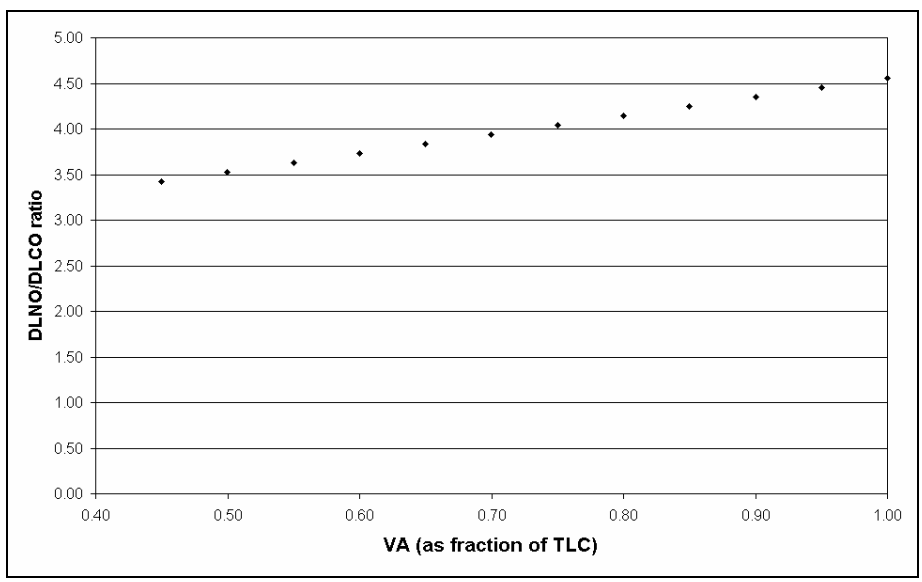


***Figure 3.*** *$DL_{NO}/DL_{CO}$ ratio versus $V_A$ depicted as the fraction of the value at TLC.*

## Discussion

The use of the $DL_{NO}$ for assessing the function of the alveolocapillary membrane is supported by several studies, that pointed out that the $DL_{NO}$ constitutes the true alveolar membrane diffusing capacity [9;15;16]. The $DL_{CO}$ is stronger influenced by the binding of CO to hemoglobin [1;17], thus it is a factor that is considerably influenced by the pulmonary microcirculation. It is therefore remarkable that many clinicians consider the $DL_{CO}$ as a function of the alveolocapillary membrane. The $DL_{NO}$ has more right to claim that title, because the $DL_{NO}$ is not or less affected by capillary blood volume, and therefore it is more informative of the function of the alveolocapillary membrane than the $DL_{CO}$.

We generated reference equations for the $DL_{NO}$ and $K_{NO}$ that can be used in clinical practice. We have tested a relative young population, mainly due to the fact that we accrued subjects working in the hospital, which is overpopulated with young personnel. In our opinion the relative young age of the study sample does not lead to major bias, because the reference equations we calculated for the $DL_{CO}$ matches with the standard ERS reference equations [7].

In the second part of this study we observed an increase in $K_{CO}$ when $V_A$ decreases in 21 healthy subjects, which is in concordance with the results of Johnson [3]. Furthermore we studied the relation between the $DL_{NO}$ and the $V_A$, which proved to be very strong, more or less to the same extent as Borland et al. [8]. The slope between $DL_{CO}$ and $V_A$ is lower than the slope between $DL_{NO}$ and $V_A$ because the relative decrease in blood volume when $V_A$ increases leads to the lowering of the $DL_{CO}$. The fact that the $DL_{NO}$ strongly depends on the height (and thus on $V_A$) raises the question what the additional value is of the $DL_{NO}$ next to the $DL_{CO}$ and $V_A$. When performing combined single breath $DL_{NO}$ and $DL_{CO}$ measurements, both values can be interpreted as percentage of predicted values. When for example the $DL_{CO}$ is decreased and the $DL_{NO}$ is normal in a subject without restriction and without anemia, this indicates that the pulmonary capillary blood volume is diminished. When both $DL_{CO}$ and $DL_{NO}$ values are decreased, a malfunction of the alveolocapillary membrane is likely.

Instead of the percentage predicted values the $DL_{NO}/DL_{CO}$ ratio can be used: in subjects without restriction a lowering of the ratio indicates a (relative) increase in capillary blood volume. Seen in this way, the $DL_{NO}$ adds information to the $DL_{CO}$ and $V_A$ measurements.

A more difficult question is what the clinical use of the $K_{NO}$ could be. In concordance with the $K_{CO}$, which is the rate constant for CO uptake from alveolar gas [17], the $K_{NO}$ is a time function of removal of NO from alveolar gas. The $K_{NO}$ is four and a half times larger than the $K_{CO}$ because of the fact that the covalesence of NO to hemoglobin is much stronger than for CO. Therefore NO-uptake is not limited by the amount of hemoglobin present in the direct proximity of the alveolar gas. The $K_{CO}$ increases when $V_A$ decreases due to the relative increase of capillary blood volume per unit lung volume. The $K_{NO}$ is not influenced by this phenomenon, and stays unchanged. Therefore the $K_{NO}$, as independent of capillary filling, can be seen as a measure for the function of the alveolocapillary membrane per unit lung volume. In fact, the $K_{NO}$ is not completely independent of $V_A$, as can be seen in Figure 2. The $K_{NO}$ slightly increases with lowering of the $V_A$. This can be caused by two factors. At first it is possible that the NO-uptake is still limited by the binding to hemoglobin, in other words $1/\theta_{NO}$ cannot be neglected. The second more likely explanation is that the matching of ventilation and perfusion changes from TLC-level to a lower inspiratory level. At TLC-level the upper lung zone areas are recruited, with a relative underperfusion [18]. In other words, the upper lung zones are areas with greater dead space than the lower lung zones. Although the $DL_{NO}$ and $K_{NO}$ are less sensitive for capillary blood volume than the $DL_{CO}$ and $K_{CO}$, of course for uptake of NO the presence of capillaries (and thus hemoglobin) is essential. In our opinion this is a likely explanation for the small increase in $K_{NO}$ with decreasing $V_A$.

The strong dependence of the $K_{CO}$ on $V_A$ has led to an ongoing debate whether or not the $K_{CO}$ is useful in restrictive lung diseases [6;19]. When restriction is similar to voluntary lowering of the inspiratory levels the answers would be yes. In our opinion, the truth is more complicated, because in restrictive pulmonary disease vascular abnormalities can play an important role [20]. It will be difficult if not impossible to determine whether the decreased $K_{CO}$ will be due to decreased microvascularity or membrane dysfunction. In subjects with a

restriction and a decreased $K_{CO}$, the $K_{NO}$ (as percentage predicted) can give additional information: when normal, membrane dysfunction seems less probable.

The classical way to measure the subdivisions ($Dm_{CO}$ and Vcap) of the $DL_{CO}$ with the high-low oxygen technique [21] is time-consuming and not available in most pulmonary function laboratories. The single breath combined $DL_{NO}/DL_{CO}$ takes no longer time than the $DL_{CO}$ measurement itself, the equipment is not very complicated, only the necessity of a chemoluminescence NO-analyzer will need a financial investment. Of course further investigations will be needed to explore the $DL_{NO}$ and $K_{NO}$ in subjects with restrictive pulmonary diseases. The $DL_{NO}/DL_{CO}$ ratio is a promising measure, because it is an easy to interpret value, which can differentiate between various causes of a diminished diffusion capacity.
The $DL_{NO}$ resembles the true membrane diffusing capacity, the $K_{NO}$ is a better representative of the function of the alveolocapillary membrane than the $DL_{CO}$ because of the relative insensitivity of the $DL_{NO}$ of capillary blood volume. Therefore the use of the $DL_{NO}$ and $K_{NO}$ next to the $DL_{CO}$ and $K_{CO}$ can have great clinical advantages. Now clinical useful reference equations for the single breath $DL_{NO}$ and $K_{NO}$ are available. The question remains whether the observed independence of the $K_{NO}$ on $V_A$ in healthy subjects can be transferred to diseased subjects.

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## References

1. Hsia CC. Recruitment of lung diffusing capacity: update of concept and application. Chest 2002; 122(5):1774-1783.
2. Stam H, Hrachovina V, Stijnen T et al. Diffusing capacity dependent on lung volume and age in normal subjects. J Appl Physiol 1994; 76(6):2356-2363.
3. Johnson DC. Importance of adjusting carbon monoxide diffusing capacity (DLCO) and carbon monoxide transfer coefficient (KCO) for alveolar volume. Respir Med 2000; 94(1):28-37.
4. Hughes JM, Pride NB. In defence of the carbon monoxide transfer coefficient Kco (TL/VA). Eur Respir J 2001; 17(2):168-174.
5. Frans A, Nemery B, Veriter C et al. Effect of alveolar volume on the interpretation of single breath DLCO. Respir Med 1997; 91(5):263-273.
6. Stam H, Splinter TA, Versprille A. Evaluation of diffusing capacity in patients with a restrictive lung disease. Chest 2000; 117(3):752-757.
7. Chinn DJ, Cotes JE, Flowers R et al. Transfer factor (diffusing capacity) standardized for alveolar volume: validation, reference values and applications of a new linear model to replace KCO (TL/VA). Eur Respir J 1996; 9(6):1269-1277.
8. Borland CD, Higenbottam TW. A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. Eur Respir J 1989; 2(1):56-63.
9. Tamhane RM, Johnson RL, Jr., Hsia CC. Pulmonary membrane diffusing capacity and capillary blood volume measured during exercise from nitric oxide uptake. Chest 2001; 120(6):1850-1856.
10. Guenard H, Varene N, Vaida P. Determination of lung capillary blood volume and membrane diffusing capacity in man by the measurements of NO and CO transfer. Respir Physiol 1987; 70(1):113-120.
11. American Thoracic Society. Single-breath carbon monoxide diffusing capacity (transfer factor). Recommendations for a standard technique--1995 update. Am J Respir Crit Care Med 1995; 152(6 Pt 1):2185-2198.
12. Cotes JE, Chinn DJ, Quanjer PH et al. Standardization of the measurement of transfer factor (diffusing capacity). Report Working Party Standardization of Lung Function Tests, European Community

for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:41-52.
13. Quanjer PH, Tammeling GJ, Cotes JE et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:5-40.
14. van der Lee I, Zanen P, van den Bosch JM et al. Pattern of diffusion disturbance related to clinical diagnosis: The KCO has no diagnostic value next to the DLCO. Respir Med 2005.
15. Phansalkar AR, Hanson CM, Shakir AR et al. Nitric oxide diffusing capacity and alveolar microvascular recruitment in sarcoidosis. Am J Respir Crit Care Med 2004; 169(9):1034-1040.
16. Harris RS, Hadian M, Hess DR et al. Pulmonary artery occlusion increases the ratio of diffusing capacity for nitric oxide to carbon monoxide in prone sheep. Chest 2004; 126(2):559-565.
17. Hughes JM. The single breath transfer factor (Tl,co) and the transfer coefficient (Kco): a window onto the pulmonary microcirculation. Clin Physiol Funct Imaging 2003; 23(2):63-71.
18. West JB, Wagner PD. Ventilation-perfusion relationships. In: Crystal RG, editor. The lung: scientific foundations. Philadelphia: Lippincott-Raven Publishers, 1997: 1693-1710.
19. Hughes JM, Pride NB. Carbon monoxide transfer coefficient (transfer factor/alveolar volume) in females versus males. Eur Respir J 2003; 22(1):186-187.
20. Renzoni EA, Walsh DA, Salmon M et al. Interstitial vascularity in fibrosing alveolitis. Am J Respir Crit Care Med 2003; 167(3):438-443.
21. Cotes JE. Measurement of Transfer Factor (Diffusing Capacity) and its Subdivisions. Lung Function. Assessment and application in medicine. Oxford: Blackwell Scientific Publications, 1993: 299-324.

# Chapter 6

# The effect of red cell transfusion on nitric oxide diffusing capacity

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## Abstract

The diffusion capacity of the lung for nitric oxide ($DL_{NO}$) is supposed to reflect the properties of the alveolocapillary membrane better than the diffusion capacity of the lung for carbon monoxide ($DL_{CO}$), due to a much stronger binding of NO to haemoglobin (Hb).
The aim of this study was to investigate the effect of Hb concentration on the $DL_{NO}$.
The $DL_{NO}$ and $DL_{CO}$ (single-breath method) were measured in 10 anaemic patients before and shortly after red cell transfusion.
The mean increase in Hb concentration was 1.6 mmol/l. Whereas $DL_{CO}$ increased as predicted by the reference equations, the $DL_{NO}$ did not change: mean $DL_{CO}$ rose from 4.5 to 5.5 mmol/min/kPa (increase of 122%), mean $DL_{CO}$ corrected for Hb rose from 6.3 to 6.4 mmol/min/kPa (103%) and mean $DL_{NO}$ rose from 25.2 to 25.9 mmol/min/kPa (103%).
We concluded that the $DL_{NO}$ is not influenced by Hb concentration.

## Introduction

The measurement of the diffusing capacity of the lungs (DL), also known as transfer factor, is an important tool in the evaluation of patients with pulmonary diseases. Carbon monoxide (CO) is frequently used as test gas. The model of Roughton and Forster [1] is the model mostly used in elucidating the measurement of the DL for CO ($DL_{CO}$) (Equation 1).

$$\frac{1}{DL_{CO}} = \frac{1}{Dm_{CO}} + \frac{1}{\theta_{CO} * [Hb] * Vcap}$$

***Equation 1.*** *Model developed by Roughton and Forster 1 to interpret the determents of the $DL_{CO}$. $Dm_{CO}$ = Diffusing capacity of the alveolar membrane; $\theta_{CO}$ = reaction rate of CO to (oxy)haemoglobin at haemoglobin concentration of 9.0 mmol/l; [Hb] = haemoglobin concentration as fraction of normal; Vcap = pulmonary capillary blood volume.*

In this model, the $1/DL_{CO}$ resistance is determined by three factors: first, the resistance of the alveolocapillary membrane (Dm), which is purely diffusion dependent; second, the reaction rate of CO to (oxy)haemoglobin ($\theta_{CO}$), and third, the capillary blood volume (Vcap). $\theta_{CO}$ is dependent on oxygen concentration, a phenomenon that is used in the determination of the Dm and Vcap with low and high oxygen levels [1]. Furthermore, $\theta_{CO}$ is standardized to a haemoglobin (Hb) concentration of 9.0 mmol/l according to ERS recommendations [2].
Due to rate-inhibiting effects of the binding to (oxy)haemoglobin, changes in the integrity of the Dm are only partially expressed in the $DL_{CO}$. Therefore, searches for other test gases have been performed. In the past 20 years, some studies used nitric oxide (NO) as test gas for the measurement of the DL [3;4]. NO has an advantage over CO because it binds approximately 280 times stronger to Hb, leading to a very high value of $\theta_{NO}$. It can be derived from Equation 1 that the division '$1/\theta_{NO}$ * Vcap' becomes smaller, meaning the $DL_{NO}$ reflects the Dm resistance much better than the $DL_{CO}$ [3]. Some authors state that the

covalence between NO and Hb is so strong that the intra-erythrocyte resistance can be neglected, i.e. $\theta_{NO}$ is infinite [5]. If this is true, the $DL_{NO}$ equals the $Dm_{NO}$. This concept has never been verified in humans, as far as we know.
We studied the combined single-breath $DL_{NO}$ and $DL_{CO}$ in anaemic patients before and shortly after red cell transfusion to determine the independency of $DL_{NO}$ on Hb concentration.

## Methods

### *Patients*

The patients were recruited from the Department of Haematology, St. Antonius Hospital, Nieuwegein, the Netherlands. All patients received leukoreduced red cell transfusions for treatment of anaemia, combined with saline. The patients had to be physically capable of performing pulmonary function testing. Patients with a history of heart failure, patients with pulmonary diseases and current smokers were excluded. All patients received diuretics intravenously, which is standard procedure at the Haematology Department of our hospital, in order to prevent possible volume overloading. The pretransfusion measurement of the combined $DL_{NO}/DL_{CO}$ and Hb concentration was performed in the morning, directly prior to the red cell transfusion; the posttransfusion measurement was performed after the transfusion, as soon as possible. Shortly before and 1 h after the transfusion, the Hb concentration was measured.

### *Combined $DL_{NO}$ and $DL_{CO}$ measurements*

For the measurement of the $DL_{NO}$, some adaptations had to be made to our $DL_{CO}$ apparatus, a MasterLab Pro (Erich Jaeger GmbH, Wurzburg, Germany). For the determination of the $DL_{CO}$, a mixture of CO 0.25%, helium (He) 9.17% with balance air (Hoekloos Medical, the Netherlands) was used. A small amount of NO in $N_2$ (750 ppm; Hoekloos Medical) was submitted to the inspiratory bag, to reach an NO concentration of maximum 10 ppm in the mixture of CO, He and air. The addition of the NO to the gas mixture took place simultaneously with the filling with the CO/He/air mixture. Directly after the inspiratory bag was filled, the subject (seated in a chair)

slowly exhaled to residual volume; then, a fast inspiration to total lung capacity from the inspiratory bag was followed by an effective breath-holding period of 10 s. The effective breath-holding period is defined as the actual occlusion time measured after one third of the inspiration time up to the middle of the sample volume, see method of Jones and Meade [6]. The first 750 ml of the expiration volume was discarded, the next 750 ml was used as sample volume, for the $DL_{NO}$ as well as the $DL_{CO}$ measurement. The MasterLab Pro sampled the He and CO concentration in the expired air, the pneumotach measured the flow and volume. For the measurement of the NO levels in the inspiratory bag and in the sample volume, a chemoluminescence analyzer (type CLD 77 AM, Eco Physics, Zurich, Switzerland) was used. The system specifications are lower detection limit 0.02 to 0.05 ppb, upper detection limit 10 ppm, reaction time 0.1 s, with continuous online measurement of NO with a sampling flow of 325 ml/min. The NO analyzer was calibrated via a two-point calibration on a weekly basis with 5 ppm NO in $N_2$ and NO-free air. All connections between the NO analyzer and the inspiratory and expiratory bags were made from Teflon, which does not interact with NO. All valves needed to switch between sampling of the inspiratory bag and expiratory bag were made of stainless steel and were controlled by an electronical switchboard that followed the processing of the MasterLab. The data from the NO analyzer were visualized on the monitor of a personal computer on a real-time basis using JSCOPE software (Erich Jaeger GmbH). The $DL_{CO}$ capacity was calculated by LABManager software version 4.53a (Erich Jaeger GmbH). The $DL_{NO}$ capacity is calculated in the same manner, according to Equation 2.

$$DL_{NO} = \ln \frac{FA_{NO}(0)}{FA_{NO}(t)} * \frac{V_A}{t(P_b - P_{H_2O})}$$

***Equation 2.*** *$V_A$ = effective alveolar volume; t = effective breath-holding time; $FA_{NO}(0)$ = alveolar [NO] at t = 0; $FA_{NO}(t)$ = alveolar [NO] at t = t; $P_b$ = atmospheric pressure; $P_{H2O}$ = vapour pressure of water at 37°C, which is 6.3 mm Hg.*

We did not account for the NO backpressure, because our exhaled alveolar NO levels were always higher than 100 ppb, so a very small influence of endogenous alveolar NO was to be expected. Recent investigation points out an alveolar NO level of approximately 2 ppb in healthy subjects [7]. At the very fast exhalation flows we used, NO production by the conducting airways can be neglected [7]. Alveolar volume ($V_A$) was calculated by LABManager software according to ERS recommendations [2], with anatomic deadspace calculated according to Cotes [2.2 × weight (kg)]. The $DL_{NO}$ was corrected to body temperature pressure saturated conditions. A minimum of two measurements were done, in which variability of 10% or less for the $DL_{CO}$ was acceptable. The addition of NO to the test gas gives a dilution of the gasses in the inspiratory bag (CO, He and air). Because of the high concentration of NO in $N_2$, the dilution is approximately 1:70; therefore, great disturbances did not take place.

### *Hemoglobin Correction Formula*

The $DL_{CO}c$ is defined as the $DL_{CO}$ corrected to standardized Hb concentration of 9.0 mmol/l using ERS recommendations [2], using the formula $DL_{CO}c = DL_{CO} \bullet (10.22 + Hb*1.61)/(2.74*Hb)$. This formula for Hb correction is recommended by ERS. It is derived from the original work of Roughton and Forster [1] assuming a Dm/Vcap ratio of 0.7 and applied to oxygen pressure of 110 mm Hg [8].

### *Statistics*

We assumed that there was no effect of Hb on $DL_{NO}$ measurements. Therefore, the pre- and posttransfusion $DL_{NO}$ values may not differ from the intra-individual variability. We assumed an intra-individual variability of $DL_{NO}$ of 10%, which seems acceptable considering the interday variability of 8.1% in 7 patients measured by Perillo et al. [9]. Concerning the $DL_{CO}$ measurement, 10% variability is considered to be acceptable according to ERS recommendations [2]. We calculated the 95% confidence interval (CI) of the mean proportional increase in $DL_{NO}$, which should include 100%, meaning no change. The pre- and posttransfusion $DL_{CO}c$ should not be significantly different in pairwise comparison either. All statistics were performed with SPSS for Windows version 11.0 (SPSS Inc., Chicago, USA).

## Results

From November 2003 until April 2004, 10 patients were included in this study. Demographics, diagnosis and pre- and posttransfusion values are expressed in Table 1. A mean increase in Hb of 1.6 mmol/l was established. The mean time between the end of the infusion and the $DL_{NO}/DL_{CO}$ measurement was 2 h (range 1-4 h). $V_A$ did not significantly change after the red cell transfusions. The mean increase in $DL_{CO}$ was 122% (95% CI 107-138%), the mean increase in $DL_{CO}c$ was 103% (95% CI 97-107%) and the mean increase in $DL_{NO}$ was 103% (95% CI 93-113%; Table 2). The mean $DL_{NO}/DL_{CO}$ ratio decreased from 5.7 to 4.8. The mean $DL_{NO}/DL_{CO}c$ ratio remains unaltered 4.1. Three patients had a larger deviation than the assumed normal variability of 10% in the posttransfusion $DL_{NO}$ measurements (Table 1), in which patients 1 and 6 had higher posttransfusion $DL_{NO}$ values and patient 3 had a value lower than 10%. Values for $K_{CO}$ ($DL_{CO}/V_A$), $K_{NO}$ ($DL_{NO}/V_A$) and $K_{CO}c$ ($K_{CO}$ corrected to standard haemoglobin of 9.0 mmol/l) are expressed in Table 2. The mean increase in $K_{CO}$ was 127% (95% CI 113-142%), the mean increase in $K_{CO}c$ was 107% (95% CI 101-113%) and the mean increase in $K_{NO}$ was 107% (95% CI 99-115%; Table 2).

| *Diagnosis* | *Sex* | *Height* | *Weight* | *Age* | $V_A$ | | *Hb* | | $DL_{CO}$ | | $DL_{NO}$ | | $DL_{COc}$ | | $DL_{NO}/DL_{CO}$ | | $DL_{NO}/DL_{COc}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | pre | post | pre | post | pre | post | pre | post | pre | post | pre | post | pre | post |
| Iron deficiency anaemia | F | 1.63 | 60 | 66 | 4.7 | 4.5 | 2.8 | 6.3 | 3.9 | 6.7 | 25.5 | 29.8 | 7.4 | 7.8 | 7.4 | 6.5 | 3.4 | 3.8 |
| AML | M | 1.85 | 92 | 72 | 5.7 | 5.2 | 5.1 | 12.0 | 4.2 | 4.6 | 25.4 | 24.9 | 5.5 | 5.6 | 5.5 | 6.0 | 4.6 | 4.5 |
| Iron deficiency anaemia | M | 1.70 | 62 | 82 | 4.9 | 4.6 | 4.8 | 6.4 | 4.3 | 5.0 | 22.3 | 18.0 | 5.8 | 5.8 | 5.8 | 5.2 | 3.8 | 3.1 |
| Iron deficiency anaemia | M | 1.80 | 79 | 82 | 4.5 | 4.6 | 5.3 | 6.3 | 6.0 | 6.3 | 18.7 | 18.4 | 3.5 | 3.6 | 3.5 | 6.9 | 5.3 | 5.2 |
| Myelofibrosis | M | 1.75 | 81 | 79 | 6.9 | 6.8 | 3.8 | 12.2 | 5.7 | 6.7 | 30.2 | 32.7 | 8.9 | 8.1 | 8.9 | 5.3 | 3.4 | 4.0 |
| Iron deficiency anaemia | M | 1.70 | 80 | 76 | 4.8 | 5.1 | 4.0 | 11.8 | 3.7 | 5.5 | 21.8 | 28.9 | 5.7 | 6.9 | 5.7 | 5.9 | 3.8 | 4.2 |
| Thalassaemia | M | 1.68 | 55 | 23 | 4.0 | 3.7 | 6.0 | 7.3 | 5.7 | 6.1 | 27.4 | 26.3 | 6.9 | 6.6 | 6.9 | 4.8 | 4.0 | 4.0 |
| Thalassaemia | F | 1.63 | 69 | 26 | 3.5 | 3.2 | 5.3 | 12.3 | 5.9 | 7.0 | 29.6 | 28.0 | 7.6 | 8.4 | 7.6 | 5.0 | 3.9 | 3.3 |
| MDS | F | 1.76 | 76 | 79 | 3.9 | 3.7 | 5.5 | 6.9 | 3.4 | 3.7 | 19.8 | 20.3 | 4.3 | 4.2 | 4.3 | 5.8 | 4.6 | 4.8 |
| MDS | M | 1.74 | 87 | 61 | 5.5 | 5.3 | 6.0 | 7.3 | 5.8 | 6.7 | 31.2 | 32.0 | 7.0 | 7.3 | 7.0 | 5.4 | 4.4 | 4.4 |
| mean | | | | | 4.8 | 4.7 | 4.9 | 6.5 | 4.5 | 5.5 | 25.2 | 25.9 | 6.3 | 6.4 | 6.3 | 5.7 | 4.1 | 4.1 |

***Table 1.*** *Demographics and results of 10 patients before (pre) and after (post) red cell transfusion $V_A$ = Alveolar volume (l); Hb = haemoglobin concentration (mmol/l); $DL_{CO}$ = diffusion capacity for carbon monoxide (mmol/min/kPa); $DL_{NO}$ = diffusion capacity for nitric oxide (mmol/min/kPa); $DL_{CO}c$ = $DL_{CO}$ corrected to standardized [Hb] of 9.0 mmol/l; MDS = myelodysplastic syndrome; AML = acute myeloid leukaemia.*

| | Pre | Post | Change % | 95% CI |
|---|---|---|---|---|
| Hb | 4.9 | 6.5 | 138 | 114–163 |
| $V_A$ | 4.8 | 4.7 | 96 | 93–100 |
| $DL_{CO}$ | 4.5 | 5.5 | 122 | 107–138 |
| $DL_{CO}c$ | 6.3 | 6.4 | 103 | 97–107 |
| $DL_{NO}$ | 25.2 | 25.9 | 103 | 93–113 |
| $DL_{CO}/V_A$ | 1.0 | 1.2 | 127 | 113–142 |
| $DL_{CO}c/V_A$ | 1.3 | 1.4 | 107 | 101–113 |
| $DL_{NO}/V_A$ | 5.4 | 5.7 | 107 | 99–115 |

***Table 2.*** *Results depicted as mean of 10 patients before (pre) and after (post) red cell transfusion Hb = Haemoglobin concentration (mmol/l); $V_A$ = alveolar volume (litre); $DL_{CO}$ = diffusion capacity for carbon monoxide (mmol/min/kPa); $DL_{NO}$ = diffusion capacity for nitric oxide (mmol/min/kPa); $DL_{CO}c$ = $DL_{CO}$ corrected to standardized [Hb] of 9.0 mmol/l.*

## Discussion

We found that $DL_{NO}$ is independent of haemoglobin concentration. That means $\theta_{NO}$ can be considered as infinite; consequently, $DL_{NO}$ equals $Dm_{NO}$, meaning that $DL_{NO}$ represents the diffusion through the Dm only and is not dependent on the pulmonary capillary blood volume.

We used somewhat lower inspiratory NO levels than other investigators. The reason for the higher NO levels used by others are mostly technical: earlier NO chemoluminescence analyzers have a higher lower detection limit than our NO analyzer [3]; others have used NO electrochemical cells, which are relative insensitive and have slow response time [10]. Earlier investigations in rabbits showed that the $DL_{NO}$ is independent of inspiratory NO concentration [11]. A possible flaw in our methodology is the neglect of the NO backpressure, which can lead to a maximum of 2% underestimation of the $DL_{NO}$.

We used a longer breath-holding time than other investigators. The reason for this is that our NO analyzer is capable of measuring very low levels of NO, whereas the apparatus of Borland [3] was less sensitive. The obvious advantage is that the 10 s of breath holding is the same as the recommended 10 s in the $DL_{CO}$. A shorter breath-holding time does lead to lower values of DL in patients with obstructive pulmonary diseases, due to inadequate mixing of gas in the

lungs [12]. In normal subjects, DL should be the same in 3- and 10-s breath-holding time.

The $DL_{NO}/DL_{CO}c$ ratio of 4.1 is equivalent to the mean ratio of 4.3 found by Borland and Higenbottam [3] and somewhat lower than the ratio of 5.2 found by Guenard et al. [4], both determined by single-breath method in healthy subjects. Tamhane et al. [5] found a ratio of 4.1 at rest with a steady-state method. An important fact is that our patients are not healthy subjects: all patients had received multiple red cell transfusion in the recent past and most patients with haematological disorders had received chemotherapy in the past, which can lead to diffusion disturbances.
Clark et al. [13] measured $DL_{CO}$ in 9 patients with haematological disorders before and shortly after blood transfusion. Using the same formula for correction of anaemia as we used, the $DL_{CO}c$ stayed unchanged. We found an increase of 8.0% in $DL_{CO}$ per increase of 1 g/dl Hb concentration, which is comparable with the value of 7.2% found by Dujic et al. [14]. We observed low $DL_{CO}$ values. Most of our included subjects are transfusion dependent, as are the 62 patients measured by Carnelli et al. [15], who found comparable $DL_{CO}$ values. An earlier publication by Moinard and Guenard [16], who measured $DL_{CO}$ and $DL_{NO}$ in patients with chronic renal failure, mentioned a significant decrease in $Dm_{CO}$ (mean 76% of predicted) in 15 patients with chronic renal failure treated with haemodialysis, and low Vcap values in 3 patients, with values for the $DL_{CO}$ and $DL_{NO}$ comparable with our values.

The combined measurement of $DL_{CO}$ and $DL_{NO}$ adds a new tool to the pulmonary function laboratory. Because $DL_{NO}$ equals $Dm_{NO}$, the properties of the Dm are better reflected by $DL_{NO}$ than by $DL_{CO}$. Therefore, a simultaneous measurement of $DL_{NO}$ and $DL_{CO}$ is a fast way of interpreting diffusing defects. The combined $DL_{NO}/DL_{CO}$ technique has an additional value next to the high/low oxygen technique used to determine the subdivisions of DL. The ratio of $DL_{NO}/DL_{CO}$ is an alternative way of expressing diffusion abnormalities, providing that good reference values are available.

## References

1. Roughton FJ, Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J Appl Physiol 1957; 11(2):290-302.
2. Cotes JE, Chinn DJ, Quanjer PH et al. Standardization of the measurement of transfer factor (diffusing capacity). Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:41-52.
3. Borland CD, Higenbottam TW. A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. Eur Respir J 1989; 2(1):56-63.
4. Guenard H, Varene N, Vaida P. Determination of lung capillary blood volume and membrane diffusing capacity in man by the measurements of NO and CO transfer. Respir Physiol 1987; 70(1):113-120.
5. Tamhane RM, Johnson RL, Jr., Hsia CC. Pulmonary membrane diffusing capacity and capillary blood volume measured during exercise from nitric oxide uptake. Chest 2001; 120(6):1850-1856.
6. Jones RS, Meade F. A theoretical and experimental analysis of anomalies in the estimation of pulmonary diffusing capacity by the single breath method. Q J Exp Physiol Cogn Med Sci 1961; 46:131-143.
7. Pietropaoli AP, Perillo IB, Torres A et al. Simultaneous measurement of nitric oxide production by conducting and alveolar airways of humans. J Appl Physiol 1999; 87(4):1532-1542.
8. Cotes JE. Measurement of Transfer Factor (Diffusing Capacity) and its Subdivisions. Lung Function. Assessment and application in medicine. Oxford: Blackwell Scientific Publications, 1993: 299-324.
9. Perillo IB, Hyde RW, Olszowka AJ et al. Chemiluminescent measurements of nitric oxide pulmonary diffusing capacity and alveolar production in humans. J Appl Physiol 2001; 91(5):1931-1940.
10. Zavorsky GS, Quiron KB, Massarelli PS et al. The relationship between single-breath diffusion capacity of the lung for nitric oxide

and carbon monoxide during various exercise intensities. Chest 2004; 125(3):1019-1027.
11. Heller H, Schuster KD. Single-breath diffusing capacity of NO independent of inspiratory NO concentration in rabbits. Am J Physiol 1997; 273(6 Pt 2):R2055-R2058.
12. Graham BL, Mink JT, Cotton DJ. Effect of breath-hold time on DLCO(SB) in patients with airway obstruction. J Appl Physiol 1985; 58(4):1319-1325.
13. Clark EH, Woods RL, Hughes JM. Effect of blood transfusion on the carbon monoxide transfer factor of the lung in man. Clin Sci Mol Med 1978; 54(6):627-631.
14. Dujic Z, Tocilj J, Ljutic D et al. Effects of hemodialysis and anemia on pulmonary diffusing capacity, membrane diffusing capacity and capillary blood volume in uremic patients. Respiration 1991; 58(5-6):277-281.
15. Carnelli V, D'Angelo E, Pecchiari M et al. Pulmonary dysfunction in transfusion-dependent patients with thalassemia major. Am J Respir Crit Care Med 2003; 168(2):180-184.
16. Moinard J, Guenard H. Membrane diffusion of the lungs in patients with chronic renal failure. Eur Respir J 1993; 6(2):225-230.

# Chapter 7

# Diffusing capacity for nitric oxide and carbon monoxide in patients with diffuse parenchymal lung disease and pulmonary arterial hypertension

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## Abstract

The passage of carbon monoxide (CO) through the alveolocapillary membrane and into the plasma and intraerythrocytic compartments determines the diffusing capacity of the lung for CO ($DL_{CO}$) as defined by the Roughton and Forster equation. On the other hand, the single-breath diffusing capacity of the lung for nitric oxide ($DL_{NO}$) is thought to represent the true membrane diffusing capacity because of its very high affinity for hemoglobin (Hb) and its independence from pulmonary capillary blood volume. Therefore, the $DL_{NO}/DL_{CO}$ ratio can be used to differentiate between thickened alveolocapillary membranes (both $DL_{NO}$ and $DL_{CO}$ are decreased, and the $DL_{NO}/DL_{CO}$ ratio is normal) and decreased perfusion of ventilated alveoli (the $DL_{NO}$ less decreased than the $DL_{CO}$; therefore, the $DL_{NO}/DL_{CO}$ ratio is high) in patients with pulmonary disease.

We measured the combined values of $DL_{CO}$ and $DL_{NO}$ in 41 patients with diffuse parenchymal lung disease (DPLD), 26 patients with pulmonary arterial hypertension (PAH), and 71 healthy subjects.

The $DL_{CO}$ (corrected to the standard Hb value) was lowered in the DPLD group (64% of predicted) and in the PAH group (64% of predicted), and was normal in the control group (105% of predicted). The $DL_{NO}/DL_{CO}$ ratio in patients with PAH (4.98) was significantly higher than that in patients with DPLD (4.56) and in healthy subjects (4.36).

The $DL_{NO}/DL_{CO}$ ratio is significantly higher in patients with PAH than in healthy subjects, although this ratio cannot be applied as a screening test to discriminate between patients with DPLD and PAH as the overlap between these groups is too large.

## Introduction

The diffusing capacity of the lung for carbon monoxide ($DL_{CO}$) estimates the amount of gas uptake by the lungs and is a valuable tool in the assessment of pulmonary diseases. According to the model of Roughton and Forster [1], $DL_{CO}$ is determined by several factors: the passage of carbon monoxide (CO) through the alveolocapillary membrane, the transfer of CO into the plasma and the intraerythrocytic compartments, and the reaction rate for the binding of CO on hemoglobin (Hb). Equation 1 enables the estimation of two components of the $DL_{CO}$ (*ie*, the diffusing capacity of the alveolocapillary membrane for CO [$Dm_{CO}$] and the pulmonary capillary blood volume [Vcap]) using duplicate measurements of the $DL_{CO}$ with high and low oxygen concentrations [1].

$$\frac{1}{DL_{CO}} = \frac{1}{Dm_{CO}} + \frac{1}{\theta_{CO} * Vcap} \quad \textbf{\textit{Equation 1}}$$

Although numerous articles have been published concerning the clinical value of the $Dm_{CO}$ and Vcap, this test has not become a standard tool in the pulmonary function laboratory for the following reasons: first, the measurement of the $Dm_{CO}$ is complicated and time-consuming; and, second, measurements of $Dm_{CO}$ and Vcap are not entirely accurate. The reason for this is that the $\theta_{CO}$-value in the Roughton and Forster equation [1] is an estimate, and the $Dm_{CO}$ is determined by two separate breathholding periods, with high and low oxygen concentrations, in which several factors can influence the measurement [2].

Nitric oxide (NO) offers a solution to these problems and can be used as a gas in testing pulmonary diffusing capacity. NO binds 400 times stronger than CO to Hb [3], therefore the diffusing capacity of lung for NO ($DL_{NO}$) is much less influenced by changes in the Vcap and reflects the properties of the alveolocapillary membrane better than the $DL_{CO}$. Borland and Higenbottam [4] measured $DL_{NO}$ and $DL_{CO}$ in one single breath maneuver, and calculated the $Dm_{CO}$ and Vcap using differences in the $\theta_{CO}$ and the $\theta_{NO}$ values [5]. Several interesting studies have been conducted in this field. Phansalkhar et al. [6] showed that the $DL_{NO}$ (using the rebreathing technique) closely relates to the $Dm_{CO}$

measured by the high/low oxygen method. Tamhane et al. [3] measured the combined $DL_{NO}$-$DL_{CO}$ values with a rebreathing technique and found that the $DL_{NO}$ directly correlates to the diffusing capacity of the pulmonary membrane. Thus the $DL_{NO}/DL_{CO}$ ratio should be able to locate the position of diffusion impairment. Assuming that the $DL_{NO}$ is not or less effected by impaired capillary filling, and thus represents the diffusing capacity of the pulmonary membrane, the $DL_{NO}/DL_{CO}$ ratio has to differ between subjects with a pure alveolocapillary membrane disturbance and subjects with microvascular disease. Subjects with a decreased $DL_{CO}$ due to lowering of the Vcap component will have undisturbed $DL_{NO}$; therefore, the $DL_{NO}/DL_{CO}$ ratio will increase. Subjects with a decreased $DL_{CO}$ due to thickening of the membrane without disturbing the pulmonary capillaries will also have a lower $DL_{NO}$, so the $DL_{NO}/DL_{CO}$ ratio will not alter. The study by Harris et al. [7] demonstrated in sheep that the occlusion of one pulmonary artery increased the $DL_{NO}/DL_{CO}$ ratio by decreasing the $DL_{CO}$ while the $DL_{NO}$ remained constant. This effect is caused by the increase in CO backpressure in stagnant capillaries. The authors concluded that the $DL_{CO}$ has a much greater sensitivity than $DL_{NO}$ in detecting a regional reduction in capillary blood flow. The aim of this study was to test the hypothesis that the $DL_{NO}/DL_{CO}$ ratio significantly differs between patients with diffusion impairment due to fibrotic disease and patients with diffusion impairment due to pulmonary vascular disease.

## Methods

### *Patients*

Subjects were recruited from the pulmonary outpatient clinic of our hospital. This study was approved by the local ethics committee. Inclusion criteria consisted of a definitive diagnosis of diffuse parenchymal lung disease (DPLD) [8] or pulmonary arterial hypertension (PAH) according to the Revised Clinical Classification of Pulmonary Hypertension [9]. A control group of healthy nonsmoking subjects was recruited from among hospital personnel.

### *Diagnostic procedure*

All patients were extensively investigated by experienced pulmonologists; the standard procedure consisted of a medical history, a physical examination, laboratory investigations, a chest radiograph, a high-resolution CT scan of the lungs, spirometry, whole-body plethysmography (6200 Autobox DL; SensorMedics; Yorba Linda, CA), the measurement of $DL_{CO}$ (MasterLab Pro; Erich Jaeger GmbH; Wurzburg, Germany), the determination of subdivisions of the $DL_{CO}$ (*ie*, $Dm_{CO}$ and Vcap, as described earlier) [10], and ECG.

Patients with (suspected) DPLD underwent bronchoscopy with bronchial lavage and transbronchial biopsies where indicated. Video-assisted thoracoscopic lung biopsy was performed in selected patients when the above-mentioned examinations did not lead to a definite diagnosis. In some patients, cardiologists or rheumatologists were consulted. Immunologic laboratory investigations and echocardiography were performed when indicated. The classification of the DPLD was based on the recommendations of the British Thoracic Society [8].

All patients with suspected PAH underwent radionuclide perfusion and ventilation scans, echocardiography with estimation of the pulmonary artery pressure by tricuspid regurgitation measurement (assessed by experienced cardiologists), CT scan of the pulmonary arteries in order to detect central thromboembolic disease, right heart catheterization with measurements of the pulmonary artery pressure with reversibility testing in most patients (epoprostenol), and pulmonary angiography when indicated. Consultation by rheumatologists included the performance of serum immunologic tests in search of collagen vascular disease and scleroderma. A definite diagnosis of PAH was made based on the Revised Clinical Classification of Pulmonary Hypertension [9].

### *$DL_{NO}$ measurement*

A combined single-breath $DL_{NO}$ and $DL_{CO}$ measurement was performed on an adapted instrument (MasterLab Pro; Erich Jaeger GmbH). The test gas contained a mixture of CO 0.25%, He 9.17%, and NO 8 ppm with balance air. The NO was added to the test gas directly before each measurement from a separate tank containing 750 ppm NO in nitrogen (Hoekloos Medical; Schiedam the Netherlands). The single-breath procedure was performed according to American

Thoracic Society recommendations [11] with an effective breathholding period of 10 seconds (the Jones and Meade method [12]), a discard volume of 750 mL, and a sample volume 750 mL. The device (MasterLab Pro; Erich Jaeger GmbH) sampled the He and CO concentration in the expired air, and a chemoluminescence analyzer (CLD 77 AM; Eco Physics; Zurich, Switzerland [lower detection limit, 0.02 to 0.05 parts per billion (ppb); upper detection limit, 10 ppm; reaction time 0.1 s]) measured the NO concentration. Once a week, the chemoluminescence analyzer was calibrated with 5 ppm NO in nitrogen and NO-free air. All connections between the NO analyzer and the inspiratory and expiratory bags were made of polytetrafluoroethylene (Teflon; DuPont; Wilmington, DE) or stainless steel, which do not interact with NO. The alveolar volume ($V_A$) and $DL_{CO}$ were calculated according to European Respiratory Society recommendations [2], and the $DL_{NO}$ was calculated according to the method described by Borland and Higenbottam [4], which is the same formula for using NO concentrations instead of CO concentrations. Endogenous NO levels and CO backpressure were ignored, and smoking was allowed until 24 hours before testing. The $DL_{CO}$ and $DL_{NO}$ were corrected to BTPS conditions, and a minimum of two measurements was performed, in which a variability of 10% or less for the $V_A$ and $DL_{CO}$ was acceptable. All $DL_{CO}$ measurements were corrected to standard Hb value according to American Thoracic Society recommendations [11]. The obtained $DL_{NO}/DL_{CO}$ ratios were compared by means of analysis of variance using a statistical software package (SPSS for Windows, version 11.0; SPSS; Chicago, IL). The relation between the $DL_{NO}/DL_{CO}$ ratio and $Dm_{CO}$ was performed with the Pearson correlation coefficient.

## Results

In a period of 1 year (April 2003 to April 2004), 71 patients were screened for study inclusion, and 67 patients were included in the study based on eligibility. Four DPLD subjects were excluded due to the presence of secondary pulmonary hypertension. In one patient, this was probably due to left ventricular failure with mitral valve regurgitation, and in the other three patients the cause of the secondary hypertension was associated with the DPLD. In the control group, 71 healthy

volunteers were included (36 female volunteers and 35 male volunteers). In the DPLD category (total, 41 subjects; female subjects, 23; male subjects, 18), sarcoidosis was diagnosed in 27 patients, 4 with nonspecific interstitial pneumonia, 5 with idiopathic pulmonary fibrosis (IPF), 2 with pulmonary alveolar proteinosis, 1 with lymphangioleiomyomatosis, 1 with respiratory bronchiolitis-associated interstitial lung disease, and 1 with cryptogenic organizing pneumonia. In the PAH category (total, 26 patients; female patients, 16; male patients, 10), primary pulmonary hypertension (PPH) was diagnosed in 4 patients, chronic thromboembolic pulmonary hypertension (CTEPH) was diagnosed in 20 patients, and pulmonary hypertension associated with scleroderma (without signs of interstitial lung disease) was diagnosed in 2 patients. The demographics of the study population are shown in Table 1 ; the mean age of the control group is lower than that in subjects with DPLD and PAH. The mean $DL_{NO}/DL_{CO}$ ratios in patients with DPLD and PAH were 4.56 and 4.98, respectively; this difference was statistically significant ($p = 0.01$). The mean $DL_{NO}/DL_{CO}$ ratio in healthy subjects was 4.36, which is significantly different from the $DL_{NO}/DL_{CO}$ ratio of subjects with PAH ($p < 0.001$) [Table 1] but is not significantly different from that in the DPLD group ($p = 0.127$). As shown in Figure 1 , the three groups had a high degree of overlap. The $DL_{NO}$ and the $Dm_{CO}$ are highly correlated ($r^2 = 0.81$), and the slope of the regression line ($DL_{NO}/Dm_{CO}$) was 2.48 (Fig 2 ).

| | Normal (n=71) | DPLD (n=41) | PAH (n=26) |
|---|---|---|---|
| Age, yr | 38.1 (10.1) * | 48.1 (13.0)* | 56.8 (11.8)* # |
| Height, m | 1.75 (0.09) | 1.71 (0.08) | 1.69 (0.07) |
| VC % predicted | 115% (16) | 92% (22)* | 97% (16)* |
| TLC % predicted | 108% (13) | 87% (20)* | 89% (16)* |
| $DL_{CO}$ (mmol/min/kPa) | 10.9 (2.4) | 6.0 (1.9)* | 5.6 (1.7)* |
| $DL_{CO}$ % predicted | 105% (16) | 65% (20)* | 65% (17)* |
| $K_{CO}$ (mmol/min/kPa/L) | 1.7 (0.3) | 1.3 (0.3)* | 1.1 (0.3)*# |
| $K_{CO}$ % predicted | 104% (15) | 85% (20)* | 76% (17)* |
| $DL_{NO}$ (mmol/min/kPa) | 47.5 (10.9) | 27.4 (9.9)* | 27.4 (10.1)* |
| $K_{NO}$ (mmol/min/kPa/L) | 7.3 (0.8) | 6.0 (1.4)* | 5.5 (1.1)* |
| $DL_{NO}/DL_{CO}$ | 4.36 (0.6) | 4.56 (0.6)* | 4.98 (0.9)*# |
| $Dm_{CO}$ (mmol/min/kPa) | | 10.5 (3.8) | 9.4 (3.6) |
| $Dm_{CO}$ % predicted | | 53% (20) | 54% (24) |
| Vcap, mL | | 48.6 (13.9) | 50.3 (14.4) |
| Vcap % predicted | | 63% (19) | 67% (17) |

***Table 1.*** *Demographics and main results of the study population. Values are given as the mean ± SD. VC = vital capacity, TLC = total lung capacity, $K_{CO}$ is $DL_{CO}/V_A$, $K_{NO}$ is $DL_{NO}/V_A$ (*p<0.03 for comparison with normals; #p<0.03 for comparison with DPLD).*

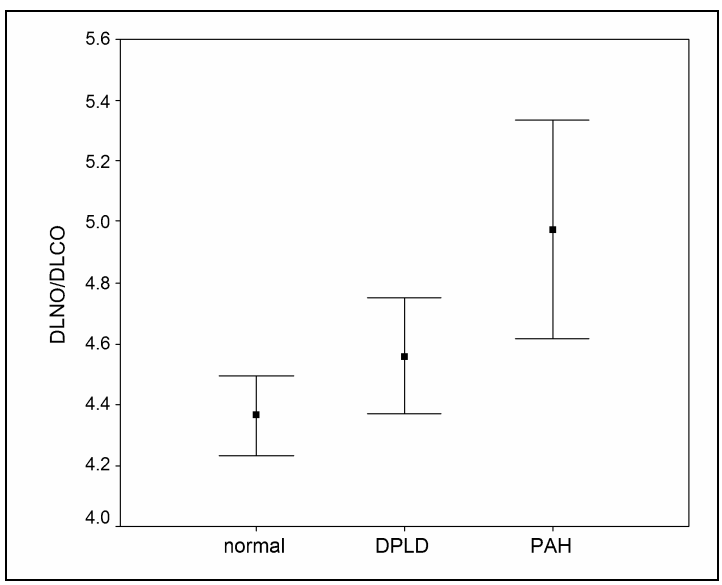


***Figure 1.*** *$DL_{NO}/DL_{CO}$ ratios in all three categories. Depicted is the mean ± 2 SEs.*

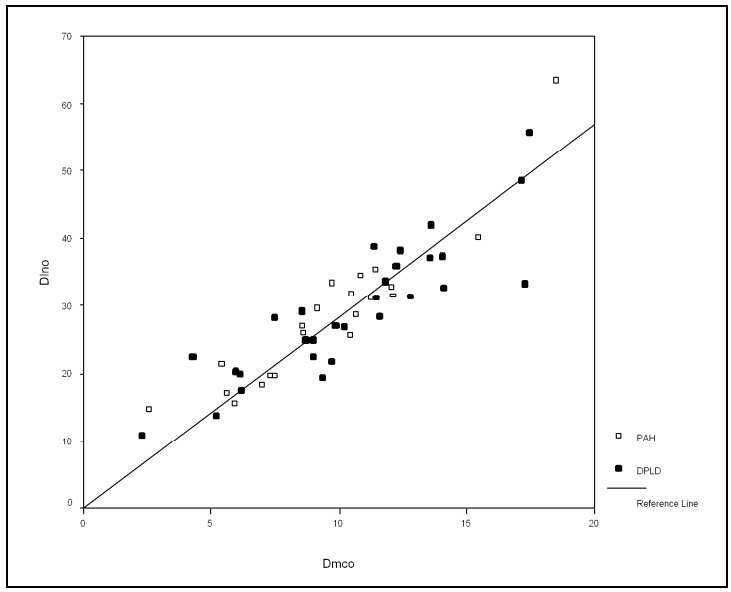


***Figure 2.*** *$DL_{NO}$ vs $D_{mCO}$ in all subjects with disease. The line of reference for total population is displayed ($r^2 = 0.81$).*

## Discussion

In this prospective study, we found a difference in the $DL_{NO}/DL_{CO}$ ratio between patients with DPLD and patients with PAH. Although this difference did reach statistical significance, the large overlap between the groups makes the $DL_{NO}/DL_{CO}$ ratio inapplicable as a clinical tool in discriminating between PAH and DPLD.

Although we used a lower inspiratory NO concentration compared to others [4], we had no reason to expect that this would influence our data as it has been shown that the $D_{LNO}$ is independent of inspiratory NO in rabbits [13]. It has been taken into account that the NO-concentration in the alveolar sample is well above the natural alveolar output, which is approximately 2 to 3 ppb in healthy subjects [14], 4.7 ppb in subjects with scleroderma-associated interstitial lung disease with or without pulmonary hypertension [15], and 4.1 ppb in subjects with hypersensitivity pneumonitis and IPF [16]. In our study the NO concentration in the sample volume was well >200 ppb; therefore, the neglect of natural alveolar NO output can only lead to a very slight

underestimation of the $DL_{NO}$. The NO production by the conducting airways can be neglected because of the very high exhalation flows used [14]. We found $DL_{NO}/DL_{CO}$ ratios of 4.36 in normal subjects, which are comparable to the ratio of 4.3 in 13 healthy subjects assessed by Borland and Higenbottam [4] by single breath method and of 4.52 in 8 healthy men (single-breath technique) measured by Zavorsky et al. [17]. In addition, the $DL_{NO}$ strongly correlated with the $Dm_{CO}$. We observed a relation of 2.48, which is similar to the value found by Tamhane et al. (2.49) using a rebreathing technique [3] and that found by Phansalkar et al. [6] (2.42). $DL_{CO}$ is often, but not always, decreased in patients with PAH [18;19]. Consequently, $DL_{CO}$ cannot be used as a screening test to exclude pulmonary hypertension in which the pre-test probability is high [20]. Borland et al. [21] found $DL_{NO}/DL_{CO}$ ratio (combined single-breath technique) to be 5.02 in 12 patients with severe PPH vs 4.51 in 10 matched healthy volunteers. This is in accordance with our results. Steenhuis et al. [18] observed decreased $DL_{CO}$ in subjects with PPH and CTEPH, mainly due to a decreased $Dm_{CO}$ (high/low oxygen method). There were no differences in the mean values of $DL_{CO}$, $Dm_{CO}$ and Vcap between the two groups. Bernstein et al. [22] measured the $Dm_{CO}$ and Vcap (high/low oxygen method) before and 3 weeks after pulmonary thromboendarterectomy in 29 subjects with CTEPH. $Dm_{CO}$ and Vcap were decreased prior to the operation and $Dm_{CO}$ decreased further after the operation. However, the short interval after the pulmonary thromboendarterectomy, the fall in $V_A$ postoperatively, and the known dependency of $DL_{CO}$ and $Dm_{CO}$ on $V_A$ [23] make it difficult to draw conclusions from this study.

There have been several studies dealing with the subdivision of the $DL_{CO}$ in interstitial lung diseases. In 1976, Saumon et al. [24] found that in patients with sarcoidosis with radiological stage I and II disease, the decrease in $DL_{CO}$ was mainly due to decreased $Dm_{CO}$, but that in stage III sarcoidosis the decrease was associated with a decrease in Vcap. The Vcap values in subjects with IPF or due to systemic sclerosis were lower than in the sarcoidosis stage III group. Phansalkar et al. [6] measured rebreathing $DL_{NO}$ and $Dm_{CO}$ values in 25 subjects with stage II-III sarcoidosis compared to 18 healthy nonsmoker subjects. They found a resting $DL_{NO}/DL_{CO}$ ratio of 4.36 in healthy subjects and 3.48 in subjects with sarcoidosis. At 80% of peak workload, the ratios were 3.70 in healthy subjects and 2.97 in subjects with sarcoidosis. Indeed,

at rest and during exercise the $DL_{NO}/DL_{CO}$ ratios were lower in subjects with sarcoidosis than in healthy subjects, as expected. The $DL_{NO}$ strongly correlated with the $Dm_{CO}$, indicating that the $DL_{NO}$ closely resembles the true diffusing capacity of the alveolar capillary membrane. The fact that Phansalkar et al. [6] found lower ratios in subjects with sarcoidosis than we did is troubling. Although Phansalkar et al. [6] included subjects with stage II-III sarcoidosis compared to our inclusion of subjects with stage I-IV disease, it is unlikely that this explains the difference. The rebreathing technique used by Phansalkar et al. [6] is performed at the functional residual capacity level, in contrast to our actual measurement at the total lung capacity level as occurred when using the single-breath method. This could explain in part the difference between the findings of Phansalkar et al. [6] and our own. In 2004, Lamberto et al. [25] measured the $Dm_{CO}$ and the Vcap in patients with stage I to IV sarcoidosis and found that the reduced $DL_{CO}$ was mainly caused by lowered $Dm_{CO}$ in all groups. Furthermore, $Dm_{CO}$ as well as $DL_{CO}$ are highly predictive of gas exchange abnormalities during exercise.
An interesting study was performed by Bonay et al. [26], who investigated whether the Vcap (determined with single breath high/low oxygen method) would be lower in subjects with DPLD and associated PAH than in subjects with DPLD without PAH. This appeared not to be the case, thus excluding the Vcap measurement as a screening test for PAH in subjects with DPLD. In this study the $DL_{NO}/DL_{CO}$ ratios differ between the different diseases, but the overlap is great.

The equation of Roughton and Forster [1] assumes that $Dm_{CO}$ and Vcap are independent components by assuming that the $1/DL_{CO}$ resistance is the sum of two resistances. The question is whether this is correct. Hypoxemia due to thickened membranes can lead to pulmonary vasoconstriction. Capillary flow is a prerequisite to measuring the $Dm_{CO}$. Some investigations [27] in patients with IPF show that capillary density is significantly decreased in diseased areas, leading to a decrease in the Vcap component of the $DL_{CO}$ in addition to the already lowered $Dm_{CO}$ component as a consequence of the diseased-thickened membranes, thus making the Vcap component dependent on the $Dm_{CO}$ component. This is of course difficult to assess *in vivo*, although some research has pointed to the dependency of the $Dm_{CO}$ on Hb concentration, and a relationship between $Dm_{CO}$ and Vcap [28]. If the

$Dm_{CO}$ and the Vcap components are dependent, the separation of the $DL_{CO}$ in these two components becomes clinically irrelevant. In other words, the lung is defined as a monoalveolar object, with a relative contribution by the Vcap and the $Dm_{CO}$ components. The $DL_{CO}$ measurement is not only a function of membrane thickness and surface area, but also (and not in the least) a function of the ventilation and perfusion inhomogeneity. In 1960, Johnson et al. [29] showed that the increase of the $DL_{CO}$ from rest to exercise is partly based on an increase in the $Dm_{CO}$. This is mainly based on improved matching of ventilation and perfusion than of recruitment of alveoli. Furthermore, ventilation and perfusion inhomogeneity is the main determinant of the $DL_{CO}$ in patients with asthma [30].

If indeed the $DL_{NO}$ is more sensitive than the $DL_{CO}$ in detecting specific disturbances of the alveolocapillary membrane, then the decreased $DL_{CO}$ in patients with PAH and DPLD is probably due to ventilation and perfusion inhomogeneity instead of to decreased passage through the alveolocapillary membrane [31;32].

In conclusion, although the overlap is large, we observed a statistically significant difference in $DL_{NO}/DL_{CO}$ ratios between patients with PAH and patients with DPLD. The $DL_{NO}/DL_{CO}$ ratio in patients with PAH was significantly higher than that of healthy volunteers.

## References

1. Roughton FJ, Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J Appl Physiol 1957; 11(2):290-302.
2. Cotes JE, Chinn DJ, Quanjer PH et al. Standardization of the measurement of transfer factor (diffusing capacity). Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:41-52.
3. Tamhane RM, Johnson RL, Jr., Hsia CC. Pulmonary membrane diffusing capacity and capillary blood volume measured during exercise from nitric oxide uptake. Chest 2001; 120(6):1850-1856.
4. Borland CD, Higenbottam TW. A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. Eur Respir J 1989; 2(1):56-63.
5. Borland CD, Cox Y. Effect of varying alveolar oxygen partial pressure on diffusing capacity for nitric oxide and carbon monoxide, membrane diffusing capacity and lung capillary blood volume. Clin Sci (Lond) 1991; 81(6):759-765.
6. Phansalkar AR, Hanson CM, Shakir AR et al. Nitric oxide diffusing capacity and alveolar microvascular recruitment in sarcoidosis. Am J Respir Crit Care Med 2004; 169(9):1034-1040.
7. Harris RS, Hadian M, Hess DR et al. Pulmonary artery occlusion increases the ratio of diffusing capacity for nitric oxide to carbon monoxide in prone sheep. Chest 2004; 126(2):559-565.
8. The diagnosis, assessment and treatment of diffuse parenchymal lung disease in adults. Thorax 1999; 54 Suppl 1:S1-14.
9. Simonneau G, Galie N, Rubin LJ et al. Clinical classification of pulmonary hypertension. J Am Coll Cardiol 2004; 43(12 Suppl S):5S-12S.
10. Zanen P, van der Lee I, van der Mark TW et al. Reference values for alveolar membrane diffusion capacity and pulmonary capillary blood volume. Eur Respir J 2001; 18(5):764-769.
11. American Thoracic Society. Single-breath carbon monoxide diffusing capacity (transfer factor). Recommendations for a standard

technique--1995 update. Am J Respir Crit Care Med 1995; 152(6 Pt 1):2185-2198.
12. Jones RS, Meade F. A theoretical and experimental analysis of anomalies in the estimation of pulmonary diffusing capacity by the single breath method. Q J Exp Physiol Cogn Med Sci 1961; 46:131-143.
13. Heller H, Schuster KD. Single-breath diffusing capacity of NO independent of inspiratory NO concentration in rabbits. Am J Physiol 1997; 273(6 Pt 2):R2055-R2058.
14. Pietropaoli AP, Perillo IB, Torres A et al. Simultaneous measurement of nitric oxide production by conducting and alveolar airways of humans. J Appl Physiol 1999; 87(4):1532-1542.
15. Girgis RE, Gugnani MK, Abrams J et al. Partitioning of alveolar and conducting airway nitric oxide in scleroderma lung disease. Am J Respir Crit Care Med 2002; 165(12):1587-1591.
16. Lehtimaki L, Kankaanranta H, Saarelainen S et al. Extended exhaled NO measurement differentiates between alveolar and bronchial inflammation. Am J Respir Crit Care Med 2001; 163(7):1557-1561.
17. Zavorsky GS, Quiron KB, Massarelli PS et al. The relationship between single-breath diffusion capacity of the lung for nitric oxide and carbon monoxide during various exercise intensities. Chest 2004; 125(3):1019-1027.
18. Steenhuis LH, Groen HJ, Koeter GH et al. Diffusion capacity and haemodynamics in primary and chronic thromboembolic pulmonary hypertension. Eur Respir J 2000; 16(2):276-281.
19. Burke CM, Glanville AR, Morris AJ et al. Pulmonary function in advanced pulmonary hypertension. Thorax 1987; 42(2):131-135.
20. Mukerjee D, St George D, Knight C et al. Echocardiography and pulmonary function as screening tests for pulmonary arterial hypertension in systemic sclerosis. Rheumatology (Oxford) 2004; 43(4):461-466.
21. Borland C, Cox Y, Higenbottam T. Reduction of pulmonary capillary blood volume in patients with severe unexplained pulmonary hypertension. Thorax 1996; 51(8):855-856.
22. Bernstein RJ, Ford RL, Clausen JL et al. Membrane diffusion and capillary blood volume in chronic thromboembolic pulmonary hypertension. Chest 1996; 110(6):1430-1436.

23. Stam H, Versprille A, Bogaard JM. The components of the carbon monoxide diffusing capacity in man dependent on alveolar volume. Bull Eur Physiopathol Respir 1983; 19(1):17-22.
24. Saumon G, Georges R, Loiseau A et al. Membrane diffusing capacity and pulmonary capillary blood volume in pulmonary sarcoidosis. Ann N Y Acad Sci 1976; 278:284-291.
25. Lamberto C, Nunes H, Le Toumelin P et al. Membrane and capillary blood components of diffusion capacity of the lung for carbon monoxide in pulmonary sarcoidosis: relation to exercise gas exchange. Chest 2004; 125(6):2061-2068.
26. Bonay M, Bancal C, De Zuttere D et al. Normal pulmonary capillary blood volume in patients with chronic infiltrative lung disease and high pulmonary artery pressure. Chest 2004; 126(5):1460-1466.
27. Renzoni EA, Walsh DA, Salmon M et al. Interstitial vascularity in fibrosing alveolitis. Am J Respir Crit Care Med 2003; 167(3):438-443.
28. Hsia CC. Recruitment of lung diffusing capacity: update of concept and application. Chest 2002; 122(5):1774-1783.
29. Johnson RL, Jr., Spicer WS, Bishop JM et al. Pulmonary capillary blood volume, flow and diffusing capacity during exercise. J Appl Physiol 1960; 15:893-902.
30. Weitzman RH, Wilson AF. Diffusing capacity and over-all ventilation:perfusion in asthma. Am J Med 1974; 57(5):767-774.
31. Agusti AG, Rodriguez-Roisin R. Effect of pulmonary hypertension on gas exchange. Eur Respir J 1993; 6(9):1371-1377.
32. Yamaguchi K, Mori M, Kawai A et al. Inhomogeneities of ventilation and the diffusing capacity to perfusion in various chronic lung diseases. Am J Respir Crit Care Med 1997; 156(1):86-93.

# Chapter 8

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

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submitted

## Abstract

The transfer factor for nitric oxide ($DL_{NO}$) 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 ($DL_{CO}$). Therefore the sensitivity for the detection of alveolar destruction is supposed to be higher.
We measured flow volumes curves, $DL_{NO}$, $DL_{CO}$, the transfer coefficients $K_{NO}$ and $K_{CO}$ and performed high-resolution computed tomography (HRCT) in 263 randomly selected heavy smokers. Subjects with areas ≥1% of the total lung volume showing an attenuation <-950 Hounsfield Units were considered to have emphysema. In 36 subjects emphysema was diagnosed with HRCT, an abnormal $K_{NO}$ was present in 94 subjects, and in 95 subjects a $FEV_1$/FVC ratio <70% was seen. The area under the ROC curve for the $K_{NO}$ was 0.894 and for the $K_{CO}$ 0.822. The $K_{NO}$ therefore showed a slightly higher sensitivity to detect emphysema, compared to the $K_{CO}$. The positive predictive value of $K_{NO}$ however was low (34.7%), while the negative predictive value of $K_{NO}$ was very high (98.2%), indicating an exclusion test. The $DL_{NO}/DL_{CO}$ ratio is significantly higher compared to normal subjects, indicating a strong influence off 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 subjects, 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 with the amount of emphysema present in lung specimens. Several investigators showed that the transfer factor ($DL_{CO}$) and transfer coefficient ($K_{CO}$) 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 $DL_{CO}$ and $K_{CO}$ are simple and cheap parameters, available in any pulmonary function laboratory, and may be used as a tool for early detection of emphysema.

The $DL_{CO}$ 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: $1/DL_{CO} = 1/Dm_{CO} + 1/\theta_{CO} * Vcap$, 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 $\theta_{NO}$ is very high, leading to a negligible value for $1/\theta_{NO} * Vcap$. Therefore, the transfer factor for NO ($DL_{NO}$) is supposed to represent the true membrane diffusing capacity [13]. A predominantly vascular disease will lower the $DL_{CO}$, but not the $DL_{NO}$, as it is not influenced by erythrocyte NO uptake (=decreased Vcap). The $DL_{NO}/DL_{CO}$ will tend to increase when predominantly vascular disease is present [14]. On the other hand [15], a predominantly membranous disturbance will affect both $DL_{CO}$ and $DL_{NO}$, and the alleged high sensitivity of the $DL_{NO}$ for membranous disturbances will tend to sharply decrease $DL_{NO}$. The $DL_{CO}$, being

partly dependent on membranous damage will not change that sharply: the ratio will tend to decrease.
The aim of this study was to see whether the $DL_{NO}$ is a better screening tool for the detection of emphysema than the $DL_{CO}$, and if the $DL_{NO}/DL_{CO}$ ratio differs from healthy subjects.

## Methods

### *Subjects*

All subjects were participating in the NELSON-project, a Dutch-Belgian multi-center lung cancer screening trial. The subjects 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 subjects 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 $FEV_1/FVC$ ratio <70% was labeled as abnormal. A simultaneous single breath $DL_{NO}$ and $DL_{CO}$ test was performed directly after spirometry, as described earlier [18]. At least two measurements per subject were obtained and a difference <10% in $DL_{CO}$ was acceptable. All pulmonary function values are given as a mean with standard deviation (SD), and as a percentage of predicted values [16;19;20]. For $DL_{NO}$ we used references equations from an earlier study (see Chapter 5). Values

<-1.64 times the standard deviation were considered abnormal. The $DL_{NO}/DL_{CO}$ and $FEV_1/FVC$ ratio's are expressed as absolute values.

### *CT Scanning and emphysema quantification*

The CT scan was 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 16x0.75mm 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 with a density mask method 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. Subjects 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, USA).

## Results

Between October 2004 and April 2005 263 male subjects were included in the study. Several characteristics of the study population are given in Table 1. Thirty-six (13.6%) subjects 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 $DL_{NO}/DL_{CO}$ ratio was 4.9, which is higher than the 4.3 value reported by Borland et al. [11] in healthy subjects 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 data of Chapter 5.
The AUC of the ROC curves showed the highest values for $K_{NO}$ (as percentage of predicted) to detect emphysema, with $K_{CO}$ (also as percentage predicted) and $FEV_1/FVC$ in second respectively in third highest position (see Table 3). The differences between the AUC ROC for the $K_{NO}$ and $K_{CO}$ 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 $K_{CO}$, $K_{NO}$ or $FEV_1/FVC$ virtually excludes an ES≥1%. The low PPV values indicate that an abnormal $K_{CO}$, $K_{NO}$ or $FEV_1/FVC$ only points at an ES≥1% in a minority of the cases.
Subjects with low $K_{NO}$ values, subjects with emphysema on HRCT and subjects with a $FEV_1/FVC$ 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 |
| $DL_{CO}$, %pred | 87.4 | 49.8-140.4 | 16.1 |
| $K_{CO}$, %pred | 84.4 | 46.6-140.2 | 15.9 |
| $DL_{NO}$, %pred | 87.5 | 45.3-121.3 | 13.5 |
| $K_{NO}$, %pred | 90.4 | 53.7-121.6 | 12.4 |
| $DL_{NO}/DL_{CO}$ 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* |
| $DL_{CO}$, %predicted | -0.26* |
| $K_{CO}$, %predicted | -0.38* |
| $DL_{NO}$, %predicted | -0.29* |
| $K_{NO}$, %predicted | -0.50* |

***Table 2.*** *Correlation coefficients of the emphysema score versus pulmonary function data (*=p< 0.01).*

| | 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 |
| $DL_{CO}$, %pred | 0.727 | <0.001 | 0.622-0.833 |
| $K_{CO}$, %pred | 0.822 | <0.001 | 0.757-0.887 |
| $DL_{NO}$, %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 |
|---|---|---|---|---|
| $DL_{CO}$ | 58.3% | 81.5% | 33.3% | 92.5% |
| $DL_{NO}$ | 50.0% | 81.9% | 30.5% | 91.2% |
| $K_{CO}$ | 88.9% | 57.3% | 24.8% | 97.0% |
| $K_{NO}$ | 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.*

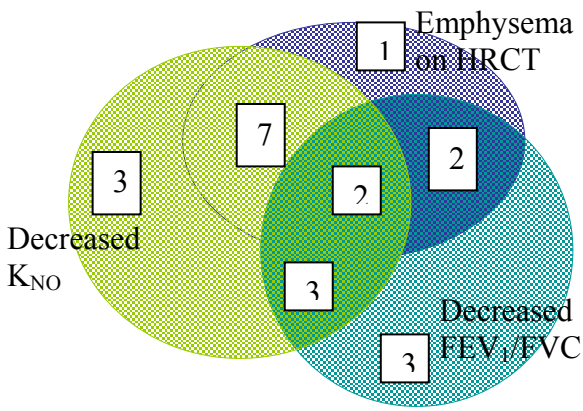


**Figure 1.** *Venn diagram: the presence of emphysema on HRCT (n=36), a decreased $K_{NO}$ (n=94) and a decreased $FEV_1/FVC$ ratio (n=95) are partially overlapping entities.*

## 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 $DL_{NO}$ would better detect emphysema on HRCT than the $DL_{CO}$, based on its alleged higher sensitivity for alveolar membrane destruction. However, this proved not to be the case.

The $DL_{NO}/DL_{CO}$ ratio in these smokers is significantly higher than in healthy subjects, and reaches values seen in subjects 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 $DL_{NO}/DL_{CO}$

ratio of 5.0 in subjects with PAH versus 4.5 in healthy subjects was reported [23].

The study outcome indicates that lung function can not detect emphysema sensitively, even if the parameter used is maximally influenced by a loss of alveolar membranes, like the $DL_{NO}$ and the $K_{NO}$. 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 $DL_{NO}$ and $K_{NO}$ are only marginally better in detecting emphysema compared to the $DL_{CO}$ and $K_{CO}$ is probably due to the fact that in our study population the majority of the tested subjects showed either no or only small amounts of emphysema. These small amounts of loss of alveolar tissue and the higher sensitivity of the $DL_{NO}$ and $K_{NO}$ 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 $DL_{NO}$ and $K_{NO}$ will lose their theoretical advantage.

As a result the correlation between CT-scan 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 subjects with COPD [24] and in candidates for lung resection or transplantation [7]. This confirms our conclusion.
In this population based study in heavy (ex) smokers the correlation between pulmonary function tests and CT based assessment of emphysema is even weaker than reported earlier [25]. This is not unexpected, because our subjects 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 subjects with emphysema on HRCT had a $FEV_1$/FVC ratio above 70%.

Similar arguments can be used to explain why the spirometric parameters ($FEV_1$ 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 $DL_{NO}/DL_{CO}$ ratio points at a significant influence of diffusion parameters on vascular damage. The $DL_{NO}$ is not influenced to the same extent by vascular damage as the $DL_{CO}$: the ratio therefore will increase if such damage is significant. Now we calculated this ratio for the entire sample, and most subjects 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 $DL_{NO}/DL_{CO}$ ratio also characterizes the non-emphysematous subjects 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-based emphysema are complementary and partially overlapping entities (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 [26]. 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.

### *Critique of methods*

Although it was recommended that the subjects 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 $DL_{CO}$ based on higher CO-backpressure. In our believe this could not alter the main results, because the smoking effect is very small: the $DL_{CO}$ decreases by 1% for each percent COHb present. So when the presence of COHb is 7%, the $DL_{CO}$ decrease by approximately 7% [27]. Such high levels of COHb are seldom.

The $DL_{NO}$ references equations are based on a previous study (see Chapter 5) in 124 subjects, of which were 65 males with a mean age of 40.1 years. The mean age in this study was higher. However, the $DL_{CO}$ values in that previous study match exactly with the ECCS vales [20], so gross deviations in estimating age, and height effects are unlikely. The extrapolation to older subjects 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 $K_{NO}$ 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.

## References

1. Chapman KR, Mannino DM, Soriano JB et al. Epidemiology and costs of chronic obstructive pulmonary disease. Eur Respir J 2006; 27(1):188-207.
2. Fletcher C, Peto R. The natural history of chronic airflow obstruction. Br Med J 1977; 1(6077):1645-1648.
3. Zielinski J, Bednarek M. Early detection of COPD in a high-risk population using spirometric screening. Chest 2001; 119(3):731-736.
4. Lundback B, Lindberg A, Lindstrom M et al. Not 15 but 50% of smokers develop COPD?--Report from the Obstructive Lung Disease in Northern Sweden Studies. Respir Med 2003; 97(2):115-122.
5. Mitchell RS, Stanford RE, Johnson JM et al. The morphologic features of the bronchi, bronchioles, and alveoli in chronic airway obstruction: a clinicopathologic study. Am Rev Respir Dis 1976; 114(1):137-145.
6. Gevenois PA, de M, V, De Vuyst P et al. Comparison of computed density and macroscopic morphometry in pulmonary emphysema. Am J Respir Crit Care Med 1995; 152(2):653-657.
7. Gevenois PA, De Vuyst P, de Maertelaer V et al. Comparison of computed density and microscopic morphometry in pulmonary emphysema. Am J Respir Crit Care Med 1996; 154(1):187-192.
8. Tylen U, Boijsen M, Ekberg-Jansson A et al. Emphysematous lesions and lung function in healthy smokers 60 years of age. Respir Med 2000; 94(1):38-43.
9. Klein JS, Gamsu G, Webb WR et al. High-resolution CT diagnosis of emphysema in symptomatic patients with normal chest radiographs and isolated low diffusing capacity. Radiology 1992; 182(3):817-821.
10. Roughton FJ, Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J Appl Physiol 1957; 11(2):290-302.
11. Borland CD, Higenbottam TW. A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. Eur Respir J 1989; 2(1):56-63.
12. Guenard H, Varene N, Vaida P. Determination of lung capillary blood volume and membrane diffusing capacity in man by the

measurements of NO and CO transfer. Respir Physiol 1987; 70(1):113-120.
13. Tamhane RM, Johnson RL, Jr., Hsia CC. Pulmonary membrane diffusing capacity and capillary blood volume measured during exercise from nitric oxide uptake. Chest 2001; 120(6):1850-1856.
14. Harris RS, Hadian M, Hess DR et al. Pulmonary artery occlusion increases the ratio of diffusing capacity for nitric oxide to carbon monoxide in prone sheep. Chest 2004; 126(2):559-565.
15. van der Lee I, Zanen P, Grutters JC et al. Diffusing capacity for nitric oxide and carbon monoxide in patients with diffuse parenchymal lung disease and pulmonary arterial hypertension. Chest 2006; 129(2):378-383.
16. Quanjer PH, Tammeling GJ, Cotes JE et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:5-40.
17. Pauwels RA, Buist AS, Calverley PM et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. Am J Respir Crit Care Med 2001; 163(5):1256-1276.
18. van der Lee I, Zanen P, Biesma DH et al. The effect of red cell transfusion on nitric oxide diffusing capacity. Respiration 2005; 72(5):512-516.
19. Cotes JE. Transfer factor (diffusing capacity). In: Standardized lung function testing. Report working party. Bull Eur Physiopathol Respir 1983; 19 (Suppl 5):39-44.
20. Cotes JE, Chinn DJ, Quanjer PH et al. Standardization of the measurement of transfer factor (diffusing capacity). Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16:41-52.
21. Gevenois PA, Scillia P, de Maertelaer V et al. The effects of age, sex, lung size, and hyperinflation on CT lung densitometry. AJR Am J Roentgenol 1996; 167(5):1169-1173.
22. Kinsella M, Muller NL, Abboud RT et al. Quantitation of emphysema by computed tomography using a "density mask" program

and correlation with pulmonary function tests. Chest 1990; 97(2):315-321.
23. Borland C, Cox Y, Higenbottam T. Reduction of pulmonary capillary blood volume in patients with severe unexplained pulmonary hypertension. Thorax 1996; 51(8):855-856.
24. Muller NL, Staples CA, Miller RR et al. "Density mask". An objective method to quantitate emphysema using computed tomography. Chest 1988; 94(4):782-787.
25. Park KJ, Bergin CJ, Clausen JL. Quantitation of emphysema with three-dimensional CT densitometry: comparison with two-dimensional analysis, visual emphysema scores, and pulmonary function test results. Radiology 1999; 211(2):541-547.
26. Traver GA, Cline MG, Burrows B. Predictors of mortality in chronic obstructive pulmonary disease. A 15-year follow-up study. Am Rev Respir Dis 1979; 119(6):895-902.
27. Sansores RH, Pare PD, Abboud RT. Acute effect of cigarette smoking on the carbon monoxide diffusing capacity of the lung. Am Rev Respir Dis 1992; 146(4):951-958.

# Chapter 9

# Summary, conclusions and view

The purpose of the studies described in this thesis was to investigate clinical aspects of the single breath diffusing capacity for carbon monoxide ($DL_{CO}$) and nitric oxide ($DL_{NO}$). The $DL_{CO}$ is a widely used measure and has been extensively investigated in the past, but despite this some controversial issues concerning the $DL_{CO}$ remain. These issues are found in the diagnostic as well as in the technical area. The research into these fields is described in the first part of this thesis. The second part compasses detailed research with the $DL_{NO}$, which is a relative new measurement and up to now has only been used by few investigators. The advantage of the $DL_{NO}$ is the supposed higher sensitivity to alveolar membrane destruction and therefore the higher sensitivity to detect disease in an earlier phase.

***Chapter 1***

In the introduction of this thesis an overview of the single breath diffusing capacity for carbon monoxide ($DL_{CO}$) is given. Some attention is given to the measurements of the diffusing capacity in the beginning of the twentieth century. An insight in the physiological process of gas uptake by the lungs is provided and technical factors influencing the $DL_{CO}$ measurement are explained.
The $DL_{CO}$ is determined by two major factors: 1] the $Dm_{CO}$, which is the diffusion of carbon monoxide through the alveolocapillary membrane, the plasma and the intraerythrocytic compartment; 2] Vcap, which is the pulmonary capillary blood volume. The traditional method to determine these subdivisions $Dm_{CO}$ and Vcap is the one where two separate measurements of the $DL_{CO}$ are made, one with a low and the other with a high $O_2$ concentration. Based on the fact that the binding of CO to hemoglobin ($\theta_{CO}$) is dependent on $O_2$ concentration, the following equation can be solved: $1/DL_{CO}=1/Dm_{CO} + 1/\theta_{CO}*Vcap$. This explanation is supplemented by a review of the literature concerning the clinical value of the $DL_{CO}$, the $K_{CO}$ (which is the $DL_{CO}$ divided by the alveolar volume or $V_A$), and the $Dm_{CO}$ and Vcap values.
Nitric oxide (NO) binds 400 times faster to hemoglobin than CO, therefore the uptake of NO by the blood is very large ($\theta_{NO}$ is very high, thus $1/\theta_{NO}*Vcap$ is neglectable). The rate limiting step of the $DL_{NO}$ is the passage through the alveolocapillary membrane. Therefore the $DL_{NO}$ equals the $Dm_{NO}$, thus the $DL_{NO}$ should be a better measure for

the alveolocapillary membrane diffusing capacity than the $DL_{CO}$. Previous research with $DL_{NO}$ is scarce, but is consistent with the above mentioned concept. This research is judged on its merits.

***Chapter 2***

In this chapter a critical assessment of the diagnostic value of the $DL_{CO}$ and the $K_{CO}$ is provided. We used the schemes provided in articles and textbooks, which describe the expected patterns of diffusion disturbances in disease. Pivotal in these schemes is the term $V_A$/TLC, in which the TLC is the total lung capacity measured by plethysmography, as an indicator for the presence of ventilatory inhomogeneity. When combining the $V_A$/TLC, the $DL_{CO}$ and the $K_{CO,}$ discrimination between different diseases should be possible. To our surprise, this concept had never been thoroughly tested. In 460 patients, the diagnosis established by the pulmonary physicians was fitted into five categories: asthma, COPD (chronic obstructive pulmonary disease), treatment effects of hematological malignancies, heart failure and diffuse parenchymal lung diseases (DPLD). We defined five patterns of diffusion disturbance: the first one is a normal $DL_{CO}$, which is expected in asthma. The second and third pattern consists of a low $DL_{CO}$ and low $K_{CO}$, which points to pathology at the level of the alveolocapillary membrane. The $V_A$/TLC ratio can subsequently discriminate between ventilation inhomogeneity (like in COPD, low ratio) and an increased thickness of the alveolocapillary membrane (in DPLD, normal or high ratio). The fourth and fifth pattern consists of a low $DL_{CO}$ and normal $K_{CO}$, which should be due to a small lung syndrome (e.g. chest cage restriction, normal $V_A$/TLC ratio), or inaccessible lung parts like in bullous emphysema (low ratio). The match between these diagnostic categories and the patterns of diffusion disturbance was inferior. Only in asthma in most cases the expected normal $DL_{CO}$ was observed, especially in DPLD the expected patterns were not observed. The DPLD cases were next categorized according to the exact diagnosis, which could not improve the match between measured and expected patterns.
In 143 subjects COPD was diagnosed, 34 of them had a normal $DL_{CO}$, 30 subjects had the pattern “belonging” to DPLD (low $DL_{CO}$, low $K_{CO}$ and normal $V_A$/TLC), 2 subjects had the pattern belonging to small

lung syndrome, the rest (77 subjects) had patterns of "emphysema" or "bullous emphysema".
The main conclusion is that the diagnostic strategy combining the $DL_{CO}$, $K_{CO}$ and $V_A$/TLC to establish a diagnosis, is of very little use in clinical practice: the use of the $K_{CO}$ has therefore no additional diagnostic value. A possible explanation for these findings is that non-perfusion of ventilated areas also leads to a low $DL_{CO}$, low $K_{CO}$ and normal $V_A$/TLC, and can be seen in several pulmonary diseases. It is in fact one of the extremes in the spectrum of ventilation/perfusion mismatching, and earlier research showed that mismatching of ventilation and perfusion is a strong determinant of the $DL_{CO}$. Another possible explanation is the fact that references equations for the $K_{CO}$ are not very reliable, especially when restriction is present.

***Chapter 3***

The $V_A$ is an important part of the measurement of the $DL_{CO}$, it is determined by a single-breath helium dilution and very sensitive to ventilatory disturbances. In subjects with COPD the emphysematous lung parts are less accessible to test gas; this is the reason that the $V_A$ is smaller than the TLC measured by plethysmography or by multiple-breath helium dilution ($TLC_{He}$). With high resolution computed tomography (HRCT) scan of the lungs, emphysematous lung parts can easily be visualized. Density mask software, which quantifies the tissue density of the lungs, separate emphysematous from non-emphysematous lung parts. The aim of this study was to correlate the $V_A$ with the non-emphysematous lung parts as assessed on HRCT scan to study the effects of emphysema of gas distribution over these diseased lungs.
Lung parts with an attenuation of <–950 Hounsfield Units (HU) were considered as emphysematous. A strong correlation was observed between the $V_A$ (mean 5.2 l) and non-emphysematous HRCT lung volume (mean 5.2 l) and between the multiple-breath $TLC_{He}$ (mean 6.6 l) and total HRCT lung volume (mean 6.4 l).
The strong correlation between the $V_A$ and non-emphysematous HRCT lung volume is a strong argument in favor of the notion that the $V_A$ is a measure of the easy-accessible lung parts. As a consequence, the $DL_{CO}$ (due to its single breath approach) only measures the function of these easy accessible lung parts. This is of importance when interpreting the

$DL_{CO}$: emphysematous and/or inaccessible lung parts are not measured with the $DL_{CO}$. Therefore HRCT scanning and the measurement of the $DL_{CO}$ are complementary approaches in assessing the severity of COPD.

***Chapter 4***

In this chapter the prevalence and extent of emphysema as assessed with low attenuation areas on HRCT scans were determined in a large group of heavy (ex)smokers. Emphysema on HRCT scans was related to the $DL_{CO}$ and spirometry with the goal to unravel the diagnostic capability of the latter to detect emphysema. The study population consisted of 545 male subjects with a smoking history of at least 20 pack-years, who underwent a low-dose chest HRCT scan as part of a lung cancer screening study. Subjects with >1% lung volume showing attenuation <-950 HU scan were considered to have emphysema: 75 subjects (14%) fulfilled this criterion. The best measure to exclude CT-assessed emphysema was a normal $K_{CO}$ (a negative predictive value (NPV) of 96%), which is slightly better than the NPV of the $FEV_1$/FVC ratio (93%). The positive predicted value of the $DL_{CO}$, the $K_{CO}$, the $FEV_1$ and the $FEV_1$/FVC are all low. Normal PFT results are useful in excluding emphysema. Many subjects without emphysema on HRCT have abnormal pulmonary function tests. Therefore pulmonary function tests can not distinguish between emphysema from other pulmonary pathology in this group of heavy smokers.

***Chapter 5***

In Chapter 5 to 8 several studies with the diffusing capacity for nitric oxide are described. First, reference values were determined in a group of 124 healthy volunteers. The single breath $DL_{NO}$ was measured simultaneously with the $DL_{CO}$, which also allowed us to compare the reference equations for the $DL_{CO}$ with the previously published ones. Fortunately, there was a good agreement. We derived reference equations for females and males and in both groups the main determinant of the $DL_{NO}$ was the height of the subject. A small age effect was seen in the $DL_{NO}$ and in the $K_{NO}$ reference equations.
In 21 subjects the dependence of the $K_{NO}$ on $V_A$ was studied, by voluntary lowering of the inspiratory maneuver. From earlier studies it was clear that the $K_{CO}$ increases when $V_A$ decreases, due to a relative

increase in capillary blood volume per unit lung volume. The $K_{NO}$ appeared almost independent on $V_A$, which is in agreement with the supposed fact that the $DL_{NO}$ is strongly determined by the alveolocapillary membrane. Because of the relative independence of the $K_{NO}$ on $V_A$, the $K_{NO}$ appears to be a much better index for the diffusion capacity per unit lung volume (transfer coefficient) than the $K_{CO}$.

***Chapter 6***

This study was performed to investigate the concept that the $DL_{NO}$ is independent of hemoglobin concentration. In ten patients with hematological diseases, the combined $DL_{NO}$ and $DL_{CO}$ were measured before and shortly after red cell transfusion. The $DL_{CO}$ rose after the transfusion perfectly in concordance with known hemoglobin correction reference equations, which means that the $DL_{CO}$ corrected for hemoglobin concentration is a valid approach. The $DL_{NO}$ did not change, and therefore the $DL_{NO}$ seems to be a better measure for the membrane diffusion capacity than the $DL_{CO}$. Because of the fact that the $DL_{NO}$ is independent of hemoglobin concentration, the $DL_{NO}/DL_{CO}$ ratio is high before the red cell transfusion and lowers afterwards.

***Chapter 7***

The use of the $DL_{NO}/DL_{CO}$ ratio in assessing the underlying defect in diffusing disturbances, is further explored in this study. Due to the dependence of the $DL_{CO}$ on capillary blood volume, the $DL_{NO}/DL_{CO}$ ratio should differ between subjects with thickened alveolocapillary membranes versus subjects with decreased pulmonary capillary blood volume. In case of thickened alveolocapillary membranes both $DL_{NO}$ and $DL_{CO}$ are decreased, and the $DL_{NO}/DL_{CO}$ ratio is normal. In case of decreased perfusion of ventilated alveoli the $DL_{NO}$ is less affected than the $DL_{CO}$, leading to a high $DL_{NO}/DL_{CO}$ ratio. We measured the combined $DL_{NO}$ and $DL_{CO}$ in 41 patients with DPLD and 26 patients with pulmonary arterial hypertension (PAH). In all subjects the $DL_{CO}$ was corrected for hemoglobin concentration. In the DPLD group a normal $DL_{NO}/DL_{CO}$ ratio was expected, while in the PAH group an increased ratio. In the DPLD as well as in the PAH group the $DL_{CO}$ was decreased, but the $DL_{NO}/DL_{CO}$ ratio was significantly higher in the PAH compared to the DPLD group or healthy subjects. Unfortunately,

the overlap in both groups was high, therefore the $DL_{NO}/DL_{CO}$ ratio can not be used as a screening test to discriminate between patients with DPLD and PAH. In all patients $Dm_{CO}$ and Vcap was measured with the 100% oxygen method. The $DL_{NO}$ and the $Dm_{CO}$ were highly correlated, as was expected.

### *Chapter 8*

The $DL_{NO}$ is a good measure for the membrane diffusion capacity, and emphysema is characterized by destruction of alveoli: therefore we determined the value of the $DL_{NO}$ in the early detection of emphysema. Spirometry, the combined $DL_{NO}$ and $DL_{CO}$ and a HRCT scan of the lungs were obtained in a population of heavy smokers, participating in a lung cancer screening study. Subjects with areas ≥1% of total volume showing attenuation below -950 HU were considered to have emphysema. A total of 263 subjects were included. In 36 subjects emphysema was present, a decreased $K_{NO}$ was present in 94 subjects, while 95 subjects had a $FEV_1/FVC$ ratio <70%. The $K_{NO}$ had a NPV of 98%, the PPV is low (35%), the NPV and PPV of the $DL_{NO}$ were comparable to that of the $K_{NO}$. The groups with a lowered $FEV_1/FVC$ or with a lowered $K_{NO}$ and the presence of emphysema on HRCT were only partially overlapping, indicating that smoking can induce different pathophysiological phenomena (obstruction and reduced gas-exchange). The $DL_{NO}/DL_{CO}$ ratio in all 263 subjects was significantly higher compared to normal subjects, which is consistent with a vascular based reduction of gas-exchange parameters.

### *Conclusion and view*

The single breath $DL_{CO}$ has earned its place in the diagnosis, prognosis and therapy of different pulmonary diseases. The $DL_{CO}$ consists of a membrane and a vascular component and it is therefore often hard to interpret. The interpretation of the $K_{CO}$ is even more difficult, especially when a restriction is present. Due to its single breath nature, the $DL_{CO}$ measures only easy accessible lung parts, therefore in COPD only the non-emphysematous lung volume is measured. The $DL_{NO}$ is independent of hemoglobin concentration, and independent on capillary blood volume. Therefore the $DL_{NO}$ is a better index for the function of the alveolocapillary membrane than the $DL_{CO}$. The $DL_{NO}/DL_{CO}$ ratio can point to the location of the decreased diffusing

capacity, and is a good candidate to replace the $Dm_{CO}$ and Vcap values determined with the 100% oxygen method. The fact that these values are derived from two or more separate breath holding periods makes the less accurate. Furthermore, the exact value of the $\theta_{CO}$ value is not well-known, therefore the $Dm_{CO}$ will always be an educated guess. The $K_{NO}$ is independent of $V_A$, and therefore is a better index for the diffusion capacity per unit lung volume than the $K_{CO}$.
In subjects with COPD, HRCT scanning, spirometry and the diffusion capacity are complementary approaches, and measure different pathophysiological entities. In heavy smokers an abnormal diffusion capacity, an abnormal spirometry and abnormal HRCT scan are frequently seen. These abnormalities are partially overlapping, therefore extensive phenotyping of COPD compasses more than only spirometry. When mass screening for COPD is performed using only spirometry, pathology will be missed. In heavy smokers the $DL_{NO}/DL_{CO}$ ratio is high, which is indicative for microvascular damage, which is often not visible on HRCT scan and not accompanied by abnormal spirometry.

Future research could be focused on the further development of the $DL_{NO}$ and $K_{NO}$. One of the first items could be a technical improvement, which could lead to a new tool in the pulmonary function laboratory, with greater efficiency in pointing to the cause of impaired gas exchange. If the $DL_{NO}/DL_{CO}$ ratio could be measured during exercise, new insights could be provided. For example, the diagnosis of pulmonary hypertension during exercise could be made easier. The development of chronic thromboembolic pulmonary hypertension after pulmonary embolism is a very grave disease; it is worthwhile to investigate whether the $DL_{NO}/DL_{CO}$ ratio can serve as a screening tool for this disease. Vascular changes in COPD can be assessed with this method, this is of particular interest considering the development of new vasoactive drugs. To answer the question whether vascular changes precede airway obstruction in COPD further research is necessary. After all, any test that can learn us more about pulmonary microvascularity and the functioning of the alveolocapillary membrane could be of great value.

# Samenvatting

De meeste patiënten die gezien worden op het spreekuur van de longarts hebben klachten van kortademigheid, al dan niet bij inspanning, met hoesten en/of slijm opgeven. Om vast te kunnen stellen wat de oorzaak van de klachten is, is bijna altijd verder onderzoek noodzakelijk. Longfunctieonderzoek is het belangrijkste onderzoeksinstrument. Het meest eenvoudige longfunctieonderzoek wordt spirometrie genoemd. De belangrijkste parameters zijn de vitale capaciteit, dat is de maximale hoeveelheid gas, die na een langzame volledige expiratie (=uitademing) ingeademd kan worden, en de één seconde waarde, in het Engels de forced expiratory volume in one second ($FEV_1$), dit is de maximale hoeveelheid gas die na maximale inspiratie (=inademing) kan worden uitgeademd in de eerste seconde na het begin van de uitademing. De $FEV_1$ is bijvoorbeeld verlaagd bij patiënten met astma of COPD (chronic obstructive pulmonary disease). Na maximale uitademing blijft er nog een residu volume achter in de longen. De totale longinhoud is gedefinieerd als de vitale capaciteit plus het residu volume, deze kan alleen op een indirecte manier gemeten worden. De meest gebruikelijke manier is de heliumverdunningsmethode: na maximale uitademing wordt een gasmengsel met een bekende concentratie van helium ingeademd. Helium is een fysiologisch inert gas is: er wordt niets van opgenomen in het lichaam. Na volledige inademing volgt na 10 seconden de maximale uitademing, de vitale capaciteit wordt bepaald, de concentratie van helium in het einde van de uitademing wordt gemeten, want die is dan gelijk aan de heliumconcentratie in de alveoli (longblaasjes). Aangezien de heliumfractie in het inspiratiemengsel vermenigvuldigt met het geinspireerde volume gelijk is aan de heliumfractie in het expiratiemengsel vermenigvuldigt met de totale longcapaciteit, is de laatste parameter eenvoudig te berekenen. Het aldus verkregen totale longvolume wordt meestal alveolair gasvolume genoemd, afgekort tot $V_A$.
De primaire taak van de long is gaswisseling, dat is de opname van $O_2$ (zuurstof) en de afgifte van $CO_2$ (kooldioxide). De opname van $O_2$ hangt af van een aantal factoren: ten eerste moet de ingeademende lucht in voldoende mate in de alveoli komen: daarover geeft het meten van de grootte van de longinhoud en snelheid van in- en uitademen

voldoende informatie. Verder hangt het af van de totale oppervlakte van alle alveoli tezamen, normaal ongeveer 80 $m^2$. Om de alveoli ligt een fijnmazig netwerk van kleine bloedvaatjes gespannen, capillairen genaamd, waarin de rode bloedcellen zich bevinden met daarin het hemoglobine, waaraan het $O_2$ bindt om vervolgens afgegeven te worden aan de weefsels in het lichaam. De dikte van de membraan tussen de alveoli en de capillairen, de alveolocapillaire membraan, is natuurlijk ook een limiterende factor voor $O_2$ opname. Verder is de hemoglobine concentratie van het bloed van belang: indien er sprake is van anaemie (bloedarmoede) kan het bloed minder $O_2$ opnemen en transporteren. Helaas is het om technische redenen niet mogelijk om de zuurstofopnamecapaciteit te meten op een eenvoudige wijze. Daarom wordt in het longfunctielaboratorium de opnamecapaciteit van koolmonoxide (CO) gemeten, wat een goede afgeleide is van de $O_2$-opname. Omdat het diffunderen van een gas door de alveolocapillaire membraan als de meest essentiële stap in de opname van een gas werd gezien, wordt deze meting meestal de diffusiecapaciteit voor CO genoemd, afgekort tot $DL_{CO}$. Deze wordt gedefinieerd als de hoeveelheid gas die wordt opgenomen door de capillairen vanuit de alveoli, per tijdseenheid en eenheid drukverschil. De procedure is in wezen eenvoudig: de patiënt ademt na maximale expiratie een bekend mengsel van CO (in een lage ongevaarlijke concentratie), helium en lucht in, na maximale inspiratie wordt de adem 10 seconden vastgehouden (apneu), waarna een snelle expiratie volgt. In het laatste gedeelte van het expiraat worden de concentraties gemeten van CO en helium, het $V_A$ kan bepaald worden volgens de boven beschreven methode. Tijdens de apneu daalt de CO concentratie exponentieel. De $DL_{CO}$ wordt gedefinieerd als de logaritme van de deling tussen de fractionele CO concentratie aan het begin en aan het einde van de apneu, vermenigvuldigt met het $V_A$ gedeeld door de gepasseerde tijd, en wordt uitgedrukt in mmol per minuut per kPa. De $DL_{CO}$ wordt gecorrigeerd voor de hemoglobine concentratie.
De op deze wijze verkregen waarde van de $DL_{CO}$ is een klinisch zeer nuttig getal, welke een belangrijk middel is om enerzijds tot een diagnose te komen, en anderzijds een prognose te schatten. Ter illustratie geef ik enkele voorbeelden: bij emfyseem is het aantal alveoli sterk verminderd, dus het totale oppervlak van de alveolocapillaire membraan is verkleind, waardoor de $DL_{CO}$ ook verlaagd is. Indien de $DL_{CO}$ meer dan 50% lager is dan de voorspelde

waarde (deze is afhankelijk van het geslacht, de lengte en de leeftijd van de patiënt) is er meestal sprake van een ernstig falen van de gaswisseling, waarbij de $O_2$-verzadiging van het bloed daalt bij geringe inspanning. Bij sommige ziekten waarbij de alveolocapillaire membraan verdikt is, zoals longfibrose, zien we ook een verlaging van de $DL_{CO}$. Bij longfibrose heeft de $DL_{CO}$ een belangrijke waarde om het effect van therapie in te kunnen schatten. Het is logisch dat mensen met kleinere longen ook een lagere $DL_{CO}$ hebben. In de dagelijkse praktijk ziet de longarts frequent patiënten met een kleinere longinhoud op basis van ziekte. Het is dan erg moeilijk om te bepalen of de verlaagde $DL_{CO}$ samenhangt met de verlaagde longinhoud sec of met een functionele beperking (dus een afwijking aan de alveolocapillaire membraan). Vandaar dat de longarts graag een waarde heeft van de $DL_{CO}$ gecorrigeerd voor het longvolume. Hiervoor wordt de term $DL_{CO}/V_A$ ook wel gebruikt, deze wordt ook wel $K_{CO}$ genoemd, waar de K afkomstig is van Krogh, een van de pioniers op dit gebied. Er is sprake van een probleem bij het maken van normaalwaarden voor de $K_{CO}$, omdat doorgaans gezonde mensen nodig zijn voor het maken van normaalwaarden. Bij een gezond persoon die vrijwillig niet maximaal inademt tijdens de $DL_{CO}$ procedure, stijgt de $K_{CO}$ bij een afname van de longcapaciteit! Dit komt doordat er relatief meer bloed in de long blijft staan ten opzichte van het longvolume. Hoewel men dit fenomeen ook ziet bij patiënten met kleine longen ten gevolge van ziekte blijft het erg moeilijk zo niet onmogelijk om een zekere uitspraak te doen of een verlaagde $K_{CO}$ komt door ziekelijk verkleinde longen of kleine longen zonder ziekte. Daardoor is de interpretatie van de $K_{CO}$ om het zacht uit te drukken een lastige zaak, omdat bij patiënten met bijvoorbeeld longfibrose de $K_{CO}$ van gezonde mensen eigenlijk niet goed te gebruiken is als referentiewaarde. Dit geeft direct ook aan waar de $DL_{CO}$ en de $K_{CO}$ afhankelijk van zijn: de longgrootte, membraanfactoren (dikte, oppervlak) en de mate van doorbloeding van de longblaasjes. In feite is de laatste factor terug te brengen op de mate van afstemming van bloeddoorstroming en ventilatie (“gasdoorstroming”) van de longen als geheel.

Al tientallen jaren is er methode in gebruik om de diffusiecapaciteit van de longen verder te onderzoeken. Om deze methode inzichtelijk te maken is verdere uitleg noodzakelijk. De opname van CO kan in feite

gezien worden als twee in serie geschakelde weerstanden: ten eerste de weerstand ten gevolge van het transport over de alveolocapillaire membraan, en ten tweede de weerstand ten gevolge van de binding aan het hemoglobine. De diffusiecapaciteit is een maat voor de conductantie, dus de reciproke waarde van de weerstand. De diffusiecapaciteit wordt dan ook weergegeven door de volgende formule:

$$\frac{1}{DL_{CO}} = \frac{1}{Dm_{CO}} + \frac{1}{\theta_{CO} * Vcap}$$

Hierin staat de $Dm_{CO}$ voor de diffusiecapaciteit van de alveolocapillaire membraan, dus $1/Dm_{CO}$ is de weerstand over de membraan, $\theta_{CO}$ staat voor de reactiesnelheid van CO met hemoglobine, Vcap is het capillaire bloedvolume, dus $1/\theta_{CO}$*Vcap kan beschouwd worden als de capillaire weerstand. De maat $\theta_{CO}$ is afhankelijk van de $O_2$-concentratie, de waarde is bekend vanuit onderzoek *in vitro* (in laboratorium omstandigheden). Indien men de diffusie capaciteit van CO tweemaal na elkaar meet, bij een lage en bij een hoge $O_2$ concentratie, krijgt men twee formules met twee onbekenden, na oplossing krijgt men een waarde van de $Dm_{CO}$ en de Vcap. Deze methode is niet op grote schaal in gebruik gekomen, om een aantal redenen. Ten eerste is de meting arbeidsintensief, en neemt zeker 30 minuten in beslag. Ten tweede wordt er in twee tempi gemeten, waarbij men zich voor kan stellen dat de omstandigheden bij die twee metingen niet volkomen identiek zijn (de mate van inspiratie kan kleine verschillen vertonen). Dit kan de uitkomst beïnvloeden. Verder is het zo dat $\theta_{CO}$ geschat wordt, omdat alleen de *in vitro* waarden bekend zijn.

Sinds 15 jaar hebben enkele onderzoekers stikstofmonoxide (nitric oxide, ofwel NO) gebruikt als testgas voor het meten van de diffusiecapaciteit. Het voordeel van NO boven CO is dat NO veel sterker bindt aan het hemoglobine, dus $\theta_{NO}$ is zeer hoog, waardoor gesteld werd dat de factor $1/\theta_{NO}$*Vcap verwaarloosbaar klein is. Dit betekent dus dat de opname van NO niet beperkt wordt door het capillaire bloedvolume of door het hemoglobine gehalte, en dus dat de diffusiecapaciteit van NO ($DL_{NO}$) gelijk is aan de diffusiecapaciteit van de alveolaire membraan. Het feit dat de $DL_{NO}$ een betere maat

voor de functie van de alveolocapillaire membraan is dan de veel gebruikte $DL_{CO}$, heeft er desondanks niet toe geleid dat de $DL_{NO}$ ingebed werd in het longfunctielaboratorium. Een mogelijke reden is dat de eerste NO-analysers ongevoelig waren, moeilijk in gebruik en erg duur. De laatste jaren zijn die punten sterk verbeterd, desondanks zijn er nog geen commercieel verkrijgbare $DL_{NO}$ apparaten op de markt.

In dit proefschrift worden twee zaken onder de loupe genomen: ten eerste is diepgravend en kritisch onderzoek verricht naar enkele aspecten van de $DL_{CO}$, verder is onderzoek verricht naar de waarde en de betekenis van de $DL_{NO}$. Hiertoe moest natuurlijk eerst een apparaat ontwikkeld worden wat in staat was om de $DL_{NO}$ te meten. Dit is in het Sint Antonius Ziekenhuis te Nieuwegein gelukt. Hiertoe werd een bestaande NO-analyser aangepast. Dit apparaat werd gebruikt voor het meten van NO in uitademingslucht, wat een marker is voor astma. De werking berust op chemolumeniscentie, in een vacuümkamer reageert NO met ozon tot $O_2$ en $NO_2$, onder afgifte van een foton, die gemeten wordt. Dit is een zeer snel reagerend en gevoelig apparaat, wat continue meet en daardoor een continue datastroom genereert met de gemeten NO-concentratie. Dit apparaat werd zodanig aangepast dat het aan een bestaand $DL_{CO}$-apparaat gekoppeld kon worden. Aanpassingen waren noodzakelijk om een kleppensysteem aan te sturen zodat op het juiste moment de NO concentratie in het inspiratiemengsel en in de uitgeademde lucht gemeten kon worden. Deze lastige klus werd tot een goed einde gebracht door de technische dienst van het Sint Antonius ziekenhuis. Het resulteerde in een eenvoudig te bedienen apparaat. Na enkele weken van metingen bleek verder dat de $DL_{NO}$ waarden reproduceerbaar waren, en goed vergelijkbaar met eerdere onderzoeken. Het onderzoek kon beginnen!

In Hoofdstuk 1 van dit proefschrift worden de fysiologische processen die betrokken zijn bij gaswisseling beschreven, en wordt zeer uitvoerig ingegaan op eerder verricht onderzoek naar de diffusiecapaciteit van CO en NO, met speciale aandacht voor de klinische waarde van de diffusiecapaciteit en de subdivisies ervan. Er worden voorbeelden gegeven van de verwachte waarde van de $DL_{CO}$, de $K_{CO}$ en het $V_A$ bij enkele specifieke ziektebeelden. Bij het literatuuronderzoek wat hieraan voorafging bleek dat deze verwachte waardes voornamelijk

gebaseerd waren op de opinies van enkele experts, beschreven in enkele artikelen en leerboeken. Er bleek geen onderzoek voorhanden wat de waarde van de $K_{CO}$ naast de $DL_{CO}$ in het diagnosticeren van ziekten onderzocht. Dat dit onderzoek op theoretische gronden wel zeer relevant is blijkt uit de eerder genoemde sterke afhankelijkheid van de $K_{CO}$ en de $DL_{CO}$ van het alveolaire volume. In Hoofdstuk 2 worden de uitkomsten beschreven van een retrospectieve studie in 460 opeenvolgende patiënten die gepresenteerd werden op de polikliniek longziekten van het Academisch Ziekenhuis te Utrecht. De uiteindelijke diagnose wordt vergeleken met het patroon van de diffusieafwijking. De diagnose bestond uit astma, COPD, haematologische maligniteiten, hartfalen en een groep met zogenaamde interstitiële longziekten (Engels diffuse parenchymal lung diseases, DPLD). De diffusie afwijking werd ingedeeld in logische, fysiologisch goed te onderscheiden patronen, zoals beschreven in de leerboeken. Hiervoor werden de $DL_{CO}$, de $K_{CO}$ en de ratio $V_A$/TLC gebruikt. De TLC is de totale longcapaciteit gemeten met plethysmografie, die exact de totale longinhoud meet, ongeacht of die inhoud deelneemt aan de ventilatie of gaswisseling. De ratio $V_A$/TLC is dus een maat voor ventilatie ongelijkmatigheid. In dit onderzoek bleek dat het patroon van diffusie afwijking zeer slecht correleerde met de diagnose. Verder bleek dat voor het stellen van de diagnose de $K_{CO}$ geen meerwaarde had naast de $DL_{CO}$. Met name werd een slechte relatie gezien tussen de verwachte diagnose en het patroon van de diffusie afwijking bij patiënten met DPLD. De conclusie is dat in de dagelijkse praktijk het patroon van gestoorde diffusie in diagnostiseren van deze longziekten beperkt is.
Hoofdstuk 3 beschrijft een studie naar het alveolaire volume ($V_A$), een belangrijk onderdeel van de bepaling van de diffusiecapaciteit. Het $V_A$ wordt bepaald met de heliumverdunnings methode, tijdens één ademteug. Hierdoor is deze bepaling erg gevoelig voor ongelijkmatigheden in de ventilatie. COPD bestaat uit emfyseem (destructie van alveoli) en irreversibele verandering van de luchtwegen. Emfysemateuze longgedeelten zijn minder toegankelijk voor testgas, daarom is het $V_A$ lager dan de totale longcapaciteit bepaalt met heliuminwas methode ($TLC_{He}$). Bij deze methode wordt helium gedurende langere tijd ingeademd, en wel net zo lang totdat het helium ook gelijkmatig verspreid is in minder toegankelijker longgedeelten, zoals bij emfyseem. Het doel van het onderzoek was

om de relatie tussen het $V_A$ en het niet-emfysemateuze longgedeelte te bepalen. Emfyseem werd gedefinieerd als een weefseldichtheid van de long van minder dan -950 Hounsfield Units (HU) op een high resolution computed tomography (HRCT) scan. De onderzoekspopulatie bestond uit 50 patiënten met COPD. Er bleek een sterke correlatie tussen het $V_A$ (gemiddeld 5.2 L) enerzijds en het niet-emfysemateuze longgedeelte op de HRCT scan (gemiddeld 5.2 L) anderzijds te bestaan. Het totale longvolume bepaald op de HRCT scan (gemiddeld 6.4 L) correleerde goed met de $TLC_{He}$ (gemiddeld 6.6 L). Er werden Bland-Altman plots vervaardigd, hierbij wordt het verschil tussen de waarden in een plot weergegeven tegen de gemiddelden van de waarden, dit is een goede manier om twee meetmethodes met elkaar te vergelijken. Deze Bland-Altman plots laten een aanzienlijke discrepantie zien tussen de twee methodes, die naar alle waarschijnlijkheid samenhangen met technische factoren; een HRCT scan wordt namelijk liggend gemaakt, er wordt niet gecontroleerd of de proefpersonen wel daadwerkelijk maximaal inademen, longfunctieonderzoek wordt zittend verricht, en er wordt wel degelijk een maximale in- en uitademing bewerkstelligd. De conclusie van dit onderzoek is dat het $V_A$ een goede maat is voor het niet-emfysemateuze longgedeelte. Dit is van groot belang bij de interpretatie van de diffusiecapaciteit: als immers het helium zich ongelijkmatig verdeeld over de longen zal het CO dat ook doen, daardoor doet de $DL_{CO}$ alleen een uitspraak over goed toegankelijke longgedeelten. Emfysemateuze gebieden in de long zijn minder toegankelijk voor de $DL_{CO}$ meting, over deze gebieden kan men dus met de $DL_{CO}$ meting geen uitspraak doen.

In Hoofdstuk 4 wordt de prevalentie en uitgebreidheid van emfyseem in een groot cohort van zware rokers of ex-rokers bepaald, door middel van het meten van de dichtheid van longweefsel op de HRCT scan. In eerder onderzoek is meestal in populaties van bekende longpatiënten (meestal patiënten onder controle wegens COPD) een goede correlatie gezien tussen de mate van weefseldichtheid van de long versus de $DL_{CO}$, de $K_{CO}$ en de spirometrische waarden voor de ernst van COPD (de $FEV_1$ en de $FEV_1$/FVC waarde). De gemeten populatie is onderdeel van een screeningsonderzoek naar de waarde van de HRCT scan bij de vroege detectie van longkanker. In totaal werden 545 mannelijke deelnemers (rokers of ex-rokers) geïncludeerd, op de

HRCT scan werd bij 258 mannen mild emfyseem (gedefinieerd als meer dan 10% longweefsel met een dichtheid kleiner dan -910 HU) gevonden, en bij 45 mannen ernstig emfyseem (gedefinieerd als meer dan 2% longweefsel met een dichtheid kleiner dan -950 HU). De correlatie tussen de weefseldichtheidsmeting en de longfunctie parameters is matig te noemen: 14% van de mannen met mild emfyseem en 13% van de mannen met ernstig emfyseem had een verlaagde $FEV_1$, 37% van de mannen met mild emfyseem en 75% van de mannen met ernstig emfyseem had een verlaagde $DL_{CO}$ of $K_{CO}$. Veel personen met afwijkende longfunctiemetingen hadden een normale weefseldichtheidsmeting. Dit leverde natuurlijk zwakke correlatie coëfficiënten op tussen de weefseldichtheidsmeting en de verschillende longfunctiemetingen, deze correlatie verbeterde echter sterk door alleen patiënten met ernstig COPD op basis van de $FEV_1$ waarde te includeren. De aldus verkregen correlatie coëfficiënten waren vergelijkbaar met die van eerdere onderzoeken. Dit suggereert een grote populatiebias in dit onderzoek. Het grote aantal personen met afwijkende longfunctiemetingen bij een normale HRCT scan suggereert dat veel schade aan de long gemist wordt met de weefseldichtheidsmeting. Het lijkt erop dat de HRCT scan enerzijds en longfunctiemeting anderzijds verschillende aspecten meten van roken-geïnduceerde longschade, ze zijn dus complementaire onderzoeken.

Het tweede deel van het proefschrift heeft de NO diffusiecapaciteit als onderwerp. In Hoofdstuk 5 worden in een groep van 124 gezonde vrijwilligers normaalwaarden bepaald. Er werden regressievergelijkingen opgesteld, met geslacht, leeftijd en lengte als variabelen. De regressievergelijking voor de $DL_{CO}$ was zeer goed compatibel met de veel gebruikte ECCS regressievergelijking, wat er op wijst dat de onderzochte groep vergelijkbaar is met eerdere onderzochte groepen. In een groep van 21 vrijwilligers werd het effect van inspiratieniveau op de diffusie meting onderzocht. De $DL_{CO}$ daalde en de $K_{CO}$ steeg bij afnemende $V_A$, zoals verwacht, en met dezelfde mate als eerder beschreven. De $DL_{NO}$ bleek zeer sterk te dalen bij afnemende $V_A$, zoals ook eerder beschreven. De $K_{NO}$ bleek nauwelijks te veranderen bij afnemende $V_A$. Regressievergelijkingen betreffende de relatie tussen de $DL_{NO}$ en de $K_{NO}$ enerzijds en het $V_A$ anderzijds werden opgesteld. Het feit dat de $K_{NO}$ niet of nauwelijks wordt bepaald door het capillaire bloedvolume is de meest voor de hand liggende

verklaring voor het feit dat de $K_{NO}$ onafhankelijk is van de mate van inspiratie, dit in tegenstelling met de $K_{CO}$, die sterk stijgt door de relatieve toename van bloedvolume ten opzichte van het oppervlak van de alveolocapillaire membraan. De afhankelijkheid van de $DL_{NO}$ van het hemoglobine gehalte wordt onderzocht in Hoofdstuk 6. Daartoe wordt in 10 patiënten die opgenomen waren voor het ondergaan van een bloedtransfusie de diffusiecapaciteit voor CO en NO gemeten, vlak voor en vlak na de transfusie. De $DL_{CO}$ (en $K_{CO}$) steeg mee met de stijging van de hemoglobine concentratie zoals voorspeld kon worden vanuit de bekende referentievergelijking. De $DL_{NO}$ en $K_{NO}$ waren voor en na de bloedtransfusie onveranderd, zoals op theoretische gronden al verondersteld werd.
In Hoofdstuk 7 wordt de klinische waarde van de $DL_{NO}$ verder onderzocht. Omdat de $DL_{NO}$ onafhankelijk is van het capillaire bloedvolume, in tegenstelling tot de $DL_{CO}$, zal de $DL_{NO}/DL_{CO}$ ratio verschillen tussen de diverse longaandoeningen. Bij patiënten met een verdikte alveolocapillaire membraan, zoals dat archetypisch gezien wordt bij patiënten met DPLD, zal zowel de $DL_{NO}$ als de $DL_{CO}$ aangedaan zijn, en dus de $DL_{NO}/DL_{CO}$ ratio normaal zijn. Indien er sprake is van een relatieve vermindering van doorbloeding van longweefsel (bij pulmonale hypertensie), zal de $DL_{CO}$ verlaagd zijn, en de $DL_{NO}$ niet, dus de $DL_{NO}/DL_{CO}$ ratio zal dan verhoogd moeten zijn. Op deze manier zou onderscheid gemaakt kunnen worden tussen verschillende oorzaken van een verlaagde diffusie meting. De $DL_{NO}/DL_{CO}$ ratio werd gemeten in 41 patiënten met DPLD, 26 patiënten met pulmonale hypertensie en deze werd vergeleken met 71 gezonde vrijwilligers. Bij alle patiënten werden tevens de klassieke $Dm_{CO}$ en de Vcap met behulp van de 100% $O_2$ methode gemeten. De $DL_{NO}/DL_{CO}$ ratio bleek bij de patiënten met pulmonale hypertensie significant hoger te zijn dan bij de groep DPLD patiënten en bij de groep gezonde vrijwilligers. De relatie tussen de $Dm_{CO}$ en de $DL_{NO}$ was zeer groot, zoals verwacht was. De spreiding van de $DL_{NO}/DL_{CO}$ ratio was in alle groepen hoog, waardoor er een flinke overlap tussen de groepen bestond, die ervoor zorgt dat de $DL_{NO}/DL_{CO}$ ratio vooralsnog niet gebruikt kan worden op individueel niveau.
Hoofdstuk 8 beziet de waarde van de $DL_{NO}$, $K_{NO}$ en de $DL_{NO}/DL_{CO}$ ratio in de diagnostiek van COPD. Hiertoe is wederom gebruikt gemaakt van de populatie van het screeningsonderzoek naar de vroegdiagnostiek naar longkanker m.b.v. de HRCT scan. Naast de

diffusie parameters werd spirometrie gemeten bij 263 proefpersonen, alle zware (ex)rokers. Emfyseem werd gedefinieerd als ≥1% longvolume met een weefseldichtheid minder dan -950 HU. Met dit afkappunt hadden 36 personen emfyseem. De $K_{NO}$ bleek een hoge negatief voorspellende waarde te hebben voor CT-gebaseerd emfyseem, dus is zeer bruikbaar om emfyseem uit te sluiten. Van de totale groep van 263 personen bleken 95 een $FEV_1$/FVC ratio lager dan 70% te hebben, wat een spirometrische maat is voor de diagnose van COPD. De $K_{NO}$ bleek een beter diagnosticum voor emfyseem te zijn dan de $FEV_1$/FVC of de $DL_{CO}$. De $DL_{NO}/DL_{CO}$ ratio was in de totale groep proefpersonen significant hoger dan in gezonde vrijwilligers, en kwam in de buurt van de waarde zoals die gezien werd bij patiënten met pulmonale hypertensie. Dit suggereert dat vasculaire problematiek een belangrijke rol speelt bij zware rokers, die al dan niet COPD hebben op spirometrische gronden. De $FEV_1$/FVC ratio, de $K_{NO}$ en CT-gebaseerd emfyseem zijn parameters die verschillende delen van het spectrum van afwijkingen aan de longen ten gevolge van roken aangegeven. Deze factoren zijn maar deels overlappend. Dit houdt in dat een strenge definitie van COPD louter op spirometrische gronden een beperking van het blikveld wat betreft de pathologie inhoudt. Afwijkingen aan de capillairen, die gesuggereerd worden door de verhoogde $DL_{NO}/DL_{CO}$ ratio, zijn een onderbelichte factor in het spectrum van roken geïnduceerde longschade, waartoe wellicht ook COPD te rekenen valt.

### *Conclusie*

De $DL_{NO}$ is een betere maat voor de functie van de alveolocapillaire membraan dan de $DL_{CO}$, omdat de $DL_{NO}$ onafhankelijk is van de hemoglobine concentratie en het pulmonale capillaire bloedvolume. De $K_{NO}$ lijkt eenvoudiger te interpreteren dan de $K_{CO}$, en is wellicht daardoor beter bruikbaar bij patiënten met restrictieve longfunctie stoornissen. De $DL_{NO}/DL_{CO}$ ratio kan richting geven aan de oorzaak van een verlaging van de diffusiecapaciteit, en is eenvoudiger en betrouwbaarder te meten dan de $Dm_{CO}$ en Vcap met de 100% zuurstof methode. Het is van belang de beperking van de diffusie capaciteitsmeting te kennen: alleen goed toegankelijke longgedeelten worden gemeten. Dat betekent dat bij patiënten met COPD ernstig bulleus gedegenereerde gebieden functioneel inactief zijn. De HRCT

scan bij deze patiënten groep laat maar een beperkt deel van de pathologie zien, zowel spirometrie als de diffusiecapaciteit hebben complementaire waarde.

Het moge duidelijk zijn dat er meer dan genoeg aanknopingspunten zijn voor verder onderzoek. In eerste instantie zijn technische verbeteringen wenselijk, met name het meten met een iets hogere inspiratoire NO concentratie kan de overigens zeer kleine verstoring door de endogene NO productie opheffen. Verder onderzoek naar de $K_{NO}$ bij meerdere groepen patiënten met restrictieve longfunctie stoornissen is noodzakelijk. De $DL_{NO}/DL_{CO}$ ratio verdient verder onderzoek, en kan wellicht (na technische verbeteringen) een duidelijke plaats krijgen in het longfunctielaboratorium. Specifieke aandachtpunten kunnen zijn de aanwezigheid van vasculaire stoornissen bij patiënten met COPD, en vroegdiagnostiek naar de ontwikkeling van pulmonale hypertensie na doorgemaakte longembolieën.

# Dankwoord

Het tot een succesvol einde brengen van promotieonderzoek vergt naast flink wat doorzettingsvermogen vooral veel hulp van anderen.

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Dr. P. Zanen, de co-promotor, is de initiator geweest van dit onderzoek. Pieter, in eerste instantie hebben we samen onderzoek verricht naar NO in de uitademingslucht. In snel tempo hebben we een aantal studie protocollen geschreven. In jouw optimistische wereldbeeld was het boekje eigenlijk al klaar. In mijn vooropleidingstijd van de interne geneeskunde bleken andere onderzoeksgroepen net zo slim maar wat sneller te zijn: "onze" studies verschenen in fraaie tijdschriften. In snel tempo ontwikkelde je een nieuw concept: het meten van de NO-diffusie capaciteit. Lovenswaardig is je eigenschap om bij tegenvallers direct met praktische oplossingen te komen, waardoor het onderzoekstempo toch redelijk hoog bleef. Als ik eens dreigde teveel te verzanden in details, trok je me uit het drijfzand omhoog. Zonder jou initiatief was dit onderzoek niet opgestart en niet afgerond.

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Lieve Marike, het afgelopen jaar is erg zwaar geweest, we zijn van Utrecht naar Haarlem verhuisd, in het nieuwe huis liepen meer

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# Curriculum vitae

De auteur van dit proefschrift werd geboren op 3 augustus 1967 te Herten. De middelbare school werd gevolgd op de Rijksscholengemeenschap te Amersfoort. De studie geneeskunde werd gevolgd aan de Universiteit van Amsterdam, het artsexamen werd in 1984 behaald. De militaire diensttijd werd verricht in het Centraal Militair Hospitaal te Utrecht. Na 1 jaar agnio-schap op de interne geneeskunde van het Sint Antonius Ziekenhuis te Nieuwegein (opleider dr. H.C.M. Haanen), volgde in 1998 de overstap naar de longziekten. In 1999 werd gestart met de vooropleiding interne geneeskunde in het Meander Ziekenhuis te Amersfoort (opleider dr. A. van de Wiel). Van 2001 tot 2004 werd de opleiding Longziekten in het Sint Antonius Ziekenhuis te Nieuwegein gevolgd (opleider prof. dr. J.M.M. van den Bosch). Het in dit proefschrift beschreven onderzoek werd gestart in 2001, en afgerond in 2005. De auteur heeft een half jaar part-time gewerkt in het MESOS Oudenrijn als chef-de-clinique. Vanaf april 2005 werkt hij als longarts in het Spaarne Ziekenhuis te Hoofddorp. Hij is getrouwd met Marike Jellema, samen hebben ze drie kinderen: Laurien, Maaike en Bart.