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## Effect of Reduction of Breath-Holding Time In The Single-Breath Method To Assess Carbon Monoxide Diffusing Capacity

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**Effect of reduction of breath-holding time in  
the single-breath method to assess carbon  
monoxide diffusing capacity**

**By**

**Elsa Magro**

**A thesis presented to The School of Physics**

**Dublin Institute of Technology**

**For the Degree of Master of Philosophy**

**June 2012**

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Mercy University Hospital, Cork

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## Abstract

Measurement of the diffusing capacity of the lung is part of the routine pulmonary function assessment in every patient and the single breath method is the most commonly used method. This method requires a subject to inspire a gas mixture followed by a 10 second (s) breath-hold. However dyspnoea may preclude measurement in patients with advanced pulmonary disease. We sought to determine if breath-hold time reduction had a significant effect on measured DLco values.

Forced spirometry and CO-diffusion by the single breath method were performed by a respiratory scientist with a **Jaeger master-screen PFT** equipment, in duplicate with breath-holding of 10 , 8 and 6 s in 30 control subjects (FEV<sub>1</sub> 107 ± 12.04% predicted), 30 severe COPD patients (FEV<sub>1</sub> 37.2 ± 7.92% predicted), and 30 patients with interstitial lung disease (ILD) (FEV<sub>1</sub> 69.5 ± 17.61% predicted).

There was no significant difference between DLcoSB and KCO measured at 10, 8 and 6 s in the control group (p=0.4431) and ILD group (p=0.5915). However, there was a significant difference between DLcoSB (p=0.0003) and DLco (VA) (p=0.0183) measured at 10, 8 and 6 s in the COPD group. In the presence of severe airway obstruction the DLco decreases with breath-hold time reduction.

In the control group and patients with ILD, there was no significant change in the DLcoSB when breath-hold time was reduced from 10 to 6 s. This could allow a reduction in breath-hold time when measuring the DLco in patients with advanced ILD who are unable to breath-hold for 10 s, but not in patients with severe COPD.

## Declaration

I certify that this thesis which I now submit for the award of Master of Philosophy, is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

This thesis was prepared according to the regulations for post graduate study by research of the Dublin Institute of Technology and has not been submitted in whole or in part for an award in any other Institute or University.

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Candidate

## **Acknowledgments**

It would not have been possible to conclude this paper without the support of all the people around me. Above all, I would like to thank my parents, my partner and closest friends for their personal support and great patience at all times. This thesis would not have been possible without the help and support and advice of my supervisors, Dr. Terence O'Connor MD, and Prof. Pat Goodman. I am extremely grateful to all the volunteers that made this research project possible and to all my colleagues of the Mercy University Hospital, for all the help and support.

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## Definitions

Anti-cholinergic: antagonist of acetylcholine drug and other muscarinic agonists. It blocks the muscarinic action of acetylcholine at receptors on postsynaptic membranes, and inhibits the response by postganglionic parasympathetic nerve activation. (Gross, 1993)

Asthma: pulmonary obstructive disease with increased responsiveness of the airways to different stimuli which produces an inflammation and widespread narrowing of the airways. It changes in severity, either spontaneously or as a result of treatment. (West, 2008b)

B<sub>2</sub>Agonist: bronchodilator drug that combines with  $\beta_2$  receptors in the lung and relax the smooth airway muscle by increasing the adenyl cyclase activity. (West, 2008b)

Bronchitis: pulmonary obstructive disease with typical excessive mucus production in the bronchial tree and sputum expectoration. (West, 2008b)

Bronchodilators: drugs that reverse or prevent the broncho-constriction of the lung. (West, 2008b)

Calibration: procedure that allows establishing the relationship between sensor-determined values of flow or volume and the actual flow or volume. (Brusasco *et al.*, 2005)



CD4 helper phenotype: helper T cell with glycoprotein CD4 on the surface and therefore, called T4 cells. (Ganong, 1995)

Capacity: composed of two or more volumes. (West, 2008a)

Cystic Fibrosis: a genetic disease affecting the function of all exocrine glands with abnormal chloride and sodium transport. (West, 2008b)

Diffusion: process by which a gas or a substance in solution expands, because of the motion of its particles into or out of an area in which it is present in high concentration, to fill all of the available volume. (Ganong, 1995)

Dyspnoea: difficulty with breathing sensation. (West, 2008b)

Emphysema: pulmonary obstructive disorder with typical enlargement of the air spaces distal to the terminal bronchiole and destruction of their walls. (West, 2008b)

Fibroblasts: synthesizer cell of collagen and elastine, which are the main components of the interstitium in the alveolar wall.

Fick's law: "The amount of gas that moves across a sheet of tissue is proportional to the area of the sheet but inversely proportional to its thickness". West (2008a)

Hypoxemia: deficiency of O<sub>2</sub> at the body tissue level. (Ganong, 1995)

Inflammatory response: localized and complex response to a foreign substance or in some situations to substances produced internally. (Ganong, 1995)

Interstitial: space filled between the alveolar epithelium and the capillary endothelium. (West, 2008a)

Lymphocytes: white blood cell, 2 cell type usually present in peripheral blood. (Ganong, 1995)

Methylxanthines: drug with bronchoactive and slight anti-inflammatory properties, only around one-fourth as potent as  $\beta$ 2-agonists. (West, 2008b)

Mitochondria: cell generating power units greater and more developed where energy requiring processes occurs. (Ganong, 1995)

Obstructive pulmonary disorder: unbalanced reduction of the maximal airflow from the lung in relation to the maximal volume, leading to airway narrowing during expiration and is defined by an FEV1/FVC ratio reduction below the 5th percentile of the predicted value. (Brusasco *et al.*, 2005)

Pulmonary Hypertension: sustained elevation of the pulmonary arterial blood pressure. (Ganong, 1995)

Non-Obstructive Pulmonary disorder: ventilatory disorder defined by a TLC reduction below the 5th percentile of the predicted value, and a normal FEV1/FVC ratio.

Shunt: blood that enters the arterial system without passing ventilated areas of the lung. (West, 2008a)

Spirometry: method used to measure lung volume changes during a forced breathing maneuver. The FVC maneuver is the most frequently performed, in which a subject inspires maximally and then expires as rapidly and completely possible. (Hyatt et al., 2003)

Vasoconstriction: constriction of the resistance vessels. (Ganong, 1995)

Ventilation: process by the air is forced to get inside the lungs, from the airways to the alveoli, from where, after releasing the O<sub>2</sub>, returns saturated of CO<sub>2</sub> being released to the atmosphere. (West, 2008a)

## **Glossary**

ATS – American Thoracic Society

B – Beta

BMI – Body Mass Index

C – Centigrade

CaO<sub>2</sub> – O<sub>2</sub> concentration in the arterial blood

CH<sub>4</sub> – Methane

cm – Centimetres

CO – Carbon Monoxide

COHb – Carboxyhaemoglobin

COPD – Chronic Obstructive Pulmonary Disease

CO<sub>2</sub> – Carbon Dioxide

CvO<sub>2</sub> – O<sub>2</sub> concentration on the mixed venous blood

dl – Decilitre

DL<sub>co</sub> – Lung diffusing capacity for carbon monoxide

DL<sub>coSB</sub> – Lung diffusing capacity for carbon monoxide by the Single breath Method

DM – Membrane conductivity

e – Electron

ECCS – European Community for Coal and Steel

Eq<sub>t</sub> – Equation

ERS – European Respiratory Society

ERV – Expiratory Reserve Volume

ESP – Epidemiologic Standardization Project

F – Female

FACO – Fraction of CO in alveolar gas

FACO<sub>0</sub> – Fraction of CO in alveolar gas at the beginning of the breath-hold,

FACOT – Fraction of CO in alveolar gas at the end of breath-hold.

FACO<sub>2</sub> – Fractional concentration of CO in the alveolar gas

FAHe – Fraction of He in alveolar gas

(fb) – Frequency

FEV<sub>1</sub> – Forced Expiratory Volume on the first second

FICO – Fraction of CO in the reservoir (usually 0.003)

Fig – Figure

FIHe – Fraction of He in the inspired gas (usually 0.10).

FIVC – Forced Inspiratory Vital Capacity

FRC – Functional Residual Capacity

FVC – Forced Vital Capacity

g – Gram

H – Hydrogen

H<sub>2</sub>O – Water

Hb – Haemoglobin

He – Helium

Hg – mercury

IC – Inspiratory Capacity

IL - Interleukin

ILD – Interstitial Lung Disease

IPF – Idiopathic Pulmonary Fibrosis

IR – Infra- Red

IRV – Inspiratory Reserve Volume

K – Diffusion coefficient

$K_{CO}$  – Transfer factor for CO

Kg – Kilogram

kPa – Kilopascals

L – Litre

Ln – Natural logarithm

M – Male

m<sup>2</sup> – Square meter

min – Minute

MMEF – Maximum mid-expiratory flow

ml – Millilitre

mm – Millimetres

mmol – Millimol

ms – Millisecond

N<sub>2</sub> – Nitrogen

O<sub>2</sub> – Oxygen

P – Pressure

PaCO<sub>2</sub> – Partial pressure of CO<sub>2</sub> in the arterial blood

PACO<sub>2</sub> – Alveolar O<sub>2</sub> pressure

PaO<sub>2</sub> – Partial pressure of O<sub>2</sub> in the arterial blood

PB – Barometric Pressure

Pco – CO pressure

PCO<sub>2</sub> – Partial pressure of CO<sub>2</sub>

PO<sub>2</sub> – Partial pressure of O<sub>2</sub>

PECO<sub>2</sub> – Expired CO<sub>2</sub> pressure

Pred – Predicted

Q – Blood flow per unit time

R – Respiratory exchange ratio

RV – Residual Volume

s – Seconds

SD – Standard Deviation

STPD – Standard temperature (273 K, 00C), pressure (101.3 kPa, 760 mmHg) and dry

T – Breath-hold interval

Th<sub>0</sub> - The T-lymphocytes

Th1 - T-helper type 1 cells

V<sub>c</sub> (theta) – Reaction rate (specific uptake of CO by the blood)

TLC – Total Lung Capacity

V<sub>A</sub> – Alveolar Volume

V<sub>A</sub> – Alveolar ventilation

V<sub>A</sub>/Q – Ratio of ventilation to blood flow

VC – Vital Capacity

V<sub>c</sub> – Pulmonary capillary blood volume

VD – Dead space

VD/VT – Ratio of dead space to tidal volume

VE – Ventilation

V/Q – Ventilation/Perfusion ratio

VT – Tidal Volume

## Background

The human respiratory system is made up of gas exchanging organs which are the lungs and a pump that ventilates the lungs. The pump consists of the chest wall, the respiratory muscles (which increase and decrease the size of the thoracic cavity), areas in the brain that control these respiratory muscles and the tracts and nerves that connect the brain to the muscles. The respiration of a subject can be assessed by pulmonary function testing, described as the process of having a subject performing specific inspiratory and expiratory manoeuvres while breathing in and out of tubing attached to equipment that measures a variety of variables. These tests allow the evaluation of the respiration of a subject and assessment of any abnormality, as well as to quantify it. There are a wide range of pulmonary function tests measuring lung volumes, gas exchange in the lungs or evaluating respiratory muscle function.

Pulmonary function tests are designed to identify and quantify defects and abnormalities in the function of the respiratory system. They can answer questions like how badly impaired is the patient lung function, if a patient has impaired gas exchange in the lung or if a treatment is of benefit. Although the majority of these procedures are non-invasive, they require cooperation from the subject and expertise from the respiratory technician. Measurement of the diffusing capacity of the lung involves the subject inhaling a low concentration of carbon monoxide (CO) and a tracer gas to determine gas exchange within the lung. Several methods of evaluating CO diffusion are available, but the single breath technique (DLcoSB) is the most commonly used. This method is called the single breath technique because the CO diffusion in the human lung is measured during a 10 seconds (s) breath-hold by a subject.



The main disadvantage of the single breath method is that not every subject is able to hold their breath for 10 s in order to measure lung diffusion. Shortening breath-hold time might allow for completion of the test in subjects with more severe respiratory impairment as clinical experience shows that longer breath-hold times lead to difficulties in cooperation in many patients. Based on limited published data on the effect of breath-hold time reduction in lung diffusion measurement, we decided to investigate if the values obtained with a shorter breath-hold time are significantly different from conventional measurements to assess human lung diffusion capacity.

This study will investigate if the reduction of breath-hold time in the single-breath CO diffusion measurement has a significant effect on measured DLcoSB in healthy subjects, patients with severe chronic obstructive pulmonary disease (COPD) and patients with interstitial lung disease compared with conventional measurements.

# Chapter 1

## INTRODUCTION

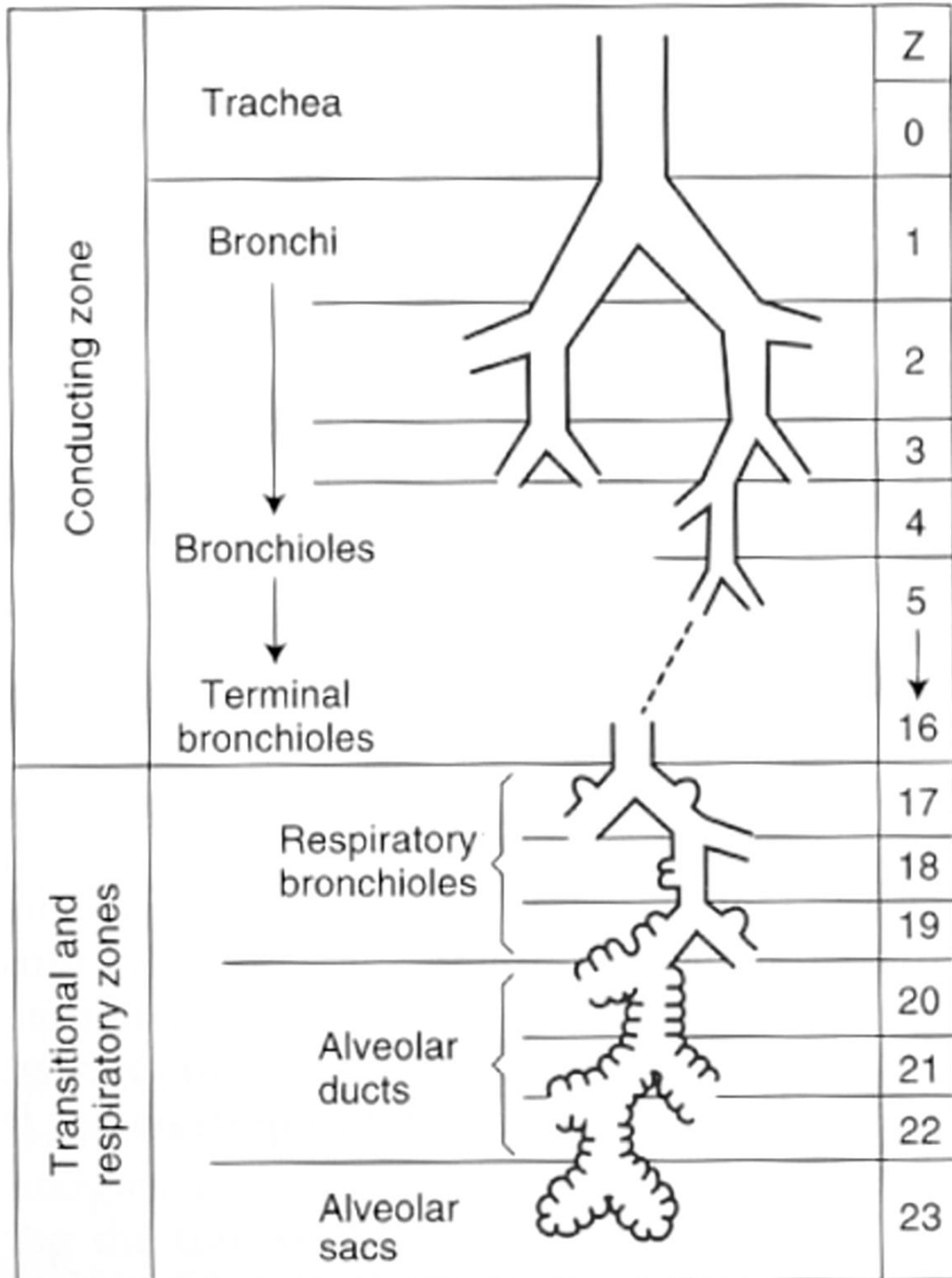
### 1.1 Aim of the Study

We sought to determine if breath-hold time reduction in the single-breath CO diffusion measurement has a significant effect on measured DLcoSB values in control group, patients with severe chronic obstructive pulmonary disease (COPD) and patients with interstitial lung disease (sarcoidosis with interstitial fibrosis or idiopathic pulmonary fibrosis (IPF)) compared with conventional measurement using ATS/ERS standards. Demonstration of the absence of a significant difference associated with breath-hold time reduction can potentially lead to a change in guidelines for breath-hold time during DLcoSB measurements and can thus increase the number of patients in whom DLcoSB can be measured, particularly for those who are unable to breath-hold for 10 s.

### 1.2 Anatomy and Physiology of the Lung

The airways of the lungs consist of a series of branching tubes which become narrower, shorter and more numerous as they penetrate deeper into the lung. Their function is to lead inspired air to the gas exchanging regions of the lung. The trachea divides into right and left main bronchi which in turn divide into lobar, then segmental bronchi, down to the terminal bronchioles. The bronchioles are the smallest airways

making up the conducting airways with no alveoli and therefore constitute the anatomic dead space of the lung, where no gas exchange occurs. The terminal bronchioles divide into respiratory bronchioles which lead to the alveolar ducts and alveoli where gas exchange occurs. This area is known as the respiratory zone of the lung and accounts for 2.5-3 L of volume during rest.



**Fig. 1.1**

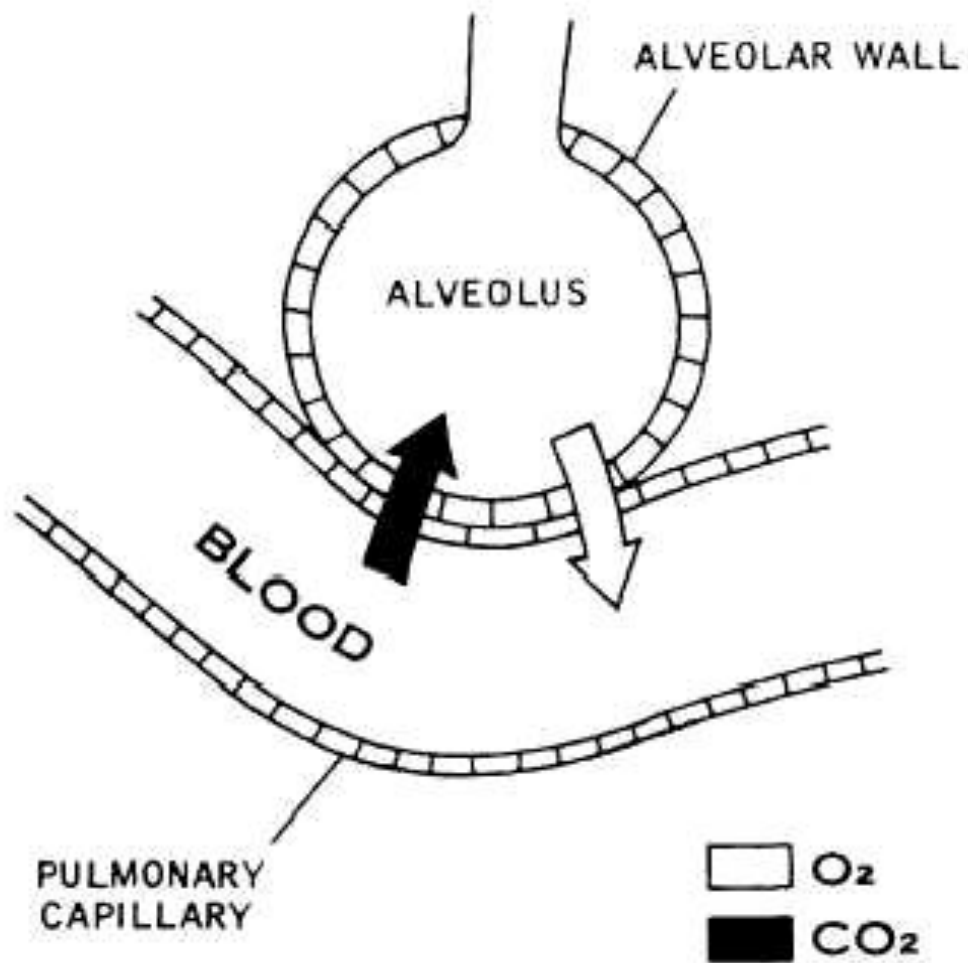
**Idealization of the human airways according to Weibel (1963),**  
 accessed 2 April 2012, <http://www.comprehensivephysiology.com>

The figure shows a model of the human airway system divided by generations of symmetric branching from trachea (0) to acinar airways (15–23), ending in alveolar sacs. The first 16 generations make up the conducting airways, and the last 7, the respiratory zone (or the transitional and respiratory zones).

The airways systematically divide over an average of 23 generations of dichotomous branching, as described by Weibel (1963), ending eventually in a blind sac (figure 1.1). The last nine generations of these airways are connected to tightly packed alveoli, airways chambers in which gas exchange takes place, whereas the central airways serve the function of conducting the air to the gas-exchange parenchyma.

According to Fishman (2007), the gas exchange parenchyma can be divided in two units: the lobes and the acini. There are three lobes on the right (upper, middle and lower) and two on the left (upper and lower), which are separated by the pleura. The acini begin with a transitional bronchiole and terminate with alveoli and are defined as parenchymal units which and participate in gas exchange.

The main mechanism of ventilation in the respiratory zone, illustrated in figure 1.2, is the diffusion of respiratory gases and the rate of diffusion of gas molecules within the airways is so rapid, as well the distances to be covered so short, that “differences in concentration are virtually abolished within a second”. (West, 2008a)



154.110

**Fig 1.2**

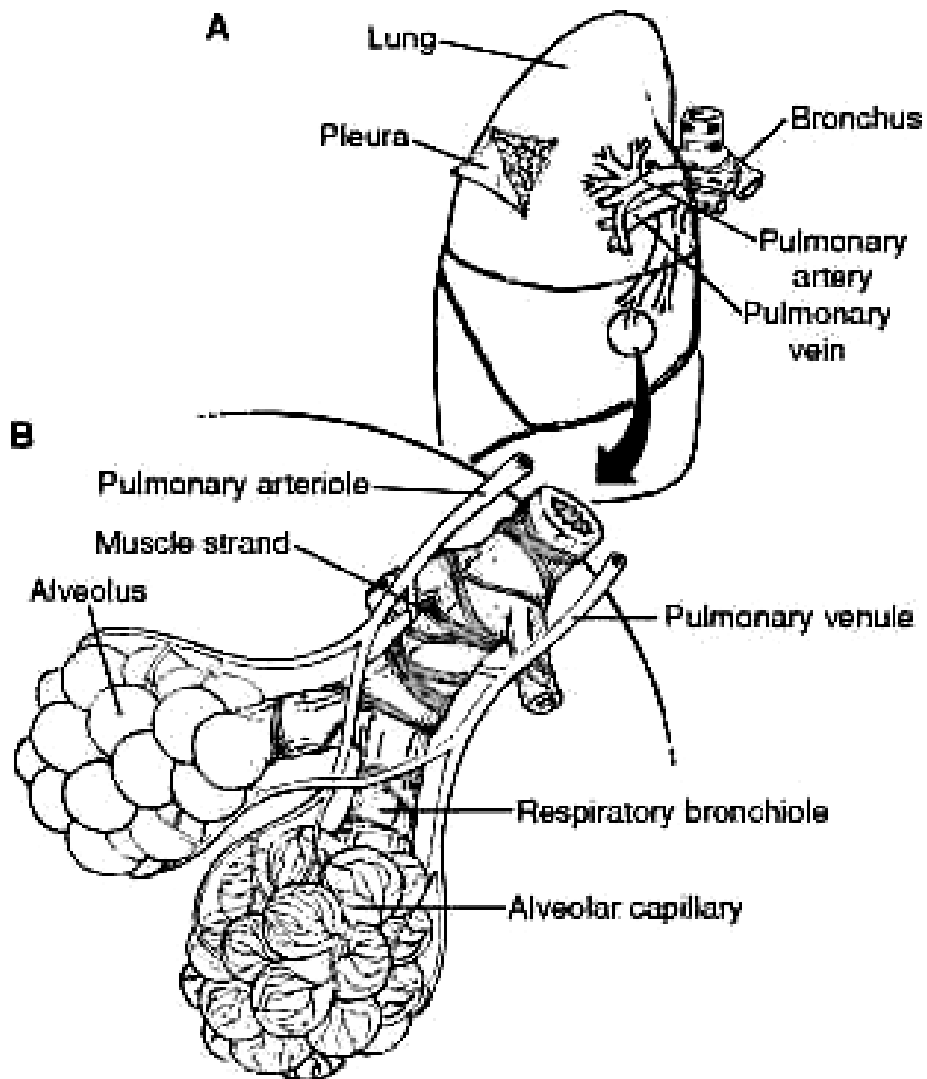
**Gas diffusion in the alveolar-capillary membrane,**

accessed 2 April 2012, [http://medical.tpub.com/10669-c/css/10669-c\\_56.htm](http://medical.tpub.com/10669-c/css/10669-c_56.htm)

The alveoli are thin, microscopic air sacs within the lungs, in direct contact with the pulmonary capillaries. At this level the gas exchanges occurs in the lung.

The pulmonary blood vessels show a characteristic relationship to these structural respiratory units, following the airway course through the centre of the units and finally ending in the capillaries located in the alveolar septa of lung parenchyma. This pulmonary blood vessels form a series of branching tubes from the pulmonary

artery into the capillaries and back to the pulmonary veins. As shown in figure 1.3, the pulmonary capillaries form a dense network around the alveoli, allowing for efficient gas exchange. (West, 2008a)



**Fig. 1.3**

**Pulmonary Circulation stratification,**

accessed 04 April 2012, <http://www.78steps.com>,

The blood is pumped from the heart right ventricle, it enters the pulmonary artery. This artery splits and takes blood to each lung. In the lungs, the blood passes through sacs called alveoli where gas exchange occurs.

Airflow occurs both by mass flow (laminar, turbulent or transitional airflow) and by diffusion. Mass flow occurs in the larger airways whilst diffusion occurs in the alveolar ducts and sacs. As the cross-sectional area of the airways increases, the velocity of the airflow decreases.

## 1.3 Measurement of Lung Volumes and gas exchange of the Lung

### 1.3.1 *Static and Dynamic Lung Volumes*

Static lung volumes and capacities (composed of two or more volumes) are an excellent tool to assess human lung function. The total lung capacity (TLC) is the maximum volume of air in the lungs after a maximal inspiratory effort. The volume of air that remains in the lungs after maximal expiration (the residual volume (RV)) cannot be measured directly.

Volume between TLC and RV, the vital capacity (VC), can be measured directly as can other volumes like the expiratory reserve volume (ERV) and inspiratory reserve volume (IRV). The tidal volume (VT) and IRV will constitute the inspiratory capacity (IC), which also is the difference between the TLC and the functional residual capacity (FRC). The FRC is the volume of air remaining at the end of a normal tidal expiration. (Cooper *et al.*, 2011)

Figure 1.4 illustrates the different static volumes and capacities of the lung.



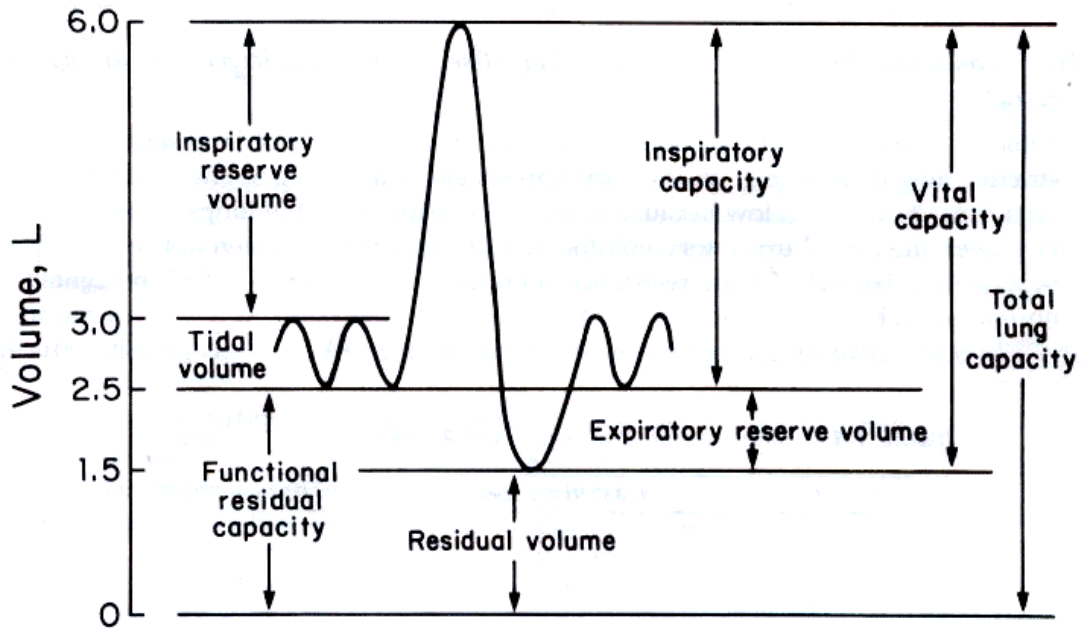


Fig. 1.4

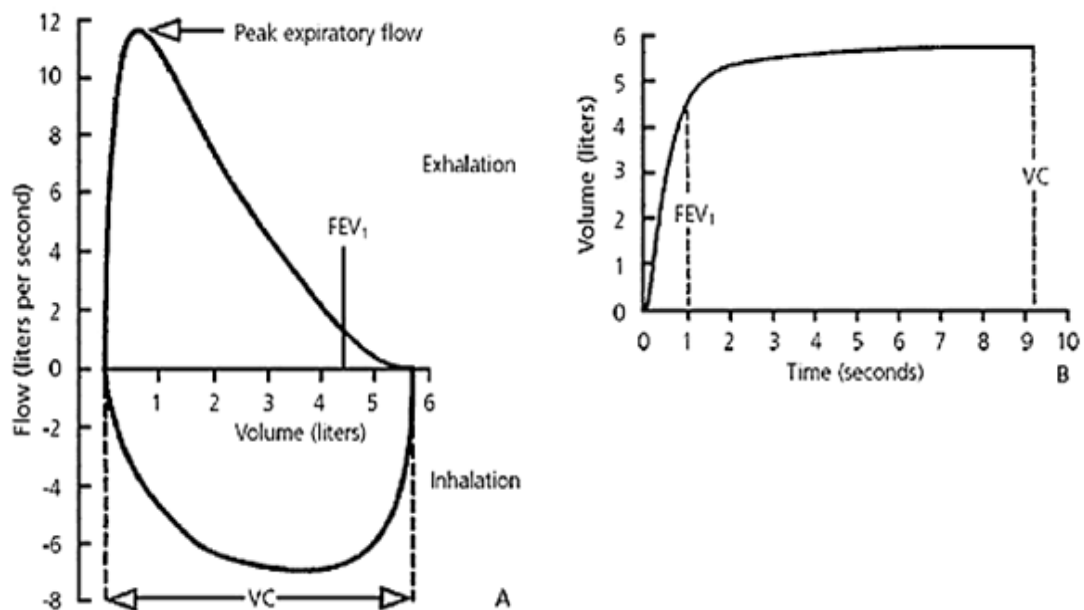
**Scheme of static lung volumes and capacities,**

accessed 4 April 2012, <http://www.cybermedicine2000.com>,

The diagram shows the changes in volume (on the vertical axis) against time (on the horizontal axis) as a subject breathes, initially tidal volumes, and then inspires and expires maximally.

When changes in volume or flow with respect to time are recorded, dynamic volumes are obtained. If a subject takes a maximal breath to TLC exhaling forcibly to RV and then inhales back to TLC, the forced vital capacity (FVC) and forced inspiratory vital capacity (FIVC) can be measured during the expiratory and inspiratory manoeuvres respectively. A series of variables can be obtained from this recording with respect to time, the most valuable of which is the forced expiratory volume in the first second ( $FEV_1$ ). It has been reported by Cooper *et al.* (2011) that “on average a normal subject can exhale about 80% or more of the FVC in the first second of a full exhalation”. They also suggest that the measurement of the  $FEV_1$  and FVC has a number of applications, such as determining if lung disease is present and quantifying the degree of impairment.

Lung volumes can be measured and recorded directly using a volume measuring spirometer which electronically reproduces the volume signal with respect to time to obtain the flow, allowing the construction of volume-time and flow-volume curves as illustrated in figure 1.5.



**Fig. 1.5**

**Volume-time and flow-volume loop,**

accessed 4 April 2012, <http://blog.ert.com/2011/04/29/spirometry>

Spirometry is one diagnostic test for pulmonary mechanics, which measures the inhalation and exhalation of air over time and represented by spirograms: flow volume loop (A) and volume time curve (B).

The flow-volume curves are measured directly using a flow measuring pneumotachograph and the shape of the flow volume curve assists in the recognition of different types of respiratory impairment.

Before performing pulmonary function tests the patient's age, height, weight and sex must be obtained for the calculation of reference values, as lung volumes are related

to body size, with height as a major correlating factor. Reference equations for spirometry are obtained from cross-sectional studies and both the European Community for Coal and Steel (ECCS) and the American Thoracic Society (ATS) have published reference equations for spirometry. (Brusaco *et al.*, 2005) Combined reference equations published by Quanjer *et al.* (1993) are often used in Europe for people aged 18-70 years old, with a height range of 155-195 cm in males and 145-180 cm in females.

### 1.3.2 Ventilation

Ventilation ( $V_E$ ) is the process by which air is drawn into the lungs and gas from the alveoli is expelled, allowing gas exchange. In this process, oxygen ( $O_2$ ) is absorbed in the pulmonary circulation and carbon dioxide ( $CO_2$ ) released from the blood into the alveoli. The primary function of ventilation is to facilitate gas exchange.  $V_E$  is the movement of air between the atmosphere and the alveoli and can be calculated from breathing frequency ( $f_b$ ) and  $V_T$  using the equation:

$$V_E = V^T \times f_b \quad \text{Eq. 1.1 From Cooper } et al., 2011.$$

The total volume of air breathed by a subject per minute and the portion of volume that reaches the alveoli, called alveolar ventilation, must be differentiated. Alveolar ventilation ( $V_A$ ) is the volume of gas that effectively participates in gas exchange and is always less than total ventilation. The anatomical dead space, which is the volume of air in the conducting airways that does not participate in gas exchange, represents the difference between total and alveolar ventilation. (Cooper *et al.*, 2011)

The lung volume that is ventilated but not perfused by pulmonary capillary

blood flow constitutes the respiratory dead space ( $V_D$ ) and can be divided into: the conducting airways (anatomic dead space) and the non-perfused alveoli (alveolar dead space). The total volume of air breathed by a subject per minute (total ventilation) can be measured directly by having the subject breathing through a valve box that separates the inspired from the expired gas, and collects all the expired gas in a bag. (Ruppel, 1994)

However, the  $V_A$  cannot be measured directly and one method to determine is by measuring the volume of the anatomic dead space and calculates the dead space ventilation using the following equation 1.2:

$$V_A = V_T - V_D \quad \text{Eq. 1.2 From Ruppel, 1994.}$$

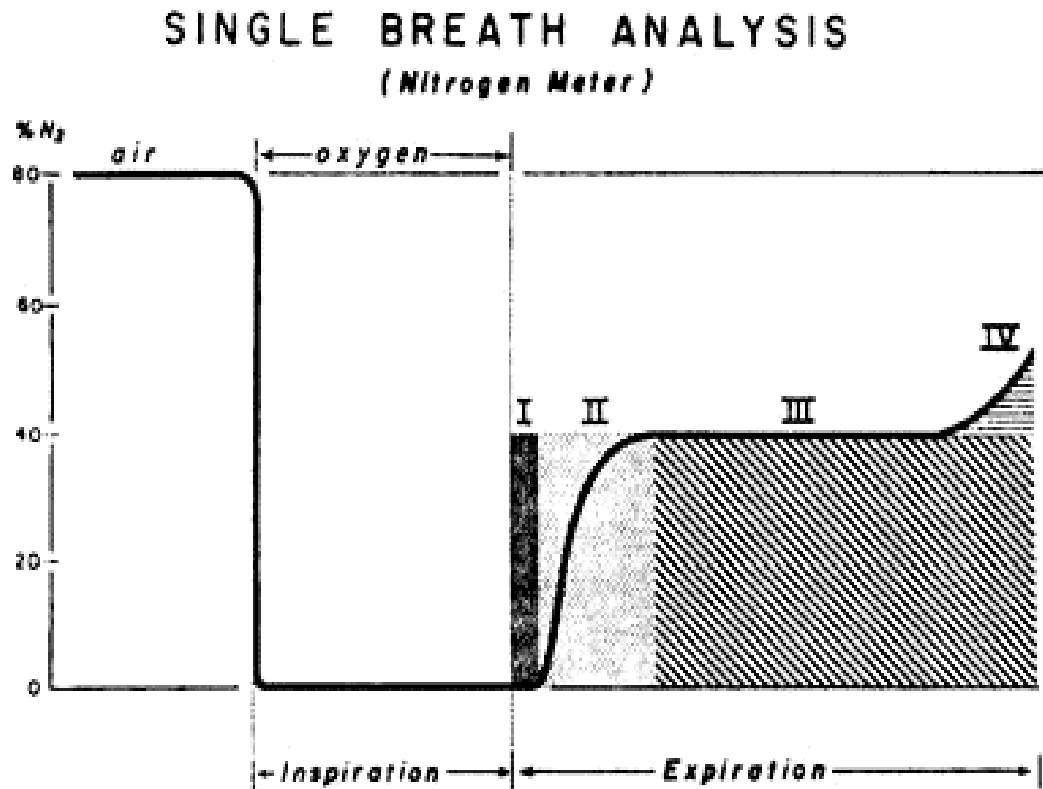
In atmospheric gas, the  $CO_2$  concentration is almost zero, so the  $V_A$  can be calculated also based on  $CO_2$  elimination from the lungs. In this method, a volume of expired gas is collected in a bag and analysed to determine the volume of  $CO_2$  and  $V_A$  calculated by the following equation:

$$V_A = \frac{V_{CO_2}}{F_{ACO_2}} \quad \text{Eq. 1.3 From Ruppel, 1994.}$$

In equation 1.3 the  $V_{CO_2}$  is the volume of  $CO_2$  produced in litres per minute and the  $F_{ACO_2}$  is the fractional concentration of  $CO$  in alveolar gas.

The volume of air in the conducting airways that does not participate in gas exchange is called anatomic dead space, usually approximately 150 ml that can be increased by large inspirations due to the traction or pull exerted on the bronchi by the

surrounding lung parenchyma. This anatomic dead space can also depend on the size and posture of the subject. One of the methods used to measure  $V_D$  is the Fowler's method, where a subject breaths a nitrogen concentration following a single inspiration of oxygen (West, 2008a) as shown in fig 1.6.



**Fig 1.6**

**Fowler's method,**

accessed 4 April 2012, <http://www.tzhealth.com>,

The figure shows a  $N_2$  analyzer monitoring continuously the  $N_2$  concentration of gas being inspired or expired. During inspiration, the  $N_2$  analyzer records 80%  $N_2$  in inspired and expired gas. The subject will take a deep breath of  $O_2$  and followed by a slow breathe out. During inspiration, the  $N_2$  analyzer records 0%  $N_2$ . At the beginning of expiration, about 50 ml of pure  $O_2$  is expired (phase I). In phase II about 200 to 300 ml of  $N_2$  concentration rises, which represents the washout of the remainder of the dead space gas by alveolar gas, and then at last by pure alveolar gas (phase III). At phase IV, the  $N_2$  concentration of the expiration end rises because of the progressive closure of the small airways at the bases of the lungs.

The principal of this method is to measure volume in the conducting airways until dilution of inspired gas occurs with gas already in the lung. The volume measured will depend on the geometry of the rapidly expanding airways, reflecting the morphology of the lung airways.

Another way of measuring the dead space is the *Bohr's method* using the Bohr's equation (Eq. 1.4). Since the expired CO<sub>2</sub> comes mainly from alveolar gas and not from dead space and also because the alveolar concentration of CO<sub>2</sub> is difficult to determine, the partial pressure is used and the equation 1.4 is written as follows:

$$\frac{V_D}{V_T} = \frac{P_{ACO_2} - P_{ECO_2}}{P_{ACO_2}} \quad \text{Eq. 1.4 From Cooper et al., 2011.}$$

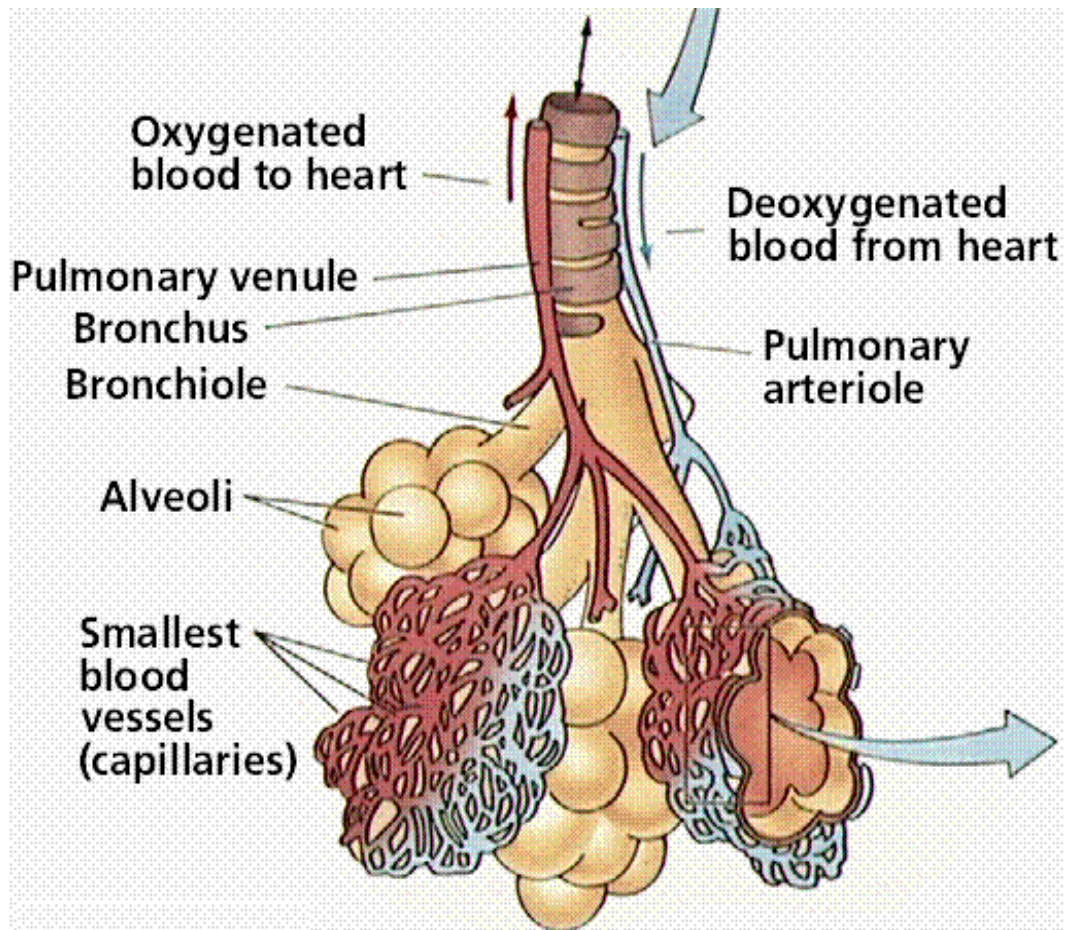
In equation 1.4, A and E refer to alveolar and mixed expired, respectively. The normal ratio of dead space to tidal volume ( $V_D/V_T$ ) is from 0.2 to 0.35 at rest.

According to Ruppel (1998) this method measures the volume of the lung that does not expel CO<sub>2</sub> and the volume measured is called physiologic dead space. In normal subjects the anatomic and physiological dead space are almost the same but in patients with pulmonary impairment, the physiological dead space may be larger because of ventilation / perfusion mismatch in the lung. Therefore, the measurement of physiological dead space represents a useful index of ventilation-perfusion in the lung since all CO<sub>2</sub> in expired gas comes from perfused alveoli. The  $V_D/V_T$  ratio will reflect the volume of ventilation not used in the conducting airways and poorly perfused alveoli. The  $V_D$  measurement provides important information regarding the ventilation-perfusion characteristics of the lungs, increasing with increased  $V_T$  during exercise and decreasing with bronchial obstruction or mucous plugging. West (2008) states: "lower

regions of the lung are better ventilated than the upper regions because of the effects of gravity on the lung”.

### *1.3.3 Perfusion*

The pulmonary circulation begins with the pulmonary artery, which collects mixed venous blood from the right ventricle. The pulmonary artery branches successively like the bronchial tree into the pulmonary arteries and arterioles following the airways to the terminal bronchioles. These arterioles supply the capillary network in the walls of the alveoli, forming a dense network in the alveolar wall as illustrated in figure 1.7 and establishing an efficient surface for gas exchange.



**Fig. 1.7**

**Alveolar circulation,**

accessed 4 April 2012, <http://www.uic.edu>,

The bronchi branch into smaller tubes called the bronchioles and then to small gas exchange units known as alveoli, covered by the smallest blood vessels, the capillaries.

Oxygenated blood is collected from the capillaries by small pulmonary veins that will unite to form four large veins that drain into the left atrium of the heart. The pulmonary circulation pressure is much lower compared with the systemic circulation. The systemic circulation is responsible for the supply of blood to various body organs and by contrast the pulmonary circulation accepts the entire cardiac output. The pressure at the pulmonary capillaries is unknown as they are surrounded by gas and they collapse or distend depending on the pressures within and around them. The pressure



difference between the inside and outside of the capillaries is called the transmural pressure.

During inspiration when the lungs expand, larger blood vessels like the pulmonary arteries and veins are pulled open by radial traction of the elastic lung parenchyma decreasing the pressure around them. Alveolar capillaries are exposed to alveolar pressure and their calibre is determined by the relationship between alveolar pressure and the pressure within them. (West, 2008a)

The extra-alveolar vessels include all the blood vessels that run through the lung parenchyma. These vessels are exposed to a lower pressure compared to the alveolar pressure and are pulled by the radial traction of the surrounding parenchyma of their walls. The lung volume constitutes an important determinant of pulmonary resistance. During deep inspiration, alveolar pressure rises compared to capillary pressure and the blood vessels will be compressed and therefore their resistance rises. Also the calibre of these pulmonary capillaries will depend on lung volume, decreasing at larger lung volumes due to stretching and thinning of the alveolar walls.

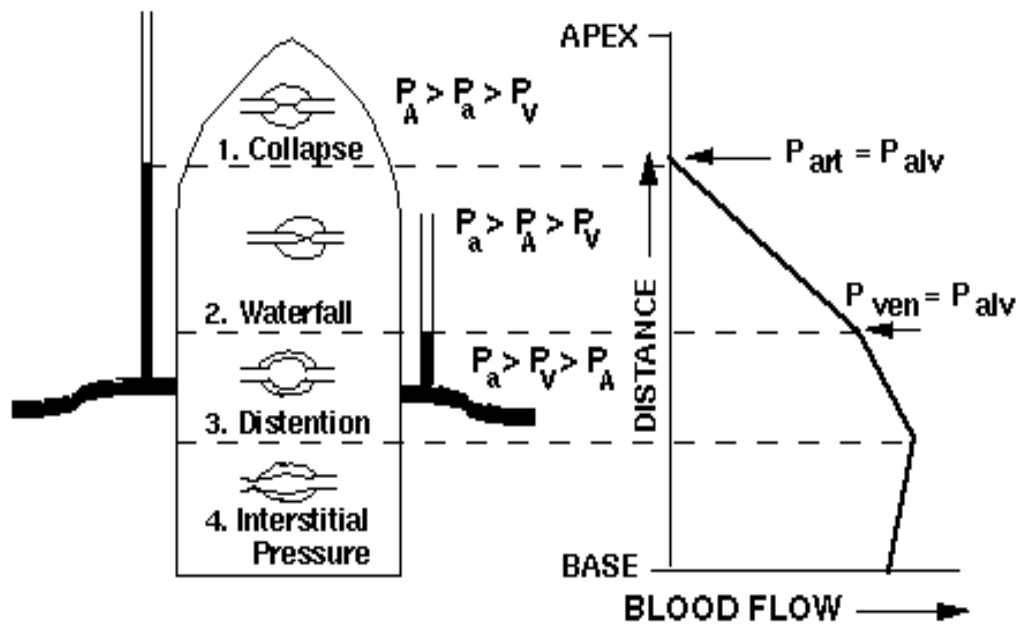


Fig. 1.8

**Four zone model of pulmonary circulation,**

accessed 4 April 2012, <http://www.coheadquarters.com>

The human lung is about 30 cm tall from apex to base therefore gravity exerts an important role in determining blood flow direction in the lung. The human lung can be divided into 4 zones represented by the figure 1.8 above. The hydrostatic arterial and venous pressures fall with increasing distances up the 30 cm height of the lung where: a=arterial, A=alveolar and V=venous.

The lung of an adult is around 30 cm from apex to base and gravity is a significant factor in determining blood flow in the lung. The lung can be divided into 4 zones of perfusion as shown in figure 1.8, illustrating that the hydrostatic blood pressure is highest at the base and lower at the apex.

The four zone model presented in figure 1.8 shows that the arterial and venous pressures at the apex may both be less than the air pressure inside the alveoli and this will interrupt the blood flow as the air pressure decreases and closes the blood vessels.

Often, there is very little, if any, blood flow in zone 1. At zone 2, the arterial pressure is higher than alveolar pressure but the venous pressure is less than alveolar pressure and the blood flow, instead of being determined by the usual arterial-venous pressure difference, is determined by the arterial-alveolar gradient. Arterial pressure increases in zone 2 due to gravity, while alveolar pressure remains relatively constant and so the arterial-alveolar difference increases in zone 2 and thus blood flow increases from the top to the bottom of this zone.

In zone 3, the arterial and venous pressures exceed alveolar pressures and here blood flow will be determined by the arterial-venous difference. In this zone of the lung, both arterial and venous pressure increase due to gravity and the pulmonary blood vessels distend leading to decreased resistance. Also the pulmonary blood flow will rise from the top to the bottom of zone 3.

Finally, at zone 4 at the base of the lung, the ratio of ventilation to blood flow is low and consequent alveolar hypoxia leads to compensatory vasoconstriction, limiting local blood flow. When the alveolar  $pO_2$  is reduced, hypoxic pulmonary vasoconstriction occurs through contraction of smooth muscle in the walls of small pulmonary arterioles. The primary function of hypoxic pulmonary vasoconstriction is to direct blood flow away from hypoxic regions of the lung. These hypoxic regions may arise from airway obstruction and this diversion of blood flow reduces the effects on gas exchange. West (2008a)

According to West (2008a) this mechanism remains obscure in spite of a great deal of research.

### *1.3.4 Ventilation – Perfusion*

The relationship between ventilation and blood flow is a major determinant of pulmonary gas exchange. The four scenarios of impairment of gas exchange that result in hypoxemia are: hypoventilation, diffusion impairment, shunt and ventilation-perfusion mismatch. West (2008b) defines hypoxemia as an abnormally low  $pO_2$  in arterial blood, with the normal value between 11 and 13 kPa. The  $pO_2$  in the alveolar gas depends on a balance between two processes: pulmonary capillary blood capacity for the  $O_2$  absorption and its continued replacement by alveolar ventilation. The rate at which  $O_2$  is absorbed from the lung is determined by its consumption by the body tissues and is almost constant at rest. Alveolar  $pO_2$  is greatly determined by the level of alveolar ventilation. If alveolar ventilation is decreased, the alveolar  $pO_2$  decreases and the  $pCO_2$  increase due to hypoventilation. If hypoventilation is present, the  $pCO_2$  increases until a steady state has been achieved and the alveolar and arterial  $PO_2$  decreases, except when the subject breaths an  $O_2$  mixture that compensates for reduced ventilation. (West, 2008b)

Diffusion impairment means that the  $pO_2$  in the pulmonary capillary blood and alveolar gas are not balanced. In some pulmonary diseases, the alveolar-capillary membrane is thickened and diffusion is decreased to the point that equilibration may be incomplete, due to a slower time course for diffusion. The presence of pulmonary diseases, such as interstitial lung diseases where the diffusion path from alveolar gas to red blood cell may be increased, may be affect time for oxygenation. (West, 2008b) Another factor that can decrease the arterial  $pO_2$  in relation to alveolar level is the presence of shunted blood. According to West (2008) shunt refers to blood that enters

the arterial system without being previously in ventilated areas of the lung. Intrapulmonary shunts can be caused as well by arterial-venous malformations, often with genetic basis. The hypoxemia induced by a shunt cannot be corrected by administering 100% O<sub>2</sub> because according to West (2008b) “the shunted blood that bypasses ventilated alveoli is never exposed to the higher alveolar pO<sub>2</sub>, so it continues to depress the arterial pO<sub>2</sub>”.

The intrapulmonary shunts can be divided in true shunt and shunt-like effect. In a true shunt, blood flows from the right to the left side of the heart without contacting an alveolus for gas exchange (perfusion without ventilation). A true shunt can be classified as an anatomic or a capillary shunt and the main difference is their origin. Anatomic shunts can be normal when the shunted blood comes from veins such as bronchial, pleural, or thebesian veins, and abnormal when shunted blood arises from congenital heart disease, pulmonary arteriovenous malformations or vascular lung tumours. Capillary shunts are caused by alveolar collapse (atelectasis), alveolar fluid accumulation (pulmonary oedema) and alveolar consolidation (pneumonia). As anatomic and capillary shunts are true shunts, the associated hypoxia cannot be treated by increasing the inspired O<sub>2</sub> concentration.

In the shunt-like effect, the blood exchanges gas with alveolar gas but the pO<sub>2</sub> does not reach the pO<sub>2</sub> of normal alveoli. This type of shunt can be caused by hypoventilation (bronchospasm or excessive mucus in the tracheobronchial tree) and alveolar-capillary membrane impairment such as in patients with pulmonary fibrosis, where diffusion does not have enough time to occur.

The ventilation-perfusion inequality is characterized by mismatch of ventilation and blood flow in various regions of the lung leading to an impaired gas transfer. In this process, both O<sub>2</sub> and CO<sub>2</sub> transfer results will be impaired and this is the main

mechanism of hypoxemia in diseases such as chronic obstructive pulmonary disease, interstitial lung disease and vascular disorders. The ventilation-perfusion inequality is commonly identified by excluding the other three causes: hypoventilation, diffusion impairment and shunt. (West, 2008b)

The O<sub>2</sub> concentration in the human lung is determined by the ratio of ventilation to blood flow (V<sub>A</sub>/Q) by the following equation (which also applies to CO<sub>2</sub>, N<sub>2</sub> and any other gas present under steady-state condition). The equation is as follows:

$$\frac{V_A}{Q} = 8.63R \frac{C_{AO_2} - C_{VO_2}}{PaCO_2} \quad \text{Eq. 1.5 From Cooper } et al., 2003.$$

Where:

- Q is the blood flow per unit time,
- R is the respiratory exchange ratio,
- CaO<sub>2</sub> the O<sub>2</sub> concentration in the arterial blood,
- CvO<sub>2</sub> the O<sub>2</sub> concentration on the mixed venous blood,
- PaCO<sub>2</sub> the partial pressure of CO<sub>2</sub> in the arterial blood.

If the V<sub>A</sub>/Q of the lung unit is decreased by airway obstruction, the O<sub>2</sub> concentration will decrease and the CO<sub>2</sub> will rise, and if the ventilation is completely ceased, this ratio will reach zero. On the other hand, if this ratio increases by impaired perfusion, the O<sub>2</sub> concentration will increase and the CO<sub>2</sub> will fall, and also if the blood flow ceases, will reach the composition of the inspired gas.

Regional gas exchange patterns are defined by the V<sub>A</sub>/Q, being high at the apex of the lung and much lower at the base. If pulmonary disease is present, this regional

pattern will disappear and at the alveolar level the normal relationships between ventilation and blood flow will be perturbed. Although ventilation-perfusion inequality will reduce the gas exchange efficiency of the lung for all gases, in many cases a normal  $p\text{CO}_2$  may occur because ventilation will be increased in the alveoli. However, the  $p\text{O}_2$  is always low and so the alveolar-arterial  $p\text{O}_2$  difference is a useful measure of ventilation-perfusion inequality. (West, 2008b)

### *1.3.5 Diffusion Capacity of the Lung*

Oxygen travels along the airways from the mouth into the alveoli by bulk movement of air (movement of air from a region of high pressure to one of low pressure) and diffusion occurs by movement of a gas when a pressure gradient in the alveoli is present. Diffusion of gases in the lung occurs by molecular movement that increases as temperature rises and occurs over a short distance of about 1mm, between the alveolar duct and the alveolar capillary membrane. (Cooper *et al.*, 2011) The  $\text{O}_2$  must cross the alveolar capillary membrane before entering the erythrocytes, moving from the gas phase to the liquid phase. Oxygen has to diffuse through pulmonary surfactant, the alveolar epithelium, the interstitium, the capillary endothelium and then the plasma before reaching the erythrocyte membrane and finally combining with haemoglobin. The blood will carry  $\text{O}_2$  to the body tissues where it diffuses through the plasma, capillary endothelium, interstitium and the mitochondrial membrane. The reverse process will occur for  $\text{CO}_2$ . (Cooper *et al.*, 2011) According to West (2008a) “the diffusion of a substance in fluids (liquids or gases) is a passive process occurring between regions of differing gases concentrations. If a membrane of constant thickness

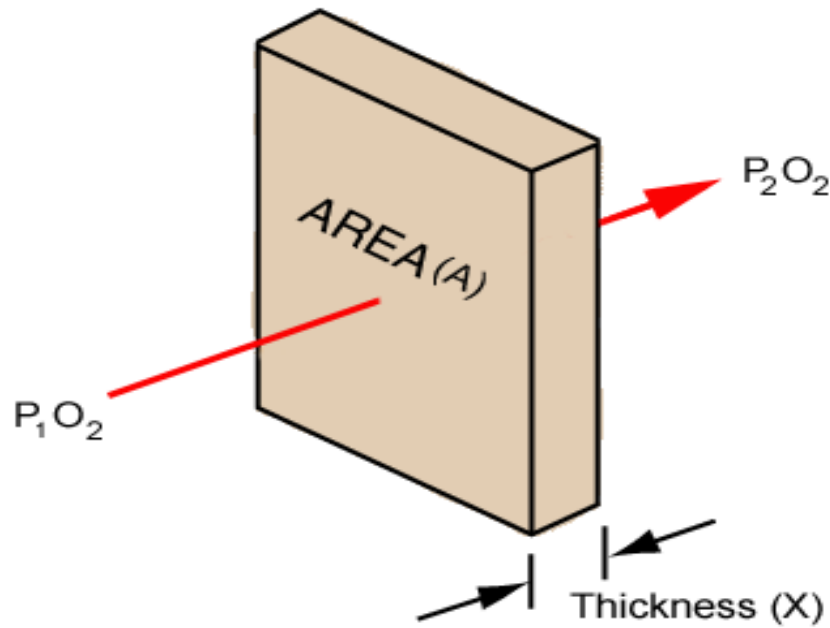
and permeable to a particular gas is placed between two fluid compartments, the diffusion of a gas across the membrane is described by Fick's law of diffusion which states that the rate of transfer of a gas through a sheet of tissue is proportional to the tissue area and the difference in gas partial pressure between the two sides, and inversely proportional to the tissue thickness" (West, 2008a). The following equation 1.6 is Fick's Law of diffusion and allows the calculation of the diffusion of a gas.

$$V_{gas} = \frac{kA}{x(P_1 - P_2)t}$$

**Eq. 1.6** From Cooper et al., 2011.

In the equation 1.6, K is the diffusion coefficient and  $P_1 - P_2$  the difference of the gas partial pressure. The larger the surface area (A), the higher will be the difference in pressure and the thinner the membrane (t), the higher will be the rate of diffusion as illustrated in figure 1.9.





**Fig. 1.9 Fick's law of diffusion,**  
accessed 4 April 2012, <http://www.elu.sgul.ac.uk>

The  $O_2$  diffuses from a place of higher partial pressure ( $P_1O_2$ , e.g. the alveolar air) to one of lower partial pressure ( $P_2O_2$ , e.g. the pulmonary capillary) through a membrane of area  $A$  and thickness  $X$ .

The density and solubility of a gas will also determine the diffusion rate. Oxygen, which is less dense than  $CO_2$ , diffuses about 1.2 times faster under same conditions in the alveoli. However, their relative solubility changes when crossing the alveolar-capillary membrane, as  $CO_2$  in the liquid phase is about 24 times more soluble than  $O_2$ , and therefore  $CO_2$  diffuses about 19 times faster than  $O_2$ . (Cooper *et al.*, 2011)

Oxygen travels easily across the alveolar capillary membrane combining with haemoglobin (Hb). Initially, this movement will depend on a pressure gradient between alveoli and capillaries for  $O_2$  which continues to diffuse from alveoli to capillary blood until Hb becomes saturated with  $O_2$ . At this point the  $O_2$  pressure rises and equilibrates

with alveolar  $O_2$  pressure ( $P_{AO_2}$ ). If the thickness of the alveolar-capillary membrane increases,  $O_2$  diffusion is impaired and the rate of rise of oxygen partial pressure ( $pO_2$ ) is reduced. If a severe abnormality is present, the  $pO_2$  may not reach the  $pO_2$  of alveolar gas. (Cooper *et al.*, 2011) The rate of diffusion will therefore depend of the physical properties of the alveolar-capillary membrane, as the surface area and thickness of the membrane will determine the rate of diffusion. The surface area for diffusion can be altered by changes in lung volume and in pulmonary artery pressure.

The Hb molecule has a major role on the gas transport and diffusion given its large capacity for  $O_2$ , as each Hb molecule can transport four  $O_2$  molecules, releasing  $O_2$  when needed. Hb is also involved in  $CO_2$  and hydrogen ion transport. The combination of  $O_2$  with Hb can be illustrated by the Oxyhaemoglobin dissociation curve in figure 1.10 and is only possible due to changes in the structure of the Hb molecule. (Cooper *et al.*, 2011)

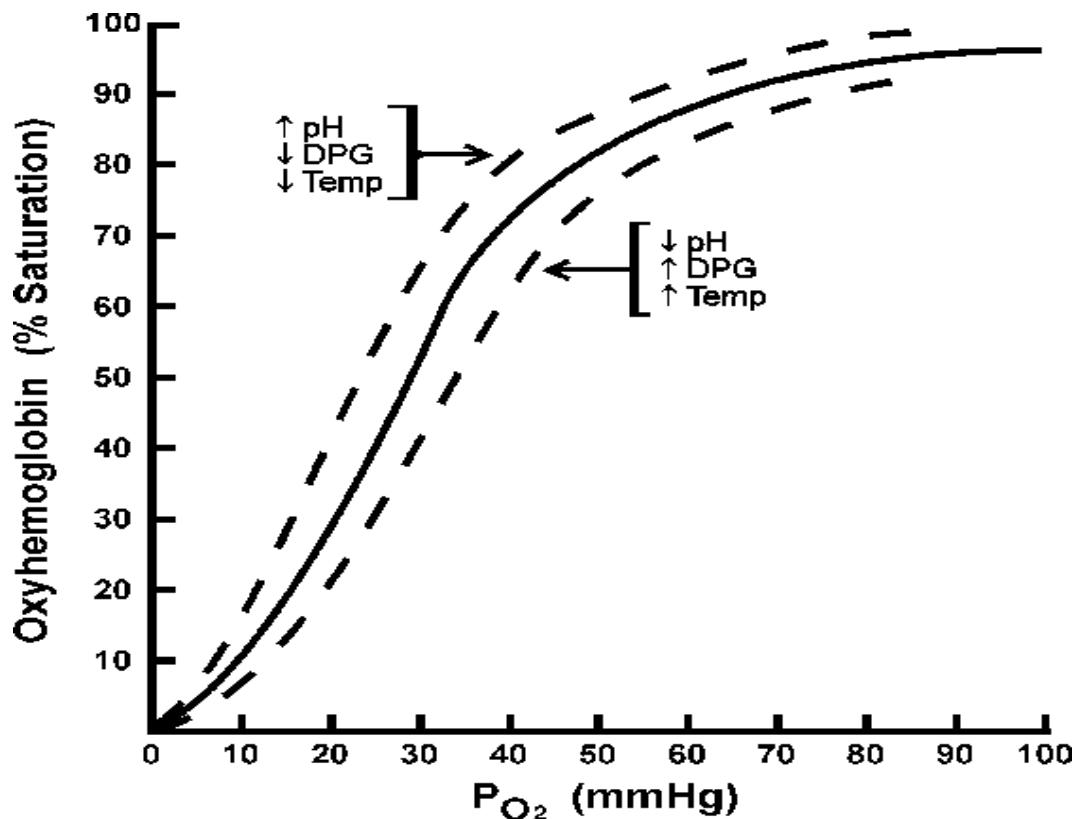


Fig. 1.10

**Oxyhaemoglobin dissociation curve**

From Cooper *et al.*, 2011

The Oxyhaemoglobin dissociation curve is a graph that shows the percent saturation of Hb various partial pressures of O<sub>2</sub>. The main goal of the dissociation curve is to show the equilibrium of oxyhaemoglobin and nonbonded Hb at various partial pressures. At high partial pressures of O<sub>2</sub>, Hb binds to O<sub>2</sub> to form oxyhaemoglobin.

The sigmoidal shape of the Oxyhaemoglobin dissociation curve is the result of chemical reactions that occur between O<sub>2</sub> and each haem group of the Hb molecule. This curve shifts position but the sigmoid shape is preserved. (Cooper *et al.*, 2011)

Figure 1.10 illustrates the percentage of haemoglobin that reacts with O<sub>2</sub> at each O<sub>2</sub> pressure. Oxygen rapidly combines with haemoglobin as the pO<sub>2</sub> rises but around 60 mmHg, despite of increase of pO<sub>2</sub> there is minimal increase in the oxygen-haemoglobin binding as Hb is already 90% saturated. At the flat portion of this curve, the pO<sub>2</sub> in the blood can drop from 100 to 60 mm Hg and the Hb will remain 90% saturated with O<sub>2</sub>

and wide changes in  $pO_2$  only will produce a small variation in the oxygen saturation ( $SaO_2$ ). Even after most of the  $O_2$  is transferred, significant partial pressure difference appears to exist between the alveolar gas and the blood, as blood continues to flow through the alveolar-capillary system which facilitates  $O_2$  diffusion across the alveolar-capillary membrane.

There are other factors that will shift the  $O_2$  dissociation curve. If the pH decreases this curve will shift to the right (decreased affinity) caused by a rise of  $pCO_2$  as  $CO_2$  moves into the blood from the cells, helping the unloading of  $O_2$ . In the opposite scenario, an increase of pH will shift the curve to the left (increased affinity), caused by decreased  $pCO_2$  as  $CO_2$  moves from the blood into the alveoli, increasing the loading of  $O_2$  in the lungs and decreasing the unloading of  $O_2$  at the tissues. Temperature is another important factor affecting binding of the  $O_2$  with the Hb. An increase in temperature will shift the curve to the right (decreased affinity), enhancing the unloading of  $O_2$  and decreasing the loading of  $O_2$  in the lungs. In the other hand, a decrease in temperature will shift the curve to the left (increased affinity), increasing the loading of  $O_2$  in the lungs.

The  $CO_2$  concentration in the blood will also affect the Oxyhaemoglobin dissociation curve. An increase in  $CO_2$  will shift the curve to the right (decreased affinity) because an increase of the partial pressure of arterial carbon dioxide ( $PaCO_2$ ) leads to an increase in hydrogen ion concentration. This will enhance the unloading of  $O_2$  in actively metabolizing tissues like muscles during exercise and decrease loading of  $O_2$  in the lungs. A decreased  $CO_2$  will shift the curve to the left (increased affinity), caused by decreased  $pCO_2$  as  $CO_2$  moves from the blood into the alveoli, as well as enhancing the loading of  $O_2$  in the lungs and reducing the unloading of  $O_2$  at the tissues. Finally the 2,3-Diphosphoglycerate (2,3-DPG), a substance that is formed by the red

blood cells during anaerobic glycolysis will shift the curve to the right (decreased affinity) if its concentration rises, as  $O_2$  will be released more efficiently at the tissues. This scenario can be caused by hypoxia, anaemia and pH increase. On the other hand, decreased 2,3-DPG will shift the curve to the left (increased affinity), enhancing the loading of  $O_2$  in the lungs and decreasing the unloading of  $O_2$  at the tissues.

The diffusing capacity of the lung is measured using carbon monoxide (CO) and referred to as DLco. The DLco is used to assess the lungs gas exchange, measuring the transfer of CO across the alveolar-capillary membrane. The CO affinity to bond with Hb is approximately 210 times more than  $O_2$  and with normal Hb concentration and normal ventilatory function, the major limiting factor to CO diffusion is the integrity of the alveolar-capillary membrane. CO concentration in pulmonary capillary blood is normally very low or inexistent and therefore the pressure gradient causing diffusion is effectively the alveolar pressure. Consequently, a small amount of CO in inspired gas will produce changes in the concentration of inspired versus expired gas that can be measured and if the partial pressure of CO in the alveoli and the rate of uptake of the gas can be measured, the DLco of the lung can be determined. (West, 2008a)

The diffusion properties of the alveolar membrane depend on its thickness and area. The diffusion is also reduced when the surface area of the blood-gas barrier is reduced. In obstructive diseases such as emphysema, the reduction in diffusing capacity is partly due to destruction of alveoli and capillaries. The rate of combination of CO with Hb is reduced when the number of red cells in the capillaries is low and also in diseases that limit the capillary blood volume. (West, 2008a)

The diffusing capacity of the lung for the CO can be measured by three methods: the single-breath method (which is the most commonly used), the Clarke Rebreathing method and the Intra-breath method. The single breath method will be further discussed

in this paper, as it was the method used in this study to assess lung diffusion. In the single-breath method, a subject takes a VC breath of CO and He, holds their breath for 10 s, and then exhales to RV. The first 750 ml are discarded because of dead space, and the next litre is collected and analysed. Another gas, helium (He), is used as a tracer gas and facilitates assessment of the dilution of the inspired gas (CO) to obtain the initial alveolar CO pressure (Pco). According to West (2008), CO is absorbed from alveolar gas proportionate to the Pco during a breath-hold; thus, the diffusing capacity can be calculated as the volume of CO taken up per minute per mmHg alveolar Pco. (West, 2008a)

The advantages of this method include standardization by the ATS/ERS task force (MacIntyre *et al.*, 2005), good reproducibility and reference values available. Disadvantages are the fact this measurement is performed at TLC, leading to difficult patient co-operation and also a minimum of 1.3 litres VC is required. The rebreathing method is a simple technique that requires a 1 litre anaesthetic bag to which the patient is connected and the gas mixture is the same as that used for the single-breath method. At the end of a normal tidal breath, a tap is turned and the subject breathes in and out only from this bag, emptying it each time, at a rate of 10 breaths in 10 s. The rebreathing time is recorded from a stopwatch and the contents of the rebreathing bag are then analysed and the alveolar gas concentrations recorded. The major advantage of this method is for patients who are unable to perform the breath-hold technique because lung volumes are too small as no VC is required. However, this method also depends of the patient co-operation and few reference values are available.

The third and last method to measure CO diffusion in the lung is called intra-breath and is a variation on the single-breath breath-holding method mentioned above. There is no breath-hold and the subject inhales the test gas mixture of CO and CH<sub>4</sub>

(Methane) from RV to TLC and then exhales gradually until RV is reached. The major advantage of this technique is the lack of requirement for a minimum VC, although no standardization for this measurement exists and only few reference values are available. (Cooper *et al.* 2011)

Therefore, the single-breath breath-holding method is the most commonly used to access the DLco of the human lung.

### *1.3.6 Historic Development of DLcoSB measurement*

The use of CO to measure diffusion capacity was introduced by Christian Bohr in 1909 when he reported that the affinity of Hb for CO was greater than the affinity of Hb for O<sub>2</sub>. He also reported that even if small concentrations of CO in alveolar gas were used, the back pressure of the carboxyhaemoglobin (COHb) in the pulmonary capillary blood could be ignored. (Cooper *et al.*, 2011)

In 1915 August and Marie Krogh (Forster *et al.* 1954) introduced the idea that the CO was transferred to the alveolar space by diffusion alone and they designed a measuring method which included a period of breath-holding. Krogh asked subjects to inhale a VC breath of CO as two alveolar samples were collected, and while the first sample was collected during an immediate exhalation to about ½ VC, obtaining the CO concentration at the beginning of the breath-hold, the second sample was collected after six to ten seconds of breath-hold measuring the CO final concentration.

For the determination of the VA, the dilution of hydrogen method was applied, where the end volumes of the two expirations represented the alveolar gas and were analyzed for CO concentration. To Krogh the CO concentration fell exponentially and

could be calculated by the following equation 1.7.

$$F_A = F_{A0} \exp(-DPbt/V_A) \quad \text{Eq. 1.7 From Forster et al., 1954.}$$

In the above 1.7 equation the  $F_A$  is the alveolar concentration of CO at time t, representing the final sample; the  $F_{A0}$  is the alveolar concentration at time zero, the initial sample; the “exp” is e; the base of the natural logarithms, raised to the power contained in the brackets following: D is the pulmonary diffusing capacity for CO;  $V_A$  is the total alveolar gas volume during the period of breath-holding; t is time in seconds between the delivery of the two gas samples; and P<sub>b</sub> is the total barometric pressure minus 47mmHg. In this equation (Forster *et al.* 1954) the CO concentration on the first sample was representative of all alveolar gas and that the CO tension in the pulmonary capillary plasma (P<sub>c</sub>) could be ignored. However, Roughton (1945) reported later that P<sub>c</sub> should be considered.

The Krogh equation describes diffusion of CO into the blood for time of breath-holding during apnoea:

$$DL_{CO}SB = V_A \cdot \ln(F_{ACO_{t0}}/F_{ACO_{t1}})/t_{BH}/P_B - 47 \quad \text{Eq. 1.8 From Qutayba, A. Shannon, J. Martin, J. 2005.}$$

Later Forster *et al.* (1954) suggested the addition of He to the Krogh method and also the reduction to a single gas collection at the end of a known period of breath-holding. (Forster *et al.*, 1955)

In 1957, a similar paper was published by Olgivie *et al.* (1957) which incorporated all the modifications mentioned by Forster and standardized the technique



for the single-breath DLco method. Ogilvie proposed the elimination of the first alveolar sample by adding He, an inert gas, to the mixture test gas and standardized the DLcoSB measurement, still in use currently, where subjects rapidly inhale a test gas mixture containing CO, He, O<sub>2</sub> and N<sub>2</sub> from RV to TLC. After a 10 second breath-hold, subjects exhale again to RV. The major modification introduced by Ogilvie was the parallel measuring of He dilution to estimate the concentration of CO at the onset of breath-holding. In this technique all the alveolar gas was collected except the first litre (used to washout the V<sub>D</sub>) and breath-hold time was measured from the start of inhalation (t<sub>0</sub>) to the beginning of the sample collection (t<sub>1</sub>).

Jones and Meade (1961) realized that Krogh equation was valid only for the breath-hold time portion during apnoea in the Ogilvie method (Ogilvie *et al.* 1957). Errors in the CO diffusion measurement could be minimized by measuring the breath-hold time from 3/10 of the time of inhalation to the time for one-half of the alveolar sample collection, and also by collecting a smaller alveolar gas sample. (Qutayba, A. Shannon, J. Martin, J. 2005)

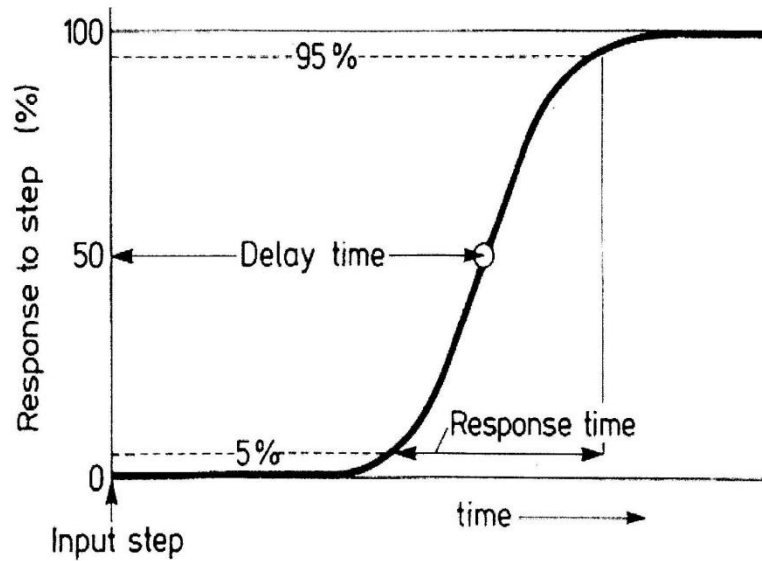
Modifications of Ogilvie's method to calculate DLcoSB are the current methods in use and VA is measured from the single breath dilution of He as proposed by McGrath and Thompson (1959) as shown by the equation 1.9.

$$VA = VC \cdot F_I He / F_A He_{t1}$$

**Eq. 1.9** From Qutayba, A. Shannon, J. Martin, J., 2005.

### 1.3.7 Respiratory Gas Analysis

Analysis of respiratory function involves the measurement of gas concentrations where precise measurements are crucial and the analyzer used should provide a rapid, accurate, linear, stable and specific response to the gas being analysed. How fast an analyzer detects a change in the gas concentration is an important factor. In order to produce a feasible measurement of gas concentration at any instant in time, the response of the analyzer should be preferably of 100ms or greater. (Cooper *et al.*, 2011) Figure 1.11 illustrates the delay time and response time of a gas analyzer.



**Fig. 1.11**

**Definition of delay time and response time of a gas analyser,**

From Cooper *et al.*, 2011.

The delay time is defined as the amount of time it takes for a sample to reach the analyser, and is the time taken by the gas to travel from the sampling end to the entrance of the analyser. The delay time is represented by the straight line portion of the response time graph. During the delay time, the response time graph remains flat, since no gas has yet reached the analyser. As soon as the test gas sample enters the sample chamber, the gas begins to rise and this will mark the rise time of the curve. The response time is the delay caused by transit time and rise time. In the response time graph, rise time is the time required for the displayed value to rise from the baseline to the maximum value.

To design and construct a gas analyser, the physical properties of gases are taken into account and consequently a variety of gas analysers exist. In respiratory function testing, the accuracy of the gas analyser of respiratory gases can be affected by the presence of other gases in the sample, typical examples being water vapour and CO<sub>2</sub>. Water vapour will dilute the gas to be analysed and should be removed from the sample. CO<sub>2</sub> is removed by granules containing barium hydroxide or sodium hydroxide, also

called soda lime. (Cooper *et al.*, 2011)

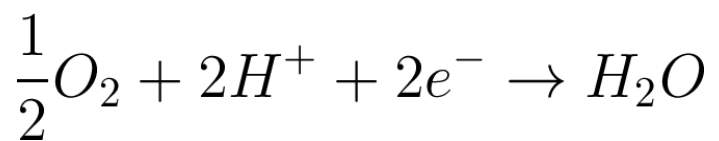
Analyzers used to measure CO gas diffusion in the lung are an Infra-Red or an Electro-chemical type analyzer. In the Infra-Red analyzer, infra-red radiation is absorbed by gases such as CO<sub>2</sub> and CO in proportion to their concentration. A single element coil with a filter will constitute the IR source, so that only the wavelength to the gas being analysed is emitted. Since CO and the CO<sub>2</sub> have close IR absorption bands, the presence of one in the sample being analysed will interfere with the analysis of the other. This interference can be minimized by the use of a filter tube manufactured to the optical pathway. (Cooper *et al.*, 2011)

CO can also be analysed using an electrochemical sensor as shown in figure 1.12, which consists of a sealed fuel cell with an aqueous electrolyte containing a sensing and reference electrode with a constant voltage supplied by a battery. CO will diffuse into the cell oxidising at the platinum anode according to the equation 1.10:



**Eq. 1.10** From Cooper *et al.*, 2011.

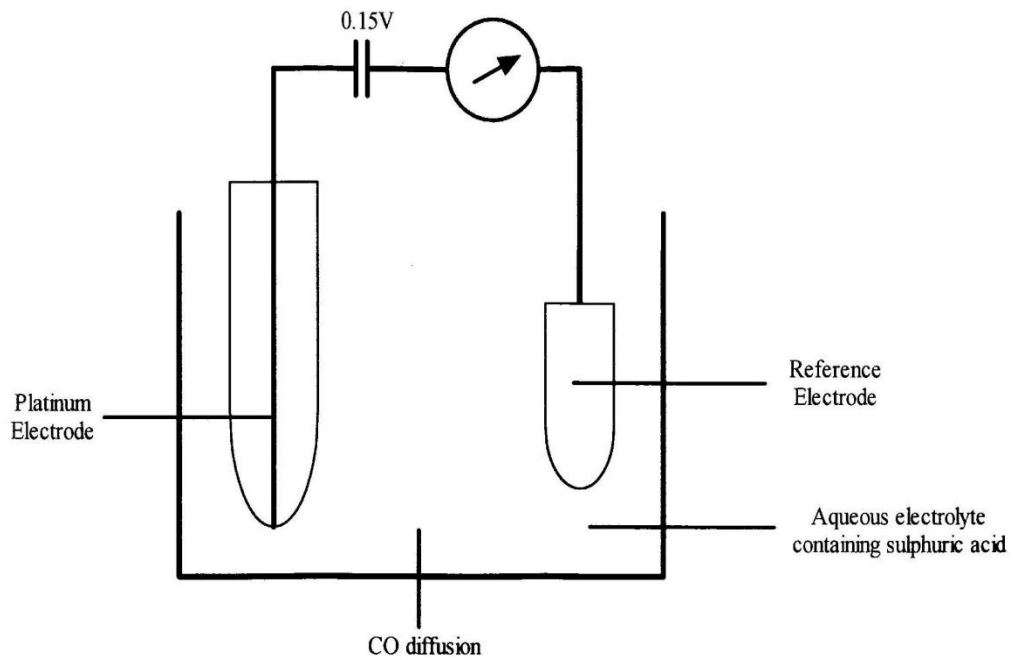
Where  $e^-$  is an electron and at the reference electrode the reaction is describe by equation 1.11:



**Eq. 1.11** From Cooper *et al.*, 2011.

The flux of  $e^-$  resulting from this electrochemical reaction is recorded and is directly proportional to the partial pressure of CO. In contrast to the IR analyzers, the electrochemical ones are not affected by CO<sub>2</sub> and only filters for water and alcohol vapour are used. Each time the analyzer is used, a full scale calibration is performed

with air and a CO/air mixture. (Cooper *et al.*, 2011)



**Fig. 1.12**

**Diagram of the fuel cell for electrochemical analysis of CO.**

From Cooper *et al.*, 2011

The fig. represents a sealed fuel cell with an aqueous electrolyte with sulphuric acid containing a platinum sensing electrode and reference electrode with a constant 0.15 voltage supplied by a battery.

Another gas analysed during lung diffusion assessment is He which is measured by a thermal conductivity analyzer. This type of analyzer measures the rate at which heat is conducted by a gas, the thermal conductivity of a gas. The thermal conductivity of He is much higher compared with other respiratory gases. This type of analyzer consists of a Wheatstone bridge comprising four resistances with a constant given bridge current, so that the temperature and the resistance remain constant. Of these four resistors, one is the reference cell sealed with a gas of known concentration or exposed to atmosphere and one the measuring cell. If the Helium concentration changes in the

reference cell, the temperature and resistance in the measuring cell will change - more heat is conducted and the resistance will fall, proportional to the He concentration change. Thermal conductivity analyzers are stable, linear, calibrated at zero with air and contain a CO<sub>2</sub> and water vapour absorber through which the sample being analysed passes. (Cooper *et al.*, 2011) Calibration should be performed daily or before the analyzers are used, the readings recorded in a logbook and the minimal requirement to obtain a quality test is a two point calibration: a zero point and a full scale deflection.

#### *1.3.8 Diffusing capacity for CO by the Single Breath Method*

The single breath breath-holding measurement is based on the measurement of CO uptake over a short period of time by the estimated difference of the partial pressure between the alveoli and the pulmonary capillary blood.

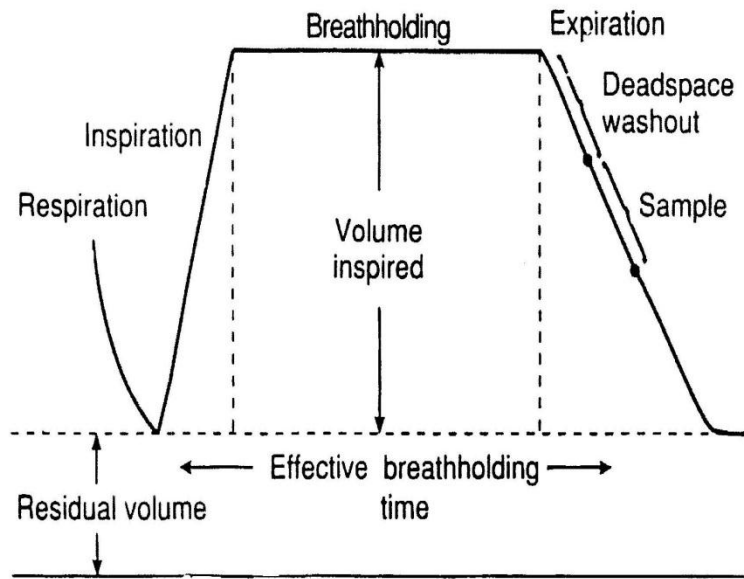
Measurement of the diffusing capacity of the lung by the single breath method requires a mixture containing the actual test gas, CO and an inert gas that allows alveolar volume assessment, He. He is a stable gas that is almost inactive and relatively insoluble and therefore is called the tracer gas due to its chemical properties. He is used to determine the CO initial concentration, as well as the VA. Diffusion of the tracer gas should be similar to the transfer gas (CO), not interfere with the measurement of CO concentration and also be absent in alveolar gas. In this method the subject inspires a vital capacity breath from a system with a spirometer containing a gas mixture of 0.3% CO, 10% He, 21% O<sub>2</sub>, and the balance N<sub>2</sub>. First, the subject exhales completely to RV and then inhales the gas mixture and holds breath at TLC for 10 s. Before the breath-hold, a valve opens allowing exhalation to the spirometer and a sample of alveolar gas

is collected in a bag, after the washout volume is discarded, as illustrated in figure 1.13. The washout volume contains gas from the anatomical and instrumental dead space and should be about 750 to 1000ml, unless the VC of the subject is less than 2000 ml in which case the washout volume should be reduced to 500ml. The instrumental  $V_D$  represents the mouthpiece, filters and connections within the valve system, and should be specified by the manufacturer. The anatomical  $V_D$  is the volume of the conducting airways that are not involved in gas exchange and the most commonly used formula for its calculation is by the equation 1.12.

$$2.2ml \times Kg \text{ subject body weight}$$

**Eq. 1.12** From MacIntyre *et al.*, 2005.

Alveolar gas sample contamination with  $V_D$  gas may interfere with measured CO uptake. Therefore, its correct estimation is particularly important, as He concentration in expired gas will indicate the effective dilution of the inspired gas with the alveolar gas and give the initial alveolar CO pressure. (Ruppel, 1998)



**Fig 1.13**

**Trace obtained during the single breath breath-hold transfer factor measurement.**

Trace shows the inspired volume breath-hold time, washout and sample volumes.

From Cooper *et al.*, 2011

The DLcoSB method assumes that both CO and He are similarly diluted in inspiration. The sample collected is analysed and the fractional CO and He concentrations in the alveolar gas obtained. The equation 1.13 illustrates how the initial concentration of CO is calculated:

$$FACO_0 = FICO \frac{FAHe}{FIHe}$$

**Eq. 1.13** From Cooper *et al.*, 2011.

Where:

- FICO is the fraction of CO in the reservoir (usually 0.003);
- FIHe the fraction of He in the inspired gas (usually 0.10);
- FACO<sub>0</sub> is the initial CO concentration in the alveoli at the



beginning of the breath-hold;

- FAHe the fraction of He in alveolar gas.

The variation of the He concentration will predict dilution by gas remaining in the lungs and allow the assessment of the FACO<sub>0</sub> before any gas is diffused from the alveoli into the pulmonary capillaries.

The DLcoSB is calculated by Krogh's standard equation 1.14:

$$DLcoSB = \frac{V_A 60}{(PB-47)(T)} \ln \frac{FACO_0}{FACO_T}$$

**Eq. 1.14** From Ruppel, 1998.

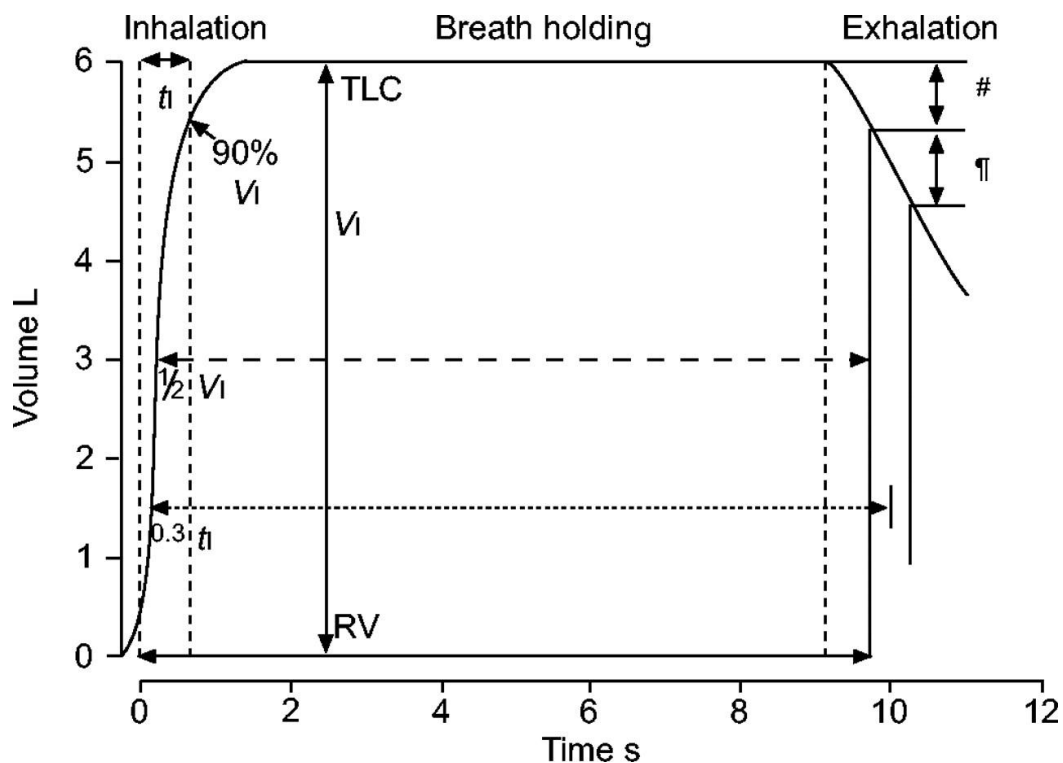
Where:

- V<sub>A</sub> is alveolar volume, ml (STPD),
- 60 is correction from seconds to minutes,
- PB is the barometric pressure, mmHg,
- 47 is the water vapour pressure (PH<sub>2</sub>O) at 37°C, mmHg,
- T the breath-hold interval, seconds,
- Ln the natural logarithm,
- FACO<sub>0</sub> the fraction of CO in alveolar gas at the beginning of the breath-hold,
- FACO<sub>T</sub> the fraction of CO in alveolar gas at the end of breath-hold.

If abnormal distribution of inspired volume is present, such as in airway obstruction, poor gas mixing can occur and this can reduce the transfer gas dilution and thus lead to a lower measured VA value.

There are several methods of measuring the breath-hold time, which estimates the time of contact between the test gas mixture and the red cells during gas exchange. In order to standardize its measurement, the ATS/ERS guidelines (MacIntyre *et al.*, 2005) recommend the Jones and Meade method (Jones and Meade, 1961) of the three available methods described in figure 1.14, in which “the breath-hold time equals the time starting from 0.3 of the inspiratory time to the middle of the sample collection time and the time when 90% of the inspired volume is achieved”. (MacIntyre *et al.*, 2005) The other two methods are the Olgivie method (Olgivie *et al.*, 1957) that measures the breath-hold time from the beginning of inspiration to the beginning of alveolar sample collection and the Epidemiologic Standardization Project (ESP) that measures the breath-hold time from the time of 50% of inspired volume ( $V_I$ ) is achieved to the beginning of alveolar sample collection.

According to Ruppel, the DLco measured with the ESP method might be higher if breath-hold time starts at the midpoint of  $V_I$ . Similarly, some diffusion occurs during washout and alveolar sampling if, as in the Jones and Meade method, the breath-hold time period is extended into the alveolar sampling phase and the real time of breath-hold is increased and the additional diffusion measured. (Ruppel, 1998)



**Fig. 1.14**

**Schematic illustration of different methods of measuring  
breath-hold time for the DLcoSB.** From MacIntyre *et al.*, 2005

The method by OGILVIE(—) measures breath-hold time from the beginning of inspiration to the beginning of alveolar sample collection. The method by JONES and MEADE(----) includes 70% of inspiratory time and half of sample time.

The Epidemiologic Standardization Project (---) measures breath-hold time from the time of 50% of VI to the beginning of alveolar sample collection. The time of inspiration (tI) defined from the back-extrapolated time 0 to the time that 90% of the VI has been inhaled.

The breath-hold time should be measured by an accurate timing device able to measure 100ms over a 10 second interval. Most modern computerized systems time the breath-hold automatically. (Ruppel, 1998)

In order to standardize the DLco measurement some precautions should be taken. According to the ATS/ERS task force guidelines (MacIntyre *et al.*, 2005), the interval between tests should be at least 4 minutes and in patients with obstructive lung disease up to 10 minutes, which will allow an adequate washout of test gas from the

lungs. Also, the patient should remain seated during this time.

MacIntyre *et al.* (2005) emphasises that some considerations must be taken before measuring DLcoSB such as diurnal variation in DLco, ingestion of ethanol (reported to decrease DLco) and administration of bronchodilators before the test (may increase DLco by up to 6%). Also supplemental oxygen should not be administered at least 10 minutes before test. Subjects should refrain from smoking 24 hours before the test (time of the last cigarette smoked should be noted), as smoking is a major source of carboxyhaemoglobin and may lead to a reduction in measured DLco reduction, due to effects on CO back pressure that decrease the Hb available to combine with CO from the test gas. (MacIntyre *et al.*, 2005) At least two acceptable tests should be recorded and meet the repeatability criteria of “either being within 3 ml CO (STPD) · min<sup>-1</sup> · mmHg<sup>-1</sup> of each other or within 10% of the highest value” in order to obtain an accurate DLco measurement. The acceptability test criteria for DLcoSB measurement, according the ATS/ERS guidelines, are the use of quality-controlled equipment, an inspired volume of at least 85% of the higher VC in less than 4 s, no evidence of leaks, a stable breath-hold for 10 s, an expiration of less than 4 s with sample collected in less than 3 s and sufficient V<sub>D</sub> clearance.

Whether Hb should be standardised, in normal subjects or patients, for DLcoSB measurement is still being debated, as the cost of measuring Hb in every patient during routine clinical assessment is high in relation to the small improvement in accuracy of the measurement. According to Cooper *et al.* (2011) unless the Hb concentration is < 11.0 g.dl<sup>-1</sup> or > 18 g.dl<sup>-1</sup>, correcting the Hb for each DLcoSB measurement will add a ± 5% difference. For that reason, Hb correction should be applied in conditions where the Hb concentration is expected to be low or changing. The standard Hb value is assumed to be 14.6g.dl<sup>-1</sup> in adult males and adolescents and 13.4g.dl<sup>-1</sup> in adult females and

children < 15 years old, but equations 1.15 for adult males and 1.16 for adult females and children <15 years old can be used for adjusting the DLco predicted:

$$Hb = DLco \text{ predicted } (1.7Hb/(10.22 + Hb))$$

**Eq. 1.15** From MacIntyre *et al.*, 2005

$$Hb = DLco \text{ predicted } (1.7Hb/(9.38 + Hb))$$

**Eq. 1.16** From MacIntyre *et al.*, 2005

CO diffusion depends mainly on two conductance properties which are the rate of combination of CO with Hb, expressed as the product of the CO-Hb reaction rate ( $\theta$ ) and the volume of Hb in alveolar capillary blood ( $V_c$ ); and the membrane conductivity (DM) which predict the diffusion properties of the alveolar capillary membrane. Several physiological and pathological processes can affect DM,  $\theta$  and  $V_c$  and interfere with the DL<sub>CO</sub>. (MacIntyre *et al.*, 2005) Conditions such as respiratory muscle weakness and thoracic deformity that can lead to a reduction in lung inflation (which consequently reduces  $V_A$ ) will decrease effort and produces changes in DM,  $\theta$  or  $V_c$  reducing DL<sub>CO</sub>. Also, diseases like anaemia and pulmonary emboli, as well as Hb binding changes and the Valsalva manoeuvre can reduce  $\theta$  and  $V_c$  and thus reduce DL<sub>CO</sub>. Lung resection, emphysema, interstitial lung disease, pulmonary oedema, pulmonary vasculitis and pulmonary hypertension reduce DM,  $\theta$  and  $V_c$  and thus reduce DL<sub>CO</sub>. Other conditions and diseases can increase  $\theta$  and  $V_c$  and increase DL<sub>CO</sub>, as such polycythaemia, left-to-right shunt, pulmonary haemorrhage, asthma, Hb binding changes, the Muller manoeuvre, exercise, the supine position and obesity (possible DM component). (MacIntyre *et al.*, 2005)

Diseases that are associated with abnormally increased alveolar-capillary membrane thickness, such as interstitial lung disease, lead to a reduction in the diffusing capacity of the lung and it is also reduced when the surface area of the blood-gas barrier is reduced, such as after a pneumonectomy. In emphysema, low diffusing capacity of the lung is caused by the destruction of alveolar walls and capillaries (West, 2008b). Thus, DLco measurement is a useful tool for the clinical assessment, diagnosis and management of both obstructive and restrictive pulmonary disorders (Ruppel, 1998).

The degree of severity of decrease in diffusing capacity for carbon monoxide is defined by the ATS/ERS guidelines (Brusasco *et al.*, 2005) as mild where DLcoSB and KCO% predicted are between the lower limits of normal and above 60%, moderate where DLcoSB and KCO % predicted are between 40 and 60% and severe where DLco and KCO% predicted are below 40%.

The DLcoSB reflects the uptake of CO per unit pressure difference between the alveoli and pulmonary capillary and if the CO diffusion within the lung is normal, abnormalities of DLco suggest disease at alveolar level. The KCO, also called transfer factor, attempts to correct alterations in the VA of the subject, due to an inadequate inspiration to TLC during DLco measurement in normal lungs or with maximal inspiration in the presence of respiratory disorders that reduce the VA. The measured DLco is divided by the VA during CO diffusion measurement. According to Brusasco *et al.* (2005) when “the  $K_{CO}$  is multiplied by the volume of gas in the lung containing CO (alveolar volume), the total uptake of CO by the lung per unit of time per unit driving pressure is obtained, this product being termed the transfer factor of the lung (KCO)”.

Interpreting DLco and KCO requires an understanding of normal physiology and of pathophysiological effects of the disease being assessed. The predicted reference values for VA, DLco and KCO should be used from the same source and factors that

interfere with DLco and KCO measurement should be considered when interpreting the results. The most commonly used predicted reference values equations for DLcoSB calculations are those from 1993 ERS document. (Jones and Meade, 1961)

## 1.4 Abnormalities of Gas Exchange in Human Lung

### 1.4.1 Chronic Obstructive Pulmonary Disease

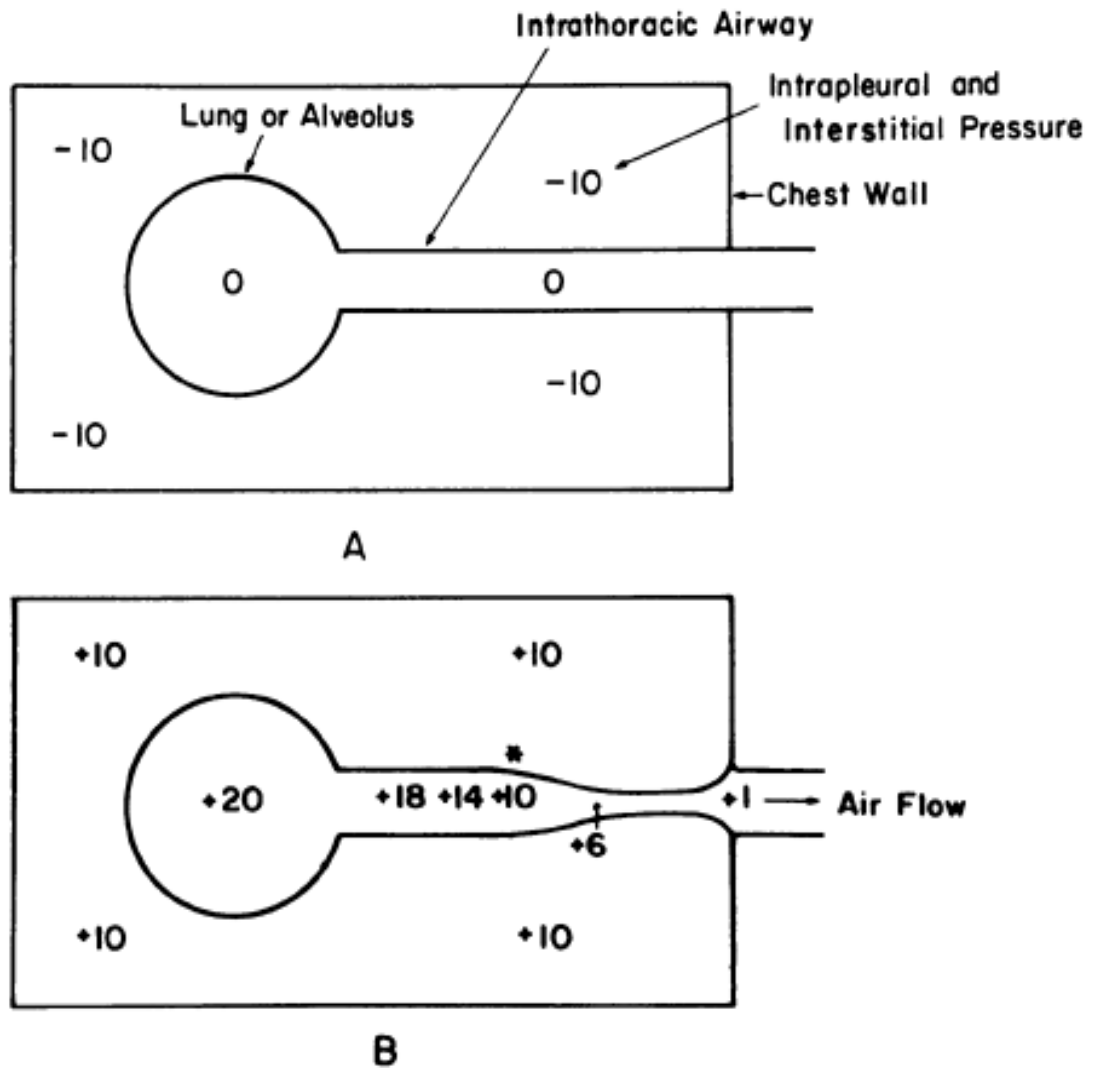
According to the ATS/ERS task force (Celli *et al.*, 2004), chronic obstructive pulmonary disease (COPD) is a “preventable and treatable disease state characterised by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and is associated with an abnormal inflammatory response of the lungs to noxious particles of gases, primarily caused by cigarette smoking”. COPD commonly produces pathological changes in four different compartments of the lungs: the central airways, peripheral airways, lung parenchyma and pulmonary vasculature, and despite being primarily a disease of the lungs, can also lead to significant systemic effects. (Celli *et al.*, 2004)

COPD is an umbrella term for a combination of emphysema and chronic bronchitis (peripheral airway obstruction). Emphysema is characterized pathologically by alveolar wall destruction which leads to enlargement of airspaces and loss of lung elasticity, obstruction of peripheral airways and destruction of the capillary bed. Chronic bronchitis is characterized by peripheral airways obstruction due to inflammation and excessive mucus production by hypertrophy of mucous glands. The abnormal amount of mucus in the airways may obstruct some of the small bronchi. (West, 2008b) Airway obstruction in patients with COPD will increase resistance to the airflow. Loss of radial

traction from destruction of the lung parenchyma will also cause airway narrowing, particularly in the peribronchial region of the airways.

The increase in airflow resistance in COPD will amplify the effect of the static recoil pressures reduction on the normal pressure gradient between the alveoli and the mouth. The loss of elastic recoil and excessive contraction of abdominal muscles will decrease intraluminal pressures during expiration, leading to airway collapse. This can be summarized in fig. 1.15 where an equal pressure point model is used.





**Fig. 1.15**  
**Model illustrating pleural pressures,**  
 accessed 4 April 2012, <http://flylib.com/books>

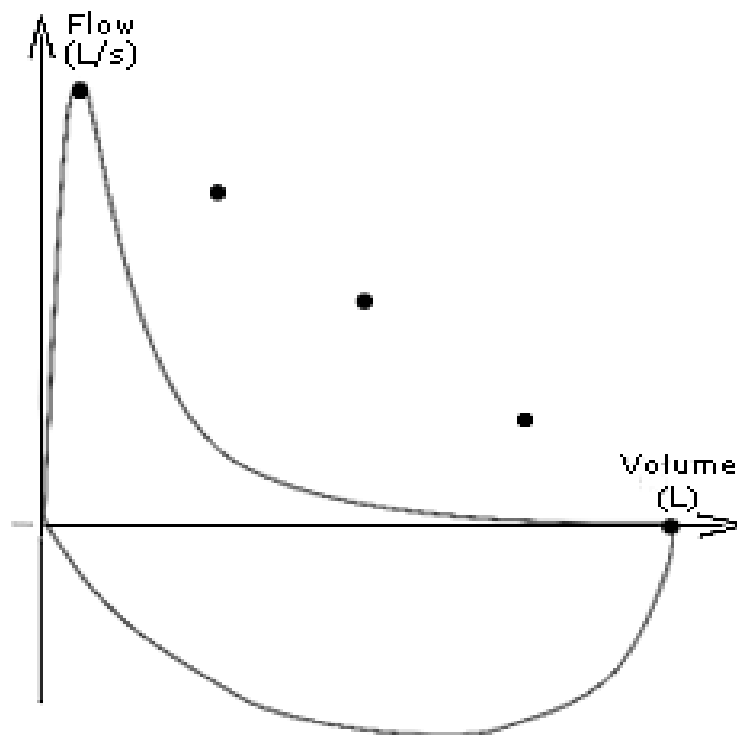
Equal pressure point model where static recoil pressure is +10 cm H<sub>2</sub>O.

In scenario A (static conditions) no flow is present and the forces of elastic recoil and chest wall recoil are balanced. As forced expiration occurs in presence of airway obstruction in scenario B (Dynamic conditions), the thoracic pressure becomes positive, frictional pressure losses within the airway occur reducing the intraluminal pressure. When the critical point is reached, airway collapse may occur and despite further increases in intrathoracic pressure, expiratory flow will remain constant.

In this diagram a critical pressure point occurs in the airway where the expiratory flow is independent of any extra applied pressure, explaining the typical

expiratory flattening of the flow-volume loop in COPD obtained by spirometry (Fig. 1.16). This explains why the FEV<sub>1</sub> and the FEV<sub>1</sub>/FVC ratio assessment are of great importance in COPD patients.

Figure 1.16 demonstrates why patients with severe COPD achieve maximum expiratory flow during tidal breathing, reducing their capacity to increase ventilation without increasing either lung volumes or inspiratory work.



**Fig. 1.16**

**COPD flow volume loop,**

accessed 4 April 2012, <http://www.spirometrie.info/fvc.html>

The air in the large airways can be expired without problems, so the PEF is will be normal.

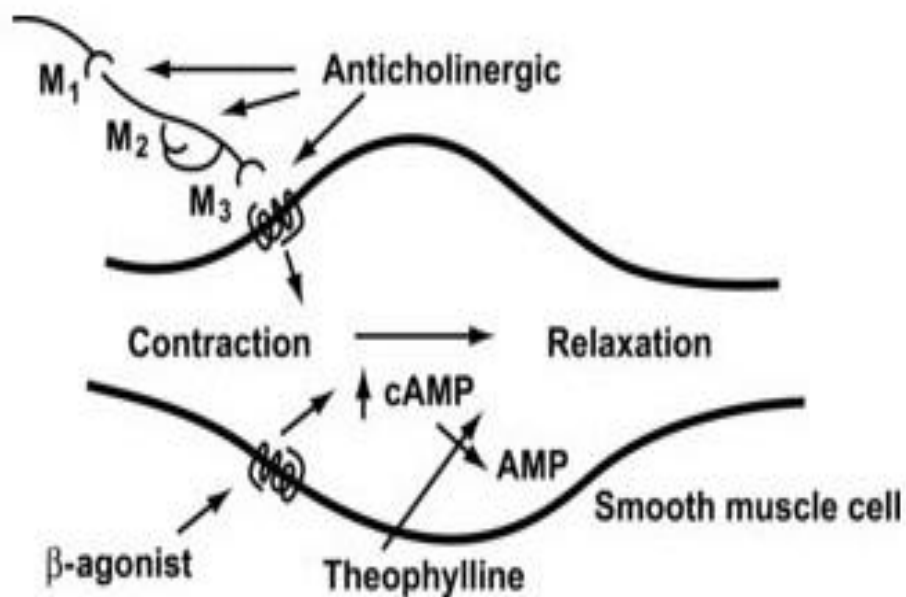
When all the air is expired from the large airways, the air from the smaller airways will be expired partially blocked and will come out slower and in a lower flow creating a sharp fall in the flow-volume.

The inflammatory response in COPD is caused predominantly by cigarette smoking. An important feature of COPD pathogenesis is the imbalance of proteinases and antiproteinases in the lungs, and oxidative stress. (Celli *et al.*, 2004)

Hyperinflation is another important feature of COPD and that leads to a significant elastic load to breathing, which becomes important as flow resistance increases. Shortening of the diaphragm length can occur, changing the muscles fibre orientation and leading to impairment of contraction. Also geometric changes will make the development of tension harder to achieve. All these respiratory muscles changes will increase the total work of breathing and oxygen consumption by respiratory muscles. The loss of vertically oriented diaphragm muscles fibres, the increased work of the pectoral muscles as accessory respiratory muscles, and the use of abdominal muscles during expiration contribute to increased work of breathing in COPD. (Gross, N. 1993) Farkas *et al.* (1983) reported that the respiratory muscles may adapt to the chronic hyperinflation by a compensatory reduction in sarcomere numbers.

The diagnosis of COPD should be considered when symptoms of cough, sputum production, dyspnoea or a history of exposure to risk factors for the disease are present. Diagnosis requires confirmation of airflow obstruction by spirometry. If spirometry after bronchodilator shows an  $FEV_1/FVC$  ratio  $\leq 0.7$ , the presence of airflow limitation is confirmed, which in COPD case is not fully reversible. The Global Initiative for Chronic Obstructive Lung Disease guidelines (Lenfant, C. Khaltsev, N. *et al.*, 2003) define COPD severity by a post-bronchodilator  $FEV_1/FVC \leq 0.7$  and mild COPD represented by  $FEV_1 \geq 80\%$  predicted, moderate COPD by  $FEV_1$  50-80% predicted, severe COPD by  $FEV_1$  30-50% predicted and very severe COPD by  $FEV_1 < 30\%$  predicted. (Celli *et al.*, 2004)

Three types of bronchodilator are commonly used for management of COPD:  $\beta_2$ -agonists, anticholinergic drugs and methylxanthines. Despite differences in their site of action in the airways, the most important effect of bronchodilator therapy is airway smooth muscle relaxation and lung emptying during tidal breathing. Figure 1.17 illustrates the different types of bronchodilators and their site of action.



**Fig. 1.17**

**Mechanisms of action of bronchodilators on airway smooth muscle,**

accessed 4 April 2012, <http://www.medscape.com>

The anticholinergics act by blocking the effect of acetylcholine on muscarinic receptors (M1, M2 and M3). Anticholinergics dissociates slowly from the muscarinic receptors, providing bronchodilation. Beta-2 agonist drugs bind to beta-2 receptors on airway smooth muscle and lead to an increase in the concentration of cAMP (cyclic adenosine monophosphate) in the muscle cell which results in smooth muscle relaxation. When airway smooth muscle relaxes, the diameter of the air passages is increased. Theophylline blocks the action of phosphodiesterases and prevents the breakdown of cAMP to AMP (adenoside monophosphate). This also has the effect of relaxing smooth muscle and allowing the airways to dilate.

The FEV<sub>1</sub> change in COPD after a bronchodilator is typically small but can produce larger changes in lung volumes, thus reducing RV and hyperinflation. (Celli *et al.*, 2004)

The DLcoSB is often reduced in COPD, as emphysema reduces the lungs surface area by loss of both alveolar walls and their associated capillary beds. The main consequence is less gas transfer per minute even if the remaining gas exchange units are structurally normal. The path length from the terminal bronchiole to the alveolar-capillary membrane in emphysema can be increased and gases must cross farther to reach the alveolar-capillary surface. Airways collapse and gas trapping also produces ventilation-perfusion abnormalities. (Ruppel, 2008)

Ventilation-perfusion inequality in COPD is caused by the disorganization of the lung architecture in emphysema causing hypoxemia with or without CO<sub>2</sub> retention. Respiratory failure in COPD can occur in 2 settings: moderate hypoxemia with normal arterial pCO<sub>2</sub> (Type 1 respiratory failure), or severe hypoxemia with an increased pCO<sub>2</sub>, typically seen in more advanced disease (Type 2 respiratory failure). A high alveolar-arterial pO<sub>2</sub> difference is always present (especially in severe bronchitis). V<sub>D</sub> is also increased in emphysema. Destruction of the capillary bed causes uneven ventilation as well as regional inequality of blood flow. However, impaired gas exchange and V/Q inequality can be minimised by collateral ventilation (physiologic communicating channels that exist between adjacent alveoli and between small airways) and hypoxic vasoconstriction, a local response to low alveolar pO<sub>2</sub> that reduces blood flow to poorly ventilated areas. (West, 2008)

Despite V/Q inequality, arterial pCO<sub>2</sub> can be normal in mild to moderate COPD, but as severity progresses, the arterial pCO<sub>2</sub> may increase. An important factor is the increased work of breathing but also reduced sensitivity of the respiratory centre to

CO<sub>2</sub>. The rise of pulmonary artery pressure in COPD as severity progresses contributes to V/Q inequality as portions of the capillary bed are destroyed, increasing vascular resistance and hypoxic vasoconstriction. (West, 2008b)

Physiologically significant changes in pulmonary circulation occur with disease progression in COPD. The normal pulmonary circulation has great capacitance with low vascular resistance, due to its ability to recruit additional capillary beds. Gross *et al.*(1993) reported that in patients with COPD at rest, the mean pulmonary artery pressure increases by “the loss of capillary bed due to emphysema, changes in blood viscosity due to polycythaemia, altered alveolar oxygen tension due to a V/Q inequality, or raised intrathoracic pressures”. Polycythaemia occurs due to daytime and/or nocturnal hypoxia which can worsen with continued cigarette smoking (Calverley *et al.*, 1982). In advanced COPD, severe pulmonary hypertension can occur, reducing the cardiac output.

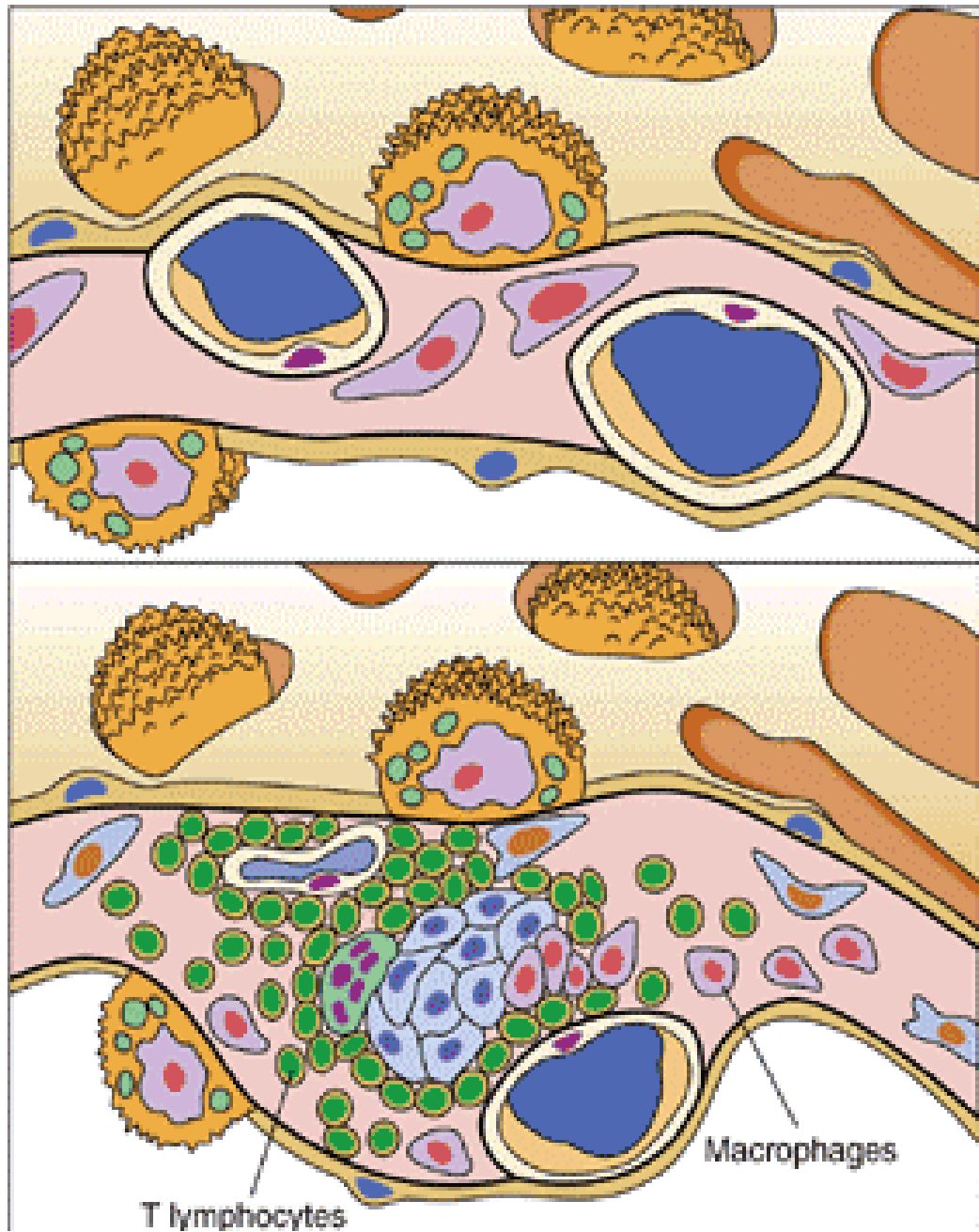
#### 1.4.2 Sarcoidosis

Sarcoidosis is defined by Fishman (2007) as a “multisystem disorder of unknown origin aetiology characterized by noncaseating granulomatous inflammation at sites of disease that frequently presents with bilateral hilar lymphadenopathy, ocular and skin lesions”. Although any organ can be involved, this disorder predominantly involves the lungs and lymph nodes. The diagnosis of sarcoidosis is frequently established from clinical and radiologic findings, in combination with demonstration of noncaseating epithelioid granulomas in affected organs and exclusion of other causes of granulomatous disease. (Fishman, 2007)

Although the cause of sarcoidosis is still uncertain, it is thought that genetically susceptible hosts develop sarcoidosis when exposed to specific environmental agents. This concept, as reported by Hunninghake *et al.* (1999), is supported the fact that the inflammatory response present in sarcoidosis is characterized by activated macrophages and CD<sub>4</sub> helper T lymphocytes that lead to a profile of cytokine production that is consistent with a Th1-type immune response triggered by an antigen. Fishman (2007) states that clinical, epidemiologic, and family studies strongly suggest that exposure to microbial agents when a genetic susceptibility is present can trigger sarcoidosis.

Sarcoidosis occurs between 20 and 40 years in 80% of cases, but also is seen in women > 50 years. (Fishman, 2007) The course and prognosis of sarcoidosis may relate to the pattern of onset of the disease. Sarcoidosis that presents acutely with erythema nodosum and bihilar lymphadenopathy commonly shows a self-limiting course, and by contrast, a more insidious onset is more likely to lead to progressive fibrosis of the lungs. (Hunninghake *et al.* 1999)

The characteristic pathologic feature in sarcoidosis is the presence of noncaseating epithelioid cell granulomas composed of differentiated mononuclear phagocytes and lymphocytes. Commonly, sarcoidosis granulomas are located in lymph nodes (especially intra-thoracic), lungs, liver, spleen and skin. These granulomas can resolve spontaneously, or produce fibrotic damage, which can lead to parenchymal fibrosis of the lung. (Hunninghake *et al.* 1999)



**Fig. 1.18**

**Pathogenesis of Sarcoidosis,**

accessed 4 April 2012, <http://highered.mcgraw-hill.com>

The normal alveoli represented in the top and the alveoli in active sarcoidosis in the bottom.

The latter are distorted by the accumulated CD4<sup>+</sup> T<sub>H</sub>1 lymphocytes, alveolar macrophages, and macrophages aggregated into granulomas. There is mild damage to alveolar epithelial and endothelial cells.



Figure 1.18 shows how alveoli in active sarcoidosis may be damaged by accumulated CD4+ Th1 lymphocytes, alveolar macrophages, and macrophages aggregated into granulomas. The trigger origin for the Th1 lymphocytes is uncertain but according to Hunninghake *et al.* (1999), a limited class of antigens or self-antigens presented in the context of class II HLA surface molecules by mononuclear phagocytes to the Th1 lymphocyte may be causal. Increased production of inflammatory mediators such as TNF-alpha, IFN-gamma, and IL-12 is present. The antigen class II HLA complex is identified by the T cell antigen receptor, and the T cell is activated. As a result, Th1 lymphocytes will release IL-2, which causes activation of more T lymphocytes. Macrophage inflammatory proteins and granulocyte-macrophage colony-stimulating factor contribute to granuloma formation. The diagnosis of pulmonary sarcoidosis is based on finding stable formed granulomas surrounded by lymphocytes and fibroblasts in a perilymphatic interstitial distribution with no alternative cause.

In sarcoidosis the lungs are commonly affected, and in 30-50% of cases, dyspnoea, dry cough and chest pain may occur. Pulmonary sarcoidosis is frequently staged by radiographic findings, as reported by Hunninghake *et al.* (1999) Five chest radiographic stages can be defined: stage zero, with no visible intrathoracic findings; stage one where bilateral hilar lymphadenopathy is present, together in some cases with paratracheal adenopathy; stage two with hilar adenopathy and parenchymal infiltration; stage three with parenchymal infiltration without hilar adenopathy; and the last, stage 4 with advanced fibrosis and “honey-combing”, hilar retraction, bullae, cysts and emphysema. Grutters *et al.* (2009) stages sarcoidosis based on data from patient studies, interpreted in the context of experimental models of immune responses and in this model, three stages are mentioned:

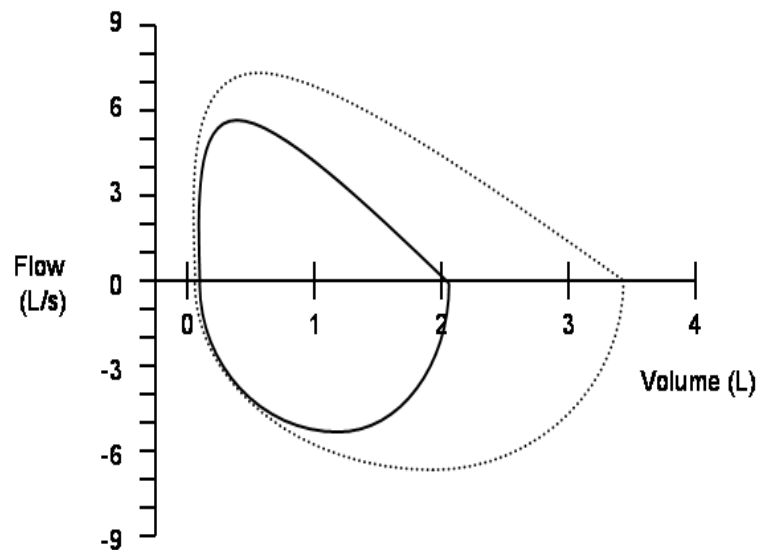
- Stage one, involves the exposure to present unknown exogenous or endogenous antigenic proteins taken by the antigen cells and processed into peptide fragments. These peptides are loaded by an histocompatibility Class II molecules and taken to the T-Cell receptors of the T-lymphocytes (Th<sub>0</sub>). The Th<sub>0</sub> is activated and polarised as T-helper type 1 cells (Th<sub>1</sub>) under the influence of an interleukin (IL).
- Stage two, is characterised by a granuloma formation due to an ongoing presentation of antigen by the lung macrophages to the Th<sub>1</sub> cells. These will start a well organized production of chemokines and cytokines by the Th<sub>1</sub> cells which leads to a high demand, migration and retention of cells such as T-lymphocytes and macrophages. All these cells organize into immune granulomas, usually non-necrotising.
- Stage three, will involve the evolution of the granulomatous inflammation that can resolve spontaneously or persist leading to a chronic course of disease and the onset of a fibrotic response. This fibrotic process may lead to end-stage sarcoidosis marked by parenchymal fibrosis and honeycombing pattern.

Commonly, pulmonary function is normal in stages zero and one of the disease, but in stages two and higher, a non-obstructive ventilatory defect can be found. In this setting, lung volumes will be decreased except for the FEV<sub>1</sub>/FVC ratio which is normal as classified by the British Thoracic Society (Bellamy, 2005) and presented in table 1.1.

Spirometry Parameters	Non Obstructive Disorders
FEV <sub>1</sub>	< 80% predicted normal
FVC	< 80% predicted normal
FEV <sub>1</sub> /FVC ratio	Normal (> 0.7)

**Table 1.1 Spirometry Classification for non obstructive disorders**

The Figure 1.19 illustrates a typical non-obstructive flow volume loop.



**Fig. 1.19**

**Non-obstructive Flow Volume loop,**

accessed 4 April 2012, <http://courses.washington.edu>

The shape of the flow volume loop is relatively unaffected in restrictive disease, but the overall size of the curve will appear smaller when compared to normal on the same scale.

Similarly, there will be a rapid upslope on the volume time curve, but such patients will reach a smaller vital capacity.

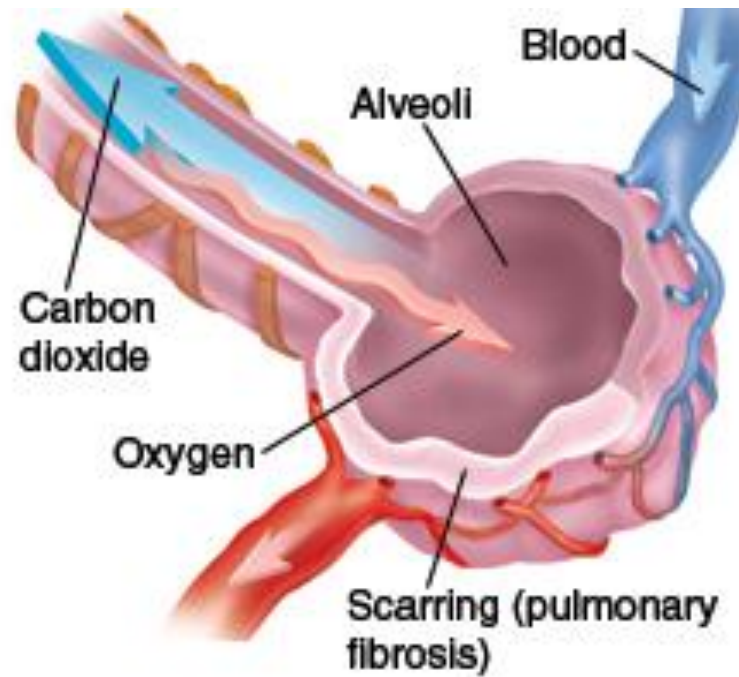
Furthermore, in stages two or three the DLcoSB and KCO can be impaired. The  $pO_2$  at rest may be low and can decrease significantly with exercise. The  $pCO_2$  can be normal or low, but will rise in advanced stages. (West, 2008b)

### *1.4.3 Idiopathic Pulmonary Fibrosis*

Idiopathic Pulmonary Fibrosis (IPF) is the most common cause of interstitial lung disease and its cause is uncertain. IPF is characterised by a specific combination of clinical, radiographic and pathological findings that lead to a progressive dyspnoea, hypoxemia and ultimately death. The IPF incidence increases with age; two-thirds of cases occur over the 60 years with a mean of 66 years at diagnosis. (Fishman, 2007)

Usually, IPF presents with gradual onset of cough and dyspnoea. Dyspnoea is progressive and commonly reported for more than 6 months before diagnosis. IPF is defined by Ruppel (1998) as a “specific form of chronic fibrosing interstitial pneumonia limited to the lung and associated with the histologic appearance of usual interstitial pneumonia on surgical lung biopsy”. The main feature is fibrosis of the interstitium, leading to subpleural changes that are seen on chest x-ray and high-resolution CT thorax, and at an advanced stage will show a “honey-combing” pattern.

In the early stages of IPF, infiltration with lymphocytes and plasma cells is present and as the disease progresses, fibroblasts appear and form a thick collagen mass. Further, the normal alveolar structure is destroyed and the typical scarring (illustrated in the figure 1.20) leads to empty cystic spaces in the terminal and respiratory bronchioles. (West, 2008b)



**Fig. 1.20**

**Alveoli with Pulmonary Fibrosis,**

accessed 4 April 2012, <http://www.mountnittany.org>

Pulmonary fibrosis is scarring of the lung tissue between the alveoli and the blood vessels. If scarring is severe,  $O_2$  can not easily move from the air in the alveoli to the blood in the vessels. As a result, the blood does not deliver enough  $O_2$  to the body.

Interstitial lung disease may arise from the use of medications such as bleomycin or amiodarone and can co-exist with autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and scleroderma, as these disorders can result in alveolar wall inflammation and fibrotic changes. (Ruppel, 1998) Therefore, other causes of interstitial lung disease should be excluded before the diagnosis of IPF is confirmed.

Pulmonary function and lung imaging studies can be normal in early IPF. (King *et al.*, 2000) However, as IPF advances, pulmonary function testing will show a non-obstructive ventilatory defect with reduced FVC and  $FEV_1$  and normal  $FEV_1/FVC$  ratio with reduced lung volumes (VC and TLC). Gas exchange is typically affected as the

alveolar-arterial pressure difference for O<sub>2</sub> is increased. The reduction in DL<sub>CO</sub> may precede lung volume impairment. (Cooper *et al.*, 2011)

The blood volume of the pulmonary capillaries decreases in IPF due to vessels destruction by the fibrotic process. The hypoxemia that results from reduced DL<sub>CO</sub> is usually mild at rest and will increase as IPF advances, where physiological V<sub>D</sub> and shunt are increased. Decreased pCO<sub>2</sub> occurs due to increased ventilation to the alveoli. The work of breathing will also be increased in IPF as hyperventilation due to high respiratory rates is present. The most important cause of hypoxemia in IPF is V/Q mismatching, not anatomic shunting or reduced O<sub>2</sub> diffusion. (Fishman, 2007)

## Chapter 2

### ***METHODOLOGY***

#### 2.1 Study Protocol

The study involved the measurement of spirometry and CO diffusion capacity by the single-breath method in healthy subjects, patients with severe COPD and patients with interstitial lung disease (IPF and sarcoidosis patients with interstitial fibrosis) recruited from the Mercy University Hospital outpatient clinic. CO diffusion was measured with a standard 10 s breath-hold time and then repeated with an 8 s and 6 s breath-hold to determine if breath-hold time reduction led to significant differences in measured DLco. The Clinical Research Ethics Committee of the Cork Teaching Hospitals approved the protocol. After detailed explanation of the reasons for the study, informed consent was obtained from the subjects for participation in the study .

#### 2.2 Study Criteria

The inclusion and exclusion criteria were identified based on the the ATS\ERS guidelines for the DlcoSB measurement (MacIntyre *et al.*, 2005). The ATS/ERS taskforce reports that some physiological and pathological changes can affect DLco measurements. Therefore, all subjects with asthma, obesity (BMI > 30), history of lung resection, anaemia (Hb<11 g/dL), recent pulmonary embolus (< 6 months), recent pulmonary haemorrhage (< 6 months), polycythemia, pulmonay oedema, pulmonary

vasculitis, left-to-right shunt, respiratory muscle weakness or thoracic deformity, severe cognitive dysfunction or psychiatric illness and unstable comorbidity (uncompensated congestive heart failure, severe pulmonary hypertension, unstable angina or recent myocardial infarction) were excluded from the study. (MacIntyre *et al.*, 2005)

The ATS/ERS guidelines also state that the use of supplemental O<sub>2</sub> 10 minutes before the DLco measurement and exercise 1 hour prior to testing testing can affect the pulmonary capillary blood volume and therefore the DLco value. Patients were instructed not to use supplemental O<sub>2</sub> 10 minutes and also not to perform exercise before measurements. The consumption of alcohol within 24 hours of testing has been reported to decrease DlcoSB measurements (MacIntyre *et al.*, 2005; Wanger *et al.* 2005) as some fuel-cell CO analysers are sensitive to exhaled ethanol and ketones. Therefore patients were excluded if alcohol consumption was not avoided.

Active smokers were excluded as COHb can decrease measured DLcoSB due to CO back pressure effects and the reported “anaemia effect” decreasing Hb binding sites for CO. (Viegi *et al.*, 1998), and consequently all study subjects were non-smokers or ex-smokers for more than one year.

### 2.3 Study Group

The study group consisted of 90 subjects who met the inclusion criteria. There were 30 healthy controls, 30 patients with COPD and 30 with interstitial lung disease (sarcoidosis with pulmonary fibrosis or IPF) all recruited from the Mercy University Hospital Outpatient clinic by a respiratory consultant. All subjects performed baseline Spirometry and DLcoSB in the respiratory laboratory of the Mercy University hospital



before recruitment, part of the routinely clinical respiratory assessment conducted by the respiratory team.

The healthy controls had no history of airway or lung disease and had normal pulmonary function ( $FEV_1/FVC > 70\%$ ,  $DL_{CO} > 80\%$  predicted and  $KCO > 80\%$  predicted).

The COPD group had a pre-existing diagnosis of COPD and were included if  $FEV_1/FVC$  was  $< 70\%$  and  $FEV_1$  was 30-50% predicted, after inhaled bronchodilator. The ILD group had a pre-existing diagnosis of sarcoidosis stage two with pulmonary fibrosis or IPF and were included if  $FVC < 80\%$  predicted,  $FEV_1/FVC$  ratio  $>70\%$  and  $DL_{CO}SB < 80\%$  predicted. (Miller *et al.*, 2005)

## 2.4 Measurements Protocol

All measurements were performed in the Respiratory Laboratory of the Mercy University Hospital by the same certified Senior Respiratory Scientist with the subject seated and at rest. All measurements were performed between 9am and 1pm, as diurnal variation caused by a combination of changes in CO back pressure and diurnal variation in Hb concentration can affect the  $DL_{CO}$  measurements. (Cinkotai and Thomson, 1966; Frey *et al.*, 1987)

All subjects performed at first spirometry according to the ATS/ERS recommendations (Miller *et al.*, 2005) allowing the  $FEV_1$ , FVC and  $FEV_1/FVC$  ratio assessment. Spirometry was recorded by a **Jaeger Master-Screen PFT** (Appendix B).

After spirometry measurement the Hb was measured in all study subjects with an **Hemocue Hb 201+** (Appendix A), necessary for the Hb value adjustment in all

following DLcoSB measurements, according to the ATS/ERS recommendations. (MacIntyre *et al.*, 2005)

The first DLcoSB measurement with a 10 s breath-hold time was performed in all subjects according to the ATS/ERS guidelines (MacIntyre *et al.*, 2005), to ensure that every subject met the minimum cooperation requirements for the study.

Before each DLcoSB measurement the analyser was zeroed against room air. Each subject was studied in the sitting position, at rest and with a nose clip in place. After a stable VT, each subject exhaled to RV and then inhaled a gas mixture containing 0.28% CO, 9% He, 19% O<sub>2</sub> and the remainder N<sub>2</sub> to TLC. The software took into account the time delay between volume and gas fraction signals. When TLC was reached, a digital clock began timing the breath-hold, and after 10 s of breath-hold, the patient exhaled again to RV and a sample was collected for analysis. The breath-hold time was measured according to the Meade-Jones method (Jones and Meade, 1961) which assumed that the breath-hold time equals the time starting from 30% of the inspiration time to the middle of the sample collection time. The inspiratory time was defined when 90% of the V<sub>I</sub> was reached determining the end point.

The DLco in this study was measured by the single breath method of Ogilvie *et al.* (1957) as modified by Jones and Meade (1961). The initial 750 ml of exhaled breath was discarded (representing the V<sub>D</sub>) and the next 750 ml of gas sample was used for analysis (or 500 ml if the subject's VC was < 2L) (MacIntyre *et al.*, 2005) The CO fraction was determined by an electrochemical analyser and the He by a thermal conductivity analyser.

Two acceptable tests for each measurement were recorded, meeting the repeatability requirements of ATS/ERS recommendations (MacIntyre *et al.*, 2005) of either being within 3 mL CO (STPD)·min<sup>-1</sup>·mmHg<sup>-1</sup> of each other or within 10% of the

highest value. At least 4 minutes were allowed between tests to provide adequate elimination of test gas from the lungs and in the COPD group a 10 minutes interval was given according to the same guidelines. (MacIntyre *et al.*, 2005). All subjects remained seated during this period.

DLcoSB was calculated according to the standard equation:

$$DL_{CO} = (VA / (t / 60 (P_B - P_{H_2O}))) \ln((F_{AHe} F_{ICO}) / (F_{IHe} F_{ACO}))$$

**Eq. 2.1** From MacIntyre *et al.*, 2005.

In equation 2.1 the VA is in ml STPD, t is the time of breath-hold and P<sub>H<sub>2</sub>O</sub> is the water vapour. The VA was measured from the He dilution as shown in the equation 2.2:

$$VA = (V_I - V_D) (F_{IHe} / F_{AHe})$$

**Eq. 2.2** From MacIntyre *et al.*, 2005.

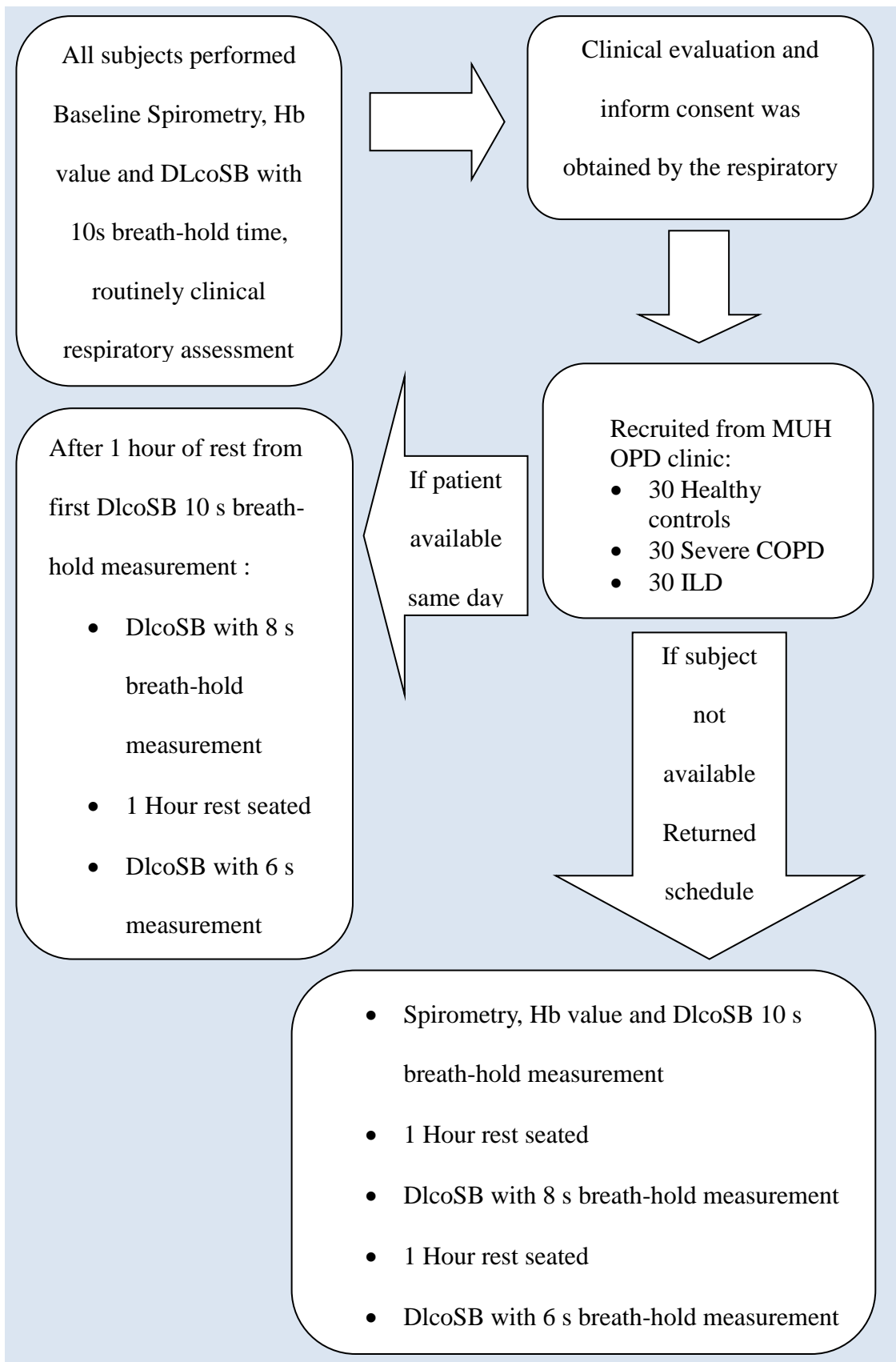
If the subject met the recruitment inclusion criteria for the study, an informed consent was obtained by a respiratory consultant at the out-patients clinic of the Mercy University Hospital for participation in the study.

After 1 hour of rest following the first DlcoSB measurement with 10 s breath-hold, another measurement with breath-hold time reduced to 8 s was repeated followed again by 1 hour of rest until the last measurement with 6 s of breath-hold. All subjects remained in the waiting area of the Respiratory Laboratory of the Mercy University Hospital between the measurements.

In the COPD group all measurements were performed after inhaled bronchodilator therapy, which was not suspended for the study. It has been reported that the bronchodilator administration may increase the D<sub>l</sub>coSB measurements up to 6% while affecting the VA and vasomotor tone. (MacIntyre *et al.*, 2005) The bronchodilator effect was taken into consideration when the results were analysed.

All measurements were part of the clinical respiratory assessment routinely conducted by the respiratory team. The only additional to the routine procedure was the breath-hold time reduction in the DLcoSB, which required two additional measurements to achieve the aim of this study.

The figure 2.1 summarizes the present study design.



**Fig. 2.1 Study Design**

## 2.5 Measurement Equipment

Two types of equipment were used during the study, both in the Pulmonary Function Laboratory of the Mercy University Hospital. A brief description of each device, the basic principle of operation and its primary function in the study are given in this chapter.

### 2.5.1 Jaeger Master Screen PFT

This is a complete system for recording spirometry, pulmonary diffusion capacity and lung volumes.

This system allows spirometry and CO-Diffusion by the single breath method measurement. Spirometry was performed in all subjects to assess FEV<sub>1</sub>, FVC and FEV<sub>1</sub>/FVC, followed by DL<sub>co</sub>SB, KCO,VD, V<sub>A</sub>, FAHe and FAco assessment at three different breath-hold times of 10, 8 and 6 s with a gas mixture of 0.28% CO, 9% He, 19% O<sub>2</sub> and the remainder N<sub>2</sub>. (Appendix A).

The volume and gas analysers used were calibrated daily according to the Irish Association Respiratory Scientists Quality Assurance guidelines (IARS, 2011). The Flow Measuring Sensor Calibration establishes the relationship between sensor-determined values of flow or volume and the real flow or volume is named calibration. (Brusasco *et al.*, 2005)

Calibration was performed twice daily with volume checked by a 3L syringe, which was within 3% or 50 ml/s. This 3L syringe should read between 2.91 and 3.09 L

in calibration mode. Also linearity checks of flow rates between 2-12 L/s were performed weekly by adjusting the speed of the syringe in the flow sensing devices to cover the range of flows produced by the patient population, as low (2L/s), medium (6L/s) and fast flows (12L/s). The calibration syringe used was periodically leak tested at more than one volume up to its maximum and had a valid calibration certification.

A second calibration was performed to the gas analyser. This calibration is called two-point calibration, where two gas concentrations are used. One gas concentration was used to zero the low end of the range and the second one was used to adjust the high end of the range. The main goal of this calibration is to adjust the analyser value in order to match the input of the known concentration of a gas. (IARS, 2011) This calibration was performed twice daily and was within  $\pm 1\%$  of initial gas concentration. Stability is of major importance in analyzers, with minimum drift ( $< \pm 0.5\%$  of full scale) between calibration and testing, therefore the reading of the analysers was checked to detect any drift during testing. Quarterly gas analysers timing should be consistent with gas flow rate at a constant 200ml/min with a response time of less than 30 s, which was checked during annual service.

Prior to and after each measurement the gas analysers were zeroed and the gas calibration was performed at the time of the volume calibration.

### *2.5.2 Hemocue Hb 201+*

This is a portable system used for the determination of the total amount of haemoglobin in whole blood (Appendix B). The system consists of a specially designed analyzer with microcuvettes containing dried reagents. The haemoglobin measurement takes place in the analyzer, which follows the progress of the reaction until the end point

has been reached. This equipment allowed the quantitative determination of haemoglobin on every patient before the CO-diffusion measurement.

No calibration process was necessary before each measurement. The system is factory calibrated against the haemoglobin cyanide method, the international reference method for the determination of the haemoglobin concentration in the blood (Davis and Jungerius, 2010), and needed no further calibration. The analyzer has an internal quality control process that automatically verified the performance of the optronic unit during each measurement.

## 2.6 Data storage and patient confidentiality

All data pertaining to the study was stored in a computerized database on an encrypted departmental computer and backed up on CD and USB drives kept in a locked press in a locked office in the Respiratory Laboratory of the Mercy University Hospital. Data was available only to the principal investigator and co-investigators.

Informed consent forms and other paper records pertinent to the study were kept by the principal investigator in a locked office. Personal data was stored, processed and analyzed in an anonymized format. This format did not include name, date of birth, address or medical record number of the subjects.



## Chapter 3

### ***DATA ANALYSIS AND RESULTS***

#### 3.1 Introduction

This chapter presents the analysis and results of the data from this study. The study limitations regarding data collection and the statistical methods employed in the analysis are described first, followed by the details of the group characteristics and the data analysis.

#### 3.2 Study Limitations

The main limitation of this study laid on the subject recruitment, as different groups were difficult to match for age and gender. This limitation justified the recruitment of 5 ex-smokers for the control group that, despite of being ex-smokers, met the inclusion and inclusion criteria for the control group, with normal spirometry ( $FEV_1/FVC > 70\%$ ,  $FEV_1 > 80\%$  pred and  $FVC > 80\%$ ), normal DLcoSB ( $DLcoSB > 80\%$  predicted and  $KCO > 80\%$  predicted) and no history of respiratory impairment or lung disease.

Despite of the required maintenance of 10 s breath-hold and inspiration to TLC for the single breath method in the DLcoSB measurement, all subjects were able to perform all measurements in the study and meet the inclusion criteria.

### 3.3 Statistical Methods

In total, 36 variables were analysed. Kolmogorov-Smirnov testing was used to determine whether data were normally distributed. Comparisons between groups were made using two-tailed parametric testing or nonparametric testing, depending on whether data were normally distributed. Comparisons across groups were made using repeated measures analysis of variance (ANOVA) or Friedman test, depending on whether data were normally distributed. Statistical significance was assumed at  $p < 0.05$ . Data were analysed using GraphPad Software (San Diego, CA).

### 3.4 Group Characteristics

Table 3.1 shows the anthropometric characteristics of the group, presented as mean and standard deviation (SD). The age of the subjects ranged between the  $52.7 \pm 11.51$  years in the control group,  $63.5 \pm 9.49$  years in the COPD group and  $61.9 \pm 13.15$  years in the ILD group. Forty three subjects never smoked (48%) in contrast with 47 ex-smokers (52%), with a predictably higher number of ex-smokers in the COPD group (100%). In the control group, 5 ex-smokers were identified and included in the study. Despite of ex-smokers they presented normal lung function confirmed by spirometry, normal DLcoSB measurements and no history of lung disease or respiratory problems.

<b>Parameter</b>	<b>Control (n=30)</b>	<b>COPD (n=30)</b>	<b>ILD (n=30)</b>
Age (years)	53 ± 11.5	63.5 ± 9.5	62 ± 13
Sex (M/F)	14/16	24/6	18/12
BMI Kg/m <sup>2</sup>	27 ± 5	25.5 ± 3	26 ± 4
Hb (g/dL)	13 ± 0.5	13.7 ± 1.2	13.6 ± 1
Non/Ex-Smoker	25/5	0/30	18/12

**Table 3.1. Group Characteristics. Values are mean ± SD (where applicable)**

### 3.5 Spirometry measurements

In the control group, the mean FEV<sub>1</sub> % predicted and FEV<sub>1</sub>/FVC ratio were normal (Table 3.2).

In the severe COPD group, the mean FEV<sub>1</sub> % predicted was 37.02 ± 7.92 and FEV<sub>1</sub>/FVC ratio 47.0 ± 9.24, indicating severe airflow obstruction.

In the ILD group, the mean FVC % predicted was 69.3 ± 14.60 and FEV<sub>1</sub>/FVC ratio 80.0 ± 10.88 showed a non-obstructive ventilatory defect. (Brusasco *et al.*, 2005)  
All the sarcoidosis patients in the ILD group were clinically and radiological classified of stage two.

Parameter	Control (n=30)	COPD (n=30)	ILD (n=30)
FEV <sub>1</sub> (L)	3.2 ± 0.653	1.05 ± 0.257	1.90 ± 0.627
FEV <sub>1</sub> % predicted	107.3 ± 12.04	37.02 ± 7.92	69.5 ± 17.61
FVC (L)	3.81 ± 0.798	2.27 ± 0.583	2.37 ± 0.735
FVC % predicted	106.5 ± 11.78	63.1 ± 10.32	69.3 ± 14.60
FEV <sub>1</sub> /FVC %	83.5 ± 4.88	47.0 ± 9.24	80.0 ± 10.88

**Table 3.2 Spirometry data by group. Values are mean ± SD**

### 3.6 DLcoSB by the single breath method

All subjects were clearly instructed before and during testing by the same certified respiratory scientist, and had no difficulty in achieving two reproducible measurements to meet ATS/ERS criteria. (MacIntyre *et al.*, 2005) The mean values for DLcoSB, VA and KCO measured with a 10 s breath-hold are described in the Table 3.3.

Parameter/Group	Control (n=30)	COPD (n=30)	ILD (n=30)
DLcoSB, mmol/min/kPa	8.10 ± 1.446	3.42 ± 1.522	3.47 ± 1.351
DLcoSB % predicted	90.6 ± 8.07	39.8 ± 16.38	40.4 ± 13.02
VA (L)	5.44 ± 0.897	4.33 ± 1.137	3.56 ± 0.857
VA % predicted	96.1 ± 10.10	73.9 ± 15.78	61.7 ± 12.38
KCO, mmol/min/kPa	1.48 ± 0.183	0.79 ± 0.360	0.97 ± 0.312
KCO % predicted	97.8 ± 10.96	57.6 ± 25.47	67.5 ± 18.07

**Table 3.3. CO Diffusion measurement by the Single Breath method with 10 s breath-hold. Values are mean ± SD**

All measurements in the control group were normal (Table 3.4). In the severe COPD group, the DLcoSB % predicted was severely decreased (<40%) and in the ILD group, moderate impairment was present (40-60%). (Brusaco *et al.*, 2005)

The DLcoSB and DLcoSB % predicted measured in severe COPD and ILD patients was very similar and differed significantly from values measured in the control group. The group with ILD had the lowest VA and VA% predicted. The lowest KCO and KCO % predicted were found in the severe COPD group.

### 3.7 DLcoSB measurement by the single breath method with breath-hold time reduction

Each group performed the DLcoSB measurements at three different breath-hold times always by the same order of 10, 8 and 6 s, not randomised, with similar inspired and expired VC.

	Breath-Hold time			p value
	10 s	8 s	6 s	
<b>Control (n=30)</b>				
DLcoSB mmol/min/kPa	8.10 ± 1.446	8.18 ± 1.428	8.02 ± 1.453	0.4431
DLcoSB % pred	90.6 ± 8.07	91.9 ± 10.35	89.8 ± 8.56	0.3943
KCO mmol/min/kPa	1.48 ± 0.183	1.51 ± 0.157	1.52 ± 0.208	0.1861
KCO % pred	97.8 ± 10.96	99.0 ± 9.15	99.3 ± 12.65	0.4369
<b>COPD (n=30)</b>				
DLcoSB mmol/min/kPa	3.42 ± 1.522	3.19 ± 1.387	2.96 ± 1.482	0.0003*
DLcoSB % pred	39.8 ± 16.38	37.3 ± 15.14	34.7 ± 16.91	0.0019*
KCO mmol/min/kPa	0.79 ± 0.360	0.75 ± 0.334	0.72 ± 0.360	0.0183*
KCO % pred	57.6 ± 25.47	55.3 ± 24.39	53.2 ± 26.41	0.0489*
<b>ILD (n=30)</b>				
DLcoSB mmol/min/kPa	3.47 ± 1.351	3.50 ± 1.342	3.44 ± 1.392	0.5915
DLcoSB % pred	40.4 ± 13.02	40.9 ± 12.84	40.1 ± 13.65	0.5251
KCO mmol/min/kPa	0.97 ± 0.312	0.99 ± 0.298	0.99 ± 0.310	0.4921
KCO % pred	67.5 ± 18.07	68.6 ± 16.31	68.2 ± 17.29	0.6177

\* Significance p<0.05

**Table 3.4 DLcoSB measurements at different breath-hold time.**

**Values are mean ± SD.**

In the control and ILD group, there were no significant differences in DLcoSB and KCO measured using the three different breath-hold times 10, 8 and 6 s (p>0.05). However, in patients with COPD, there was a significant difference in the DLcoSB and KCO measurements across the 3 breath-hold times (p<0.05). As breath-hold time was

reduced from 10 to 8 and 6 s, the DLcoSB and KCO in severe COPD patients decreased gradually. The comparison of the DLcoSB and KCO measured in the three study groups at different breath-hold times is illustrated by Figures 3.1 and 3.2.

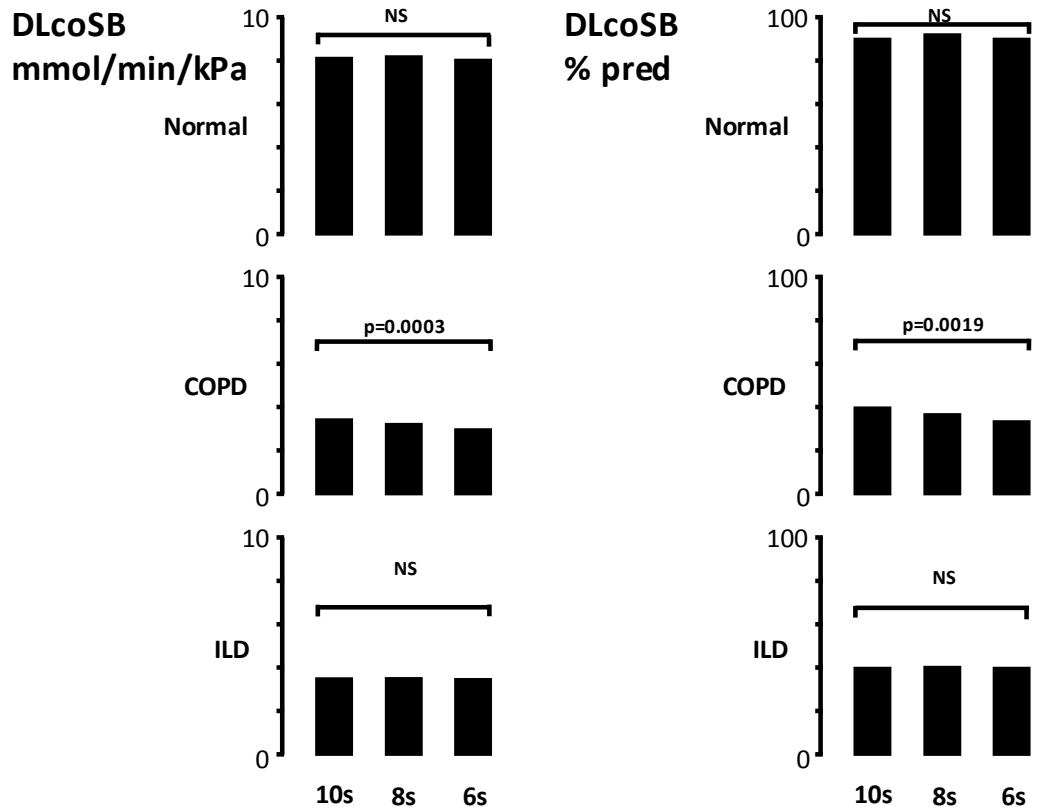
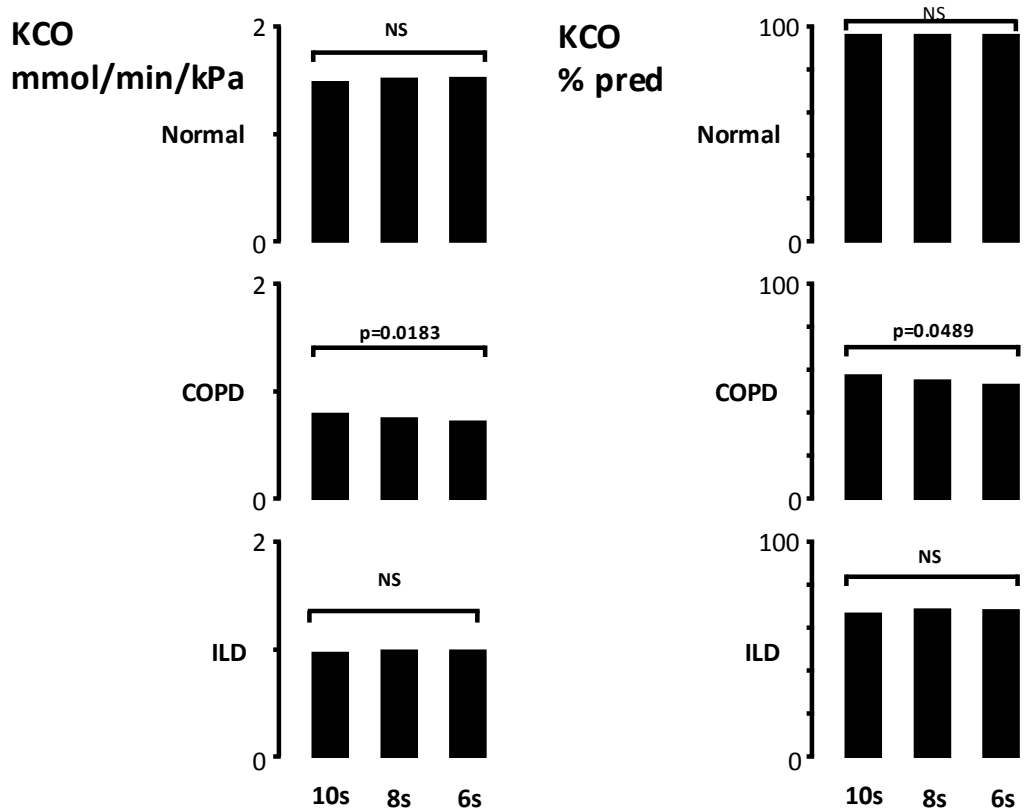


Fig. 3.1

Comparison of DLcoSB at different breath-hold times across groups.



**Figure 3.2**  
**Comparison of KCO at different breath-hold times across groups.**

Analysing the DLcoSB measurements at different breath-hold times was detected that in the severe COPD group, a significant difference in DLcoSB measured and DLcoSB% predicted with reduction of breath-hold from 10 to 8 s, however there was no significant difference detected in KCO measured from 10 to 8 s breath-hold time also stated by MacIntyre *et al.* (2005) previous report.



<b>COPD</b>	<b>Breath-hold time</b>		<b>p value</b>
	<b>10 s</b>	<b>8 s</b>	
DLcoSB, mmol/min/kPa	3.42 ± 1.522	3.19 ± 1.387	0.0110*
DLcoSB % pred	39.8 ± 16.38	37.3 ± 15.14	0.0242*
KCO, mmol/min/kPa	0.79 ± 0.360	0.75 ± 0.334	0.0636
KCO % pred	57.6 ± 25.47	55.3 ± 24.39	0.1410

\* Significant difference p<0.05

**Table 3.5 – Diffusion capacity in patients with COPD after 10 and 8 s of breath-hold time.**

**Values are mean ± SD**

Table 3.6 describes the effect of reduction in breath-hold time on measured  $V_D$  across the 3 groups. These measurements are relevant as  $V_D$  is the air in conducting airways and alveolar space that does not participate in the gas exchange and will therefore produce changes in the DLcoSB measurement.

	<b>Breath-hold time</b>			<b>p value</b>
	<b>10 s</b>	<b>8 s</b>	<b>6 s</b>	
$V_D$ ml Control (n=30)	155.74 ± 7.681	155.3 ± 8.242	153.95 ± 8.628	0.0442*
$V_D$ ml COPD(n=30)	161.42 ± 9.694	154.92 ± 7.822	155.58 ± 8.880	0.1563
$V_D$ ml IPF (n=30)	164.25 ± 11.046	150.12 ± 28.588	155.57 ± 2.235	0.0434*

\*Significant difference p<0.05

**Table 3.6 Total dead space volume measured by the single breath method at different breath-hold times. Values are mean ± SD**

The measured  $V_D$  values at different breath-hold times were significantly different in the control and IPF group but no significant difference was found in the severe COPD group.

Also, as He is used as a tracer gas to determine the initial CO concentration and measure VA in the single breath method by the dilution of He, the He and CO fraction were measured at different breath-hold times and mean results compared, in order to determine if the breath-hold time reduction will affect the fraction of He and CO in the final sample analysed. The results are presented in table 3.7.

In the control and ILD groups, no significant difference was found in the FAHe measured as breath-hold time reduced, but significant differences were seen in the severe COPD group. Regarding the FAco, in all three groups studied there was a significant difference with breath-hold time reduction, which increased as the breath-hold time decreased in all study groups.

		Breath-Hold time			p value
		10 s	8 s	6 s	
<b>Control (n=30)</b>	FAHe	5.62 ± 1.192	5.40 ± 1.533	5.86 ± 0.700	0.4437
	FAco	0.099 ± 0.048	0.107 ± 0.048	0.113 ± 0.034	<0.0001*
<b>COPD (n=30)</b>	FAHe	4.62 ± 0.801	4.78 ± 0.773	4.78 ± 0.794	0.0090*
	FAco	0.093 ± 0.018	0.104 ± 0.035	0.109 ± 0.018	<0.0001*
<b>ILD (n=30)</b>	FAHe	5.16 ± 0.963	5.27 ± 0.828	5.33 ± 0.835	0.1345
	FAco	0.101 ± 0.036	0.106 ± 0.019	0.116 ± 0.019	<0.0001*

\* Significance  $p < 0.05$

**Table 3.7 FAHe and FAco measured by the single breath method with different breath-hold time. Values are mean ± SD**

The VA measurement depends on He dilution during the single breath method and also adjusts for the KCO calculation. Comparisons were made between the VA and VA % predicted in the three study groups as breath-hold reduced and table 3.8 reports the data obtained. There was no significant difference between the VA measured in all study groups with breath-hold time reduction, except in the measured VA % predicted in the COPD group as shown in figure 3.3, which significantly reduced with breath-hold time reduction.

		Breath-hold time			p value
		10 s	8s	6s	
<b>Control (n=30)</b>	V <sub>A</sub>	5.44 ± 0.897	5.43 ± 0.956	5.32 ± 0.928	0.1769
	V <sub>A</sub> % pred	96.1 ± 10.10	96.0 ± 12.90	93.9 ± 10.43	0.2281
<b>COPD (n=30)</b>	V <sub>A</sub>	4.33 ± 1.137	4.35 ± 0.786	4.19 ± 0.761	0.3114
	V <sub>A</sub> % pred	73.9 ± 15.78	72.6 ± 14.18	70 ± 14.25	0.0107*
<b>ILD (n=30)</b>	V <sub>A</sub>	3.56 ± 0.857	3.53 ± 0.858	3.47 ± 0.847	0.3050
	V <sub>A</sub> % pred	61.7 ± 12.38	61.2 ± 12.88	60.2 ± 12.73	0.2499

\* Significance p<0.05

**Table 3.8 VA and VA % predicted measured by the single breath method with different breath-hold time. Values are mean ± SD**

## Chapter 4

### ***DISCUSSION OF RESULTS***

#### 4.1 Anthropometric Data

Previously published studies (Magnussen *et al.*, 1979; Graham *et al.*, 1985; Dressel *et al.*, 2008) analysing the breath-hold time effect on DLcoSB measurement, presented different subject demographics compared to the present study and also the population age was younger when compared with the present study. The breath-hold time effect on DLcoSB measurement in previous reports (Magnussen *et al.*, 1979; Graham *et al.*, 1985; Dressel *et al.*, 2008) was studied in patients with asthma, emphysema and cystic fibrosis, contrasting with COPD and ILD in the present study. The population size studied in the present study was also considerably larger than previous studies (Magnussen *et al.*, 1979; Graham *et al.*, 1985; Dressel *et al.*, 2008) (Table 4.1).

Previous studies (Magnussen *et al.*, 1979; Graham *et al.*, 1985; Dressel *et al.*, 2008) have analyzed the effect of DLcoSB breath-hold reduction in healthy subjects and patients with respiratory obstructive disorders, but not in non-obstructive pulmonary disorders such as ILD. The BMI was not calculated in any of the previous studies (Magnussen *et al.*, 1979; Graham *et al.*, 1985; Dressel *et al.*, 2008) and may be relevant in DLcoSB interpretation, as obesity, according to MacIntyre *et al.* (2005) increases the  $\theta V_c$  and thus increases the DLcoSB value. In the present study, the mean BMI was increased in all 3 study groups, which may have led to a higher DLcoSB measured.

<b>Studies (n=population number)</b>	<b>Study Group/Age</b>	<b>BMI</b>	<b>Sex (M/F)</b>	<b>Non/Ex/Smoker</b>
Magnussen et al., 1979 (n=47)	Normal 36.9 Emphysema 61.7 Asthma 47.1	No data	No data	No data
Graham et al., 1985 (n=36)	Normal 35±15 Emphysema 54±11 Asthma 40±16	No data	9/6 8/2 8/3	10/0/0 1/6/3 4/7/0
Dressel et al., 2008 (n=20)	Normal 33±9 CF 31±9	No data	14/6	20/0/0
Present Study 2012 (n=90)	Control 53±11.5 COPD 63.5±9.5 ILD 62±13	27±5 25.5±3 26±4	14/16 24/6 18/12	25/5/0 0/30/0 18/12/0

**Table 4.1 Anthropometric data in 3 studies analysing the breath-hold time effect on the CO diffusion measurement by the single breath method**

Age in years, Body mass index in Kg/m<sup>2</sup> (BMI), Sex (M/F = number of males and females in study population), Non/Ex/Smoker = number of Non-smokers, ex-smokers and smokers in the study population. Values are mean ± SD where applicable.

In the present study, 62% of subjects were males and 38% of females and in previous studies (Magnussen *et al.*, 1979; Graham *et al.*, 1985; Dressel *et al.*, 2008) there was similar gender distribution of subjects studied.

Regarding the population smoking habits of the present study, 48% never smoked in contrast with 52% ex-smokers, with a higher prevalence of ex-smokers on

the COPD group (100%). Similar population smoking habits were reported by Graham *et al.* (1985), despite the presence of three current smokers recruited in this study. Active smoking was an exclusion criterion in the present study, as COHb decreases DLco mainly due to the effects on CO back pressure and also decreasing the Hb binding sites for CO test gas. (MacIntyre *et al.*, 2005) None of the other previous studies (Magnussen *et al.*, 1979; Dressel *et al.*, 2008) had smokers among the population.

## 4.2 Spirometry measurements

To identify and recruit the present study population, spirometry was performed in order to determine normal, obstructive and non-obstructive ventilatory defects. Spirometry was also performed in previous studies (Magnussen *et al.*, 1979; Graham *et al.*, 1985; Dressel *et al.*, 2008) to determine the FEV<sub>1</sub> and FVC of the population (Table 4.2).

The degree of airflow obstruction present in our study population in the severe COPD group may have been more severe when compared with previous studies (Magnussen *et al.*, 1979; Graham *et al.*, 1985; Dressel *et al.*, 2008). In Dressel *et al.* (2008), the CF group had mild to moderate airway obstruction (Pellegrino *et al.*, 2005) and in Magnussen *et al.* (1979) and Graham *et al.* (1985), only the FEV<sub>1</sub>/FVC ratio was reported. However, the FEV<sub>1</sub>/FVC ratios of these two study groups were similar to the FEV<sub>1</sub>/FVC ratio of patients with severe COPD in the present study (Table 4.2).

Study (n=population number)	Group	FEV <sub>1</sub> % pred	FVC% pred	FEV <sub>1</sub> /FVC% pred
Magnussen <i>et al.</i> , 1979 (n=47)	Normal Emphysema Asthma	No data	No data	77.1±14.2 42.0±14.2 46.6±14.1
Graham <i>et al.</i> , 1985 (n=36)	Normal Emphysema Asthma	No data	No data	81±9 44±16 54±7
Dressel <i>et al.</i> , 2008 (n=20)	Normal CF	108±8 69±28	114±10 84±5	No data
Present Study 2012 (n=90)	Control COPD ILD	107.3 ± 12.04 37.02 ± 7.92 69.5 ± 17.61	106.5± 11.78 63.1 ± 10.32 69.3 ± 14.60	83.5 ± 4.88 47.0 ± 9.24 80.0 ± 10.88

**Table 4.2 Spirometry measurements in 3 studies analysing the breath-hold time effect on the CO diffusion measurement by the single breath method. Values are mean ± SD; n=number of subjects per group.**

Another important variable to consider is the bronchodilator effect on spirometric values measured, as well as on measured DLcoSB. In the present study, all spirometry and DLcoSB measurements were performed after inhaled bronchodilator, which may have led to a higher measured FEV<sub>1</sub> and DLcoSB. Inversen *et al.* (1999) reported that in obstructive lung diseases after bronchodilator administration, the DLco may increase up to 6%. Similarly, Chinn *et al.* (1988) reported that bronchodilators may affect VA and the vasomotor tone. In the Graham *et al.* study, all patients avoided bronchodilators for at least 8 hours prior to testing. In the Dressel *et al.* (2008) study, no mention was made of inhaled bronchodilator use by the study population.

### 4.3 DLcoSB by the single breath method measurements

#### 4.3.1 DLcoSB in the control group

The diffusion capacity is defined by Magnussen *et al.* (1979) as a conductance, consisting of transport of a gas divided by its pressure gradient between alveolar gas and capillary blood.

Jones & Meade (1979) suggested that the decrease of DLcoSB with increasing breath-hold time, also reported in healthy subjects by Forster *et al.* (1954), will be less significant if corrections for the time of breathing were applied, thus allowing a better estimate of the alveolar-capillary diffusion barrier. No such corrections were made in the current study as was aimed to detect from DLcoSB measurements the resistance to diffusion across the alveolar capillary membrane and the influence of respiratory disorders on these measurements.

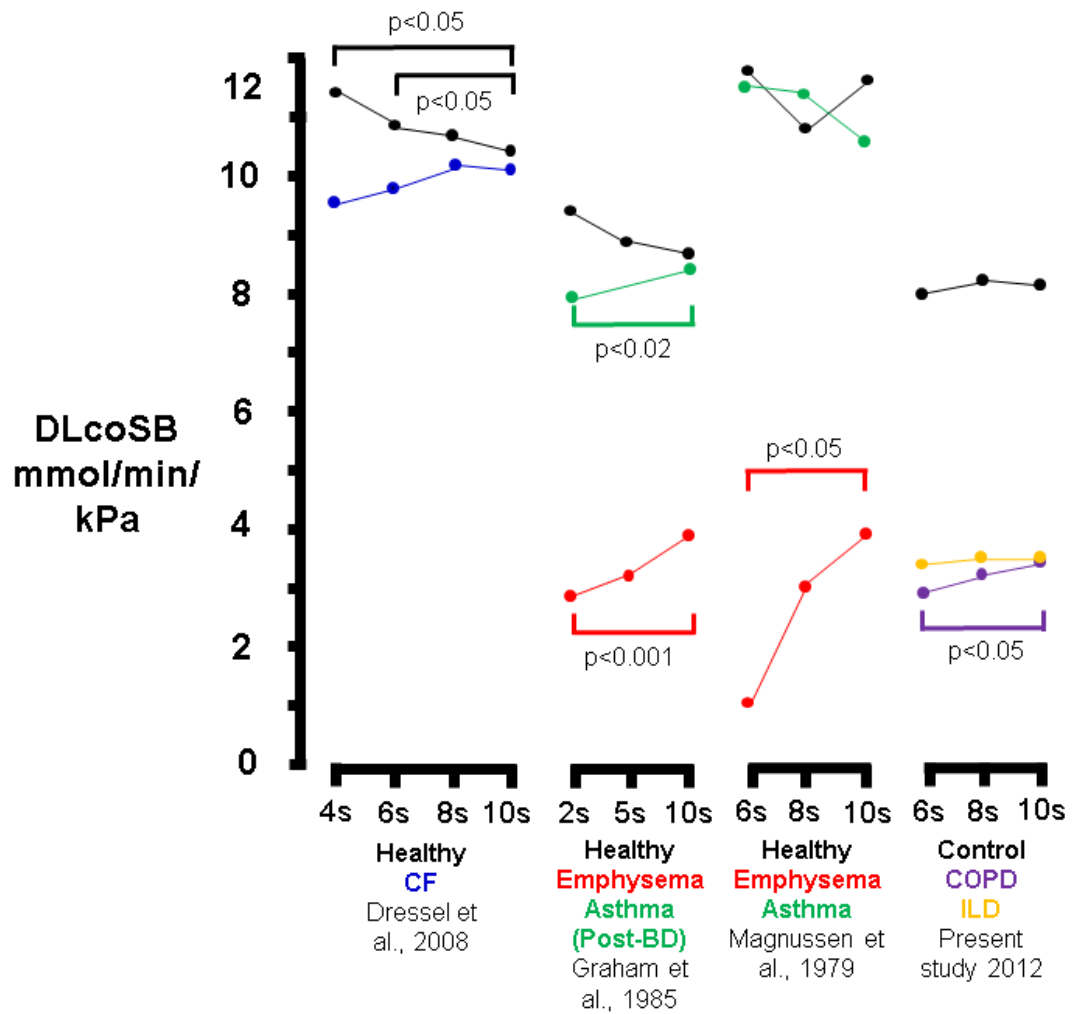
The DLco in the present study was measured by the single-breath method of Ogilvie *et al.* (1957) modified by Jones and Meade (1961). The VA was measured during the single breath measurement from the dilution of He according to McGrath and Thomson (1959). The values obtained for the DLcoSB in the control group did not change with reductions of breath-hold time, as no significant differences were found between DLcoSB and KCO measured at 10, 8 and 6 s ( $p>0.05$ ). These results were consistent with Graham's previous study (Graham *et al.*, 1985), which reported no significant difference ( $p>0.05$ ) in DLcoSB values measured in healthy subjects at different breath-hold times (10, 5 and 2 s). On the other hand, other previous studies (Jones and Meade, 1961; Graham *et al.*, 1980; Graham *et al.*, 1981; Graham *et al.*, 1984) reported that DLcoSB increases during short and long breath-hold periods in



healthy subjects. Graham *et al.* (1985) used the three-equation calculation method for DLcoSB measurement, which differs from the conventional single Krogh equation used in the present study and all other studies previously mentioned (Magnussen *et al.*, 1979; Dressel *et al.*, 2008). Despite of different calculation methods, the results for DLcoSB measurements were similar in Graham *et al.* (1985) and the current study. Graham *et al.* (1985) justified the conflicted results for DLcoSB measured obtained with other previous studies, with the calculation method used (the three equation method), arguing that the increase in DLcoSB with short/long breath-hold times using the conventional single equation method in normal subjects will ignore CO uptake during inhalation and exhalation phases of the single breath manoeuvre, increasing errors as breath-hold time becomes shorter. The DLcoSB rise, according to Graham *et al.* (1985), may be explained by the inefficiency of the method rather than any impairment in lung diffusion. MacIntyre *at al.* (2005) suggests in the ATS/ERS statement that the three-equation method can improve the accuracy of DLcoSB measurement, as it allows for volumes changes over time during inspiration and expiration when a measurement is being performed. This method might be more useful in subjects unable to rapidly inspire or expire rather than in healthy subjects. Despite the advantages of this calculation method, clinical experience with this approach is limited and need further standardization.

The DLcoSB results of the control group in the present study (with considerably more subjects when compared to Graham *et al.* (1985) study), demonstrated no change in measured DLcoSB associated with breath-hold time reduction by the conventional single equation calculation method, suggesting that both methods may be suitable for DLcoSB assessment. The single equation method for DLcoSB calculation was also used in the other two previous studies (Magnussen *et al.*,1979 and Dressel *et al.*, 2008),

where DLcoSB was reported to decrease with increasing breath-hold time in 14 healthy subjects (Magnussen *et al.*, 1979) and in 10 healthy subjects (Dressel *et al.*, 2008). Dressel *et al.* (2008) also observed a significant difference between the DLcoSB measured at 4, 6, 8 and 10 s in healthy subjects ( $p < 0.05$ ), however similarly to the present study, no significant difference was identified between the DLcoSB measured at 8 and 6 s. The Magnussen *et al.* (1979) study measured DLcoSB at 10, 8, 6, 4 and 2 s and only reported a significant difference for DLcoSB measurements when breath-hold time was reduced to 6, 4 and 2 s in all groups, and in the emphysema group at 8 and 6 s. No significant effects were seen in DLcoSB measured at 10, 8 and 6 s for the healthy and asthma group.



**Fig. 4.1**

**DLcoSB measurement in 4 studies analysing the breath-hold time effect on DLcoSB measurements with different breath-hold times.**

Closed circles joined by solid lines represent DLcoSB mean measured different breath-hold times. Square brackets and p value indicate significant differences within DLcoSB values measured at different breath-hold times using two-tailed paired t test.

These data strongly suggest that the breath-hold time for DLcoSB measurement could be reduced to 8 or 6 s as standard for healthy subjects, and still produce reliable, feasible and valid DLcoSB values.

#### 4.3.2 DLcoSB measurement in obstructive and non-obstructive pulmonary disorders

The diffusion capacity of the lung for a gas is directly proportionate to the size of the alveolar capillary membrane and inversely proportionate to its thickness. Any process that decreases the surface available for diffusion or thickens the alveolar-capillary membrane will decrease the DLco.

In obstructive pulmonary disorders such as COPD lung volumes are increased by air trapping with consequent hyperinflation, the alveolar walls and capillaries are typically destroyed reducing the total surface area available for gas exchange in the lung. According to MacIntyre *et al.*, (2005), the CO during DLcoSB measurement may only reach better ventilated regions of the lung and therefore the measured CO uptake will be determined predominantly by the capacity of those regions. On the other hand, in asthma (West, 2008b), the DLcoSB is reported to be normal or increased possibly due to a more uniform distribution of the pulmonary blood flow. (Hyatt *et al.*, 2003)

In the severe COPD group of the present study, there were significant differences in the DLcoSB and KCO ( $p < 0.05$ ) measured at 10, 8 and 6 s of breath-hold, suggesting a correlation of the DLcoSB with breath-hold time reduction when severe airway obstruction is present. Analysis of results indicated that less than 10 s of breath-hold time was insufficient to allow complete diffusion of inspired CO. This might be explained either by the alveolar destruction present in this disorder decreasing the surface area for diffusion or by air trapping preventing complete CO washout. A significant difference between the DLcoSB measurement at 10 and 8 s was also noted; however, there was no significant change in KCO.

Dressel *et al.* (2008) demonstrated no influence of breath-hold time on DLcoSB in 10 patients with CF (figure 4.1). In contrast, Graham *et al.* (1985) found that DLcoSB decreased with breath-hold time reduction in patients with asthma and emphysema. The authors explained this finding by suggesting stratification of inhaled gas within the gas-exchanging lung regions prior to the effective alveolar-capillary gas exchange. Equilibration of inspired and alveolar gas might take longer when airflow obstruction is present. (Chang *et al.*, 1973) The longer time needed for complete mixing of CO with alveolar air may cause a reduction in measured DLcoSB when airflow obstruction is present. The results obtained by Graham *et al.* (1985) are similar to the results obtained in the present study for patients with severe COPD, despite of the different calculation method used for DLcoSB. Graham *et al.*, (1985) also reported that a lower DLcoSB at shorter breath-hold time when airway obstruction is present, may lead to higher resistance to gas exchange due to a longer path that the inspired gas has to diffuse to reach the alveolar-capillary membrane.

Magnussen *et al.* (1979) similarly observed that DLcoSB decreased with breath-hold time reduction in patients with emphysema; however in patients with asthma, the DLcoSB results were comparable to normal subjects and increased with the breath-hold time reduction. Magnussen *et al.* (1979) suggested that the reduction of DLcoSB with lower breath-hold time in airway obstruction may be due to loss of gas exchange area but also to stratification of the gases mixing in the lung. Sikand *et al.* (1976) and Kawashiro *et al.* (1976) also suggested that gradients of intrapulmonary gas concentration are better explained by the stratified inhomogeneity present in the lungs. Magnussen *et al.* (1976) and Nieding *et al.* (1978) demonstrated that in patients with emphysema, stratification is an important factor limiting intrapulmonary gas mixing. As emphysema is a key feature of COPD, can also be inferred that the decreased DLcoSB

in the present study with breath-hold time reduction in the severe COPD group, may be due to poor He and CO intrapulmonary mixing limited by stratification.

All subjects in the present study population were non-smokers or ex-smokers, which minimized the “CO back pressure” influence in DLcoSB measurements. According to Qutayba *et al.* (2005), COHb exists in small concentrations, around 1% in non-smokers but increases with repeated DLcoSB measurements, up to 10% or higher in chronic smokers. If DLcoSB is not adjusted for the COHb increase, the so-called “anaemia effect” can occur, reducing the effective Hb available to bind with CO in the test gas mixture, leading to DLcoSB underestimation. The DLcoSB measured can be underestimated if COHb is not adjusted for the ambient alveolar CO concentration before each single breath measurement, which is assumed to be zero but is increased in smokers according to Graham *et al.* (2002). Abstinence from cigarette smoking 24 hours prior to DLcoSB testing can help to reduce COHb and consequent CO back-pressure (MacIntyre *et al.*, 2005). In the present study, all subjects were non-smokers or ex-smokers, which eliminated the effect of CO back pressure and the need for COHb correction during DLcoSB measurement.

To summarise the results of our study and previous studies in relation to COPD, the breath-hold time for DLcoSB measurement in patients with COPD should be retained at 10 s and certainly should not be reduced to less than 8 s.

The main reason to assess the effect of breath-hold time reduction on measured DLcoSB in patients with ILD was the absence of published data in this clinical context. We decided to study patients with sarcoidosis with pulmonary fibrosis and patients with IPF to evaluate the effect of the breath-hold time reduction when thickening of the alveolar-capillary membrane is present.

In the ILD group, no significant difference was observed in DLcoSB and KCO

measured at 10, 8 and 6 s ( $p>0.05$ ), despite the expected baseline reduction of DLcoSB. The mean DLcoSB % of predicted was  $40.4 \pm 13.02$  for 10 s of breath-hold in the ILD group, indicating a moderate to severe impairment of diffusing capacity according to ATS/ERS criteria. Despite of a similar impairment degree of DLcoSB in patients with ILD compared with the severe COPD group, there was no significant difference between the DLcoSB and KCO measured at 10, 8 and 6 s in the ILD group. From the 30 patients of the ILD group, 15 were clinically diagnosed stage two sarcoidosis patients with radiologic hilar adenopathy and parenchymal infiltration reflecting a non-obstructive ventilatory defect on spirometry measurements (table 3.2) and also moderate impairment was present when DLcoSB and KCO were measured (40-60%). (Brusaco *et al.*, 2005)

Roughton and Forster (1957) reported that the resistance to diffusion of a gas in the lung will depend of the resistance offered by the alveolar-capillary membrane to the gas transferred and also on the resistance of the chemical combination with Hb. In other words, the resistance to diffusion of CO in the lung will depend of the features of the alveolar-capillary membrane and the quantity of Hb in the pulmonary capillaries available to combine with CO. Therefore, the DLco measured will depend of membrane factors and circulatory factors. According to Weinberger *et al.* (1980) the alveolar-capillary membrane can be quantitatively impaired referring to the surface area per lung volume, and qualitatively impaired, referring to a change in the membrane thickness. In ILD, thickening of the alveolar wall will increase the resistance to diffusion of gas across the alveolar-capillary membrane. However, Weinberger *et al.* (1980) reported that the relative contribution of the membrane thickness in lowering the DLco in disorders such as interstitial fibrosis may be overestimated. Other pathological changes,

like alteration of alveolar architecture, can reduce the alveolar-capillary membrane surface area for gas exchange, which appears to be more relevant to reductions in DLco.

When breath-hold time was reduced from 10 to 8 and 6 s, no significant difference was observed in measured DLcoSB as shown in figure 3.1. These results suggested that despite of quantitative and qualitative changes in the alveolar-capillary membrane in patients with ILD, breath-hold time reduction will not produce significant changes in the DLcoSB measured, similarly to the control group already discussed.

McNeill *et al.* (1958) reported that in pulmonary fibrosis due to sarcoidosis, the membrane component (DM) is significantly more reduced than capillary volume and Johnson *et al.* (1961) observed that the reduction of DM was the major change and Vc was relatively unaffected. According to Hamer (1963), the reduction in DM after several years of pulmonary infiltration may be due to fibrotic changes reducing the area available for diffusion and Vc reduction is only commonly observed in patients with prolonged disease. Reduction of the Vc was not observed without DM reduction, which suggests that Vc changes are more frequently associated with increased severity of fibrosis. Additionally, Marshall *et al.* (1958) suggested that in pulmonary sarcoidosis, diffusion changes are mainly due to perivascular lesions rather than relative changes in the mechanical properties of the lungs.

The loss of the alveolar-capillary membrane surface area in patients with COPD through destruction of alveolar walls when compared with the loss of alveolar-capillary membrane surface area in patients with ILD through scarring and thickening of the alveoli, seemed to generate more significant changes in DLcoSB measurement with reduced breath-hold time. This suggests that airflow obstruction may be more responsible for decrease of the DLcoSB measured with breath-hold time reduction.



Krogh (1915) originally reported that the DLco decreased with VA reduction until FRC was reached and the progressive decrease in the size of the membrane would explain this change. Other studies (Ferris, 1978) have confirmed Krogh's observation and similarly noted that changes in VA are responsible for DLco measurement variability. Forster Farhi, L. Tenney, S. (1987) suggested that: "If the normal lung was a collection of spheres and if DLcoSB was directly proportional to the surface area of the spheres, then DLcoSB would vary with alveolar volume". In contrast, Cotton and Qutayba *et al.*, (2005) suggested that the capillary surface area is preserved with a decreased VA, as the alveolar-capillary membrane tends to fold, rather than shrink, when lung volume decrease.

In the present study, the VA measured by He dilution during breath-hold in the DLcoSB measurement was reduced in patients with severe COPD and ILD, with a higher reduction in patients with ILD. As breath-hold time was reduced in DLcoSB measurements from 10 to 8 and 6 s, no significant difference was observed in VA measured in all study groups, except in the measured VA % predicted in the severe COPD group. According to Fishman in Farhi and Tenney (1987), the resistance of the vascular alveolar segment decreases with VA reduction from TLC to FRC, while pulmonary capillaries widen and Vc increases. Graham *et al.*, (2002) reported that a VA reduction from TLC to 50% of IC will decrease DLcoSB minimally. Fishman (2007) states that DLco decreases with reduction of VA along with the reduction of available surface area and Vc for diffusion. The idea from Fishman in Farhi and Tenney (1987) that the Vc may increase with VA reduction while the capillary bed is still preserved may help to understand why in the present study, even with a low VA in the ILD group, the breath-hold time reduction did not produce significant changes in measured DLcoSB and KCO ( $p < 0.05$ ). This might suggest that 6 s of breath-hold time can be as

valid as 10 s to measure DLcoSB in patients with ILD, in the absence of concomitant airflow obstruction.

During the single breath method to measure DLco, after breath-hold and during expiration, the first 750 ml of volume is discarded and the next 750 ml of gas is used for analysis. In the severe COPD group in the present study, due to low VC (<2L) only 500 ml was collected for sampling. (MacIntyre *et al.*, 2005) The first 750 ml of expired breath contains gas from the anatomical  $V_D$  (volume in the conducting airways that does not participate in gas exchange) and instrumental  $V_D$  (mouthpiece, filters and connections of the system). (MacIntyre *et al.*, 2005) As  $V_D$  corresponds to the portion of expired volume in the DLcoSB measurement that does not participate effectively in gas exchange during breath-hold, was decided to investigate if the measurement of  $V_D$  was affected by the breath-hold time reduction.

Therefore,  $V_D$  was measured at different breath-hold times (10, 8 and 6s) and results are presented in Table 3.6. Significant differences were found in the control and ILD groups when breath-hold time was reduced. However, no significant difference was found in the  $V_D$  measured in the severe COPD group.

According to Morton *et al.*, (1965) the alveolar gas sample, when contaminated by  $V_D$  gas, may lead to CO uptake underestimation. Was observed that in the control group and patients with ILD, breath-hold time reduction led to a significantly reduced  $V_D$  ( $p < 0.05$ ) however the measured DLcoSB and KCO were not affected. Therefore, the effect on  $V_D$  was not significant. On the other hand, no significant difference was found in the severe COPD group for the  $V_D$  measured at different breath-hold time and yet, significant differences were detected in the measured DLcoSB and KCO with breath-hold time reduction.

Morton *et al.* (1965) stated that the initial and final CO concentrations in DLcoSB measurement are affected by dead space washout, as initial CO is determined by the dilution of He. If dead space is not efficiently washed out, the final concentration of He in the expired sample will be higher and lead to higher initial CO concentration. Morton *et al.* (1965) observed patients with normal and impaired alveolar gas distribution and noted that differences in expired volumes led to less dilution of dead space He and CO. He also noted that the size of the expired sample could have been reduced by air trapping since subjects with uneven alveolar ventilation had severe obstructive airways disease. Based on these data, it is not clear whether the time for complete washout of He and CO in the severe COPD group was sufficient to provide complete elimination of the gas mixture in the alveolar space between measurements and thus avoid changes in measured DLcoSB.

The table 3.7 shows that there were significant differences ( $p < 0.05$ ) in the FAHe and FAco alveolar fraction measured with breath-hold time reduction from 10 to 8 and 6 s in the severe COPD group, as reported by Morton *et al.* (1965). This might have been a significant factor accounting for the reduction in measured DLcoSB at progressively reduced breath-hold time in patients with severe COPD. However, in the control and ILD groups no significant difference was found for the FAHe measured, despite of a similar FAco difference. This results indicated that the time between measurements for the He washout from the alveolar space for the control and ILD group was adequate, as per ATS/ERS guidelines (MacIntyre *et al.*, 2005) 4 minutes between manoeuvres and 1 hour between measurements. In the severe COPD group, the time recommended of 10 minutes in between DLcoSB manoeuvres and 1 hour in between measurements may not to be sufficient for He and CO alveolar washout as significant differences were found. This might suggest that time in between measurements should be extended when severe

airflow obstruction is present, as the expired sample is reduced by air trapping in COPD, prolonging the test gas mixture washout and interfering with the DLcoSB measured. (Hyatt *et al.*, 2003)

## Chapter 5

### ***CONCLUSION***

#### 5.1 Conclusions / Recommendations

The present study is the largest report analysing the effect of breath-hold time on measured DLco measurement by the single breath method to date. Furthermore, our data show some conflicting results when compared to previously published reports (Magnussen *et al.*, 1979; Graham *et al.*, 1985; Dressel *et al.*, 2008)

The present study indicates that in healthy subjects, measured DLcoSB and KCO is not affected with breath-hold time reduction, as has been previously reported by Magnussen *et al.* (1979) and Graham *et al.* (1985). However, in the presence of severe COPD, the DLcoSB is influenced by breath-hold time, where DLcoSB and KCO significantly decrease with breath-hold time reduction. These data led to conclude that in patients with airflow obstruction, the time necessary for mixing of inspired gas with alveolar gas will reduce the CO uptake when breath-hold time was decreased from 10 s, as suggested by the ATS/ERS guidelines (MacIntyre *et al.*, 2005).

In contrast, in patients with ILD, despite moderate to severe baseline DLcoSB impairment, there was no significant influence of breath-hold time reduction on measured DLcoSB, suggesting that in patients with ILD who are unable to breath-hold for 10 s, DLcoSB measurement using a 6 second breath-hold time may provide an acceptable and clinically meaningful result.

Was also conclude that the washout time, necessary to clear He and CO inhaled in each DLcoSB measurement, is of major importance to produce valid results, as impaired clearance of these gases from the alveolar space will interfere with the final results for measured DLcoSB.

Can be useful further studies involving the population size increase of each analysed group to determine whether results remain consistent, studying also the effect of breath-hold time reduction on measured DLcoSB in patients with other types of ILD, as well as, the effect of breath-hold time reduction on measured DLcoSB in patients with different COPD stages. Also assess the dyspnoea degree of the study population using the Borg Scale at the time of the DLcoSB measurements can provide useful information. Furthermore, arterial blood gases assessment in addition to DLcoSB measurements in patients with obstructive and non-obstructive ventilatory disorders could help to assess ventilation/perfusion mismatching and its influence on DLcoSB measurements. Day to day variability of the DLcoSB measurements should as well be considered in further studies.

## 5.2 Publications

An abstract for this work has been accepted for poster discussion at the European Respiratory Society meeting and was presented in Vienna on September 2012. Abstracts will be submitted to the Irish Thoracic Society (2012) and American Thoracic Society (2013) meetings and we hope to submit a manuscript to a peer-reviewed journal for publication, after review of the thesis.

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# Appendices

## *Appendix A*

### **JAEGER MASTERSCREEN PFT**

- **Power supply**

Input range 230V, 50/60 Hz, 1.8 A

- **Dimensions**

Length\*Width\*Height: 78 cm \* 100 cm \* 155 cm

- **Technical data**

#### **Flow measurement JAEGER pneumotacograph**

Range  $0 \pm 20$  l/s

Accuracy  $0.2 - 12$  l/s  $\pm 2\%$

Resistance  $<0.05$  kPa/(l/s) at 10l/s

#### **Volume determination digital integration**

Range  $0 - \pm 20$  l

Accuracy 5 ml

#### **Mouth pressure piezo-resistive**

Range  $\pm 20$  kPa

Accuracy  $< \pm 2 \%$

**CO analyser electrochemical cell**

Range 0 - 0.4 %

Accuracy 0.0003 %

**He analyser thermal conductivity**

Range 0 - 9.5 %

Accuracy 0.05 %

Calibration CAL-Pack, automatically

- **Premixed gas**

Diff-SB and Diff-RB 0.28 % CO, 9 % He, 19 % O<sub>2</sub>, Balance N<sub>2</sub>

- **Computer**

Pentium computer, printer and monitor on an ergonomic, smoothly-running trolley. Lab Manager Software version 5.3.0.4

## ***Appendix B***

### **HEMOCUE HB 201+**

- **Parameters recorded**

Hb g/L

- **Performance Hb**

Measuring Range: 0-256 g/L

- **Dimensions (HxWxD)**

85x160x43 mm

- **Weight**

350 gr

- **Power supply**

4 Batteries type AA Or R6, 1.5 V

- **Environmental conditions**

Operating temperature: 15-30<sup>0</sup>C

Analyser Storage Temperature: 0-50<sup>0</sup>C

Microcuvettes Storage Temperature: 15-30<sup>0</sup>C

Relative Humidity: should not be operated at high humidity (i.e.>90% non-condensing)



## ***Appendix C***

### **UNIVERSITY COLLEGE CORK**

#### **CONSENT BY SUBJECT FOR PARTICIPATION IN RESEARCH PROTOCOL**

##### **Section A**

Protocol Number: \_\_\_\_\_ Patient Name: \_\_\_\_\_

**Title of Protocol:** Effect of reduction of breath-holding time in the single-breath method to assess carbon monoxide diffusing capacity.

**Doctor Directing Research:** Dr. Terry O'Connor, Mercy University Hospital, Cork  
Phone: 021-4935325

You are being asked to participate in a research study. The doctors at University College Cork study the nature of disease and attempt to develop improved methods of diagnosis and treatment. In order to decide whether or not you want to be a part of this research study, you should understand enough about its risks and benefits to make an informed judgment. This process is known as informed consent. This consent form gives detailed information about the research study, which will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate.

##### **Section B**

## I. NATURE AND DURATION OF PROCEDURE(S):

This study aims to investigate alternative ways of performing a test that we use to assess your lung function. During this test, you are asked to perform spirometry and CO-Diffusion which measure the lung volumes and gas exchange. CO-Diffusion requires that you empty all the air in your lung and take a deep breath during which a small amount of carbon monoxide will be inspired. When your lung is full you will have to hold your breath for 10 s followed by a complete expiration. **This procedure is part of our routine assessment of lung function and is performed routinely irrespective of your participation in the study. The additional testing that follows is the study part of the assessment.** After 1 hour of rest you will be asked to repeat the CO-Diffusion test but holding your breath for 8 s, followed again by 1 hour of rest until the last measure with 6 s of breath-holding. This test will be conducted in the Respiratory Laboratory of the Mercy University Hospital and by a qualified Senior Respiratory technician also involved in this study. All records involving your participation in this research will be kept in complete confidentiality.

## II. POTENTIAL RISKS AND BENEFITS:

All the measurements involved in this study are part of the clinical respiratory assessment routinely conducted by the respiratory team. The amount of carbon monoxide inspired during the measurements will be according with the American Thoracic Society and the European Respiratory Society guidelines minimizing any risk of injury to patients. This research may influence future guidelines for the performance

of the CO Diffusion test and may reduce the breath-holding time required to perform this test.

### III. POSSIBLE ALTERNATIVES:

You may choose not to participate in this research protocol or withdraw from the study at any time after consenting to participate. You will be given a copy of the consent form for your records.

## **Section C**

## **AGREEMENT TO CONSENT**

The research project and the treatment procedures associated with it have been fully explained to me. All experimental procedures have been identified and no guarantee has been given about the possible results. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedures involved. I am aware that participation is voluntary and that I may withdraw my consent at any time. I am aware that my decision not to participate or to withdraw will not restrict my access to health care services normally available to me. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner. When required by law, the records of this research may be reviewed by government agencies and sponsors of the research.

I, the undersigned, hereby consent to participate as a subject in the above described project conducted at the Cork Teaching Hospitals. I have received a copy of this consent form for my records. I understand that if I have any questions concerning

this research, I can contact the doctor(s) listed above. If I have further queries concerning my rights in connection with the research, I can contact the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Lancaster Hall, 6 Little Hanover Street, Cork.

After reading the entire consent form, if you have no further questions about giving consent, please sign where indicated.

**Doctor:** \_\_\_\_\_

**Signature of Subject:** \_\_\_\_\_

**Witness:**

**Date:**

**Time:** \_\_\_\_\_