PULMONARY TRANSFER FACTOR IN HEART TRANSPLANT PATIENTS

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LIST OF SYMBOLS AND ABBREVIATIONS

The use of symbols and abbreviations in this thesis follows the recommendations of the European Respiratory Society (1). The following out-line defines the key symbols and abbreviations used throughout the thesis.

Lung Volumes and Forced Expiration

FVC *Forced vital capacity*: the volume of gas which is exhaled during forced expiration starting from a position of full inspiration and ending at complete expiration.

FEV_1 *Forced expiratory volume in one second*: the volume of gas exhaled in the first second of the FVC manoeuvre.

FRC *Functional residual capacity*: the volume of gas present in the lung and airways at the average end-expiratory level.

RV *Residual volume*: the volume of gas remaining in the lung at the end of a full expiration.

TLC *Total lung capacity*: the volume of gas in the lung at the end of full inspiration.

Diffusion

TL_{CO} *Pulmonary transfer factor for carbon monoxide (CO) - also called pulmonary diffusing capacity*: the rate of carbon monoxide uptake from alveolar gas to pulmonary capillary blood; expressed in $mmol.min^{-1}.kPa^{-1}$.

K_{CO} *Transfer coefficient - also referred to as* T_L/V_A: TL_{CO} per unit alveolar volume (V_A); expressed in $mmol.min^{-1}.kPa^{-1}L^{-1}$.

$\mathbf{D_M}$ *Diffusing capacity of the alveolar-capillary membrane*; expressed in $mmol.min^{-1}.kPa^{-1}$.

$\mathbf{V_C}$ *Pulmonary capillary blood volume available for gas exchange* (also expressed as Q_C).

θ *Reaction rate of CO with oxyhaemoglobin.*

$1/TL_{CO}$ *Reciprocal of TL_{CO}*; the total resistance to CO transfer from the alveolar spaces to pulmonary capillary blood.

$1/D_m$ *Resistance of the alveolar-capillary membrane.*

$1/\theta V_C$ Resistance of the total mass of erythrocytes in the pulmonary capillary blood *"intra-capillary resistance".*

Exercise and Gas Exchange

V'_{O2} *Oxygen (O_2) consumption*; expressed in $ml.min^{-1}$.

V'_{CO2} *Carbon dioxide (CO_2) production*; expressed in $ml.min^{-1}$.

V'_E *Expiratory minute ventilation* ($L.min^{-1}$.).

V'_E/V'_{CO2} *Ventilatory equivalent for CO_2* - also called the ventilatory response.

$P_{(A-a)}O_2$ *Alveolar-arterial O_2 gradient* in kPa.

DEDICATION

To my family

SUMMARY

A number of studies have shown a consistent reduction in pulmonary transfer factor (TL_{CO}) after heart transplantation despite improvement in lung volumes and cardio-pulmonary haemodynamics. The mechanism and causes of this decline have not been determined and the clinical significance of TL_{CO} reduction in heart transplant recipients is unknown. The aims of the studies comprising this thesis were to describe prospectively the longitudinal changes in TL_{CO} after heart transplantation, determine the mechanisms and causes of its decline and to evaluate the effect of TL_{CO} reduction on gas exchange and exercise performance.

During 2.5 years of follow-up, pulmonary function tests including the single breath TL_{CO} were performed in 81 patients before and at specific intervals after heart transplantation. Fifty seven of these had results before and at least once after transplantation, 61 had 4 serial post-transplant assessments and 37 had both pre-transplant and 4 post-transplant assessments. Results were compared with data from 28 normal subjects. Before transplantation there was a mild restrictive ventilatory defect with reduction in both TL_{CO} (72.1% of predicted) and TL_{CO} per unit alveolar volume; K_{CO} (90.1% of predicted). At 6 weeks after transplantation, there was a further reduction in all lung function parameters with greater reductions in TL_{CO} and K_{CO} (declined by 18% and 26% of predicted, respectively). However, lung volumes and flow rates increased in the subsequent measurements, to exceed their pre-transplant values at about one year after transplantation. In contrast, TL_{CO}

and K_{CO} did not change after the initial decline up to three years after transplantation.

TL_{CO} and its components (the diffusing capacity of the alveolar-capillary membrane, D_M and the pulmonary capillary blood volume, V_C) were measured in 75 heart transplant recipients using the Roughton and Forster method. The procedure of estimating TL_{CO} components was used after performing several validation studies which showed that the applied technique yielded reproducible estimation of TL_{CO} components with values comparable to previous reports. Results were compared with data from 38 heart transplant candidates and 26 normal subjects. The % predicted TL_{CO} and V_C were significantly lower in recipients (56.9% and 52.8%) compared to candidates (69.9% and 80.2%) which were themselves significantly lower than normal (97.7% and 102.3%). D_M was similarly reduced in recipients (77.7%) and candidates (81.4%) compared to normal controls (100.1%). Correction for haemoglobin increased TL_{CO} in recipients to 63.5% of predicted, but this was still significantly lower than that of candidates (71.1%). In recipients, the intra-capillary resistance ($1/\theta V_C$) formed 60% of $1/TL_{CO}$ compared to 50% in candidates and normal subjects. In 9 patients assessed before and at 6 weeks after heart transplantation, the changes in TL_{CO}, D_M and V_C were similar to those of 7 patients with mitral stenosis assessed before and at 6 weeks after mitral valve replacement.

The potential responsible factors for TL_{CO} decline were analysed in 57 heart transplant recipients. The change in TL_{CO} adjusted for haemoglobin was inversely correlated with the pre-transplant TL_{CO} ($r = -0.55$) and mean pulmonary capillary

wedge pressure (r = -0.27). There was no relationship between the change in TL_{CO} and any of the other variables which included pre-transplant static and dynamic lung volumes, pre-transplant cardiac status as assessed by the duration and severity of symptoms and by left ventricular ejection fraction, cardio-pulmonary bypass time, post-transplant pulmonary complications, cytomegalovirus infection, cardiac allograft rejection and cyclosporin blood levels. The potential role of cardio-pulmonary bypass was further evaluated by comparing the results in heart transplant patients with those of 15 patients who had lung function tests before and at 6 weeks after coronary artery bypass graft (CABG). There was a significant reduction in lung volumes, flow rates, TL_{CO} and K_{CO} after CABG. However, the decline in TL_{CO} and K_{CO} was less marked in CABG patients than in recipients and it was entirely explained by post-operative anaemia (corrected TL_{CO} was 84.3% before surgery and 87.9% afterwards).

The relationship between haemoglobin-corrected TL_{CO} and exercise performance after heart transplantation was determined in 53 recipients. Results were compared with data from 53 heart transplant candidates and 28 normal subjects. Maximal symptom-limited oxygen uptake (V_{O2}) was higher in recipients than in candidates (39.8% and 46.0% of predicted respectively, $p<0.05$), but it was substantially lower than that of normal controls (92.9% of predicted, $p<0.001$). The ventilatory and gas exchange responses (V_E/V_{CO2}, V_D/V_T, and $P_{(A-a)}O_2$) at maximum exercise were significantly improved in recipients compared to candidates, but were still abnormal. In recipients, TL_{CO} was correlated positively with V_{O2} (r = 0.60) and inversely with ventilatory and gas exchange responses; V_E/V_{CO2} (r = -0.43), V_D/V_T

(r = -0.29) and $P_{(A-a)},O_2$ (r = -0.38), but there was no relationship between TL_{CO} and any of these variables in the other 2 groups.

In conclusion, the studies comprising this thesis have demonstrated that TL_{CO} decline is very common after heart transplantation and is present by 6 weeks with no further changes up to three years after transplantation. This decline was shown to be due to an increase in the intra-capillary resistance caused by a combination of anaemia and reduced V_C. After correcting for haemoglobin concentration, pre-transplant TL_{CO} and mean pulmonary capillary wedge pressure were identified as the only factors which predicted TL_{CO} decline after heart transplantation. Since increases in these two factors reflect elevated pulmonary venous pressure and because of the similarity in both TL_{CO} and pulmonary haemodynamic changes before and after heart transplantation and mitral valve replacement a causal link between TL_{CO} changes and pulmonary haemodynamics is suggest. The reduction in pulmonary vascular pressures may be responsible for V_C reduction and therefore TL_{CO} decline after heart transplantation. The decline in V_C with persistence of reduced D_M after heart transplantation are likely to be markers of persisting pulmonary vascular dysfunction caused by pre-transplant chronic pulmonary congestion rather than being due to a new damage to the lungs from heart transplantation and its associated complications. The relationship between TL_{CO} and gas exchange abnormalities on exercise is further evidence of pulmonary vascular dysfunction which appears to contribute to exercise limitation in heart transplant recipients.

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DECLARATION

The work described in this thesis was performed during a three year period of research in the Department of Respiratory Medicine and the University Department of Cardiac Surgery, Glasgow Royal Infirmary. Except where specifically stated in the acknowledgement and in the text of the thesis, I have carried out all the experimental work, including the overall planning and design, all cardio-pulmonary exercise tests, some of the resting lung function tests, all the bronchoalveolar lavage studies and data collection and analysis. The conclusions and concepts advanced throughout the various parts of the thesis represents my interpretation of the results in the light of previous knowledge in the field.

PART ONE

INTRODUCTION AND REVIEW OF LITERATURE

CHAPTER 1

INTRODUCTION AND AIMS

1.1 Introduction

Gas exchange, the primary function of the lung is carried out by the physical process of diffusion between the alveolar spaces and the pulmonary capillary blood across the alveolar-capillary membrane. The pulmonary transfer factor for carbon monoxide (TL_{CO}) is a non-invasive physiological measurement which is used to assess the integrity of the alveolar-capillary membrane and the pulmonary capillary bed. It is quantitatively defined as the rate of carbon monoxide (CO) uptake into the pulmonary capillary blood per unit of driving pressure across the alveolar-capillary membrane (2).

TL_{CO} is determined by the thickness, physical properties and the surface area of the tissue across which the diffusion takes place (3). The effective surface area depends on the number and size of functioning alveoli and pulmonary capillaries in contact with each other. TL_{CO} is also dependent on the rate of reaction of CO with haemoglobin in the capillary blood (4). The usual methods used to determine TL_{CO} do not differentiate between these factors. Since TL_{CO} is a measure of conductance (flow/pressure), its reciprocal ($1/TL_{CO}$) represents the resistance to CO transfer between the alveolar spaces and haemoglobin in the red blood cells of the pulmonary capillary blood. $1/TL_{CO}$ can be partitioned into two major components which can be estimated using the Roughton and Forster relationship(5). These are: the alveolar-capillary membrane resistance ($1/D_M$) and the "intra-capillary"

resistance ($1/\theta V_C$), where D_M is the diffusing capacity of the alveolar-capillary membrane, V_C is the pulmonary capillary blood volume and θ is the reaction rate between CO and haemoglobin. Theta (θ) depends on the prevailing alveolar oxygen tension and the concentration of haemoglobin in the pulmonary capillary blood. The two components of TL_{CO} can be estimated by measuring TL_{CO} at two different inspired oxygen concentrations (5). The relationship between TL_{CO} and its components is described by the equation of Roughton and Forster (5);

$$1/TL_{CO} = 1/D_M + 1/\theta V_C$$

From the relationship between TL_{CO} and its components in Roughton and Forster model, it is clear that TL_{CO} may be normal in diseases which cause abnormal changes in both of its components in opposite directions. For example, in heart disease with mild to moderate left heart failure, pulmonary venous hypertension tends to increase V_C whereas interstitial and alveolar oedema tends to decrease D_M. As a result, the overall TL_{CO}, may be normal or only slightly decreased despite significant abnormalities in both of its components (2).

The heart and lungs are connected in series by the pulmonary circulation. The normal matching of pulmonary blood flow and ventilation in the lungs is essential for efficient gas exchange. Because of the interdependence between the heart and lung, a dysfunction in one frequently affects the function of the other (6).

Congestive heart failure, the primary indication for heart transplantation, is characterised by a restrictive ventilatory defect with small lung volumes and reduced lung compliance (7). TL_{CO} is usually normal or only slightly reduced in

mild to moderate chronic congestive heart failure (8,9), but TL_{CO} impairment is common in heart transplant candidates with severe chronic heart failure (10,11). Lung function abnormalities in patients with heart failure are commonly attributed to the well-recognised derangement of the pulmonary circulation and the associated lung water imbalance that cause pulmonary congestion and oedema (12). Heart transplantation restores pulmonary haemodynamics, lung volumes and airway function toward normal (13,14). In contrast, TL_{CO} and TL_{CO} per unit alveolar volume (K_{CO}) have been consistently shown either to deteriorate or remain sub-normal following heart transplantation (15-21).

The cause of TL_{CO} decline following heart transplantation is unknown. Cyclosporin pulmonary toxicity (15,16) and cytomegalovirus infection (21) have been proposed as possible causes. Other potential causes in the setting of heart transplantation include; pre-operative pulmonary dysfunction, sternotomy and cardio-pulmonary bypass procedure, pulmonary haemodynamic changes following transplantation, post-operative pulmonary complications and cardiac allograft rejection and dysfunction.

The mechanism of decline in TL_{CO} following heart transplantation is obscure. It has been postulated that it may result from a reduction in the D_M component, caused by damage to the alveolar-capillary membrane (16). However, the effects of heart transplantation on the components of TL_{CO} have not been studied.

The reported studies on the TL_{CO} changes following heart transplantation are limited in being retrospective and based on cross-sectional analysis of patients

assessed at different intervals after transplantation (15,17-21). They all lack control groups for the possible effects of sternotomy, cardio-pulmonary bypass procedure and pulmonary venous congestion on lung function. In the only longitudinal study (16), the number of patients was small (21 patients), and the post-operative assessments were performed yearly for three years. There are, therefore, no longitudinal data available on lung function changes within the first year after heart transplantation. In addition, the clinical significance of TL_{CO} impairment in these patients has not been determined.

TL_{CO} studies in patients with congenital and valvular heart disease, before and after surgery, provide important pointers to the potential causes and possible mechanisms of TL_{CO} decline following heart transplantation. A detailed account of these studies is presented in chapter 3 of this thesis. Patients with congenital heart disease and increased pulmonary blood flow associated with mild to moderate pulmonary arterial hypertension have a significant increase in TL_{CO} due to a proportional increase in both of its components (D_M and V_C) (22). Surgical correction of the malformations in these patients is associated with a reduction in TL_{CO}, D_M and V_C toward normal (23,24).

Mitral valve surgery is an excellent model for the analysis of the changes in TL_{CO} after heart transplantation for the following reasons:

1. The effects of mitral valve disease on the lung have been extensively studied both before and after surgery and there is a relatively well-defined natural history of pulmonary dysfunction in this disorder (25-27).

2. The changes of TL_{CO} and other indices of lung function in chronic heart failure (the primary indication for heart transplantation) and in mitral valve disease are very similar (28).

3. Pulmonary haemodynamic disturbance is also similar in both mitral valve disease and congestive heart failure. Both conditions are associated with low cardiac output and raised pulmonary venous pressure and both can lead to secondary pulmonary arterial hypertension and high pulmonary vascular resistance as well as pulmonary fibrosis (27).

4. Both mitral valve surgery and heart transplantation have been shown to restore all pulmonary haemodynamics towards normal early after surgery (13,29).

TL_{CO} in mitral valve disease is variable and appears to parallel the functional capacity and pulmonary haemodynamics (30). Except in severe cases, it is usually normal or only slightly reduced and in a few cases it may be higher than predicted (31). V_C is often normal or elevated, but D_M is usually low (32). In advanced cases with severe pulmonary hypertension TL_{CO} is reduced and this is usually associated with a proportional decrease in D_M and V_C (32,33). TL_{CO} changes after mitral valve surgery are also variable and appear to be related to the preoperative severity of the disease and the interval between surgery and lung function assessment and there are three patterns:

1. No significant change; this is the most common pattern and has been explained by the counterbalanced effects of relieving pulmonary congestion (increased D_M), and the reduction in pulmonary vascular pressures (reduced V_C) (26,34-36)

2. Decline; this was reported in studies performed early after surgery, and this might be due to the adverse effects of thoracotomy and cardio-pulmonary bypass (37,38).

3. Improvement; this is uncommon and mainly found in cases with the lowest TL_{CO} before surgery (39,40). It might be due to a greater improvement in D_M due to relief of pulmonary congestion.

The changes in TL_{CO} following surgery for congenital heart disease and mitral valve disease serve to highlight two important points that should be considered in the investigation of TL_{CO} changes following heart transplantation:

1. The measurement of TL_{CO} components may clarify the mechanisms underlying the reported decline in TL_{CO} following heart transplantation. The relationship between TL_{CO} and pulmonary haemodynamics in patients with congenital and valvular heart disease suggests an important role for pulmonary haemodynamic changes in the decline in TL_{CO} following heart transplantation. In heart transplant candidates with advanced heart failure, the long-standing pulmonary congestion and the associated chronic pulmonary vascular and parenchymal changes would be expected to lead to a reduction in both D_M and V_C accounting for the common occurrence of TL_{CO} impairment (10). The normalisation of pulmonary haemodynamics along with the relief of pulmonary oedema following heart transplantation, would be expected to decrease V_C and increase D_M, respectively. A disproportionate decrease in V_C secondary to the normalisation of pulmonary pressures may be an important contributory factor in the observed decline in TL_{CO}

following heart transplantation. In addition, a significant decrease in haemoglobin levels after transplantation could lead to reduction in the measured TL_{CO} by reducing θ (41).

2. Lung function before transplantation and the time of assessment after transplantation may be important. Most of the potential causes of TL_{CO} decline in the setting of heart transplantation occur within the first few weeks after transplantation (42). It is therefore important to define the time course of TL_{CO} changes following heart transplantation by longitudinal measurements of TL_{CO} and other lung function tests before and serially after transplantation at specific intervals, including the early post-transplant period.

TL_{CO} is a sensitive index of alveolar-capillary membrane integrity (43), and its impairment in asymptomatic patients may be due to a sub-clinical injury to the pulmonary capillaries, alveolar membrane or both (43). TL_{CO} impairment has been shown to predict arterial oxygen desaturation and exercise limitation in various lung disorders (44). In pulmonary sarcoidosis, isolated TL_{CO} impairment in patients with normal chest x-rays and no respiratory symptoms is associated with abnormal ventilatory and gas exchange responses to exercise (45). It is, therefore, clinically important to define the time course of TL_{CO} changes following heart transplantation, and to determine the cause of its decline. In addition, the clinical significance of TL_{CO} impairment in these patients needs to be evaluated.

1.2 Aims

The objectives of the studies comprising this thesis were to:

1. Measure prospectively the longitudinal changes in TL_{CO} following heart transplantation.
2. Determine the mechanism of TL_{CO} impairment in heart transplant recipients in terms of the relative contribution of its components.
3. Analyse the factors that could potentially cause TL_{CO} reduction following heart transplantation
4. Determine the effects of reduced TL_{CO} on exercise capacity and pulmonary gas exchange in heart transplant recipients.

1.3 Layout of the thesis

The work of this thesis is presented in 11 chapters grouped in three parts.

Part 1: Consists of three chapters including this introductory chapter. Chapter 2 reviews the physiological principles underlying TL_{CO} measurement and provides a brief outline of the various factors affecting it. A review of the limitations and clinical applications of TL_{CO} measurements is also included. Chapter 3 is devoted to a literature review of lung function in heart disease with particular emphasis on TL_{CO} changes following cardiac surgery in general and heart transplantation in particular.

Part 2: This part presents the general methods used in the studies comprising this thesis in three chapters. These include: general design and protocols (chapter 4), material and methods of various lung function tests including the broncho-alveolar

lavage study (chapter 5) and validation studies of TL_{CO} and its components measurements (chapter 6).

Part 3: Consists of the last 5 chapters which investigate TL_{CO} changes in heart transplant patients. The longitudinal changes in TL_{CO} and other lung function tests following heart transplantation during a follow-up period of 2.5 years are described in chapter 7. Chapter 8 evaluates the mechanisms of TL_{CO} reduction in heart transplant recipients in terms of the relative contribution of each of its components and chapter 9 investigates the various factors that could theoretically be responsible for TL_{CO} decline in the setting of heart transplantation. Chapter 10 evaluates the significance of reduced TL_{CO} in heart transplant recipients in terms of its effects on exercise capacity and on pulmonary gas exchange on exertion. Finally, the results of the studies comprising this thesis are summarised in chapter 11. The significance and contribution of these studies to previous knowledge in the field are discussed. The chapter concludes by highlighting the research opportunities in the topic.

CHAPTER 2

THE PULMONARY TRANSFER FACTOR: REVIEW OF METHODS AND PHYSIOLOGICAL PRINCIPLES

2.1 Fundamentals of gas diffusion in the lungs

In the early years of this century there was a vigorous debate surrounding the mechanisms of gas transfer across the alveolar-capillary membrane. Bohr and Haldane believed that pulmonary gas transfer occurred by active secretion as well as by passive diffusion, whereas the Kroghs and Bancroft argued that gas transfer occurred by pure diffusion alone. It is now accepted that gas transfer across the alveolar-capillary membrane occurs by passive diffusion according to physical laws (46).

The factors that determine the diffusion of gases through tissues are described by Fick's law (3). This states that the rate of transfer of a gas (V_g) through a sheet of tissue is directly proportional to the surface area of that tissue (A), and the difference in the partial pressures of the gas across the two sides of the tissue (P_1-P_2). It is also proportional to a diffusion constant (D), and inversely proportional to the tissue thickness (T). Mathematically, Fick's law is expressed as:

$$V_g = (A \times D/T)/(P_1 - P_2) \text{ -------- } 1$$

For the lung, P_1 corresponds to the alveolar partial pressure of the gas (P_A), P_2 is equivalent to the pulmonary capillary partial pressure of the gas (P_C) and (P_A-P_C) is the effective driving pressure across the alveolar-capillary membrane. The diffusion constant (D) for a gas is directly proportional to its solubility in the diffusion barrier and inversely proportional to the square root of the molecular weight of the gas (47). In a complex structure such as the lung it is not possible to measure parameters A, D, or T individually and therefore the value (A×D/T) in equation 1 was collectively termed the "diffusing capacity of the lung" and was given the symbol (DL) with a subscript to denote the gas under consideration. In Europe the preferred term is the "Lung transfer factor (TL)". By substituting TL for (A×D/T), equation (1) becomes: $V_g = TL_g/P_A\text{-}P_C)$, and by rearrangement;

$$\mathbf{TL_g = V_g/(P_A\text{-}P_C)} \text{ -------- } \mathbf{2}$$

This equation forms the basis for all the physiological methods used to evaluate the diffusion characteristics of the lung, and it shows that the value of the lung transfer factor for a gas (g) can be readily determined from a knowledge of the rate of the gas uptake (V_g) and the partial pressure difference of the gas across the alveolar-capillary membrane (P_A-P_C).

The lung transfer factor can be measured for oxygen (TL_{O2}), carbon monoxide (TL_{CO}), and nitric oxide (TL_{NO}). TL_{O2} is the most relevant physiological measurement, but the methods used to derive it are very complex and have very poor reproducibility. This is mainly due to the difficulties in estimating the mean partial pressure of oxygen in the pulmonary capillaries, and TL_{O2} measurements

have been abandoned (3). TL_{NO} measurement has been recently introduced and its clinical application is still being evaluated (48). TL_{CO} measurement is the method of choice used to evaluate the pulmonary gas transfer factor (3).

Carbon monoxide (CO) has the advantage of being similar to oxygen in its reaction with haemoglobin. In addition, it has very high affinity for haemoglobin - about 230 times that of oxygen (3). As a result, when a small quantity of CO is used, virtually all of it is taken up by the haemoglobin of the red blood cells in the pulmonary capillaries, and the P_{cCO} remains constant and negligible. In the calculations of TL_{CO}, P_{cCO} is assumed to be zero, and equation 2 is therefore simplified to:

$$\mathbf{TL_{CO} = \dot{V}_{CO}/P_{ACO}} \text{ -------- } \mathbf{3}$$

where:

- TL_{CO} is the lung transfer factor for CO,
- $\dot{V}_{CO}$ is the rate of CO uptake and
- P_{ACO} is the alveolar CO tension.

The traditional units for TL of any gas are millilitres of gas per minute per millimetre of mercury ($ml.min^{-1}.mmHg^{-1}.$). The corresponding international units (SI) are millimoles of the gas per minute per kiloPascal ($mmol.min^{-1}.kPa^{-1}.$). The relationship between the two systems is given by the following equation (49):

$$TL_{CO}\ (ml.min^{-1}.mmHg^{-1}.) = 2.986 \times TL_{CO}\ (mmol.min^{-1}.kPa^{-1}.).$$

The elimination of the need to estimate P_{cCO} which was first suggested by Bohr and applied by Marie Krogh has greatly simplified the physiological methods of

assessing the diffusion characteristics of the lung and CO tests became the usual way of assessing this index of gas exchange (3,47).

2.2 The single-breath transfer factor for carbon monoxide (TL_{CO})

In the process of disproving the theory of active secretion, Marie Krogh described the single breath-holding method for the measurement of TL_{CO} in 1914 using equation 3 (50). In this original method, the subject first exhaled to residual volume, then inhaled a test gas containing 1% CO in air to near total lung capacity, then exhaled part away, held the breath for six seconds followed by complete expiration to residual volume. The alveolar gas was sampled twice during this two-stage expiration technique and these samples were used by M. Krogh to estimate the alveolar CO concentrations before and after the breath-hold period (46). In this original work, M. Krogh used the test in normal subjects and in patients with various respiratory diseases: But, this method did not become clinically applicable until the 1950s when Forster et al. (51), Ogilvie et al. (52), and others (53) developed modifications for its use in clinical practice. The most important modification was the addition of helium to the inspired test gas which allowed the easy determination of the initial alveolar concentration of CO, as well as the lung volume during the breath-holding time (54).

In 1954, Forster and associates introduced the first modification of Krogh's method by adding helium to the test gas (51). Helium is an inert gas and does not diffuse across the alveolar-capillary membrane, and the change in its concentration before and after the breath-holding period was used as a factor for CO dilution in the

residual volume. This allowed the estimation of the alveolar CO concentration at the start of the breath-holding manoeuvre. The technique was therefore, simplified to a single alveolar gas collection at the end of breath-holding period.

In 1957, Ogilvie et al. (52) used the modified Krogh single breath technique described by Forster to produce a standardised single breath-holding method for the clinical measurement of TL_{CO}. In this work, many of the factors that affect TL_{CO} measurement were presented, and a standardised technique to account for these factors was described. TL_{CO} values in normal subjects and in patients with various respiratory disorders were also presented. In this technique, the subject was asked to make a full expiration after which, he made a maximal inspiration of a test gas mixture containing 0.3% CO and 21% O_2 in nitrogen. The subject then held his breath for about ten seconds and then rapidly expired. The first litre of the expired gas was discarded and the remainder was collected for analysis. The initial CO concentration, before any CO had been transferred across the alveolar-capillary membrane was calculated from the dilution of the inspired helium according to the following equation:

$$\textbf{Initial alveolar [CO] = inspired [CO] } \times \textbf{ (expired [He] / inspired [He])}$$

From the change in CO concentration in the alveolar sample over the breath-holding time, TL_{CO} was calculated using Krogh's equation which is based on the assumption that the rate of CO uptake across the alveolar-capillary membrane is exponential;

$$\mathbf{TL_{CO} = (V_A \times 60/ t \times P_{ACO}) \times \ln (initial\ [CO] / final\ [CO])}$$

where:

- V_A was the alveolar volume,
- t was the breath-hold time in seconds and
- 60 was used to convert from seconds to minutes.

Ogilvie and associates measured V_A separately using either the helium closed circuit technique or the nitrogen wash-out method according to the following equation;

$$\mathbf{V_A = V_I + RV}$$

where:

- V_I was the inspired volume and
- RV was the residual volume.

The duration of breath-holding was timed from the start of inspiration to the start of the alveolar sample collection.

In 1959 McGrath and Thomson (53) introduced the use of helium dilution simultaneously to estimate V_A as suggested by Forster (51). The two methods of V_A estimation give similar values in normal subjects and in patients with interstitial lung disease. In patients with chronic airway disease associated with maldistribution, the simultaneous method may be unreliable (52).

In 1961, Jones and Meade (55) suggested to inclusion of two thirds of the inspiratory time and one half of the alveolar sampling time as parts of the breath-holding period. This modification was based on a mathematical model and supported experimentally and subsequently recommended by Cotes (56), and by both the European Respiratory Society and the American Thoracic Society (41,57).

2.3 TL_{CO} components

The value of TL_{CO} derived from equation (3) was originally thought to represent the entire process of diffusion from the alveolar spaces to the haemoglobin of the red blood cells in the capillary blood. In 1945, Roughton demonstrated that the lung transfer for CO was limited not only by the resistance of the alveolar-capillary membrane, but also significantly by the relatively slow reaction of CO with haemoglobin (4). In 1957, Forster and Roughton further advanced this concept and derived a mathematical model to describe the relationship between the determinants of the pulmonary transfer factor (5). This model is described by the following equation:

$$1/TL_{CO} = 1/D_M + 1/\theta \times V_C \text{ -------- } 4$$

where:

- $1/TL_{CO}$ is the total resistance to gas transfer for CO between the alveolar gas and haemoglobin in the pulmonary capillaries,
- $1/D_M$ is the resistance to gas transfer across the alveolar-capillary membrane,
- $1/\theta \times V_C$ is the resistance of the instantaneous total volume of red blood cells in the pulmonary capillaries "intra-capillary resistance",
- V_C is the volume of the pulmonary capillary blood in millilitres and
- θ is the standard rate at which 1 ml of whole blood combines with CO in millilitres STPD per minute per millimetre of mercury of partial pressure.

In this model, TL_{CO} was called "the apparent diffusing capacity" of the entire lung, and the term "D_M" was called the "true diffusing capacity" of the alveolar-capillary membrane (58). It was also demonstrated that TL_{CO} was inversely proportional to the prevailing alveolar oxygen partial pressure and that was shown to be due to

changes in θ. The values of θ at different oxygen partial pressures were obtained by the same investigators from *in vitro* CO uptake in a suspension of human erythrocytes at 37C (59).

The mathematical derivation of equation 4, and the principles underlying the estimation of D_M and V_C have been fully described by Roughton and Forster (5,58). The values of D_M and V_C can be derived by measuring TL_{CO} using single breath-holding method or any other method, at two different alveolar oxygen tensions and using the two corresponding values for θ to obtain two equations which are then solved simultaneously for D_M and V_C. Alternatively, the values of D_M and V_C may be obtained graphically by plotting the two values of $1/TL_{CO}$ against the corresponding $1/\theta$ as shown in Figure 5.1. The slope of the line equals $1/V_C$ and its intercept with $1/TL_{CO}$ axis equals $1/D_M$.

In this model, D_M and V_C are assumed to be independent of the alveolar oxygen tension. It is also assumed that the haemoglobin concentration in the pulmonary capillary blood is identical to that of venous blood and the rate of reaction of CO with haemoglobin (θ) *in vitro* is identical to that occurring in the pulmonary capillary blood (5).

The Roughton and Forster model has been supported by many recent clinical and experimental studies, and it has become the standard physiological model which describes the transfer of CO and O_2 between the alveolar gas and the haemoglobin of the red blood cells in the pulmonary capillaries (59). The clinical application of this model has contributed to the understanding of the changes in pulmonary gas

transfer under different physiological and pathological conditions (41). In particular, it has been used extensively in clinical research as a non-invasive index of pulmonary capillary bed function in health and disease with consistent results (2,60).

2.4 Determinants of TL_{CO}

From the equations used to calculate the lung transfer factor for CO and its components, there are five major determinants. These are: 1) the length of diffusion path, 2) the surface area available for diffusion, 3) the characteristics of the alveolar-capillary membrane, 4) the driving pressure across the alveolar-capillary membrane, and 5) the "diffusion" within the pulmonary capillary blood.

1. Length of diffusion path

Before entering blood, oxygen and other gases such as CO must first cross all the tissues interposed between the alveolar spaces and the erythrocytes in the pulmonary capillary blood. These tissues are collectively referred to as the alveolar-capillary membrane and include: the alveolar surface lining (surfactant), the alveolar wall and its basement membrane, the pulmonary capillary wall and its basement membrane, and the interstitial space between the alveoli and the capillaries (61). Normally, the thickness of the alveolar-capillary membrane is about 0.5 micrometers, but it can become much thicker when there is pulmonary fibrosis, oedema or an inflammatory exudate into the interstitial space (61). Thickened alveolar-capillary membrane causes a characteristic reduction in TL_{CO} (43). However, isolated increase in the thickness of the alveolar-capillary membrane is

believed to be very rare and indeed some investigators believe that this state does not exist in isolation as most pulmonary disorders affect more than one determinant of gas exchange (43).

2. Effective surface area

As stated before, the pulmonary gas transfer factor is directly proportional to the effective surface area of diffusion. This area is estimated to be about 50-100 m^2 in a healthy adult at rest (43). The effective surface area is determined by the lung volume and by the distribution of alveolar ventilation and pulmonary perfusion. A decrease in the number of ventilated alveoli, or the number of patent capillaries, as in ventilation-perfusion mismatch, reduces the effective surface area for diffusion and can reduce the measured TL_{CO} even when the diffusion distance is normal (62).

3. Characteristics of the alveolar-capillary membrane

The rate of gas transfer across the alveolar-capillary membrane is proportional to the solubility of the gas in the pulmonary tissue, but there are no information about the influence of this factor on the measured TL_{CO} in either health or disease. It is possible that fibrotic lung tissue might resist gas transfer more than normal tissue of similar thickness and surface area.

4. Driving pressure across the alveolar-capillary membrane

The effective driving pressure for gas transfer across the alveolar-capillary membrane is the difference between the alveolar partial pressure of the gas and its partial pressure in the pulmonary capillaries. For carbon monoxide, the pulmonary capillary partial pressure is assumed to be zero. In heavy smokers, the pulmonary

capillary partial pressure for carbon monoxide increases significantly and if it is not taken into account, it leads to underestimation TL_{CO} (41,63).

5. "Diffusion" within the pulmonary capillary blood

The diffusion path through plasma is believed to be very short as the diameters of erythrocytes and the pulmonary capillaries are similar (about 7 μm each) (59,61). The overall "intra-capillary resistance, $1/\theta \times V_C$" is determined by two factors; 1) the total haemoglobin in the pulmonary capillaries which depends on the haematocrit and the instantaneous pulmonary capillary blood volume (V_C), and 2) the rate of reaction (θ) between CO and haemoglobin (5,43). Any factor that reduces the total haemoglobin concentration in the pulmonary capillary bed causes a fall in the measured TLco (59). In normal subjects, the "intra-capillary resistance" has been shown to be equivalent to the resistance caused by the alveolar-capillary membrane (5).

Thus, the measured value of TL_{CO} may be altered by abnormalities in the ventilation-perfusion relationship, in the alveolar-capillary membrane, or in the pulmonary capillary blood. Most disorders of gas exchange involve more than one of these, sometimes with opposing effects on TL_{CO} (43).

2.5 Factors affecting TL_{CO} measurement

Several physiological and technical factors have been identified as influencing the measurement of TL_{CO} and its components (59). Understanding the effects of these factors is not only important for the accurate interpretation of TL_{CO} changes, but also essential for standardising the methods of TL_{CO} measurement (41). These

factors include: 1) age, sex, and body size, 2) body position during the measurement, 3) lung volume at which TL_{CO} is measured, 4) haemoglobin concentration, 5) carboxyhaemoglobin blood level, and cigarette smoking, 6) alveolar oxygen tension, 7) exercise and 8) ventilation-perfusion relationship. In addition, there are several important technical factors specifically related to the single breath-holding method. The influence of these factors on TL_{CO} measurements has been extensively evaluated by many investigators (5,52,53,64), and the results of these studies form the basis of the recent recommendations of the American Thoracic Society and the European Respiratory Society for the standardisation of TL_{CO} measurements (41,65). The following is an outline of the influence of these factors on TL_{CO} measurement and interpretation.

1. Age, sex, and body size

TL_{CO} increases with growth in children. In adults it decreases with age and is proportional to body size. The TL_{CO} is less in normal females than in normal males. This is believed to be due the difference in body size and the alveolar volume between the two sexes (66,67). Body weight appears to have no significant effect on TL_{CO}. Prediction equations are used to correct for age and height (41).

2. Body position

TL_{CO} increases by about 10-20% in the supine compared to sitting position. D_M and V_C show similar trends with V_C increasing more than D_M on assuming the supine position. These changes are believed to be secondary to the gravitational effect on lung volumes and pulmonary ventilation-perfusion distribution (68).

3. Lung volume

TL_{CO} increases with increasing lung volume. This observation lead Marie Krogh to consider the TL_{CO} per unit lung volume (TL_{CO}/V_A), which she called the diffusion constant (K_{CO}), to be a more useful index of diffusion because she found it constant at various lung volumes (50). Further studies have confirmed that TL_{CO} increases with increasing lung volumes. However, K_{CO} is not constant; when single breath TL_{CO} was measured at various lung volumes, K_{CO} decreased as lung volume increased until the lung volume reached 80 % of the total lung capacity (TLC) and above this K_{CO} became constant (41,69).

The changes in D_M with increasing lung volume parallel that of TL_{CO}, but in normal subjects V_C decreases at high lung volumes (70); the expansion of the lungs is believed to increase the surface area for diffusion, reduce the thickness of the alveolar-capillary membrane, and flatten the pulmonary capillaries and as a result TL_{CO} and D_M increase, while V_C and K_{CO} decrease with increasing lung volumes (41).

4. Haemoglobin concentration

TL_{CO} decreases in anaemia and increases in polycythaemia. This is a function of the rate CO reaction with haemoglobin which is directly proportional to haemoglobin concentration (66). The measured TL_{CO} can be normalised for actual haemoglobin using standard equations and this is recommended when haemoglobin levels are outwith the normal range (41).

5. Carboxyhaemoglobin (Co-Hb) and cigarette smoking

TL_{CO} is inversely proportional to the level of Co-Hb (41,63). Co-Hb reduces the measured TL_{CO} by two different mechanisms. Firstly, the increase in the venous partial pressure of CO creates a back pressure in the pulmonary capillary blood and because the pulmonary capillary CO partial pressure (P_{cCO}) is assumed to be zero, the increase in P_{cCO} reduces the effective driving pressure for CO across the alveolar-capillary membrane thereby causing a reduction in the measured TLco (65). Secondly, Co-Hb reduces the number of the available haemoglobin binding sites causing a reduction in the functional haemoglobin concentration (the anaemic effect) (71). The magnitude of Co-Hb effect on the measured TL_{CO} is about 1% decrease in TL_{CO} for every 1 % increase in Co-Hb (72). Repeated measurements of TL_{CO} increase the level Co-Hb, but this is usually not clinically significant (59,63).

TL_{CO} is reduced by both acute and chronic cigarette smoking (73), and in heavy smokers Co-Hb can reach significant levels and adjustment for this may be necessary (41). Cigarette smoking is also associated with accelerated decline in TL_{CO} even after allowing for the effects of Co-Hb (71). Smoking cessation is associated with rapid improvement in TL_{CO} (74). Reference equations that include adjustment for Co-Hb and smoking are available (41). The acute effects of smoking are best avoided by asking the patients to stop smoking overnight before to being tested (63)

6. Alveolar oxygen tension (PA_{O2})

TL_{CO} is inversely proportional to P_{AO2} and this varies with the inspired oxygen making standardisation of P_{AO2} essential (41,75). The European Respiratory Society recommended the use of a gas mixture containing 17 to 18% oxygen, because at this range P_{AO2} is nearly constant (41). The American Thoracic Society, on the other hand recommended the use of gas mixtures containing 21% oxygen (57,65). At other inspired oxygen concentrations, correction to the standard P_{AO2} should be done using Roughton and Forster equation (5).

7. Exercise

TL_{CO}, D_M, and V_C increase with exercise. This increase is believed to be due increases in the surface area for diffusion and the pulmonary capillary blood volume either by the distension of patent vessels, or by the opening of previously closed vessels (recruitment) or by both (66).

8. Ventilation-perfusion relationship

All methods of estimating TL_{CO} consider the lungs as a homogeneous system with a uniform distribution of ventilation and perfusion. However, the normal lungs have an uneven distribution of ventilation and perfusion and this usually worsens in disease (2,76). The effects of ventilation-perfusion mismatch on the measured TL_{CO} depends on the method used; it has relatively little effect on TL_{CO} measured by either single breath-holding or rebreathing techniques, but significantly influence the steady-state estimation of TL_{CO} especially at rest (77). The discrepancy between single breath-holding and steady state TL_{CO} in different diseases is believed to be

due to the greater effect of ventilation-perfusion mismatch on steady state measurements (62).

9. Technical factors specific to single breath-holding method

There are several technical factors specifically related to the single breath-holding method which could alter the measured TL_{CO} (78). These include the inspiratory and expiratory manoeuvres, the timing and duration of breath-holding, the timing and volume of washout and alveolar samples, the variability in the alveolar pressure during the breath-holding manoeuvre and the time interval between and the number of repeated measurements in a single session (52,55). Standardisation and good control of these factors have been shown to decrease the variability of single breath TL_{CO} (79).

2.6 Comparison between the principal methods of TL_{CO} measurement

TL_{CO} can be measured using several techniques, but the principal methods in use are: a) single breath-holding method, b) steady-state method, and c) rebreathing method (79). All of these physiological methods are based on the general equation:

$$\mathbf{TL_{CO} = V_{CO}/P_{ACO}}$$

Therefore each method involves two basic steps: 1) measuring the rate of CO uptake (V_{CO}), and 2) estimating the mean alveolar partial pressure of CO (P_{ACO}).

The details of these methods have been well described (2,77,80). The single breath-holding technique as described by Ogilvie and associates (52) is the most commonly used method, and is the method used in studies comprising this thesis. However,

some of the previous studies relevant to the work of this thesis were performed using other methods, especially steady state and rebreathing methods. The following is a brief account of the advantages and disadvantages of the three principal methods (2).

1. Single breath-holding method

The advantages of the single breath-holding method are that it is widely available, relatively simple to perform, less affected by ventilation-perfusion abnormalities and relatively insensitive to CO back pressure in blood. In addition, it is the most standardised method and has been computerised and widely studied in normal subjects and patients with reproducible results (41). The disadvantages of single breath TL_{CO} include the criticism that the breath-holding manoeuvre is not a physiological state and therefore the obtained TL_{CO} values do not reflect the gas exchange function during tidal breathing. The requirement to hold their breath for about ten seconds may be difficult for some patients with severe breathlessness and a vital capacity of at least 1.3 litres is necessary to allow adequate dead space wash out and adequate alveolar samples. It is also difficult to apply during exercise (80).

2. Steady state methods

In all steady state methods, the subject breathes a low concentration of carbon monoxide (0.1-0.3) for several minutes to reach a steady state of CO exchange. A mixed expired gas sample is collected over several more minutes and the rate of CO uptake is estimated from the difference between the quantities of the inspired and

expired CO per unit time. The alveolar partial pressure for CO ($P_{A,CO}$) is estimated using one of the following different ways:

- Filley's Technique : This method requires an arterial blood sample to measure the arterial carbon dioxide tension (P_{aCO2}) which is then used to estimate P_{ACO} using Bohr equation.
- End-tidal CO determination technique : The P_{ACO} is determined by taking an average end-tidal CO tension from the instantaneous analysis of multiple breaths. The average end-tidal value is taken to equal the mean P_{ACO}.
- Dead space technique: This technique uses an assumed dead space ventilation (V_D) and the measured tidal volume (V_T) to derive P_{ACO} using the Bohr equation.
- Mixed venous P_{CO2} technique: In this method, the P_{aCO2} is derived indirectly by estimating the partial pressure of mixed venous CO2 using an equilibrium technique. The derived P_{aCO2} is then used to calculate P_{ACO} using the Bohr equation as in Filley's technique.

The advantages of steady state-methods include measurement during tidal breathing which is physiological and can be applied at rest, during sleep, exercise, and general anaesthesia. They also require less equipment, less calculations, and less patient co-operation. One of the most important limitations of these methods is their sensitivity to ventilation-perfusion abnormalities. This is mainly due to the use of the V_D/V_T ratio, and P_{aCO2} in the calculation of P_{ACO}, and small errors in the estimation of these parameters can lead to wide variations in the measured TL_{CO}.

The requirement for arterial blood sample in Filley's technique is another disadvantage. In general, the different steady-state methods for estimating TL_{CO} give similar results (81), although values for TL_{CO} are consistently less than those obtained using single breath-holding method. This is believed to be related to the greater lung volume at which the single breath-holding TL_{CO} is made (80). In normal subjects, there is good agreement between single breath-holding and steady-state methods, but in patients the disparity between the two methods can be considerable (82).

3. Rebreathing Technique

In this method the subject rebreathes from a bag containing a gas mixture (0.3% CO, 10% He, 21% O2 with the remainder being nitrogen) for 30 to 60 seconds at a rate of 24 to 30 breaths per minutes. The rapid rebreathing allows continuous mixing of the test gas so that the bag gas concentrations can be considered equivalent to the alveolar gas concentrations. Gas concentrations are continuously monitored during the test using rapidly responding gas analysers. The uptake of CO is calculated from the system volume and from the rate of carbon monoxide disappearance from the bag. TL_{CO} is calculated using the modified Krogh equation, except that the alveolar volume is replaced by the system volume which includes the alveolar volume and the gas volume in the bag and the apparatus (83).

The advantage of this method is that it can be performed during exercise and in patients with vital capacities of less than 1.3 litres. In addition, it is the method least affected by ventilation-perfusion abnormalities. However, some patients find it

difficult to rebreathe rapidly at a fixed rate (80). This method is not recommended for routine use (41), but it is considered to be the method of choice when the single breath-holding method cannot be used (84).

2.7 Predicted normal values and reproducibility of TL_{CO} measurement

The single breath-holding method is the most reproducible technique and it is the method of choice (41,57,65). The reported normal intra-individual coefficient of variation for single breath TL_{CO} is 3.7 % (range; 2.6% - 10.7 %) (79,80). Under good control of measurement conditions, the reported coefficients of variation for D_M and V_C are 12 % and 7 % respectively (79).

Predicted normal values for TL_{CO} have been derived from many population studies and there are many prediction equations for TL_{CO} which are corrected for age, height, and gender (49,85). Like most other lung function tests, there is a wide range of variability in these equations (85). This variability is primarily attributed to differences in the techniques and different criteria for population selection (63,80). The importance of all the possible technical and physiological factors affecting the measurement of TL_{CO} has been emphasised by both the American Thoracic Society and the European Respiratory Society in their recommendations for the standardisation of TL_{CO} measurements (41,57,65). The adherence to these recommendations is hoped to improve the reproducibility of TL_{CO} measurements and reduce the variability between the published results (41,57).

Like most other lung function tests, the normal range of normal TL_{CO} is commonly defined as the predicted TL_{CO} +/- 20% (86). However, this arbitrary definition is

only valid when the scatter about the regression line of the prediction equation is proportional to the value of the predicted index (86). In common with most lung function tests in adults, the scatter about the prediction equation for TL_{CO} is independent of its value (86,87). Therefore, the practice of using a fixed percentage of predicted as a lower limit of normal TL_{CO} in adult is not recommended (41,57,63,65). The recommended approach is to define the lower limit of normal by the lower 5th percentile of the reference population (41,63,65,88).

2.8 Clinical applications

The measurement of TL_{CO} gives information about the mass of functioning pulmonary capillaries in contact with ventilated alveoli and reflects dysfunction of gas exchange mechanisms in certain pulmonary vascular and parenchymal diseases. TL_{CO} measurement has become an established routine test in most respiratory laboratories and is used in the screening, monitoring and differential diagnosis of many lung disorders (89).

Impaired TL_{CO} is one of the earliest objective indices in extrinsic allergic alveolitis (54). It is also used for early detection of pulmonary drug toxicity (e.g. amiodarone (90,91)). Serial measurements of TL_{CO} have been used to monitor progress and response to treatment in many interstitial lung diseases with variable results (54).

Like all other non-invasive lung function tests, TL_{CO} is not diagnostic of any disease, but it may be useful in the differential diagnosis. TL_{CO} estimation is particularly helpful in the differential diagnosis of chronic obstructive pulmonary disease; usually being normal and sometimes elevated in asthma, normal in chronic

bronchitis and reduced in emphysema (63,92). TL_{CO} is frequently abnormal in pulmonary vascular diseases, but it has very little value in the differential diagnosis of these disorders (54). The values of TL_{CO} and its components have been shown to correlate with severity of pulmonary vascular disease including congenital and acquired heart disease (30).

2.9 Limitations of TL_{CO} measurements

From the previous sections, it is clear that TL_{CO} tests have important limitations. These include lack of specificity, the wide range of normal values and the lack of correlation with the severity of disease (80). In addition, the use of different methods and different types of equipment along with the lack of standard values make it difficult to compare results obtained in different laboratories. The adoption of standardised methods is expected to reduce the differences in the TL_{CO} values obtained in different centres (41,65). The estimation of D_M and V_C requires very precise control of measurement conditions as these parameters are very sensitive to changes in the breath-holding time and the alveolar pressure during this time which must kept constant and close to the atmospheric pressure (79).

In spite of all of its limitations, TL_{CO} estimation remains a valuable index of the gas exchange function. It is simple, sensitive and non-invasive and may become abnormal before dysfunction is reflected by changes in arterial blood gases or by the other non-invasive lung function tests (93).

CHAPTER 3

LUNG FUNCTION IN HEART DISEASE - REVIEW

The lungs are interposed between the right and left sides of the heart and are connected to the heart in series by the pulmonary circulation. The matching of pulmonary blood flow and ventilation in the lungs is of prime importance to the efficiency of gas exchange. Because of the interdependence between the heart and lung in both structure and function, compromise in one adversely affects the function of the other (6).

The overall process of gas exchange at the alveolar-capillary interface consists of 4 interdependent processes (28). These processes are: ventilation of the alveoli, perfusion of the pulmonary capillaries, matching of ventilation and perfusion, and diffusion of oxygen and carbon dioxide across the alveolar-capillary membrane. Impairment of all of these processes has been reported in various forms of heart disease (6,12,26). The purpose of this section is to review lung function changes in heart disease with particular emphasis on the diffusing characteristics of the lung before and after various forms of cardiac surgery.

3.1 Mechanisms of pulmonary dysfunction in heart disease

Heart disease disturbs lung function through one or more of the following mechanisms (12): 1) increased pulmonary venous pressure, 2) lung compression, 3)

low cardiac output, 4) altered pulmonary to systemic blood flow ratios, and 5) pulmonary complications of heart disease treatment, e.g. drugs and cardiac surgery.

1. Increased pulmonary venous pressure

Pulmonary venous hypertension is the most common cause of altered lung function in heart disease (12). Regardless of the cause of left heart failure, the resulting increase in left ventricular end-diastolic pressure is transmitted backwards into the left atrium and is in turn transmitted to the pulmonary venous system. The effects of increased pulmonary venous pressure on lung function are well documented and the extent of pulmonary dysfunction appears to depend on the severity and the duration of the pulmonary venous hypertension (94-96). The increased pulmonary venous pressure causes transudation of fluid out of the capillaries which appears first as an increase in the pulmonary lymph flow. Then it accumulates in the interstitial space surrounding the peripheral vessels and airways and later in the alveolar walls. In severe cases it floods the alveoli (95). Mildly elevated pulmonary venous pressure is associated with a corresponding increase in the pulmonary arterial pressure to maintain a normal pressure gradient along the pulmonary capillaries. With increasing severity, pulmonary artery pressure increases out of proportion to the increase in the pulmonary venous pressure indicating the development of established active pulmonary hypertension (97). Histological studies have shown that severe chronic pulmonary venous hypertension is complicated by medial and intimal hyperplasia in the small pulmonary arteries and veins and by interstitial fibrosis (30,98,99). These pathological processes are reflected by characteristic abnormalities of lung function (30).

2. Lung compression

Cardiomegaly, pleural effusions, and elevated diaphragm resulting from hepatomegaly and ascites are common in congestive heart failure of any cause and are believed to contribute to the restrictive ventilatory defect that is commonly observed in patients with this condition (12,14).

3. Low cardiac output

Low cardiac output leads to uneven distribution of perfusion and ventilation resulting in impairment of pulmonary gas exchange (28). Respiratory muscle fatigue is also common in heart failure and has been attributed to low perfusion secondary to low cardiac output (100-102).

4. Altered pulmonary blood flow

In congenital heart disease with a left to right shunt, persistent massive pulmonary blood flow leads to structural changes in the pulmonary vascular bed which can be irreversible resulting in arterial pulmonary hypertension and raised pulmonary vascular resistance (27). On the other hand, congenital heart disease with right to left shunt is characterised by low pulmonary blood flow, hypoxaemia, and cyanosis (103).

5. Pulmonary complications of heart disease treatment

Some of the drugs used in the treatment of heart disease have been shown to cause or exacerbate lung disease (12). Beta-adrenergic blockers such as propranolol exacerbate bronchoconstriction in patients with asthma and chronic obstructive

pulmonary disease (104). Amiodarone-induced pulmonary toxicity has been documented clinically and pathologically in patients taking this anti-arrhythmic drug, and include pneumonitis, pulmonary fibrosis, and sub-clinical abnormalities of lung function (91). Lung function is also altered by cardiac surgery, including heart transplantation (10,38).

3.2 The pulmonary circulation in heart disease

As has been noted above, the pulmonary vascular bed is frequently disturbed by disorders of cardiac output such as congestive heart failure and congenital heart disease. Initially, there are changes in the pulmonary vascular pressures, the pulmonary blood flow or both, followed by pathological changes in lung structure (30,95). The functional consequences of these change include: abnormal ventilation-perfusion relationships (26,94), altered diffusing properties of the lungs (31,105) and abnormal lung volumes and mechanics (25). A review of the normal structure and function of the human pulmonary circulation is essential for the understanding of lung function changes in heart disease. A detailed account of the functional anatomy of the pulmonary circulation is beyond the scope of this thesis. The following is a brief out-line of the structure and function of the human pulmonary circulation both in health and in heart disease (27,61,106,107).

The lungs receive blood from both the bronchial and the pulmonary circulations. The bronchial blood flow constitutes a very small portion of the output of left ventricle (about 2%) and it supplies parts of the tracheo-bronchial tree with systemic arterial blood. The pulmonary blood flow constitutes the entire output of

the right ventricle and supplies the lungs with mixed venous blood draining the tissues of the entire body in order to undergo gas exchange at the alveolar-capillary interface. The walls of large pulmonary arteries are thin and consist mainly of elastic tissue. The media of the pulmonary arteries is about half as thick as in the systemic arteries of corresponding size. At a diameter of about 1000 μm, the pulmonary arteries become muscular with a distinct media muscularis. These muscular arteries are believed to be the resistance vessels of the pulmonary circulation and they branch down to a diameter of 100 μm giving rise to the pulmonary arterioles. In contrast to systemic arterioles, pulmonary arterioles have no muscular layer. They terminate by supplying the pulmonary capillaries which arise beyond the terminal bronchioles. The pulmonary capillaries have an average luminal diameter of about 7 μm and form a dense network which is enclosed by the alveoli to form the functional unit of pulmonary gas exchange. It is estimated that there are about 280 billion pulmonary capillaries supplying about 300 million alveoli, leading to an estimated surface area for gas exchange between 50 and 100 m^2. The pulmonary capillaries have no smooth muscle or any contractile elements surrounding them. The change in their calibre is believed to be achieved by mechanical means, such as the changes in the surrounding alveolar pressure, the pulmonary arterial and venous pressures and the movement of adjacent structures. The pulmonary capillary blood is collected into the pulmonary venules which have similar structure to the arterioles and as they increase in size they take up the appearance of veins which unite to form the pulmonary veins which eventually drain oxygenated blood into the left atrium.

Because of the structural differences, pulmonary arteries have greater compliance and provide less resistance to blood flow than do the systemic arteries. The pulmonary vascular resistance (PVR) is about a tenth of the systemic resistance (20 to 130 and 700 to 1600 dynes.sec.cm^{-5}, respectively). Under normal resting conditions, some of the pulmonary capillaries are under-perfused, and as pressure rises, they take up more blood (vascular recruitment). With further increases in pulmonary vascular pressures the patent capillaries increase their diameter (vascular distension). Vascular recruitment and distension act together to reduce the overall PVR in the face of increasing blood flow and pressures.

The pulmonary blood flow is equivalent to that of the systemic blood flow (about 3.5 L.min^{-1}.m^{-2} of body surface area at rest). It is estimated that about 250 to 300 ml/m^2 of body surface area is in the pulmonary circulation at any instant, and 60 to 70 ml.m^{-2} of this blood is in the pulmonary capillaries. A red blood cell takes about 4 to 5 seconds to travel through the pulmonary circulation at rest and 0.75 seconds of this is spent in the pulmonary capillaries where gas exchange takes place. In normal resting conditions gas exchanging reaches equilibrium in about 0.25 seconds. This gives the gas exchange unit of the lung a large physiological reserve at rest.

Chronic left heart failure from any cause can potentially raise the pulmonary artery pressure with reactive changes in the pulmonary capillary bed (12). However, most of the knowledge concerning the pulmonary consequences of left heart failure has come from studies on patients with mitral valve disease, especially those with mitral stenosis (27). In mitral stenosis, the rise in left atrial pressure is transmitted to the

pulmonary veins, capillaries and arteries. Initially the increased pulmonary artery pressure is passive, and at this stage, patients usually develop symptoms and signs of left heart failure. With long standing mitral stenosis, the pulmonary vascular resistance increases along with pathological changes in the vascular and parenchymal lung tissue, and patients develop symptoms and signs of right heart failure (30,94). Isotope studies have revealed a characteristic early redistribution of pulmonary blood flow in mitral stenosis (26,94). Initially there is an abolition of the normal pulmonary blood flow gradient from upper to lower lobes with subsequent reversal of the normal pattern as the severity of mitral stenosis increases. Mitral valve surgery is associated with rapid relief of symptoms and resolution of most of the cardio-pulmonary haemodynamic abnormalities (27). Left atrial pressure and pulmonary artery pressure fall immediately after surgery and pulmonary congestion is also relieved. The cardiac output improves and the PVR decreases to near normal (25,29).

Histological studies have demonstrated frequent pulmonary vascular and parenchymal abnormalities in patients with mitral valve disease (30,98,99). These include medial and intimal thickening of the muscular arteries and arterialisation of the veins. In advanced cases, there is marked fibrosis and thickening of the alveolar walls and interstitium. The structural changes in patients with increased pulmonary blood flow due to congenital heart disease are more severe and are also related to the duration and severity of the cardio-pulmonary haemodynamic disturbance. In addition to the structural changes seen in patients with chronic pulmonary venous hypertension, the pulmonary vasculature of patients with severe pulmonary

hypertension secondary to congenital heart disease is also characterised by loss of smooth muscle cell nuclei and fibrin deposition in the arterial walls (fibrinoid necrosis) and by aneurysmal dilatation of the arteries (plexiform lesions) (27,107).

3.3 TL_{CO} changes in heart disease

The introduction and development of different forms of corrective surgery for heart disease in the late 1950s and early 1960s, was accompanied by a substantial interest in the pulmonary consequences of chronic heart disease (27). In this respect, particular attention was given to the possible reversibility of lung function abnormalities commonly attributed to heart disease, especially those found in congenital and valvular heart disease. This resulted in many reports on the relationship between the non-invasive measurements of lung function, including TL_{CO}, and the functional status and pulmonary haemodynamics in patients with these disorders both before and after surgery (30). A review of these studies provides an insight into the possible mechanisms underlying the reported decline in TL_{CO} following heart transplantation.

3.3.1 Valvular heart disease

Lung function changes in mitral valve disease have been extensively studied both before and after surgery. Severe lung dysfunction is uncommon, but mild to moderate abnormalities of various lung function indices have been reported (6,26). The most common abnormality is a restrictive ventilatory defect with small lung volumes and reduced pulmonary compliance (25,30). Variable degrees of airway obstruction and increased bronchial hyper-reactivity have also been reported (108).

Mitral valve surgery leads to resolution of most of these abnormalities (26,108,109).

TL_{CO} in mitral valve disease is variable and ranges from higher than predicted normal to very low values depending on the haemodynamic and functional severity of the disease (30,32,38,110,111). In 1960, Bates and associates (112) using a steady state TL_{CO} method ($TL_{CO,SS}$) during exercise in 6 patients with mitral stenosis showed that V_C was significantly increased with a concomitant reduction in D_M, but TL_{CO} was within normal limits. Two years later, Flatley et al. (93) reported TL_{CO} changes in 59 patients with different heart diseases using the single-breath method ($TL_{CO,SB}$). There was no significant difference in mean TL_{CO} in normal subjects and in patients with aortic valve disease, mitral incompetence and functional class II mitral stenosis. In contrast, TL_{CO} was significantly reduced in patients with severe mitral stenosis (New York Heart Association functional classes III and IV). V_C was within normal limits in patients with aortic valve disease and mitral incompetence, but was elevated in patients with functional class II and III mitral stenosis. There was no consistent relationship between the diffusion parameters and the pulmonary vascular pressures.

In 1963, Ried et al. (113) reported TL_{CO} changes in 53 patients with mitral valve disease studied at rest and during exercise using $TL_{CO,SS}$. TL_{CO} was normal both at rest and during exercise in patients with normal pulmonary arterial pressure. In patients with mild to moderate pulmonary hypertension, TL_{CO} was normal at rest, but failed to rise normally on exercise. In patients with severe pulmonary hypertension, TL_{CO} was reduced at rest and did not rise on exercise. Using the

same method at rest, McCredie (33) found a significant reduction in mean TL_{CO} in 18 patients with mitral valve disease compared to normal controls (4.34 and 8.34 mmol. $kPa^{-1}.min^{-1}$. respectively), and this difference was mainly due to a reduction in D_M (6.5 and 16.7 $mmol.kPa^{-1}.min^{-1}$ respectively). In contrast, there was no significant difference in V_C between the 2 groups (V_C was 69.7 ml in patients and 89.6 ml in normal controls). V_C reduction was only found in patients with increased PVR.

In 1965, Aber and co-workers (30) reported the changes in $TL_{CO,SB}$ in 79 patients with predominant mitral stenosis. TL_{CO} changes were analysed in relation to the degree of functional capacity, the pulmonary haemodynamic disturbance, and the pathological lesions in the pulmonary arteries as assessed by lingular biopsies taken during mitral valve replacement surgery. Their findings indicated that TL_{CO} impairment parallels both the functional capacity and the pulmonary vascular changes. Mean TL_{CO} was less than 60% of predicted in patients with high pulmonary vascular resistance and this was associated with considerable thickening of the intima of the small peripheral pulmonary arteries.

Two years later, Gazioglu and associates (32) reported the changes in total pulmonary blood volume (PBV), TL_{CO} and its components in relation to pulmonary haemodynamics in 62 patients with mitral valve disease and in 44 patients with aortic valve disease using $TL_{CO,SB}$ in the supine position. In patients with mitral valve disease, mean TL_{CO}, D_M and V_C were 7.7, 16.7 and 80.1, compared to 11.3, 22.6, and 96.1 in normal controls, respectively. There was a progressive decline in D_M and TL_{CO} from functional class II to IV. There was no significant difference

between the mean values of V_C for functional classes II and III and normal controls (84.5, 87.2, and 96.1 ml, respectively). In contrast, V_C was markedly reduced in patients in functional class IV (48.1 ml). The diffusing characteristics of the lung in aortic valve disease were similar to those of the normal controls irrespective of severity.

In 1974, Burgess reported TL_{CO} changes in various pulmonary vascular diseases (31). In this study, $TL_{CO,SB}$ and pulmonary haemodynamics were determined in 48 patients with pulmonary vascular disease associated with either abnormal pulmonary vascular pressures, abnormal pulmonary blood flow or both. Patients were subdivided into 4 groups based on their pulmonary haemodynamics: 1) in-flow obstruction (9 patients with isolated pulmonary hypertension), 2) in-flow and out-flow obstruction (17 patients with long-standing mitral valve disease), 3) out-flow obstruction (12 patients with mitral valve disease and heart failure with normal pulmonary artery pressure) and 4) increased pulmonary blood flow (10 patients with intra-cardiac septal defects). In all groups there was an inverse relationship between TL_{CO} and the pulmonary vascular resistance (PVR). Patients with mitral valve disease and normal PVR had a normal mean TL_{CO} (96% of predicted). In contrast, TL_{CO} was significantly impaired (68%) in those with increased PVR.

In 1978, Jebavy and associates (114) reported the relationship between supine $TL_{CO,SS}$ and the pulmonary haemodynamics in 12 patients with severe mitral stenosis (NYHA functional class IV). Mean TL_{CO} was normal both at rest and during exercise. In the entire group, there was no correlation between TL_{CO} and the pulmonary vascular pressures. However, there was a significant positive correlation

between TL_{CO} and mean left atrial pressure up to a value of 22.5 mmHg (r = 0.71). With further increases in the left atrial pressure, the correlation between these 2 variables became negative (r = - 0.44). These findings were later confirmed by Yernault and associate (111). In this study, TL_{CO} was normal in patients with mitral valve disease (MVD) and pulmonary capillary wedge pressure (PCWP) less than 12 mmHg whereas it was significantly reduced in those with elevated PCWP. The results of these 2 studies are consistent with a biphasic relationship between TL_{CO} and pulmonary vascular pressures and might explain the lack of a consistent linear correlation between TL_{CO} and the pulmonary haemodynamics in previous studies. It was suggested that in mild pulmonary venous hypertension, without any significant pulmonary arterial hypertension, there is recruitment and distension of the pulmonary capillaries, which increases V_C, and this acts to maintain a normal TL_{CO} which would otherwise decreases due to reductions in D_M caused by pulmonary oedema. In advanced cases with severe pulmonary venous hypertension and established pulmonary arterial hypertension, there is progressive pulmonary vascular and parenchymal fibrosis resulting in progressive destruction and narrowing of the pulmonary capillary bed resulting in increased PVR. At this stage, V_C starts to decrease along with a progressive decline in D_M causing a reduction in the overall TL_{CO} (33).

In 1982, Rhodes and co-workers (115) further explored the relationship between $TL_{CO,SB}$ and cardio-pulmonary haemodynamics in 25 patients with MVD. Mean TL_{CO} was reduced (74% of predicted), but mean K_{CO} was normal (93%). TL_{CO} was negatively correlated with PCWP (r = - 0.44) and PVR (r = - 0.53), and positively

correlated with the cardiac index (r = 0.46). K_{CO} was also negatively correlated with the PCWP (r = - 0.45), but it had no consistent relationship with any of the other cardio-pulmonary haemodynamics.

The changes in TL_{CO} and components following mitral valve surgery have been reported by many investigators (34-40,105,112,116-118). Earlier studies involved patient with various cardio-pulmonary disorders, with only small numbers with valvular heart disease (37,112). In the study of Bates and associates (112), TL_{CO} and its components were measured in one patients with mitral stenosis before and 3 weeks after valvotomy. At similar levels of exercise V_C decreased from 275 to 85 ml, whereas D_M increased from 6.7 to 10.4 $mmol.kPa^{-1}.min^{-1}$. These opposite changes resulted in a relatively unchanged TL_{CO} before and after surgery (6.3 and 7.1 $mmol.kPa^{-1}.min^{-1}$.). In one patient with mitral incompetence studied by Howatt and co-workers (37), TL_{CO} values were 6.0, 3.1, 4.3, and 4.8 $mmol.kPa^{1}.min^{1}$., before and at 5 days, 10 days, and 5 months after mitral valve replacements respectively.

In 1964, Reid et al. (39) reported TL_{CO} changes in 40 patients with mitral stenosis before and at 6 to 9 months after valvotomy. In mild cases with normal pulmonary arterial pressure, TL_{CO} was normal at rest and during exercise before surgery and remained unchanged afterwards. In severe cases with significant pulmonary arterial hypertension, TL_{CO} was reduced pre-operatively, and failed to increase despite improvement in pulmonary haemodynamics, lung volumes, and symptoms after surgery. Analysis of the data presented for 6 of these patients who had symptoms of chronic bronchitis, revealed that the mean TL_{CO} before surgery was reduced both

at rest and during exercise (4.9 and 6.3 mmol.kPa^{-1}.min^{-1}. respectively) and decreased further after surgery (4.0 and 5.1 mmol.kPa^{-1}.min^{-1}. respectively). Whereas TL_{CO} declined in 4 out of the 6, it increased in one only. This patients had the lowest pre-operative TL_{CO} (TL_{CO} was 3.2 and increased to 4.0 mmol.kPa^{-1}. min^{-1}. post-operatively).

The effects of valvotomy on TL_{CO} was also reported by McCredie in 1966 (105). Eleven patients were studied before and at varying intervals (2 weeks to 18 months) after surgery. The was no significant difference between the pre- and post-operative results, but there was a tendency for mean D_M to increase (D_M increased from 6.7 to 7.1 mmol.kPa^{-1}.min^{-1}.) and for mean TL_{CO} and V_C to decrease following surgery (TL_{CO} decreased from 4.9 to 4.3 mmol.kPa^{-1}.min^{-1}., and V_C from 84 to 71 ml). Analysis of individual results showed that V_C decreased in 9 patients, did not change in one and increased in one. The latter 2 patients had the lowest pre-operative V_C. There were no consistent changes in the individual results of D_M and TL_{CO}.

In 1970, Singh and associates (34) reported the effects of valvotomy on TL_{CO} in a larger group of patients. Eighty patients with mitral stenosis were studied a few days before and at 3 to 24 months following closed mitral valvotomy. Before surgery, mean TL_{CO} was reduced (4.8 mmol.kPa^{-1}.min^{-1}.). After surgery, patients were subdivided into 3 groups based on the time of post-operative assessment. Although, TL_{CO} declined in 14 patients at 3 to 5 months and in 43 patients at 6 to 11 months after surgery, this decline did not reach statistical significance. In the 24 patients who were assessed at 12 to 24 months after surgery TL_{CO} decreased by 1.6

mmol.kPa^{-1}.min^{-1}., and this was statistically significant. These findings were taken to suggest progressive lung damage even after valvotomy.

In 1973, Gazioglu (35) measured TL_{CO} and its components in 12 patients with mitral valve disease before and at an average of 17 months after surgery. Mean TL_{CO} and D_M were 8.5 and 17.1 mmol.kPa^{-1}.min^{-1}. before, and 8.5 and 13.4 mmol.kPa^{-1}.min^{-1}. after surgery. Mean V_C was unchanged by surgery (82 ml before and after surgery). Individual TL_{CO} changes were mostly in the direction of V_C.

In 1984, the longitudinal changes in TL_{CO} following mitral valve surgery were first reported by Mustafa and associates (38) in 40 patients with mitral valve disease studied before and serially after surgery. Mean pre-operative TL_{CO} was 65% of predicted and was inversely correlated with PVR ($r = - 0.46$). Post-operatively mean TL_{CO} declined to 56% of predicted at 10 weeks, but progressively improved in the subsequent intervals reaching 71% at 25 weeks, 72% at 50 weeks, and 79% of predicted at 125 weeks after surgery.

Despite improvement in symptoms, exercise performance, and lung volumes in 50 patients studied by Rhodes et al. (36) before and 6 months after mitral valve surgery, TL_{CO} and K_{CO} failed to increase (mean TL_{CO} and K_{CO} were respectively 64% and 86% before and 63% and 84% of predicted after surgery). It was suggested that these changes may indicate irreversible damage to the pulmonary capillary bed. Alternatively, it was argued that the relief of pulmonary vascular engorgement with corresponding decrease in Vc might have masked any expected post-operative improvement in D_M resulting from relief of pulmonary oedema.

In 1987, Ohno and associates (40) showed that TL_{CO} changes following mitral valve surgery (open mitral valve commissurotomy or mitral valve replacement) were related to the pre-operative severity of the disease as judged by the degree of pulmonary arterial pressure and the presence of tricuspid valve regurgitation (TR). Twenty three patients were studied before and 14 to 20 months after surgery. In 7 patients with normal pulmonary arterial pressure, pre-operative lung function was normal and did show any significant change after surgery (mean TL_{CO} was 104% and decreased slightly to 95% of predicted). In 11 patients with pulmonary arterial hypertension (PAH) without TR, pre-operative TL_{CO} was 87% of predicted and despite improvement of the mild ventilatory dysfunction following surgery, it remained relatively unchanged (85% of predicted). In the 5 patients with PAH and TR, the ventilatory dysfunction was more severe and was unchanged by surgery. TL_{CO} impairment was also more severe (62% of predicted), but increased slightly (67% of predicted) after surgery.

In 1990, in attempt to determine the effects of pulmonary haemodynamics on lung function without the confounding effect of thoracotomy, Yoshioka and associates (116) studied 25 patients with mitral stenosis before and one week after percutaneous transvenous mitral commissurotomy (PTMC). Despite marked improvement of lung volumes and airway function, mean TL_{CO} declined significantly from 105% to 96% of predicted. It was suggested that this was probably due to the normalisation of the pulmonary vascular pressures which occurred immediately after the procedure (mean left atrial pressure decreased from 14.7 to 7.9 mmHg).

The effect of PTMC on TL_{CO} was further evaluated by 2 groups of investigators in 1994 (117,118). In 46 patients studied by Kim and associates (117), mean TL_{CO} decreased significantly from 95% of predicted before to 86 % of predicted 10 days after the procedure. In a study of 9 patients, Ray and co-workers (118) reported that mean TL_{CO} was 78% of predicted before PTMC, and did not change following the procedure (TL_{CO} was 75% of predicted both at 48 hours and at 3 months following PTMC). Similarly, D_M was reduced before surgery (56% of predicted) and remained unchanged afterwards (59%). In contrast, V_C was elevated before (118% of predicted) and decreased significantly to 111% of predicted at 48 hours and to 98% at 3 months after the procedure. It was concluded that in patients with mitral stenosis associated with moderate PAH, the reduction in TL_{CO} is primarily due to a reduction in D_M, and that successful PTMC reduces V_C , but does not improve D_M implying persistent abnormalities of the pulmonary vascular bed.

In summary, TL_{CO} in mitral valve disease is variable and appears to parallel the functional capacity and pulmonary haemodynamics. In mild disease, there is a tendency to an increase in V_C. At this stage D_M is usually slightly reduced, with an overall normal or slightly elevated TL_{CO}. With increasing severity, secondary pulmonary hypertension develops and the pulmonary vascular resistance increases leading to a reduction in V_C, D_M and TL_{CO}. The failure to demonstrate a consistent relationship between TL_{CO} and pulmonary haemodynamics may be due to the biphasic nature of this relationship; in mild disease they are positively correlated whereas in severe disease the correlation is negative (111,114). The effects of heart surgery on TL_{CO} are also variable and appear to be influenced by the pre-operative

severity of the disease and the interval between surgery and TL_{CO} measurement. Most patients show no significant change in TL_{CO} despite improvements in symptoms and lung volumes. However, there is a tendency for TL_{CO} to improve following surgery in patients with the lowest pre-operative TL_{CO}. The overall effect of chronic venous hypertension is therefore a composite of the opposing effects of raised pulmonary venous pressure and the increased pulmonary vascular resistance together with the pulmonary oedema and fibrosis which reduce D_M. In this context, it is clear that TL_{CO} may be normal despite significant abnormalities in both of its components when they change in opposite directions.

3.3.2 Congenital heart disease

The influence of pulmonary haemodynamics on TLco and its components is further supported by studies in patients with congenital heart disease. Congenital heart disease with left to right shunts is associated with increased TL_{CO} and this has been shown to be due to increases in both D_M and V_C (22-24,31,93,110,119,120). In 1958, Rankin and Callies (22) reported increased TL_{CO} in patients with atrial septal defects (ASD) or ventricular septal defects (VSD) when the pulmonary arterial pressure was normal and this was due to proportionate increases in D_M and V_C. In presence of pulmonary hypertension, TL_{CO} was reduced due to disproportionate decrease in D_M. Auchincloss (110) also reported elevated TL_{CO} in 16 patients with increased pulmonary blood flow and this increase was attenuated by the presence of pulmonary hypertension (TL_{CO} was 142 and 110% of predicted in the absence and presence of pulmonary hypertension respectively).

In 1961 Bedell (119) showed that TL_{CO} was significantly correlated with pulmonary blood flow (PBF) and was elevated in patients with ASD (165% of predicted), but was within normal limits in those with VSD (110% of predicted). In the same year, Bucci and associates (23) found that TL_{CO}, D_M and V_C were significantly elevated in patients with increased PBF (143%, 138%, and 160% of predicted respectively). In patients with ASD associated with increased PBF and PCWP, V_C was even higher (225% of predicted). It was concluded that the increase in V_C in these patients was due to increased PBF augmented by the associated increase in PCWP. These findings were subsequently confirmed by several investigators (24,31,93,120).

TL_{CO} and its components have also been shown to return to normal following corrective surgery (23,24). In the study of Bucci and associates (23), 10 patients with ASD were assessed before and at least 3 months after surgical closure of their lesions. Mean TL_{CO}, D_M and V_C declined from 144%, 130%, and 166% to 104%, 110% and 110% of predicted respectively).

In 1985, Schofield and co-workers (24) studied TL_{CO} and other lung function tests in 33 patients with ASD before and at an average of one year after surgery. Pre-operatively, TL_{CO} was elevated (127% of predicted) and was negatively correlated with the pulmonary arterial pressure ($r = - 0.52$). Despite unchanged ventilatory function, TL_{CO} returned to normal (100% of predicted) following surgery with the greatest decline occurring in those with the highest pre-operative TL_{CO} values.

3.3.3 Coronary artery bypass graft surgery

Cardiac surgery has a complex impact on lung function. The surgical correction of different forms of cardiac lesions usually improves most of the lung function abnormalities specifically related to these lesions (38). However, new abnormalities of lung function after cardiac surgery, including coronary artery bypass graft (CABG), have also been reported (121). The cause of these abnormalities is not clear, but they have been variously attributed to general anaesthesia (122), the mechanical effects of thoracic surgery (123), cardio-pulmonary bypass (CPB) procedure (37) and post-operative pulmonary complications including infection and drug toxicity (121). Patients undergoing cardiac surgery are subjected to the combined effects of all of these factors and it is often difficult to separate the individual influence of each factor on lung function. With this in mind, the following account is a review of the effects of general anaesthesia, sternotomy, and CPB on lung function following CABG.

1. General anaesthesia: General anaesthesia results in approximately 20% reduction in the functional residual capacity (124). The cause of this is not fully understood, but it is generally believed to be due to the cephalic shift of the diaphragm and the decrease in the transverse cross-sectional area of the chest which occur immediately after induction of anaesthesia (121,124). Pulmonary atelectasis has also been shown to occur immediately after induction (124). The overall effects of these changes is to increase the proportion of lung units with low ventilation-perfusion ratios (intra-pulmonary shunt) which results in a widened

alveolar-arterial oxygen gradient (121). It is not clear how long these abnormalities persist after general anaesthesia, but they are generally believed to be transient (125). The effect of general anaesthesia on TL_{CO} is not well documented. In 12 patients studied by steady state method, before and after halothane anaesthesia, Bergman (126) found no significant change in TL_{CO}. In contrast, Zebrowski and associates (127) using a rebreathing technique reported a significant decline in TL_{CO}, D_M, and V_C in 9 patients undergoing lower abdominal or lower limb surgery under controlled ventilation and halothane anaesthesia. In another study, halothane general anaesthesia was shown to cause a reversible decrease in the lung transfer factor for oxygen in dogs (128).

2. The surgical procedure: Most cardiac operations are performed through a standard median sternotomy. During operation, the sternum is spread open for several hours, and during the harvest of the internal mammary artery (IMA), the pleural space may be opened. The use of IMA is associated with increased incidence of pleural effusions, atelectasis, and greater decrease in lung volumes (129-131). In addition, bone scan evidence of rib cage fractures has been demonstrated in more than 50% of patients undergoing sternotomy (132). It has been suggested that these mechanical changes may contribute to lung function deterioration following cardiac surgery (132). However, Barnas et al. (133) showed that open heart surgery using median sternotomy does not affect the mechanical property of the chest wall and demonstrated that lung dysfunction in this setting is largely due to changes in mechanical properties of the lung which are similar to those found in pulmonary oedema.

The effect of sternotomy alone on TL_{CO} in humans is not known. In 1959, Schramel et al. (134) studied the effect of thoracotomy alone (4 dogs), CPB alone (4 dogs), and thoracotomy with CPB (2 dogs) on TL_{CO} using the steady-state method. All dogs had an immediate decline in TL_{CO} and this was of longer duration in animals with the combined procedures.

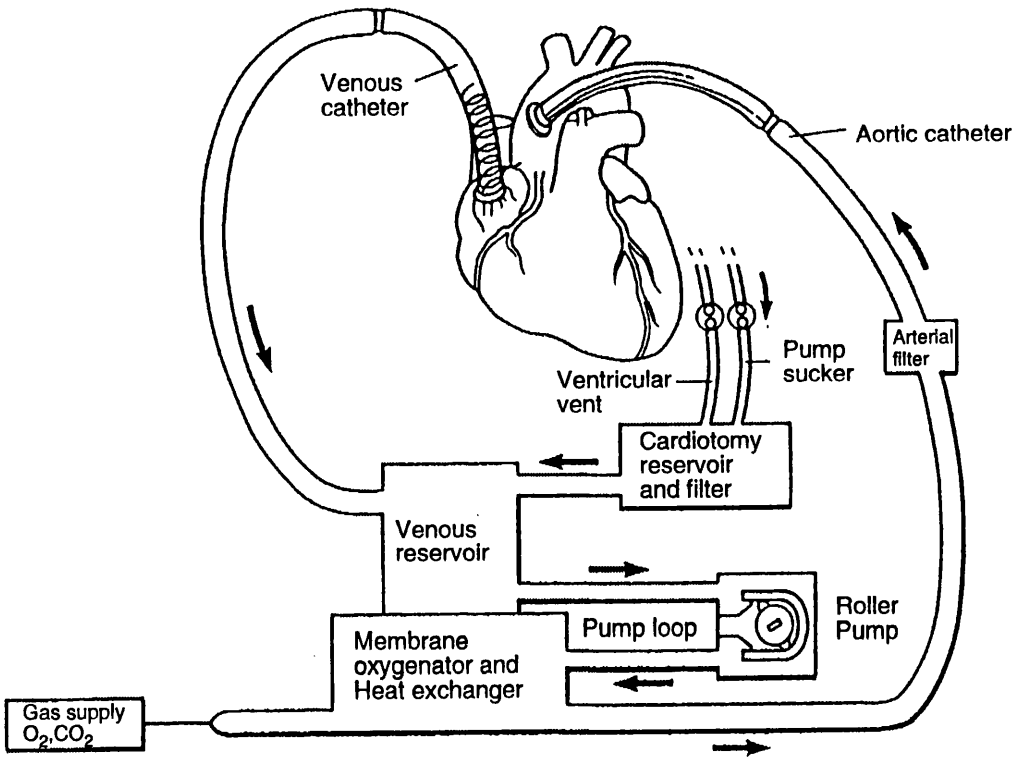
3. The cardio-pulmonary bypass (CPB) procedure: Most heart operations require the heart to be immobilised or opened. The goal of total CPB is to divert all the venous return to the bypass circuit, so that the bypass machine provides total perfusion and gas exchange for the entire body. Detailed discussion of the CPB is beyond the scope of this thesis. The following is an outline of the basic components and concepts of the CPB (135,136).

Figure 3.1 illustrates a typical CPB circuit (135). The CPB machine consists of large diameter plastic tubes, a reservoir, a mechanical pump, a gas exchanger (oxygenator), heat exchanger, and an arterial filter. The plastic tubes carry blood from the right atrium or the venae cavae to the reservoir, from which it is pumped and passed through the oxygenator where oxygen is added and carbon dioxide is removed. The blood (perfusate) is then passed through the heat exchanger which controls its temperature. The oxygenated blood is then filtered and returned to the aorta via plastic tubes connecting the CPB machine to a cannula inserted into the proximal aorta. Systemic heparinisation is essential to prevent the activation of the coagulation cascade when blood comes in contact with the artificial membranes of the bypass machine. After separation of the patient from bypass, heparin effect is reversed by protamine. The preparation of the bypass machine requires filling

(priming) with a fluid (prime). The prime fluid is usually a clear crystalloid solution. In addition to systemic cooling, myocardial protection during bypass is achieved by cold cardioplegia solution, and topical myocardial cooling using iced saline solution.

After heparinisation, CPB is established by cannulation of the ascending aorta and the venae cavae. CPB is initiated with systemic cooling in the range of 25 to 28 ^{o}C with a flow rate of about 1.7 L.m^{2} body surface area, and a mean systemic pressure between 50 to 70 mmHg. Once systemic arterial blood pressure has been adequately controlled, ventilation is stopped and the ascending aorta is cross-clamped. Electromechanical cardiac arrest (asystole) is then achieved by cold cardioplegia solution (St.Thomas at 4 ^{o}C) infused into the aortic root with topical cooling of the heart using iced normal saline. Surgery on the heart is usually performed during a single period of aortic cross-clamping (complete bypass).

Figure 3.1: Schaematic diagram of a typical CPB circuit (from reference 135)



Adequacy of perfusion and gas exchange while on bypass is assessed by monitoring direct arterial blood pressure, haematocrit, arterial blood gases and pH, central and peripheral temperature. Separation from bypass is a gradual process and starts with systemic rewarming followed by removal of the aortic cross-clamp. This usually leads to spontaneous reappearance of sinus rhythm, otherwise defibrillation is used. Ventilation is then started and cardiac output is initiated by gradual restriction of the venous return to the bypass machine directing blood to the right ventricle. Once complete separation from bypass is established, heparin is reversed using protamine sulphate.

Pulmonary complications following CPB

The development and continuous refinement of CPB techniques have made cardiac surgery for various forms of heart disease one of the most common major surgical procedures world-wide (136). However, since the early days of its development, it was recognised that CPB is associated with significant complications, including pulmonary dysfunction, bleeding tendency, renal and cerebro-vascular complications (121,135). These multi-organ complications have been called the "post-pump syndrome" (121). Although the advances in various aspects of heart surgery, have minimised these complications, they still occur, and in some patients contribute significantly to post-operative morbidity and mortality (137). In addition, transient sub-clinical physiological disturbance of various body systems is almost universal (138). The underlying mechanisms of these disturbances appear to be complex and are not completely understood (138). There is increasing evidence

that these complications result from whole body inflammatory responses to the exposure of foreign material in the bypass circuit leading to pan-endothelial damage (136,138,139). After exposure of blood to the artificial surfaces of the CPB circuit, various inflammatory response are activated the including complement, coagulation, fibrinolytic and kallikrein cascades. These changes are accompanied by impaired platelet function, leucopenia and histological evidence of leucocyte sequestration in the capillaries with endothelial cell damage (139,140). In addition, a variety of inflammatory mediators has been identified in association with CPB including endotoxins, cytokines and reactive oxygen species (139). Despite extensive research, the precise role of these mediators and responses remains obscure (138).

Pulmonary dysfunction is the most common complication of CPB (121). This ranges from sub-clinical abnormalities of lung function to severe respiratory failure (121). Almost all patients undergoing CPB experience transient deterioration of lung function during the first few days of the operation (135). Lung function impairment following CPB is characterised by impaired oxygenation, loss of lung volume, and reduced TL_{CO} (141-144).

TL_{CO} changes following CPB have been reported by several investigators (123,141,144-146). In 1963, Ellison and associates (123) measured the steady-state TL_{CO} in 146 patients undergoing thoracic (81 patients) and non-thoracic (65 patients) operations. Twenty eight of the thoracic operations were open heart surgery requiring CPB. On the first post-operative day, TL_{CO} decreased significantly in all types of operations and persisted for at least 5 days. In thoracic

operations, especially those involving CPB, the decline in TL_{CO} was more marked and took longer to return to its pre-operative values.

The short-term effect of CPB on TL_{CO} was also evaluated by Turnbull and co-workers (145) in 19 patients using the steady-state method. In this study, pulmonary dysfunction was maximal on the first post-operative day and gradually improved on subsequent measurements. Mean pre-operative TL_{CO} was 5.9 $mmol.kPa^{-1}.min^{-1}$. and was 3.1, 4.2, and 4.6 $mmol.kPa^{-1}.min^{-1}$ on the first, third, and seventh post-operative days respectively. These immediate post-operative changes in lung function were confirmed by Macnaughton et al. (144). Using the rebreathing method, TL_{CO} was measured immediately before induction and 2 hours after the operation. Haemoglobin corrected TL_{CO} declined by 27% and this was partly due to simultaneous reduction in lung volumes as K_{CO} decreased by 18% only. There was no correlation between any of the lung function parameters and pulmonary endothelial permeability, as assessed by a protein accumulation index. It was suggested that the decline in lung function following CPB is not mediated by increased pulmonary capillary permeability.

The long-term changes in lung function following CABG were assessed by 2 groups of investigators with similar results (141,146). In 19 patients, Braun et al. (141) reported a significant decline in P_aO_2, lung volumes and TL_{CO} at 2 weeks after surgery. Although there was a significant improvement at 3 to 4 months, all parameters remained significantly lower than their pre-operative values. Mean pre-operative TL_{CO} was 82% of predicted and was reduced by 28% and 11% at 2 weeks and 4 months after surgery respectively. There was no significant change in

K_{CO} at any of the measurements suggesting that the decline in TL_{CO} following CABG was due to the reduction in lung volumes. In 40 patients studied by Dubios and associates (146), mean pre-operative TL_{CO} was 100% of predicted and declined to 94%, 6 months after surgery ($p<0.05$). In contrast there was no significant change in K_{CO} (104% to 107% of predicted).

In summary, CABG surgery results in immediate deterioration in lung function characterised by loss of lung volume, reduction in TL_{CO} and widening of the alveolar-arterial oxygen gradient. These abnormalities tend to improve with time, but remain below their pre-operative values at 4 to 6 months after surgery. The changes in TL_{CO} and K_{CO} following CABG suggest that TL_{CO} impairment in the immediate post-operative period is only partly explained by the reduction in lung volumes; reversible alveolar-capillary membrane damage may be an additional contributory factor. Beyond 2 weeks, TL_{CO} impairment appears to be due to the loss of lung volume. Since most of these studies did not report the changes in haemoglobin and its effects on the measured TL_{CO} before and after surgery, it is possible that the transient reduction in TL_{CO} after CPB may be partly due to reductions in haemoglobin concentration which is common after cardiac surgery.

3.3.4 Congestive heart failure

Ischaemic heart disease is the most common cause of congestive heart failure (CHF). Other causes include valvular heart disease, systemic hypertension and primary myocardial disease. In contrast to valvular heart disease, there are few studies on lung function changes in CHF due to other causes. The results of these

studies indicate that lung function abnormalities are common in CHF and are similar to those described in patients with mitral valve disease (28). A restrictive ventilatory defect is the most common abnormality, but obstructive ventilatory abnormalities are also common especially during acute episodes of left ventricular failure (7,147). Bronchial hyperresponsiveness to cholinergic agonists is also well documented (148-150). Studies in non-heart transplant candidates (i.e. patients with less severe CHF) showed that TL_{CO} is usually normal or only slightly reduced even during episodes of acute heart failure (7,151,152). In 28 patients admitted with acute CHF, the initial mean TL_{CO} was within normal limits (91% of predicted) and showed no significant change after treatment (99% of predicted). In contrast, all indices of lung volumes and airway function were markedly reduced before treatment and all improved by greater than 20 % following medical treatment. These findings were similar to those of Frand and associates (152) who reported mean TL_{CO} of 91% of predicted in 6 patients with acute pulmonary oedema due to CHF and those of Bedell et al. (151) who found no changes in TL_{CO} in 9 patients with acute CHF over 2 weeks of treatment despite improvement of lung volumes. It was suggested that the increased pulmonary capillary blood volume in acute CHF probably compensates for the effects of pulmonary oedema which would be expected to reduce TL_{CO} (7). It was also suggested that the presence of abnormally low TL_{CO} in patients with CHF should raise the possibility of co-existing primary lung disease such as emphysema or pulmonary vascular disease (7).

In 34 stable patients with mild to moderately severe chronic CHF, Siegel and associates (8) showed that mean TL_{CO} in the entire group was 73 % of predicted

and was significantly lower in patients with rales compared to those without (53% vs. 83% of predicted). In addition, TL_{CO} was found to be strongly correlated with the left ventricular ejection fraction (LVEF) in patients with rales (r = 0.81; $p<0.001$), but not in those without rales. It was concluded that TL_{CO} was a useful predictor of clinically evident CHF. The presence of rales on auscultation was presumed to indicate pulmonary oedema which reduces TL_{CO}, and in the absence of rales TL_{CO} is usually normal.

When the work of this thesis was started, there were no data available on changes of TL_{CO} components in patients with CHF secondary to ischaemic or dilated cardiomyopathy. In 1994, Puri and associates (9) provided the first report on the changes of TL_{CO} and its components in stable patients with mild to moderately severe CHF (NYHA functional classes II and III). In the entire group (38 patients) mean TL_{CO} was reduced compared to controls (6.8 and 9.7 $mmol.kPa^{1}.min^{-1}$, respectively; $p<0.001$). TL_{CO} impairment was primarily due to a reduction in D_M (mean D_M was 9.0 in patients and 16.7 $mmol.kPa^{1}.min^{1}$ in normal controls; $p<0.001$). TL_{CO} and D_M were significantly lower in patients in NYHA functional class III compared to those in class II. In contrast, mean V_C was not significantly different in functional class II patients and normal controls (61 and 66 ml, respectively), and was significantly greater than normal controls in functional class III patients (95 ml). These finding were similar to those found in patients with mitral valve disease (32). In 1995, the same group of investigators (153) showed that the reduction in D_M in patients with CHF was significantly correlated with maximal exercise oxygen uptake and was inversely correlated with the pulmonary

vascular resistance. It was concluded that reduction in D_M was the major cause of TL_{CO} impairment in patients with CHF and it appeared to contribute to exercise intolerance. It was also suggested that D_M may be a useful marker for the alveolar-capillary membrane damage caused by pulmonary hypertension.

3.4 The lungs in heart transplant patients

3.4.1 Background

Heart failure is a serious condition with significant morbidity and high mortality. The reported mortality rates in patients with heart failure range from 24 to 35%, reaching greater than 50% in patients with NYHA functional class IV (154). Although drug therapy with angiotensin converting enzyme (ACE) inhibitors and vasodilators has been shown to improve survival in heart failure, patients with NYHA class IV and some patients with class III continue to have very poor life-expectancy (154). In addition, these patients remain severely limited by dyspnoea and fatigue even when on maximal medical therapy (155). For these patients, heart transplantation has become the treatment of choice (156).

Since the introduction of cyclosporin immunosuppressive therapy, the reported survival rate has reached 80 to 90 % at one year and 60 to 70% at 5 years after heart transplantation (154). In addition to increased life expectancy, heart transplant recipients report a remarkable improvement in symptoms and functional capacity (157). The success of heart transplantation is attributable to the substantial improvements in many fields of medicine. These include pre-operative management of heart failure, surgical techniques, donor management and organ preservation

techniques, prevention and treatment of rejection and early, aggressive treatment of medical complications after transplantation (156). Despite these improvements, heart transplant recipients continue to be at risk for a number of complications that may limit the overall success of heart transplantation (42). Infectious complications are very common, occurring in 40 to 80% of patients and at least 25% of deaths in these patients are due to infection (158). Other complications include cardiac allograft rejection, accelerated graft atherosclerosis and increased incidence of malignancies (42). Pulmonary infections account for more than 50% of all infectious complications following heart transplantation (159). Other pulmonary complications include atelectasis, oedema, thrombo-embolism, pleural effusions, and malignancy (42,159). Pulmonary complications are particularly common in the early post-operative period and contribute significantly to the morbidity and mortality in these patients (42,158).

Pulmonary function tests are part of routine assessment of potential heart transplant candidates in most centres, and are primarily performed to screen for any significant co-existing primary lung disease and to assess the risk for the surgery of heart transplantation (160). In addition, studies of lung function before and after transplantation provide a unique opportunity to evaluate pulmonary function abnormalities specifically related to heart failure (14). The abnormalities of lung function that resolve following heart transplantation may reasonably be assumed to have been due to the effects of heart failure before transplantation. On the other hand, persistent or progressive pulmonary dysfunction following heart transplantation may be due to one or more of several possibilities with different

clinical and therapeutic implications. These include: co-existing primary lung disease before transplantation, irreversible pulmonary damage due to long-standing heart failure and new lung injury from the procedure of heart transplantation or its complications. Lung function measurements before and serially after transplantation in conjunction with clinical correlates may help to distinguish between these various possibilities.

3.4.2 Cardio-pulmonary haemodynamics

The primary indication for heart transplantation is severe congestive heart failure due to ischaemic and dilated cardiomyopathy (156,161). Other indications include end-stage valvular heart disease, severe angina, and life threatening arrhythmias not responsive to conventional medical or surgical therapy. The majority of these patients have mild to moderate pulmonary arterial hypertension secondary to chronically elevated end-diastolic left ventricular pressure (161). Heart transplantation is associated with an almost immediate improvement in cardio-pulmonary haemodynamics (13,162). Haemodynamic studies several weeks to months after transplantation have reported normalisation of resting cardio-pulmonary haemodynamics including, the mean pulmonary arterial pressure (PAP), mean pulmonary capillary wedge pressure (PCWP), transpulmonary gradient (TPG) and the pulmonary vascular resistance (PVR), but they all remain in the upper limit of normal (13,163-165). Bhattia and associates (13) reported the time course of the cardio-pulmonary haemodynamic changes in 24 heart transplant recipients. In this study, right and left heart filling pressures and pulmonary arterial pressures declined rapidly reaching normal levels at about 2 weeks. The pulmonary vascular resistance

took longer to decrease and was still higher than normal at one year after transplantation. During exercise, abnormal increases in PAP, PCWP and PVR have been reported by several investigators (165-167). The lack of complete resolution of resting pulmonary haemodynamics together with their abnormal responses to exercise after heart transplantation has been suggested to indicate incomplete reversibility of the pulmonary vascular dysfunction caused by the pre-transplant severe chronic heart failure (167).

3.4.3 Lung function

Lung function abnormalities are common in heart transplant candidates, but they are usually mild with a restrictive ventilatory defect being the most common abnormality (11,14,16-18). TL_{CO} impairment is also common and is usually mild (10,11,16-19). Airway obstruction is uncommon (10,14), but has been reported in this selected group of patients (11,18). Heart transplantation restores lung volumes and airway function towards normal. In 1990, Hosenpud et al. compared the spirometric results in 17 patients before and 15 +/- 10 (mean +/- SD) months after transplantation (14). Mean values for FVC increased from 3.34 to 3.89 L ($p<0.01$) and FEV_1 increased from 2.63 to 2.95 L, ($p<0.05$), but FEV_1/FVC did not change. These findings were subsequently confirmed by several investigators (16-18,20,21). In contrast, TL_{CO} and K_{CO} have been consistently shown to deteriorate or remain sub-normal following heart transplantation (15-21).

Casan et al. (15) were the first to report the changes in lung function following heart transplantation. In their study, lung function tests including $TL_{CO,SS}$ were

measured in 10 patients before and at 6 to 12 months after transplantation. The comparison between data before and after transplantation revealed an overall improvement of lung function indices except TL_{CO} and K_{CO} which deteriorated. Mean TL_{CO} and K_{CO} declined from 83% and 97% of predicted before transplantation to 69% and 73% of predicted after transplantation respectively. The decline of K_{CO} was positively correlated with whole blood levels of cyclosporin determined within few days of lung function assessment ($r = 0.87$; $p<0.001$). It was suggested that the decline in TL_{CO} and K_{CO} may be due to new pulmonary vascular or interstitial damage. In absence of thrombo-embolic disease, graft rejection, and pulmonary infection, Casan and associates suggested cyclosporin pulmonary toxicity as a possible cause.

In a longitudinal study, Groen and associates (16) reported lung function changes before and serially after heart transplantation. In this study, 34 patients were followed for at least one year, with 27 of them for 2 years, and 21 for 3 years. These patients were selected from 124 transplant recipients. The selection criteria were based on the ability to perform pulmonary function tests at the time of follow-up, pre-transplant chest radiograph with no abnormalities other than changes of heart failure and a normal chest radiograph in the subsequent post-transplant intervals. In this selected group, the mean value of pre-transplant K_{CO} was 98% of predicted with 33 of the 34 patients having K_{CO} values within the normal predicted range. In the first post-operative year, lung volumes and flow rates (TLC, VC, FEV_1) tended to normalise. In contrast, K_{CO} decreased by a mean of 12% ($p<0.01$). In the following 2 years, K_{CO} tended to improve, but remained below its base line

pre-operative value (89% and 88 % of predicted in the second and third year after transplantation respectively). There was a weak correlation between K_{CO} and the mean pulmonary capillary wedge pressure (PCWP), both before and after transplantation. Using multiple regression analysis, it was found that the factors most strongly related to the percentage of change in K_{CO} in the first year after transplantation were the pre-operative K_{CO}, mean cyclosporin blood levels in the first post-operative year and the change in diastolic PAP in that year. There was no correlation between cyclosporin levels and the pulmonary vascular resistance (PVR). It was suggested that the decline in K_{CO} may represent alveolar-capillary membrane damage secondary to high levels of cyclosporin.

In 1993, Ohar et al. (19) reported lung function studies in 22 patients before and 3 to 7 months following heart transplantation. In this study 21 (95%) patients had abnormal lung function before transplantation. The mean values for static and dynamic lung volumes did not change, but TL_{CO} and K_{CO} decreased significantly after transplantation. TL_{CO} decreased from 68% to 54% of predicted. The values of TL_{CO} and K_{CO} decreased in 19 (86%), and 18 (82%) patients respectively. There was no significant correlation between TL_{CO} and the pre-transplant left ventricular ejection fraction, mean PAP, PCWP, or PVR. The conclusions of this study were that lung function abnormalities were common after heart transplantation and that the diffusion abnormalities were not related to indices of cardiac function measured before transplantation.

In another selected group of heart transplant patients, Ravenscraft et al. (18) reviewed pulmonary function data in 38 patients before and one year after

transplantation. Mean TL_{CO} was sub-normal before transplantation, and deteriorated after transplantation (from 82.3% to 76.8% of predicted; $p<0.05$). With improvement in lung volumes, the decline in K_{CO} was more pronounced (from 106.2% to 85.0% of predicted).

In a retrospective study, Egan and associates (21), analysed lung function data in 22 patients before and at an average of 14 months after heart transplantation (range 1 to 42 months). Despite normal cardiac function and in the absence of respiratory symptoms, mean percent predicted TL_{CO} and K_{CO} declined from pre-operative values of 80.0% and 94.1% to 57.8% and 70.6% of predicted respectively. The decline in TL_{CO} did not show any correlation with cardiac allograft rejection, cyclosporin levels, or cardio-pulmonary haemodynamics. The mean percentage reduction in TL_{CO} in asymptomatic patients with serological evidence of cytomegalovirus (CMV) infection was greater than that of those who had no evidence of CMV infection (31 vs. 16%; $p = 0.06$). It was suggested that the decline in TL_{CO} following heart transplantation may be due sub-clinical lung injury caused by CMV infection. In another retrospective study, Niset et al. (17) analysed lung function data in 47 patients before and one year after transplantation. In this study, the mean % predicted TL_{CO} and K_{CO} were impaired before transplantation (64% and 75%, respectively), and despite improvement of lung volumes after transplantation, TL_{CO} remained sub-normal at 67% and K_{CO} decreased to 64% of predicted. There were no correlations between TL_{CO} and the duration of congestive heart failure or the pre-transplant pulmonary haemodynamics.

The long-term change in lung function following heart transplantation were reported by Degre et al. (20) in a study of 100 transplant patients. Before transplantation, patients had a significant restrictive ventilatory and mean TL_{CO} was 67% of predicted. The long-term changes following heart transplantation in these patients were assessed by cross-sectional analysis of lung function data from one month up to 10 years following transplantation. At one month after transplantation, lung volumes improved slightly, but the restrictive defect was still present. Lung volumes continued to improve and at one year they were normal and remained so in the subsequent years. In contrast, TL_{CO} declined significantly following transplantation with no significant difference between the different post-operative interval. At 10 years post-transplantation, mean TL_{CO} was 59% and this was significantly lower than its pre-transplant value.

The time course of pulmonary function changes following heart transplantation was also assessed by Jahnke and associates (168) in 21 heart transplant recipients. Patients were selected randomly and divided into 2 groups based on the time interval between transplantation and lung function assessment. The short-term group (11 patients) was assessed at 12.9 +/ -7.0 months (mean +/-SD), and the long-term group (10 patients) was assessed at 53.9 +/- 14.8 months after transplantation. In this cross-sectional study mean TL_{CO} was reduced in both groups, but it was significantly lower in the short-term group compared the long-term one (70% and 47% of predicted, respectively). It was suggested that the difference may be due to a gradual reconstruction of the alveolar-capillary membrane secondary to the normalisation of the pulmonary haemodynamics.

PART TWO

METHODS

CHAPTER 4

GENERAL DESIGN AND PROTOCOLS

4.1 Design

A prospective descriptive longitudinal study consisting of one primary study group and 3 control groups. The primary group consists of all heart transplant patients who were transplanted between January 1992 and March 1996 at the Scottish Cardio-pulmonary Transplantation Unit. The control groups include normal subjects, coronary artery bypass graft patients and mitral valve replacement patients.

4.2 Setting

University Department of Cardiac Surgery and Department of Respiratory Medicine, Glasgow Royal Infirmary.

4.3 Study Population

4.3.1 Heart Transplant Patients

Background

The first heart transplantation in the Scottish Cardio-pulmonary Transplantation Unit (SCPTU) was performed in January 1992. Since the start of the transplantation programms, the unit has established routine protocols for the various aspects of heart transplantation management. All heart transplant patients were managed according to the same protocols. Information derived the protocols serves to define the clinical characteristics of the patients and are relevant to the

understanding of the changes in lung function following heart transplantation. These include; the pre-operative condition, the heart transplant surgery, immunosuppressive therapy, and post-transplant complications such as cardiac allograft rejection and pulmonary infection.

Selection criteria

Like any other heart transplant centre, the primary indication for heart transplantation is severe heart failure of any cause not amenable to conventional medical or surgical therapy. Other indications include severe angina or life threatening arrhythmias unresponsive to conventional treatment. Potential candidates must have a life expectancy substantially worse than that which would be expected after heart transplantation. Table 4.1 lists the primary criteria for heart transplant recipients selection in the Scottish Cardio-pulmonary Transplantation Unit.

Table 4.1: The primary criteria for recipients selection in the Scottish Cardio-pulmonary Transplantation Unit.

Inclusion criteria

- Age; over 10 year and under 65 years.
- Severe symptoms and poor life expectancy.
- High motivation for transplantation.

B. Exclusion criteria

- Raised pulmonary vascular resistance defined as fixed transpulmonary gradient greater than 14 mmHg.
- Severe disease which may itself compromise life-expectancy or limit the success of transplantation (e.g., malignancy, insulin dependent diabetes with significant extra cardiac complications, irreversible renal dysfunction, severe liver disease, and active systemic infection.
- Severe obesity, alcohol or drug abuse, and active cigarette smoking.
- Poor prospect of compliance with post-transplant regimes.
- Presence of intra-aortic balloon pump or other mechanical circulatory assistance.

Pre-operative Management

Table 4.2 lists the pre-transplant screening tests for heart transplantation candidates. Many of these tests are performed by the referring cardiologists. These investigations are aimed at identifying patients most likely to benefit from heart transplantation and to identify any systemic disease which may limit the success of transplantation. If clinical assessment and specialised investigations suggest that the

patient is indeed a potential candidate for heart transplantation, the patient is put on the active waiting list. While on the waiting list, patients are followed up regularly and their treatment is optimised.

On acceptance of a donor call, a recipient is chosen and contacted as soon as possible. The recipient is admitted to the transplant ward. History, clinical examination, and routine pre-operative investigations are performed to identify any recent changes in the recipient condition.

Table 4.2: Pre-transplant screening test for heart transplantation candidates

- Chest x-ray and 12-lead electrocardiogram.
- Left ventricular (LV) angiography with LV pressure measurements.
- Nuclear and echocardiographic assessment of LV function.
- Right heart catheterisation
- Pulmonary function and cardio-pulmonary exercise tests.
- Selective coronary angiography in those with angina as the main problem.
- Serum urea, electrolytes and creatinine clearance.
- Liver and thyroid function tests.
- Full blood count and white blood cell differential count.
- Viral antibodies (including, HIV, hepatitis, and CMV).
- Immunocompatibilty tests.

The heart transplant operation

The timing of operation in the recipient is carefully co-ordinated with that of the donor. During the donor heart operation, one litre of St. Thomas cardioplegia

solution is infused at 4 ^{o}C under pressure via an aortic cannula. After excision, the donor heart is packed within a plastic bag containing cold saline and placed in a box packed with ice for transport.

Anaesthesia and cardio-pulmonary bypass: Once suitability of the donor heart has been confirmed, the patient is moved to theatre about one hour before the estimated time of the donor heart arrival. A standard anaesthetic regimen similar to that for conventional heart surgery is followed. The techniques and dynamics of cardio-pulmonary bypass procedure are also very similar to conventional heart surgery with 2 notable differences. The first is that systemic cooling is maintained at 25 ^{o}C during transplantation instead of the usual 28 to 30 ^{o}C in conventional heart surgery. The second is that heart transplant recipients are given one litre of warm reperfusion solution just before removing the aortic cross clamp. The perfusate solution contains glucose, citric acid, buffered amino acids and potassium, and it is administered mixed with warm oxygenated blood through the bypass circuit over at least 30 minutes.

Operative technique and immediate postoperative management: The technical details of heart transplant surgery is beyond the scope of this work. The following is a brief outline of the standard heart transplantation procedure as abstracted from the unit protocols. The recipient heart is exposed by a standard median sternotomy and longitudinal excision of the pericardium taking care not to open the pleural spaces. After heparinisation, CPB is established by cannulation of the ascending aorta and the venae cavae. Once the donor heart has arrived in theatre, CPB is

started with cooling to 25 ^{O}C. The aorta is cross-clamped and a left ventricular vent is inserted and a standard cardiectomy is performed.

After appropriate preparation, the donor heart is anastomosed to the remaining cuffs of the left atrium, right atrium, aorta and the pulmonary artery in that order. At this stage a left ventricular vent is inserted and the cardioplegia solution is infused into the ascending aorta immediately proximal to the cross clamp with simultaneous de-airing. The left ventricular vent is turned on and the heart is gradually allowed to become reperfused without being distended. Temporary epicardial pacing wires are attached to the right atrium and ventricle. When normothermia and at least 30 minutes of reperfusion have been attained, the patient is slowly weaned from the bypass, maintaining haemodynamic stability by the use of isoprenaline, glyceryl trinitrate, and low dose dopamine infusions with or without adrenaline. The heart is then decannulated, and protamine is administered. Haemostasis is then secured, pericardial and mediastinal drains are inserted and the sternal wound is closed. The patient is then transferred to the intensive care unit.

The intensive care management of heart transplant recipients is virtually identical to that of patients undergoing conventional open heart surgery. The aim is to monitor and maintain haemodynamic stability, prevent infection and to wean from the ventilator as quickly as possible. In general, patients are weaned from the ventilator within 24 to 48 hours. When stable the patients are transferred to the transplant ward where they are nursed and monitored in single cubicles.

Follow up

After discharge, patients are routinely followed up at the outpatient clinic according to a specific protocol. During each outpatient attendance, all patients have clinical assessment, chest x-rays, and blood taken for full blood count, urea and electrolytes, liver function tests, and cyclosporin levels. Other investigations are performed as indicated in the follow up protocol. Table 3 shows the outpatients timetable and lists the routine tests at each visit.

Table 4. 3: Outpatients follow-up timetable and routine investigation

Time	Clinic	E.M.B*	ECG & cardiac scan*	PFTs	CPX*
2 - 4 weeks	twice a week	weekly			
4 - 6 weeks	weekly	fortnightly	at 4 weeks	at 6 weeks	
6 - 12 weeks	fortnightly	fortnightly	at 12 weeks	at 12 weeks	
3 - 6 months	monthly	monthly	at 6 months	at 6 months	at 6 months
6 -12 months	3 monthly	3 monthly	at 12 month	at 12 month	at 12 months
1- 2 years	6 monthly		6 monthly	6 monthly	6 monthly
> 2 years	yearly		yearly	yearly	yearly

* E.M.B = endomyocardial biopsy, resting left and right ventricular ejection fractions were determined using Technitium ventriculogarphy and CPX = cardio-pulmonary exercise

Immunosuppression

Immunosupression is maintained using triple therapy with steriods, cyclosporin A, and azathioprine. Methylprednisolone (500mg intravenously) is routinely given with induction anaesthesia and again when on bypass, and additional 3 doses of 125 mg are given 8 hourly after the operation. Thereafter, 2.5 $mg.kg^{-1}$ of oral prednisolone is given twice daily until reduced to 0.2 $mg.kg^{-1}$ daily on day 14 post-transplantation. Further reductions in steroids are considered at 3 months after transplantation on an individual basis.

The first dose of cyclosporin is given pre-operatively (3 $mg.kg^{-1}$). Post-operatively, cyclosporin is given orally in a dose adjusted to maintain the whole blood trough level within a specific target range. The initial target level is 400 to 600 $mcg.L^{-1}$ reduced to 400 $mcg.L^{-1}$ at 1 month, 350 $mcg.L^{-1}$ at 3 month, 300 $mcg.L^{-1}$ at 6 months, 250 $mcg.L^{-1}$ at 1 year, and 150 to 200 $mcg.L^{-1}$ at 2 years. Azathioprine is given in a dose of 3 mg/kg on induction, followed by 2 mg/kg daily after the operation reducing to 1 to 1.5 $mg.kg^{-1}$ daily.

Cardiac allograft rejection and its management

Surveillance endomyocardial biopsies are routinely performed in all heart transplantation patients according to the clinical protocol. Allograft rejection was diagnosed on histological grounds and is graded from 0 to 4 with grade 0 being no rejection, and grades 1, 2, 3, and 4 denoting mild, focal moderate, diffuse moderate, and severe rejection respectively. Grades 2 to 4 are routinely treated with augmented immunosuppression. Augmented immunosuppression in stable

patients consists of a standard high dose steroid taper. Severe and resistant rejection require admission and may require treatment with intravenous OKT3 (Muronab CD3, Murine-derived monoclonal antibodies directed against the CD3 antigen of human T lymphocytes).

Infection and its management

The following antimicrobial agents are routinely given to all heart transplant recipients:

1. Cefuroxime; 1.5 g during induction anaesthesia and 8 hourly after the operation for 3 doses.
2. Acyclovir; 200mcg orally 3 times daily for the first 6 weeks after transplantation as a prophylaxis for herpes simplex virus.
3. Septrin; 960 mg twice a week continued indefinitely as prophylaxis against pneumocystis pneumonii and toxoplasma gondii.

CMV-negative recipients who receive hearts from CMV positive donors receive CMV prophylaxis with ganciclovir. This is given by intravenous infusion in a dose of 5 $mg.kg^{-1}$ twice daily for the first post-operative 2 weeks, reduced to 5 $mg.kg^{-1}$ once daily for 4 subsequent weeks.

Patients with symptoms or signs of infection (e.g. pyrexia) undergo standard screening tests for infection. These include a chest x-ray, specimens of blood and urine and a throat swab. Specialised tests such as broncho-alveolar lavage and CT scan are also used when indicated. The aim is to identify the causative organism before starting any anti-microbial therapy.

4.3.2 Control Groups

A. Normal subjects

Normal controls were recruited from the medical and laboratory staff of Glasgow Royal Infirmary who volunteered to participate in the study.

Inclusion criteria - The inclusion criteria in this group were:

1. Age less than 65 years
2. No history of cardio-pulmonary disease
3. Normal spirometry and lung volumes

B. Patients undergoing coronary artery bypass grafting (CABG)

This group was studied to control for the possible effects of sternotomy and the cardio-pulmonary bypass procedure.

Inclusion criteria - The inclusion criteria for this group were:

1. Age less than 65 years
2. No history of primary lung disease
3. No symptoms or clinical signs of heart failure
4. No previous cardiac surgery
5. No co-existing valvular heart disease

Anaesthesia and CPB management: A standard anaesthetic regimen was followed. Anaesthesia was induced with midazolam, fentanyl and propofol, and intubation was performed after administration of a muscle relaxant (pancuronium or Vencuronium). Anaesthesia was maintained with propofol, fentanyl, and the muscle relaxant. CPB was performed with roller pump, membrane oxygenator, and arterial line filter. The CPB circuit was primed with 2 litres of Hartmann's solution and

systemic hypothermia was maintained at 28 C. Myocardial protection was achieved using cold potassium cardioplegia (St. Thomas Solution), and topical cooling with iced saline.

Surgical technique and post-operative management: The heart was exposed by standard median sternotomy. The left internal mammary artery was used to construct one of the coronary artery bypass grafts in all patients with the saphenous vein being used for the remainder of the grafts. Post-operatively, all patients were transferred to the intensive care unit paralysed and under controlled mandatory ventilation. Patients were extubated when haemodynamically stable, usually within 10 to 12 hours after the operation.

C. Patients undergoing mitral valve surgery

This group was included to control for the effects of pulmonary congestion on TL_{CO} and its components.

Inclusion criteria - The inclusion criteria for this group were:

1. Age less than 65 years
2. No history of primary lung disease
3. No co-existing coronary artery disease

Anaesthesia, CPB procedure, exposure of the heart, and the post operative management of mitral valve replacement patients were similar to that of CABG. The mitral valve was exposed through an incision in the left atrium.

4.4 Study Protocol

4.4.1 Heart transplant patients

A. Pulmonary function Tests

Before the start of this study in October 1993, most heart transplant patients had resting pulmonary function tests and cardio-pulmonary exercise as part of their routine assessment for possible heart transplantation. From October 1993, all transplant patients performed pulmonary function and cardio-pulmonary exercise tests according to a protocol specifically designed for the purpose of this study. Pulmonary function tests were performed during the assessment for heart transplantation and serially after transplantation. In order to include a maximum number of heart transplant patients during the period of study and to evaluate both the short-term and long term changes in TL_{CO} following heart transplantation, patients were divided into 2 groups based on the interval between heart transplantation and the first post-transplant pulmonary function assessment. The early assessment group included all patients who were transplanted since the start of the study (October 1993), and in these, resting pulmonary function assessment was carried out at 6 weeks, 3 month and 6 months after transplantation, and at 6 monthly intervals, thereafter, until 2 years after transplantation. Patients who were transplanted before October 1993, were previously assessed at 12 monthly intervals and this was changed so that they had their first assessment at 12 months after transplantation, and at 6 monthly intervals thereafter up to 2 years after transplantation. Beyond 2 years after transplantation, pulmonary function

assessment was performed at yearly intervals for all patients until the end of the study period (March 1996).

For the purposes of validation and reproducibility studies, a random sample of heart transplant patients performed repeat measurements of TL_{CO} and its components either on the same day or at the same time on the next day. In addition, pulmonary function tests including TL_{CO} were repeated in patients awaiting heart transplantation for more than one year after their first assessment. The purpose of this was to assess the changes in lung function while on the waiting list for transplantation.

The appointments for pulmonary function assessment were co-ordinated with the transplant co-ordinator and the transplant clinic nurse on weekly basis. The tests were performed during patients' routine visit for the out-patient follow-up. The tests were performed in patients who were stable, ambulatory and who had not suffered from any recent respiratory symptoms. Patients who were admitted for treatment of rejection or systemic infection were not tested until at least 2 weeks after recovery.

During each assessment the following measurements were performed:

Lung volumes and forced ventilatory flows: These were performed first during each visit and included forced vital capacity (FVC), forced expired volume in the first second (FEV_1), residual volume (RV) and total lung capacity (TLC).

TL_{CO} and its components: The TL_{CO} measurement was performed after spirometry and lung volume measurement. From August 1994, the measurement of

TL_{CO} components (D_M and V_C) was introduced as part of the assessment of the pulmonary diffusion characteristics to determine the contribution of each component to the observed decline in TL_{CO} following transplantation.

B. Cardio-pulmonary exercise:

Cardio-pulmonary exercise tests were performed before transplantation, and at 6 to 12 months after transplantation.

C. Broncho-alveolar Lavage (BAL) Study

This study was undertaken to determine the role of sub-clinical pulmonary CMV infection in the observed decline of TLco following heart transplantation. The study was conducted during the period between July 1994 and January 1996, and involved serial bronchoscopy with BAL in all consenting heart transplant recipients during the first 3 months following transplantation. The procedure was discussed with patients towards the end of the first week after transplantation and if they agreed to participate the procedure was performed at 2, 6 and 12 weeks after transplantation. The protocol was approved by the Ethics Committee of the hospital and all patients gave consent before each test. The contra-indications to the test in these patients were acute rejection requiring admission for treatment, haemodynamic instability, and significant arrhythmias. Specimens obtained from BAL along with blood, urine and throat swabs were sent to the Regional Virology Laboratory at Ruchill Hospital for tests of CMV activity. In addition, one BAL specimen was sent the Department of Cytology in the Royal Infirmary for cytological evidence of CMV infection.

D. Clinical and laboratory data

The case notes of all heart transplant patients were regularly reviewed and the relevant data were retrospectively recorded in specifically prepared data sheets.

1. Clinical data: This included the patient characteristics, cardiac diagnosis, primary indication for transplantation, co-existing diagnoses, medications and pre-operative haemodynamic data. It also included CXR findings, haemoglobin and functional status at the time of pulmonary function assessment. Smoking status was based on history obtained during the first pulmonary function assessment and patients were categorised into current smokers, smokers who had stopped smoking (ex-smokers), and those who never smoked (non-smokers). Ex-smokers were defined as those who had stopped smoking at least one year before the first pulmonary function assessment. The number of pack-years of smoking (average daily consumption of cigarettes multiplied by the number of years smoked) for current smokers and ex-smokers, and the length of time since stopped smoking for ex-smokers were recorded.

2. Peri-operative data: Peri-operative events that might interfere with lung function were also recorded. These included the duration of surgery, cardio-pulmonary bypass, mechanical ventilation, and the donor heart total ischaemic time.

3. Post-operative pulmonary complications: These included any documented lower respiratory tract complication occurring within the first 6 weeks after transplantation. Analysis was confined to this period because most of pulmonary complications after transplantation occur within this interval (42). In addition, and

as detailed in chapter 3, TL_{CO} decline was evident on the first assessment at 6 weeks after transplantation. For each episode, the interval after transplantation, diagnosis, method of diagnosis, treatment, and the outcome were recorded.

4. Endomyocardial biopsy data: The results of all endomyocardial biopsies were reviewed and the grade for each biopsy and its interval were recorded up to one year after transplantation. An episode of rejection was defined as grade 2 or greater.

5. Cyclosporin levels: These included all routine cyclosporin levels up to one year after transplantation. For each measurement, the time from transplantation and whole blood trough levels were recorded.

6. Cytomegalovirus data: In addition to the Broncho-alveolar Lavage Study, data from surveillance specimens for CMV infection during the first year after transplantation were collected for all patients. For each episode of CMV infection, the interval, presentation, affected organs, and treatment if required were recorded. The pre-operative CMV status of both the donor and the recipients were also recorded.

4.4.2 Control groups

A. Normal subjects

All normal subjects performed one base-line measurement of lung function including TL_{CO} and its sub-divisions and were asked to repeat the measurements at variable intervals from the base-line.

B. Coronary artery bypass and mitral valve surgery

Coronary artery bypass graft and mitral valve replacement patients were recruited from the weekly theatre list. They were interviewed on the day before the operation and if the inclusion criteria were satisfied and the patients agreed to participate in the study, resting lung function tests, including TL_{CO} and its sub-divisions, were performed on that day and at 6 weeks after the operation (at the routine post-operative follow-up clinic). In a small number of these patients additional measurements were made at one week and 3 months after the operation.

4.5 Data handling and analysis

All data were sampled and analysed using the SPSS statistical package (SPSS for Windows, Release 6.0, SPSS Inc. USA). Details of the different statistical methods used are presented in the respective chapters. The following statistical methods were applied throughout the process of analysis as appropriate:

1. **Checks for normality:** All relevant data were checked for normality of distribution before applying any statistical test based on this assumption. Unless otherwise stated, results were presented as mean +/- standard error of the mean (Mean +/- SEM).
2. **Comparison between means:** For normally distributed data, a two independent-sample (unpaired) Student's t-test was used for comparison between 2 means for separate groups and a paired Student's t-test was used for comparison between 2 means from the same group (e.g. TL_{CO} before and after heart transplantation). When there were more than 2 means from separate groups, comparisons were made using the one way analysis of variance.

3. **Assessment of the relationship between various factors:** The relationship between 2 or more variables was assessed using the Pearson's correlation coefficient and stepwise multiple linear regression analysis.

4. **Statistical significance:** A level of $p < 0.05$ was considered significant.

CHAPTER 5

LUNG FUNCTION - MATERIALS AND METHODS

5.1 TL_{CO} and its components

5.1.1 Equipment and quality control

Between January 1992 and August 1994, TL_{CO} measurements were performed using a standard single breath transfer test system (Transfer Test, Model B, P.K. Morgan, Kent, England). From August 1994 onwards, measurements were made using new transfer test equipment (Transflow Test, Model 540, P.K. Morgan, Kent, England). The calculations used to determine TL_{CO}, K_{CO}, and alveolar volume are identical in both systems. In the Transflow model, volume measurements, gas analysis, and all calculations were performed automatically using a software system which conforms to the recommendation of the British Thoracic Society and the Association of Respiratory Technicians and Physiologists (87). In addition, the Transflow model has facilities to estimate the individual components of TL_{CO} using the classic Roughton and Forster equation (5). Before changing to the Transflow model, the concordance between the 2 systems was assessed in a group of patients with various cardio-pulmonary disorders (chapter 6).

The Transflow model consists of the main transfer test instruments, video display unit, computer keyboard, and pure oxygen administration kit comprising a 6-litre anaesthetic bag, controlling tab and connecting tubing. The main transfer test

instruments consist of the subject breathing valve, a pneumotachograph, gas analysers, absorption chamber (soda lime for CO_2 absorption, and calcium chloride as a desiccant), and metalised bags for inspiratory and expiratory gas samples. The breathing valve includes a mouthpiece port for connecting the subject and 4 additional ports. One of these ports is controlled manually by a ring-sealed plug and it is kept occluded during the transfer test. The remaining 3 ports are controlled by pneumatic valves which allow rapid switching of the breathing gas to the appropriate port. One of these ports opens to room air (or the pure oxygen administration set in the case of high oxygen measurement). Of the remaining two ports one opens to the bag of inspired gas mixture and the other opens to the bag which collects the post-dead space alveolar gas sample. The dead space of the breathing valve is 170 ml and this includes 30 ml due to the dead space of the disposable filter fitted to the mouthpiece for each subject. Gas analysis was established by an infra-red gas analyser for CO, a thermal conductivity meter for helium and a polarographic analyser for oxygen.

Gas analysers were calibrated before each measurement with 2 certified gas mixtures (BOC Special Gases) by an automatic calibration routine. The 2 gas mixtures correspond to the actual mixtures used during the TL_{CO} measurements. The pneumotachograph was calibrated daily with a 3-litre syringe stroked 10 times at variable emptying velocities (from 15 $L.s^{-1}$ to 0.1 $L.s^{-1}$). Verification of the volume calibration was performed using the same 3-litre syringe. In addition, TL_{CO}, K_{CO} and the alveolar volume were routinely performed on alternate days by one of

the laboratory staff to ensure the integrity of the system and the repeatability of measurements.

5.1.2 Procedure

A. Standard TL_{CO} measurement:

Before each test was performed, each subject was carefully instructed in all the required manoeuvres. The following points were emphasised:

- Rapid inhalation to total lung capacity
- Breath holding at full inspiration without straining
- Rapid exhalation without interruption

After preparing the system, the subject wearing a nose clip was connected to breathing valve with a mouthpiece fitted with a filter (Spiroguard, Air Safety, Ltd.). After a few tidal breaths, the subject was instructed to breathe out as fully as possible, and at full expiration, the subject was connected to the test gas mixture and asked to breathe in as fully as possible. He was then encouraged to hold this breath for about 10 seconds, without straining, after which he was asked to breathe out rapidly without interruption. The contents of the test gas were as recommended by the European Respiratory Society, and consisted of CO = 0.28 %, helium (He) = 14 %, O_2 = 18 % with the remainder nitrogen (41). After a pre-set wash out volume of 750 ml, the expired air was directed to the expired gas bag with a sample collection time of 1.5 seconds. On completing the expiration, the subject was disconnected from the breathing valve and gas analysis started automatically followed by a display of the test results which were then inspected for acceptability.

The test was then repeated after a 5 minute interval. The criteria for acceptable measurements were:

1. Inspiration should be rapid and the inspired volume should be at least 90 % of the previously measured vital capacity (obtained during ventilatory flow measurements)
2. Breath holding should be maintained at full inspiration, and the final breath-hold time should be between 9 and 12 seconds
3. The values of TL_{CO} and K_{CO} in 2 technically acceptable measurements should be within 10 % of each other

The means of the 2 technically acceptable and repeatable TL_{CO} and K_{CO} values were reported as the subject's TL_{CO} and K_{CO}.

B. Measurement of TL_{CO} components

Duplicate measurements of single breath TL_{CO} were made using 2 different test gas concentrations. The low oxygen gas mixture was that used for standard TL_{CO} measurement described above. The high oxygen gas mixture consisted of CO = 0.28 %, He = 14 %, and O2 = 85.72 %. The sequence of measurements was in the following order:

TL_{CO} at low oxygen concentration was measured in duplicate as previously described. The subject was then allowed 5 minutes of room air breathing followed by another 5 minutes of pure oxygen breathing while wearing a nose clip. The single breath TL_{CO} at high oxygen concentration was then measured using the same steps described for the standard TL_{CO} measurement, except for the use of the high oxygen mixture in the inspired gas mixture. The steps of room air breathing and pure oxygen breathing were repeated and a second high oxygen TL_{CO} measurement

was made. Thus, the interval between the sets of low and high oxygen measurements and between each of the high oxygen measurements was at least 10 minutes.

5.1.3 Calculations

A. TL_{CO} calculations:

The single breath TL_{CO} was calculated using the basic Krogh's equation (50) as described by Cotes (2) and recommended by the European Respiratory Society (41). The tension of CO in the pulmonary blood was assumed to be zero.

$$\mathbf{TL_{CO}\ (mmol.min^{-1}.kPa^{-1}) = V_{A,\ BTPS} \times b/t \times \log_{10} (F_{ACO0} / F_{ACOt})}$$

where:

- $V_{A,\ BTPS}$ is the effective alveolar volume corrected to body temperature and pressure saturated with water vapour,
- **b** is a composite factor with a value of 53.6 reflecting the conversion from natural to decimal logarithm, from seconds to minutes, from millilitres to millimoles and from millilitres STPD to litres BTPS,
- **t** is the effective breath holding time in ~~minutes~~ seconds, and
- F_{ACO0} and F_{ACOt} are the fractional alveolar concentration of CO, at the start and the end respectively of the breath holding period.

It is assumed that helium (He) and CO are uniformly distributed throughout the lungs so that the initial alveolar concentration of CO (F_{ACO0}) can be calculated from the following relationship:

$$F_{A,CO0} = F_{I,CO} \times (F_{EHe}/F_{IHe})$$

where:

- F_{ICO} is the known inspired concentration of CO and

- F_{EHe} and F_{IHe} are the known fractional concentration of helium in the expired and inspired gas samples respectively.

The effective alveolar volume (V_A) is calculated as follows:

$$V_A = K \times (V_I - V_{Dan} - V_{ds}) \times (F_{IHe} / F_{EHe})$$

where:

- V_I is the inspired volume at BTPS,
- **K** is a constant to correct for the change in the concentration of expired helium due to absorption of carbon dioxide before analysis with a value of 1.05,
- V_{Dan} is the anatomical dead space (2.2 × body weight in kilograms) and
- V_{ds} is the instrument dead space.

The transfer coefficient (K_{CO}) was calculated from TL_{CO} and V_A

$$K_{CO} \text{ (mmol.min}^{-1}\text{.kPa}^{-1}\text{.L}^{-1}) = TL_{CO}/V_A$$

B. Calculation of TL_{CO} components:

The means of each of the duplicate values of TL_{CO} with their corresponding θ values obtained at low and high alveolar oxygen tensions, were used to derive the diffusing capacity of the alveolar-capillary membrane (D_M) and the pulmonary capillary blood volume (V_C) using the classic Roughton and Forster equation (5);

$$1/TL_{CO} = 1/D_M + 1/\theta V_C$$

where:

- θV_C is the "diffusing capacity" of the total mass of the erythrocytes in the pulmonary capillary blood available for gas exchange at any instant.
- θ is the standard rate at which one ml of blood takes up the gas CO, and its value depends on the prevailing alveolar oxygen tension and the concentration of haemoglobin in blood (5,58).

The values of θ were derived from the original data of Roughton and Forster obtained from *in vitro* CO uptake in a suspension of human erythrocytes at 37°C (5). This parameter was first reported in ml of CO per minute per ml of blood at an average haemoglobin of 14.6 $g.dL^{-1}$. After correction for actual haemoglobin blood concentration and conversion to SI units, the values of θ at different alveolar oxygen partial pressures were obtained using the equation described by Cotes (2);

$$1/\theta = (\alpha + \beta \times P_{cO2}) / ([Hb] \times \{1 - S_{cCO}/100\})$$

The various terms and parameters appearing in this equation are described below:

1. α - is a constant reflecting the diffusion barrier of the erythrocyte and its value depends on the value chosen for the partition coefficient between the membrane of the red blood cell and its interior (λ). Roughton and Forster demonstrated *in vitro* that λ has a wide range of values with a range between 1.5 and infinity (5). However, most studies using the Roughton and Forster model assume the red cell membrane to have infinite permeability (λ = infinity), and at this value of λ, α = 1.0 min.kPa. ml of blood.$mmol.^{-1}$ of CO (2).
2. β - is a temperature dependent factor. At temperature of 37°C, β = 0.134 min.kPa. ml of blood.$mmol.^{-1}$ of CO.
3. P_{cO2} - is the mean capillary O_2 tension assumed to be 0.67 kPa (5 mmHg) less than the alveolar oxygen tension (P_{AO2}) (5). P_{AO2} was derived by analysis of post-dead space alveolar sample collected after the breath hold period; $P_{AO2} = F_{EO2} \times (P_B - P_{H2O})$, where F_{EO2} is the fractional expired O_2 concentration, P_B is the atmospheric barometric pressure, and P_{H2O} is the water vapour pressure.
4. **[Hb]** - is the haemoglobin concentration as a fraction of normal (the standard normal haemoglobin was defined as 14.6 $g.dL^{-1}$). To obtain conventional TL_{CO} values, not corrected for haemoglobin, it was assumed that all subjects had normal haemoglobin concentration (14.6 $g.dL^{-1}$). Where indicated, the effect of haemoglobin variability on TL_{CO} values was determined using a version of the

classic Roughton and Forster equation as described by Cotes and recommended by both the European Respiratory Society and the American Thoracic Society (41,88);

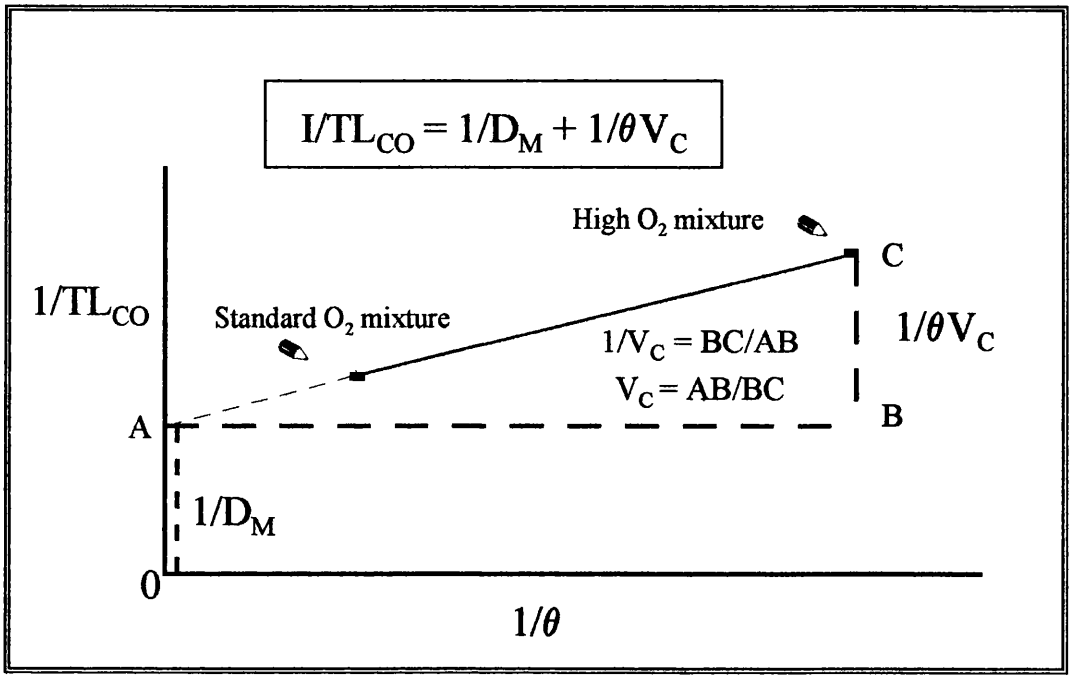
$$1/TL_{CO} = 1/D_M + 1/([Hb] \times \theta V_C)$$

where [Hb] is the haemoglobin concentration of the subject as a fraction of normal (i.e. actual haemoglobin divided by 14.6).

5. S_{cCO} - is the mean percentage saturation of haemoglobin with CO (assumed to be 1.5 %) (2).

The process described for TL_{CO} measurement at high and low oxygen concentrations yields duplicate values for TL_{CO} at each of the oxygen concentrations. The mean of each of these duplicate values with the means of their corresponding θ values were used for the determination of D_M and V_C by solving the Roughton and Forster equation graphically (Figure 5.1). A plot of $1/TL_{CO}$ against $1/\theta$ yields a straight line which intersects the ordinate $1/ TL_{CO}$ at point A. At this point, the value of $1/\theta$ equals zero and therefore the value of $1/ TL_{CO}$ at point A equals $1/D_M$. The triangular area above the intersection represents a plot of $1/\theta V_C$ against $1/\theta$. V_C can therefore be obtained by dividing $1/\theta V_C$ by $1/\theta$ (i.e., $1/V_C$ = BC/AB), which is the slope of the line AC. In short, the intersect of the plotted line with $1/TL_{CO}$ ordinate equals $1/D_M$ and its slope equals $1/V_C$.

Figure 5.1 The graphical derivation of TL_{CO} components (D_M and V_C) using the Ruoghton and Forster method



5.1.4 Normal values

Results of the diffusion parameters were expressed in absolute values and as percentage of predicted. Predicted normal values for TL_{CO} and K_{CO} were determined using the European Community for Steel and Coal equations (ECSC) which were recommended by the European Respiratory Society (41) and endorsed by both the British Thoracic Society (BTS) and the Association of Respiratory Technicians and Physiologists (87). The predicted values of D_M and V_C were taken from Cotes (49). The lower limit of normal was defined by the lower 5^{th} percentile of the reference population. Mathematically, this was obtained as follows:

Lower limit of normal = Predicted value - 1.64 × RSD, where RSD is the residual standard deviation of the prediction equation and 1.64 RSD represents the limit at 90 % confidence interval (41,87).

5.2 Static and dynamic lung volumes

5.2.1 Equipment and procedures

Standard spirometry and lung volumes were measured using a body plethysmograph (PK. Morgan Ltd, Kent, UK). Measured variables included vital capacity (VC), forced vital capacity (FVC), force expiratory volume in one second (FEV_1), FEV_1/FVC ratio, residual volume (RV), and total lung capacity (TLC). Quality control and procedures of testing were performed according to formal guidelines established by the European Respiratory Society (ERS) (169) and recommended by the British Thoracic Society (BTS) and the Association of Respiratory Technicians and Physiologists (87). Results of at least 3 satisfactory

manoeuvres were analysed and the reported values were the highest value for FEV_1 and FVC and the mean of the 3 results for each of the remaining indices.

5.2.2 Calculations and normal values

Static and dynamic lung volumes were compared with normal predicted values of ECSC which were recommended by the ERS and BTS (87,169), and results were expressed as a percentage of predicted. The lower limit of normal for each index of lung function was defined as described for TL_{CO} (87).

5.3 Cardio-pulmonary exercise

5.3.1 Equipment

Symptom-limited exercise tests were performed using an electrically braked bicycle ergometer with the patient breathing through a low dead space, low resistance valve box. The valve box incorporates a flexible pneumotachograph on the inspired limb for the measurement of inspired minute ventilation (Flexiflow, PK Morgan Ltd, Kent, UK). The expired limb is fed through a mixing chamber from which samples of expired air are analysed for the fractional concentrations of carbon dioxide and oxygen by an infra red spectrometer and zirconium cell analyser, respectively (Benchmark System, PK Morgan, Kent, UK). Gas analysers were calibrated with certified gas mixtures, and the pneumatograph system was calibrated and verified using a 3-litre calibration syringe before each exercise test.

Arterial blood gas values were monitored throughout exercise testing using a transcutaneous system (TCM3, Radiometer Ltd, Copenhagen) heated to 45^{o}C with the electrode attached to the flexor aspect of the forearm (170). The use of the

transcutaneous system during exercise in patients with dyspnoea due to various cardio-pulmonary disorders has been previously validated in our laboratory (171,172).

5.3.2 Procedure

Before each test, subjects were seated in a comfortable chair and a brief history was taken aimed at identifying any recent respiratory illnesses or cardiac decompensation and estimating the functional status at the time of assessment. After explaining the procedure, a transcutaneous electrode was attached to the flexor surface of the forearm and standard continuous 12-lead electrocardiogram monitoring was started. Following a period of an *in-vivo* calibration using a sample of arterial or arterialised ear lobe capillary blood, subjects were initially monitored for 2 minutes at rest whilst seated on the bicycle ergometer with a nose clip in place. They were then instructed to cycle with no additional load for 2 minutes. Then the work-load was automatically increased by increments of 25 watts every 2 minutes until a symptom limited-maximum and the primary symptom limiting exercise was recorded. Blood pressure was measured using standard cuff sphygmomanometer at the end of each stage. In addition, Borg scale scores were recorded for both dyspnoea and leg fatigue. The criteria for terminating the exercise before patients reached a maximum symptom-limited point were: ischaemic changes on ECG, ventricular arrhythmia, systemic hypotension (resting systolic BP $<$ 90 mm Hg or falling BP during exercise), or severe systemic hypertension (systolic BP $>$ 220 mm Hg).

5.3.3 Calculations and normal values

Throughout each test, minute ventilation (V_E), oxygen consumption (VO_2), and carbon dioxide production (VCO_2) were measured by on line ventilation and expired gas analysis (PK Morgan Ltd, Kent, UK) using standard equations (173).

The ventilatory anaerobic threshold on exertion was calculated by the curve fitting method, using a plot of oxygen consumption against carbon dioxide production (174).

The transcutaneous values of oxygen ($tcPO_2$) and carbon dioxide ($tcPCO_2$) tension were used to calculate alveolar-arterial oxygen gradient ($P_{(A-a)}O_2$) and dead space to tidal volume ratio (V_D/V_T) using standard equations (173). The following cardio-respiratory responses to exercise were also derived (175):

Maximum voluntary ventilation (MVV) = $37 \times FEV_1$.

Ventilatory response at maximum exercise = the change in minute ventilation (ΔV_E) divided by the change in carbon dioxide output (ΔV_{CO2}).

Heart rate response ($beats.L^{-1}$) = the change in heart rate (ΔHR) divided by the change in oxygen uptake in litres (ΔV_{O2}).

Oxygen pulse ($ml.beats^{-1}$) at maximum exercise = V_{O2} in millilitres at maximum exercise divided by the maximal heart rate.

Maximal exercise values were compared to the predicted normal values of Jones and Campbell (175).

5.4 Broncho-alveolar lavage (BAL) study

5.4.1 Procedure

On the day of the BAL, consenting patients were asked to take a light breakfast no later than 8 o'clock in the morning, after which they take nothing orally until at least 3 hours after the procedure. They were admitted as day cases to the heart transplant ward. During admission a written consent was obtained and the required specimens were taken. These included:

- 10 ml of clotted venous blood
- 10 ml of heparinised venous blood
- A urine specimen in a universal container
- A throat swap placed in a standard viral transport medium

BAL was performed as part of the routine Tuesday afternoon bronchoscopy list starting at 13:30 hours with the study patients placed first on the list. The procedures of bronchoscopy and BAL were performed using standard techniques (176,177). During the procedure, all patients were given oxygen (2 L. min^{-1}), and oxygen saturation and heart rate were monitored using continuous pulse oximetry. Patients were pre-medicated with intravenous atropine (600 mcg) and diazepam (5-10 mg) with or without fentanyl (25-100 mcg) just before the procedure. Local anaesthesia was initiated using 4 % topical lignocaine spray applied to the nose and throat. Fibreoptic bronchoscopy was performed with an Olympus T10 bronchoscope. After lubricating its tip with lignocaine gel, the bronchoscope was inserted via the transnasal route. Additional local anaesthesia was obtained by instilling 2 ml aliquots of 4 % lignocaine through the bronchoscope channel on to

the vocal cords and the bronchial tree as necessary, keeping the total dose of lignocaine below 800 mg. BAL was performed by wedging the tip of the bronchoscope in a segment of the right middle lobe, and 5 to 6 30-ml aliquots of sterile warm normal saline were instilled. Each aliquot was immediately aspirated by gentle suction, and BAL was directly received into 3 sealed sterile universal containers (15 - 20 ml each) numbered sequentially. The first BAL aliquot was considered contaminated with lignocaine and was not used. and the remaining 2 aliquots were used for viral diagnostic tests of CMV activity. After the procedure, all patients were reviewed in the ward, and they were discharged when completely alert and had had a light meal.

5.4.2 Specimens and diagnostic tests

Specimen were sent to the virololgy laboratories within one hour of their collection and were processed in the same day.

1. Serology

CMV IgM and complement fixation tests were performed on 10 ml clotted blood.

2. Direct Early Antigen Fluorescent Foci Test (DEAFF)

DEAFF is used as a rapid test for detection of CMV in various types of clinical specimens. In conventional tissue cultures, the detection of virus depends on the identification of a specific cytopathic effect in the infected cell, and this takes about 6 to 8 weeks to produce a definitive result (178). DEAFF can detect CMV in shell-vial cultures before the development of the cytopathic effect (CPE) with sensitivity and specificity approaching that of the conventional CMV cultures (179). In this

test, immediate early proteins of CMV can be detected in the nuclei of MRC-5 cells by immunofluorescence within 24 to 48 hours of CMV infection, well before CPE becomes apparent. DEAFF was performed on BAL specimens, throat swab, urine, and buffy coat cells. Buffy coats (leukocyte suspensions) were prepared by gradient density centrifugation using 10 ml heparinised blood layered onto a polysucrose solution.

3. CMV cultures:

Conventional CMV cultures on human fibroblast cells were also routinely performed on BAL, throat swabs, and the buffy coats.

CHAPTER 6

VALIDATION AND REPRODUCIBILITY STUDIES

6.1 Introduction

In contrast to standard single breath TL_{CO} measurement, there are no specific guidelines for the measurement of its components (41,65). Although many aspects are covered by the guidelines for the standardisation of single breath TL_{CO}, several technical questions remain to be answered. These include the effect of CO back pressure at high alveolar oxygen tensions, the sequence of oxygen mixtures used and the time interval between repeated attempts of measurements at each of the inspired oxygen concentrations. As noted in chapter 2, repeated measurements of TL_{CO} can increase the level of carboxyhaemoglobin, but this is usually not significant especially if the number of TL_{CO} measurements is limited to a maximum of 5 per session (41,59). In heavy smokers, especially when using high inspired oxygen concentration, the effects of CO back pressure and carboxyhaemoglobin (CO-Hb) may be significant and a correction for these effects is recommended (41).

In order to minimise the effects of CO back pressure and CO-Hb during the measurement of TL_{CO} components, Cotes recommended starting with the high oxygen mixture and allowing at least 30 minutes between each measurement (2). Since at least 4 measurements (2 at each of the inspired oxygen mixtures) are required for reproducible results, a single test will take at least 2 hours. This recommendation is based on theoretical considerations and has not been evaluated

against shorter time intervals. In addition, investigations involving the measurement of TL_{CO} components rarely state the sequence of measurement steps or the time interval between repeat measurements. In the few studies which provide information, measurement was started with the standard oxygen mixture and the time interval between measurements was 5 to 10 minutes (32,70,93).

As a result of these uncertainties, it was necessary to perform some preliminary studies on the measurement of TL_{CO} components. The general purpose of these studies was to validate the methods, check the reproducibility of the results and compare results obtained in normal subjects with published predicted normal values.

6.2 Study 1: Validation of the Transflow model against the Transfer test model B

Between January 1992 and August 1994, TL_{CO} measurements were performed using a standard single breath transfer test system (Transfer Test, Model B, P.K. Morgan, Kent, England). From August 1994 onwards, measurements were made using new transfer test equipment (Transflow Test, Model 540, P.K. Morgan, Kent, England).

Aim:

To validate the use of the Transflow Test by comparing its performance with the Transfer Test, Model B.

Subjects and Methods:

In July 1994, 42 consecutive patients referred from respiratory and general medical clinics for pulmonary function tests were studied. The group consisted of patients with a range of cardio-pulmonary disorders of varying degrees of severity and included heart transplant patients.

TL_{CO} and K_{CO} values were obtained for each patient using the 2 systems in 2 separate sessions. The 2 sessions were allocated randomly and were separated by at least 30 minutes during which spirometry and lung volume measurement were performed. The technique of measurements and calculations were identical in both systems and were as described in chapter 5.

The degree of agreement between results obtained by the 2 systems was assessed using Bland and Altman statistical analysis (180).

Results:

Table 6.1 shows the results of TL_{CO} and K_{CO} using the 2 transfer test systems. Using Model B, mean TL_{CO} and K_{CO} were 7.54 and 1.43 $mmol.min.^{-1}kPa^{-1}$ respectively, with a wide range of values for each, reflecting the wide spectrum of patients. The corresponding values using the Transflow Model were almost identical. In addition, For each individual, the difference between the duplicate values of both TL_{CO} and K_{CO} were within 10 % of their mean.

Table 6.1: Summary statistics of TL_{CO} and K_{CO} using the 2 transfer test systems

		Mean	SD	SEM	Range
TL_{CO}	Model B	7.54	3.21	0.49	1.9 -14.7
	Transflow	7.49	3.17	0.49	1.7 -14. 0
K_{CO}	Model B	1.43	0.41	0.06	0.44 - 2.21
	Transflow	1.42	0.40	0.06	0.41 - 2.11

Figure 6.1 is a scatterplot of individual TL_{CO} values as measured by the 2 systems. TL_{CO} values obtained by the 2 systems were highly correlated ($r = 0.99$, $p<0.001$) with similar results for K_{CO} values (Figure 6.2).

Figure 6.1: Scatterplot of TL_{CO} values obtained by the 2 transfer test systems (Transflow Model and Transfer test, Model B)

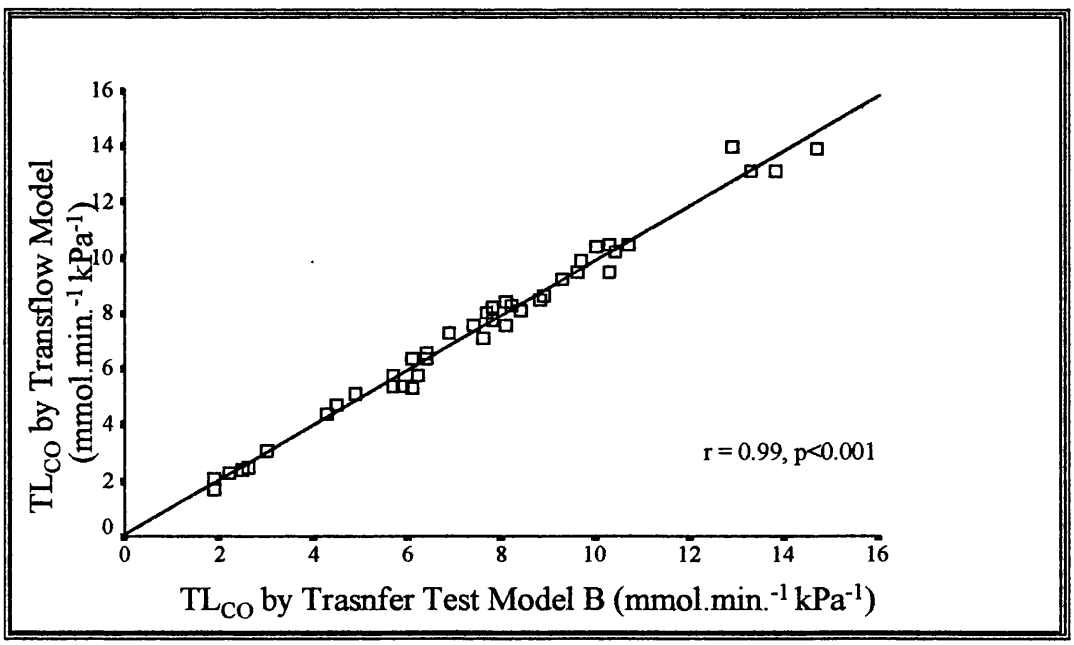


Figure 6.2: Scatterplot of K_{CO} values obtained by the 2 transfer test systems (Transflow Model and Transfer test, Model B)

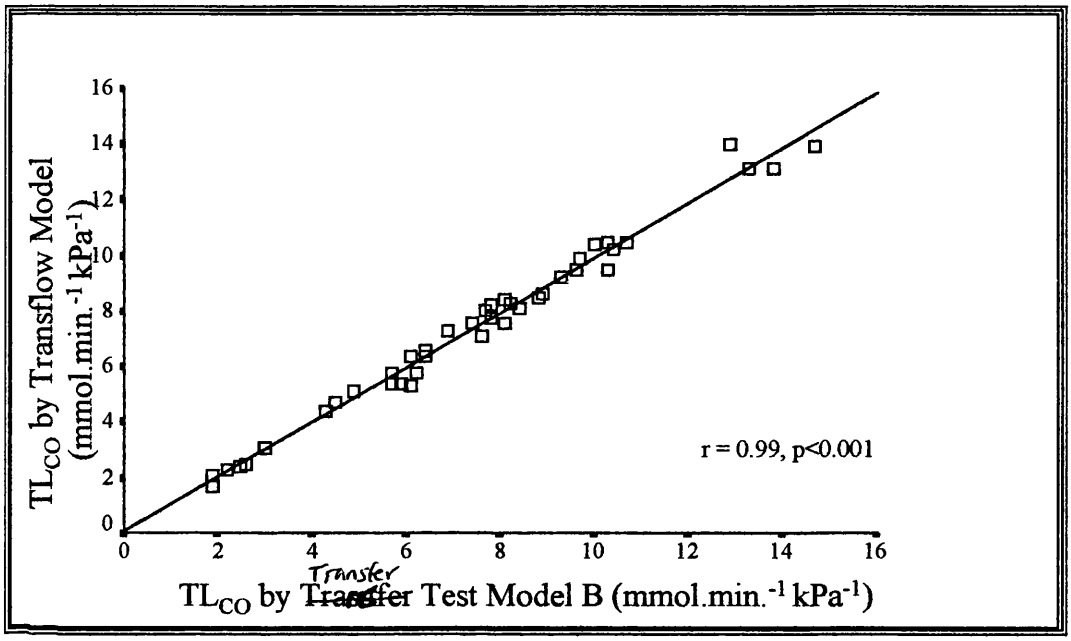


Figure 6.3 is a plot of the difference between the TL_{CO} results obtained by the 2 systems against their mean. There was a excellent agreement between the values obtained by the 2 systems. The mean difference was 0.05 mmol.min.$^{-1}$kPa^{-1} with very small limits of agreement (- 0.03 and 0.13). For K_{CO} (figure 6.4), the mean difference was 0.01 mmol.min.$^{-1}$kPa^{-1}, and limits of agreement were 0.00 to 0.02.

Figure 6.3: Plot of the difference between TL_{CO} values obtained by the 2 transfer test systems against their mean

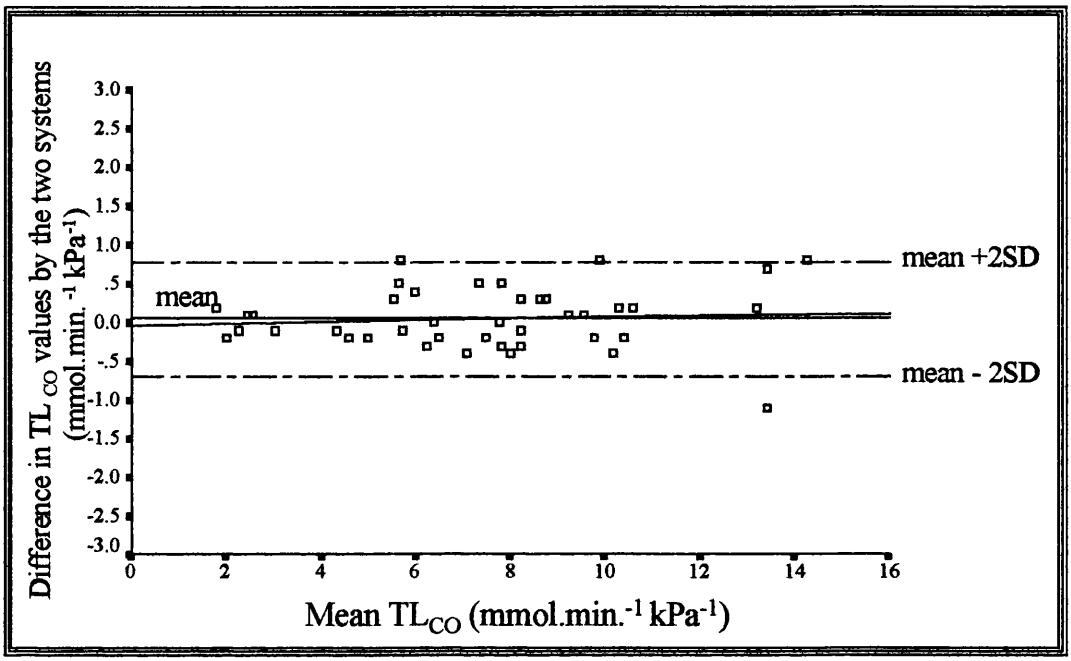
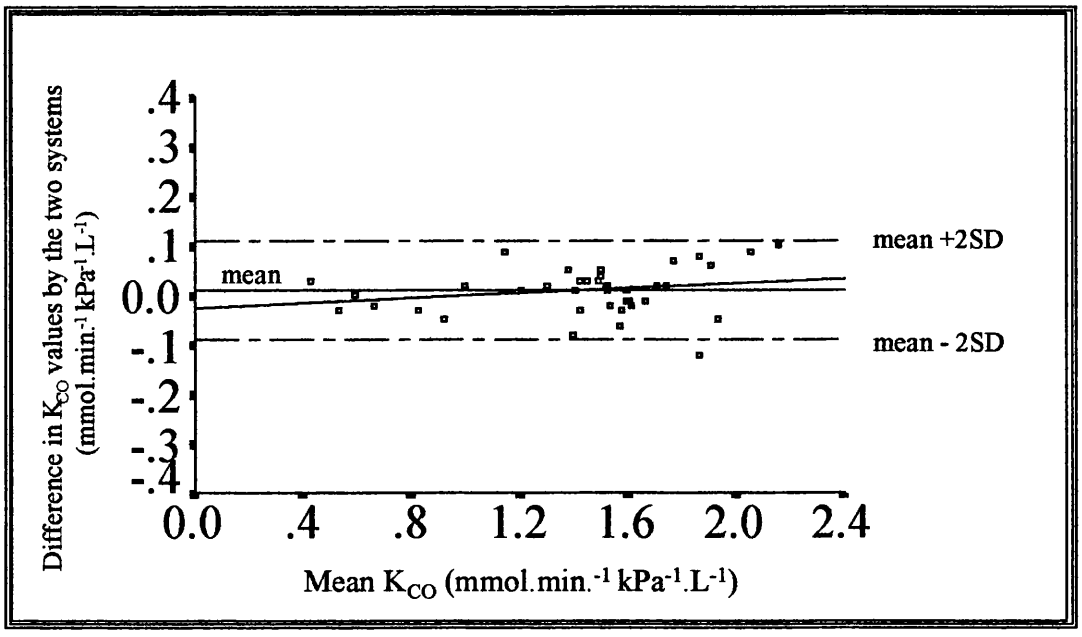


Figure 6.4: Plot of the difference between K_{CO} values obtained by the 2 transfer test systems against their mean



Comments

The results show good agreements between TL_{CO} and K_{CO} results obtained by the 2 systems across a wide range of values in both parameters. In addition, the difference between values obtained by the 2 systems in individual patients is comparable to the expected intra-individual variability of repeated measurements using the same system in one session (41). It can, therefore, be concluded that the Transflow system gives similar results to those obtained by the Transfer Test Model B.

6.3 Study 2: The sequence of inspired oxygen mixtures

As mentioned previously, the usual sequence of inspired oxygen mixtures used in the determination of TL_{CO} components starts with the standard oxygen mixture followed by a high oxygen mixture. However, the use of high inspired oxygen concentration shortly after exposure to CO in the standard oxygen mixture, can potentially increase both CO tension in the pulmonary capillaries and the concentration of carboxyhaemoglobin (CO-Hb) in blood. As both CO back pressure and CO-Hb are assumed to be negligible in the calculations of TL_{CO} and its components, a significant increase in either of them could result in underestimation of TL_{CO} (41,59). If this effect were significant, a protocol starting with standard oxygen mixture would consistently yield results lower than that of a protocol starting with the high oxygen mixture.

Aim

The aim of this study was to determine the effect of the sequence of inspired oxygen mixtures on the results of TL_{CO} and its components.

Subjects and Methods

Ten randomly selected heart transplant recipients were studied. All patients performed 2 sets of TL_{CO} and components determinations. Three patients were life-long non-smokers and 7 were ex-smokers (stopped smoking for more than one year). For each patient, the 2 sets of measurements were separated by at least one hour and were performed in random order. One set was started with the standard

inspired oxygen concentration and the other with the high oxygen concentration. Apart from this difference, the procedures and timing of the different stages of measurements were identical and were as described previously (chapter 5).

The degree of agreement between results obtained by the 2 protocols was assessed using Bland and Altman statistical analysis (180).

Results

Table 6.2 shows that the 2 sequences of measurements yielded results with virtually identical means.

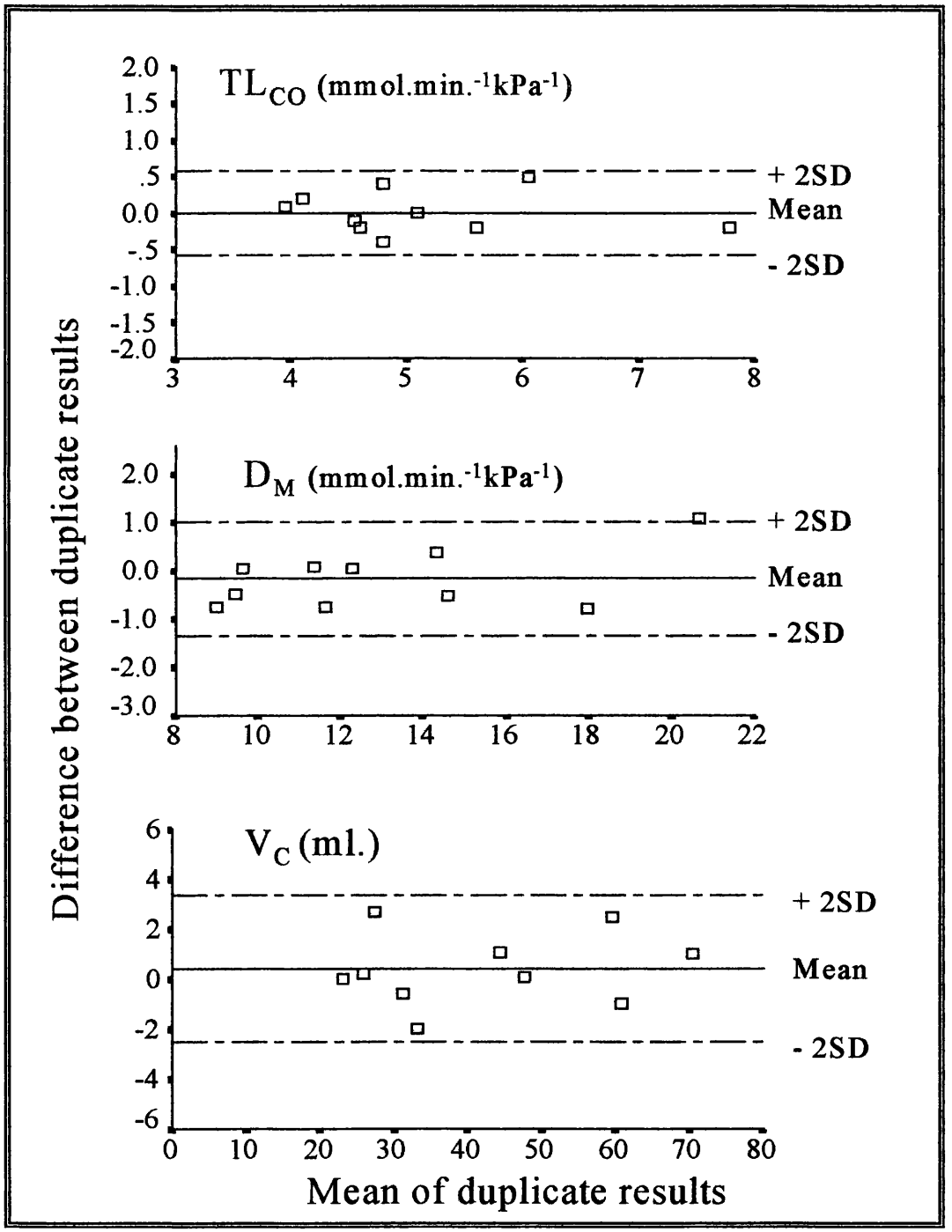
Table 6.2: Mean values of TL_{CO} and its components obtained by the 2 'sequence' protocols

	Mean (SD)		
	High O_2 first	Standard O_2 first	Difference
TL_{CO} (mmol.min.$^{-1}$kPa^{-1})	5.14 (1.12)	5.13 (1.15)	0.01 (0.29)
D_M (mmol.min.$^{-1}$kPa^{-1})	13.02 (3.99)	13.18 (3.72)	-0.16 (0.59)
V_C (ml.)	42.62 (16.89)	42.22 (16.65)	0.40 (1.47)

Figure 6.5 displays plots of the difference between the duplicate values of TL_{CO} and its components obtained by the 2 protocols against their means. Using Bland and Altman analysis, the limits of agreement between duplicate results were: - 0.57 and 0.59 mmol.min.$^{-1}$kPa^{-1} for TL_{CO}, - 1.34 and 1.02 mmol.min.$^{-1}$kPa^{-1} for D_M, and - 2.54 and 3.34 ml. for V_C. Inspection of individual results on the graphs, shows that

the means of difference between duplicate measurements are nearly zero with similar scatter above and below the mean for all parameters.

Figure 6.5: Plots of the difference between duplicate values of TL_{CO} and its components obtained by the 2 'sequence' protocols against their mean



Comments

The results of this study suggest that in non-smokers and ex-smokers there is no advantage of starting with high inspired O_2 mixture compared to starting with the standard mixture. The lack of any significant difference between results of the 2 protocols, under the conditions of measurement, indicates that there is no differential effect of the sequence of oxygen mixtures on CO back pressure and CO-Hb. However, an equally significant effect of both methods on CO back pressure and CO-Hb cannot be ruled out.

6.4 Study 3: The time interval between measurements

Aim

To compare the time interval between measurements used in the studies of this thesis during the estimation of TL_{CO} components (short interval protocol) with that recommended by Cotes (long interval protocol).

Subjects and Methods

Ten normal volunteers performed TL_{CO} measurement at high and standard inspired oxygen concentrations using 2 different time protocols, in random order. In the short protocol, the time intervals between measurements within the same session were:

- 5 minutes between each of the measurements at standard O_2,
- 10 minutes between the sets of measurements at standard and high O_2 and

- 10 minutes between each measurement at high O_2 concentration.

The corresponding time intervals for the long protocol were: 5 minutes, 30 minutes and 30 minutes. The total duration of a session was about 30 minutes for the short protocol and about 70 minutes for the long protocol. The 2 sessions were performed on the same day, but separated by at least one hour. All subjects were non-smokers and had no history of any cardio-respiratory disease. The degree of agreement between results obtained by the 2 protocols was assessed using Bland and Altman statistical analysis (180).

Results

Table 6.3 shows the summary statistics of TL_{CO} and its components obtained by the 2 protocols. The mean values of duplicate measurements were identical for TL_{CO} and D_M and nearly identical for V_C. Figure 6.6 displays scatterplots of the results of individual subjects using the 2 protocols. The values obtained using the 2 timing protocols were strongly correlated for all parameters with all points being close to their regression line.

Table 6.3: Mean values of TL_{CO} and its components obtained by the 2 'interval' protocols

	Mean (SEM)		
	Short interval	Long interval	Difference
TL_{CO} (mmol.min.$^{-1}$kPa^{-1})	8.0	8.0	0.0
D_M (mmol.min.$^{-1}$kPa^{-1})	18.4	18.4	0.0
V_C (ml.)	61.8	61.7	0.1

Figure 6.6: Scatterplots of duplicate results from individual subjects obtained by the 2 'interval' protocols

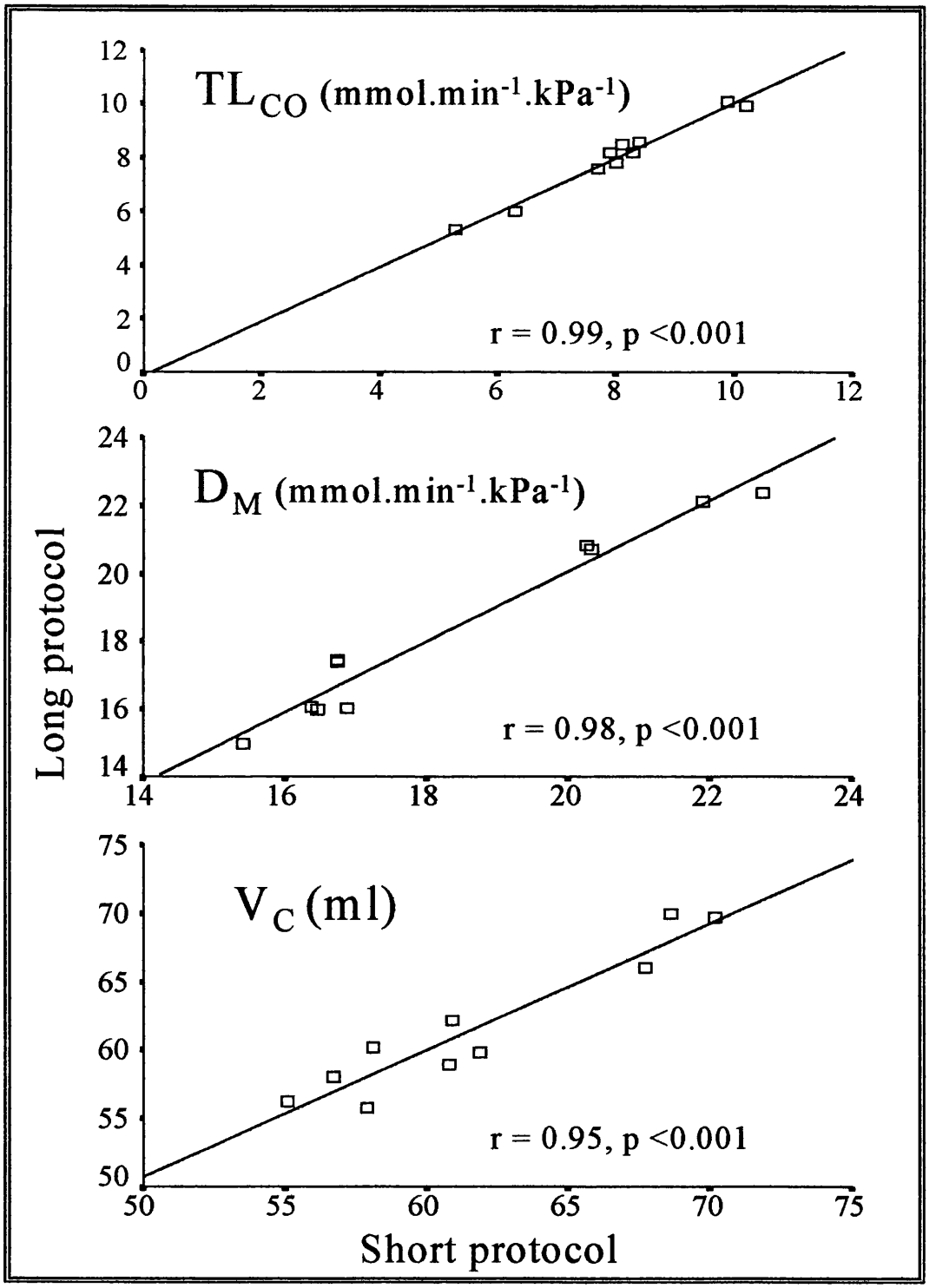
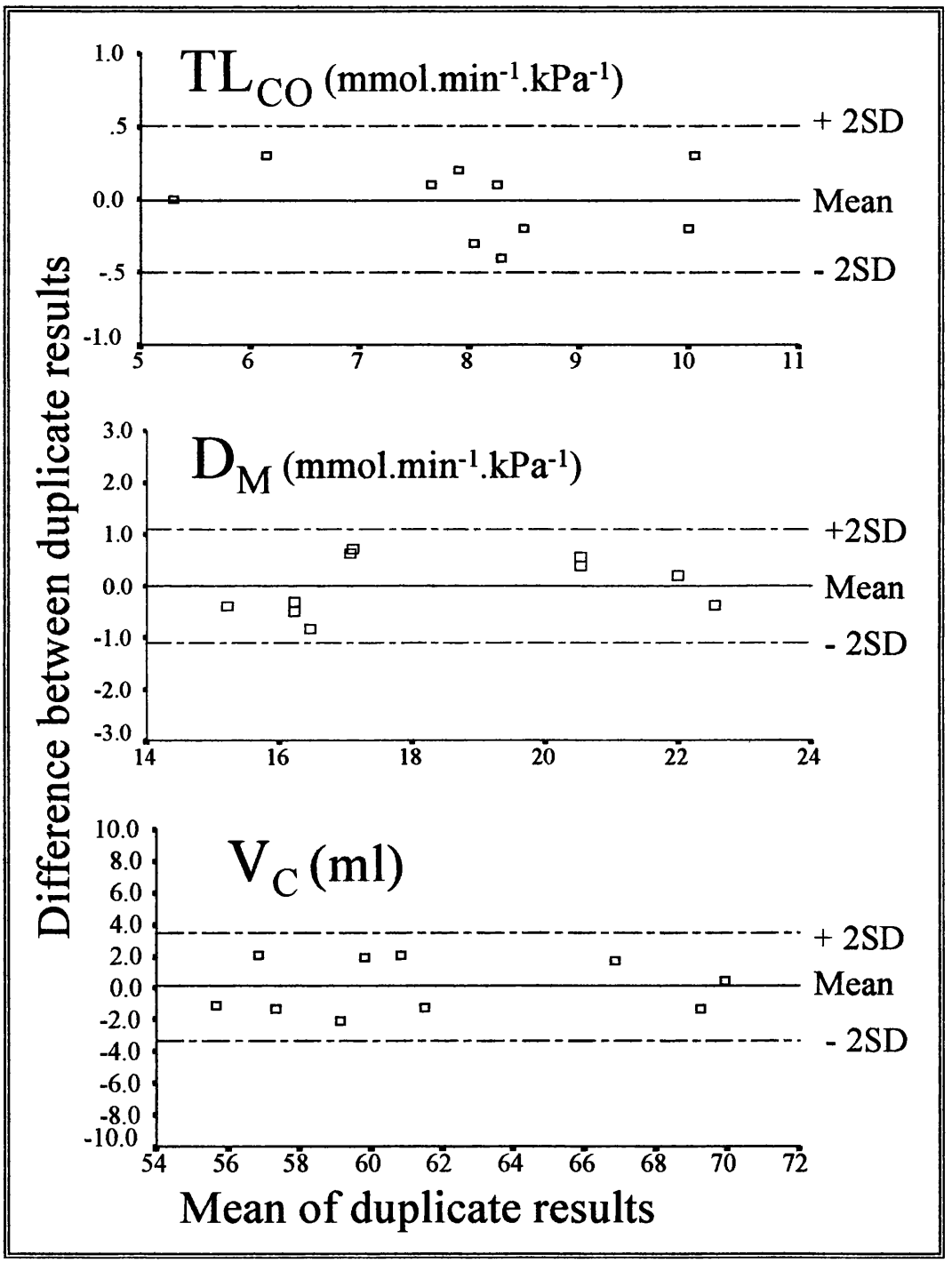


Figure 6.7 shows plots of Bland and Altman analysis. There was good agreement between the values obtained by the 2 protocols for all parameters. The limits of agreement between duplicate results were: - 0.5 to 0.5 $mmol.min^{-1}.kPa^{-1}$ for TL_{CO}, -1.1 to 1.1 $mmol.min^{-1}.kPa^{-1}$ for D_M and -3.2 to 3.5 ml for V_C. There was no systematic difference between duplicate results of individual subjects (equal points above and below the line of mean difference which was zero for TL_{CO} and D_M and 0.1ml for V_C).

Comments

Under the conditions of measurement described in chapter 5, the use of 10 minute intervals between successive manoeuvres during the estimation of TL_{CO} and its components is practical and provides results similar to those obtained when longer intervals are used.

Figure 6.7: Plots of the difference between duplicate values of TL_{CO} and its components obtained by the 2 'interval' protocols against their mean



6.5 Study 4: Reproducibility of the measurements of TL_{CO} and its components

Aim

To determine the reproducibility of results of TL_{CO} and its components as measured by the method applied in the studies of this thesis

Subjects and Methods

As part of routine quality control assurance, TL_{CO} and its components were routinely measured in one of the laboratory staff on alternate days (biological control). Repeat measurements of these parameters were also performed by a randomly selected group of subjects on 2 consecutive days. The group consisted of 9 heart transplant recipients and 5 normal volunteers.

Methods of measurement were as described in chapter 5. Variability in measurement in each of the diffusion parameters was assessed in percentage terms as the coefficient of variation (CV), where:

- For multiple measurements in one subject

  $CV = 100 \times$ SD of results / mean of largest and smallest values

- For 2 measurements in several subjects

  $CV = 100 \times$ SD of the difference / mean of both measurement means

Results

Figure 6.8 displays plots of results of 12 measurements of TL_{CO} and its components performed by one subject on alternate days over a period of month. All values were within 10% of their means and there was no evidence of drift with time. The

coefficients of variation of these measurements were 2.5%, 2.7%, 3.0% and 2.9% for TL_{CO}, K_{CO}, D_M and V_C, respectively.

Figure 6.8: Variability of TL_{CO} and its components over 12 measurements performed by one subject on alternate days

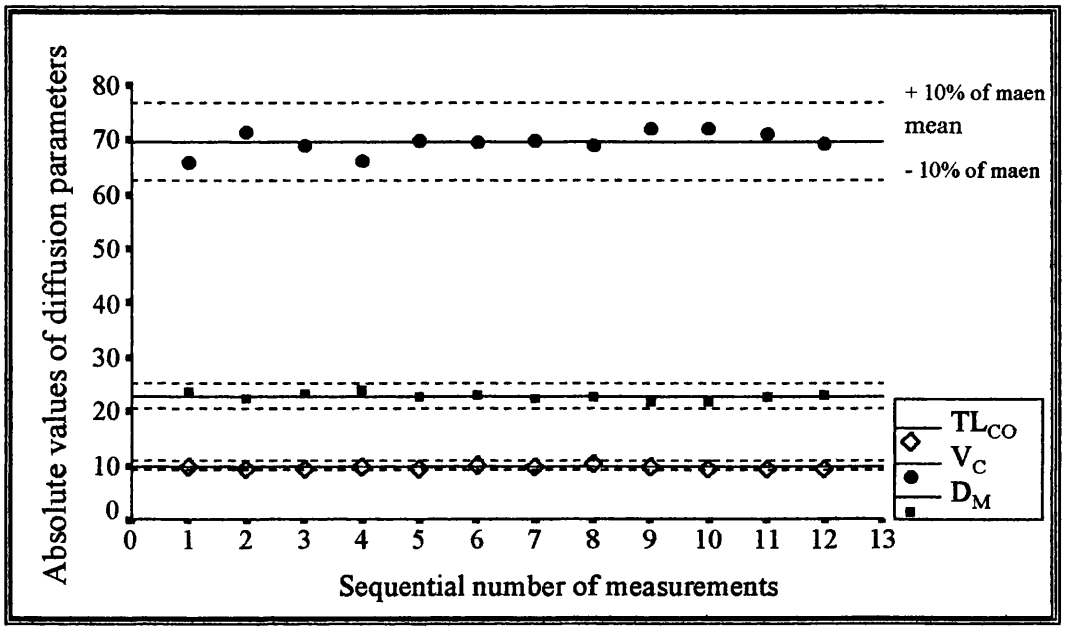
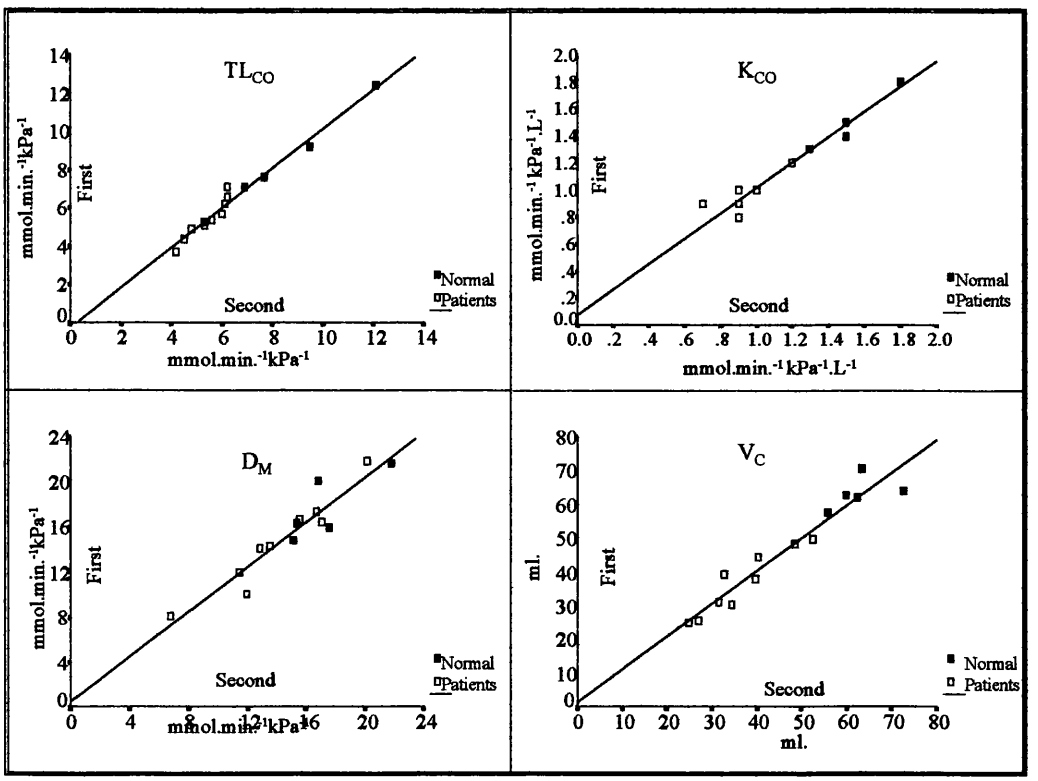


Table 6.4 shows summary statistics of the duplicate measurements performed in the 14 subjects. The coefficients of variation were 5.6, 6.6, 8.7 and 8.9 for TL_{CO}, K_{CO}, D_M and V_C, respectively. The variability of individual values is shown in figure 6.9. There is no obvious difference in the variability between heart transplant recipients and normal subjects.

Table 6.4: Summary statistics of reproducibility results of TL_{CO} and its components in 14 subjects (9 heart transplant recipients and 5 normal subjects)

	Means of absolute values				SD	Coefficient
	first	second	both	difference	of difference	of variation
TL_{CO}	6.48	6.46	6.47	0.02	0.36	5.6%
K_{CO}	1.21	1.21	1.21	0.00	0.08	6.6%
D_M	15.73	15.20	15.47	0.53	1.35	8.7%
V_C	46.46	46.18	46.32	0.28	4.07	8.8%

Figure 6.9: Variability of duplicate measurements of TL_{CO} and its components (9 heart transplant recipients and 5 normal subjects)



Comments

The reproducibility of results of TL_{CO} and its components as measured by the method used in this thesis is good and similar to published results (79,181). In 18 patients receiving bleomycin treatment for testicular tumours, the coefficients of variation of D_M and V_C from duplicate measurement within one session were 7.1% and 8.9%, respectively (181).

6.6 Study 5: Normal values of TL_{CO} components

Aim

To compare values of TL_{CO} components obtained using the method outlined in chapter 5 of this thesis with the reference values of Cotes (49).

Subjects and Methods

Thirty one normal subjects recruited as volunteers from the general population were studied (22 males and 9 females). Their mean age was 48.6 years (range 27 to 65 years) and the mean height was 173.4 cm (range 165 to 187) for men and 161.1 cm (149 to 172) for women. They all had normal resting pulmonary function and none had a history of cardio-pulmonary disease. Seven subjects were former smokers and 24 were lifelong non-smokers.

The relationship between actual values obtained in this study and reference values (49) was assessed by calculating the % of predicted for each subjects and by linear regression of the measured absolute values against their predicted values.

Results

The mean of measured D_M was 98.7 % of predicted (range; 87% to 111%), and that of V_C was 102.1% of predicted (range; 81% to 112%). Figures 6.10 and 6.11 display regression plots of D_M and V_C against their reference values. There is a high correlation between measured and reference values for both parameters.

Figure 6.10: Scatterplot comparing measured values of D_M against their reference values by Cotes.

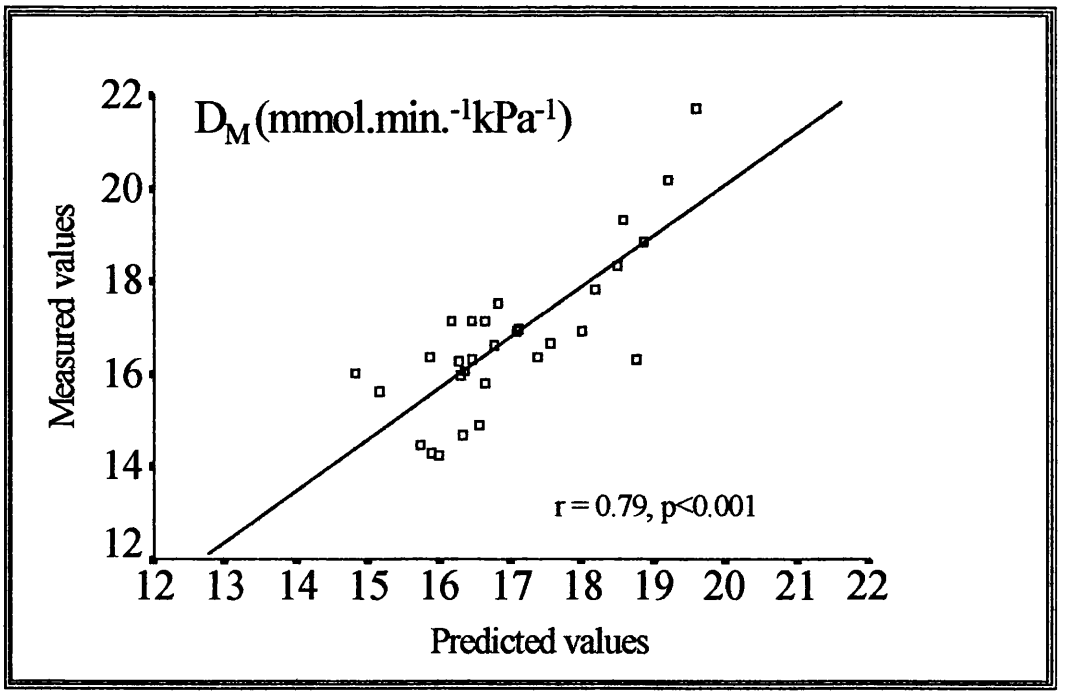
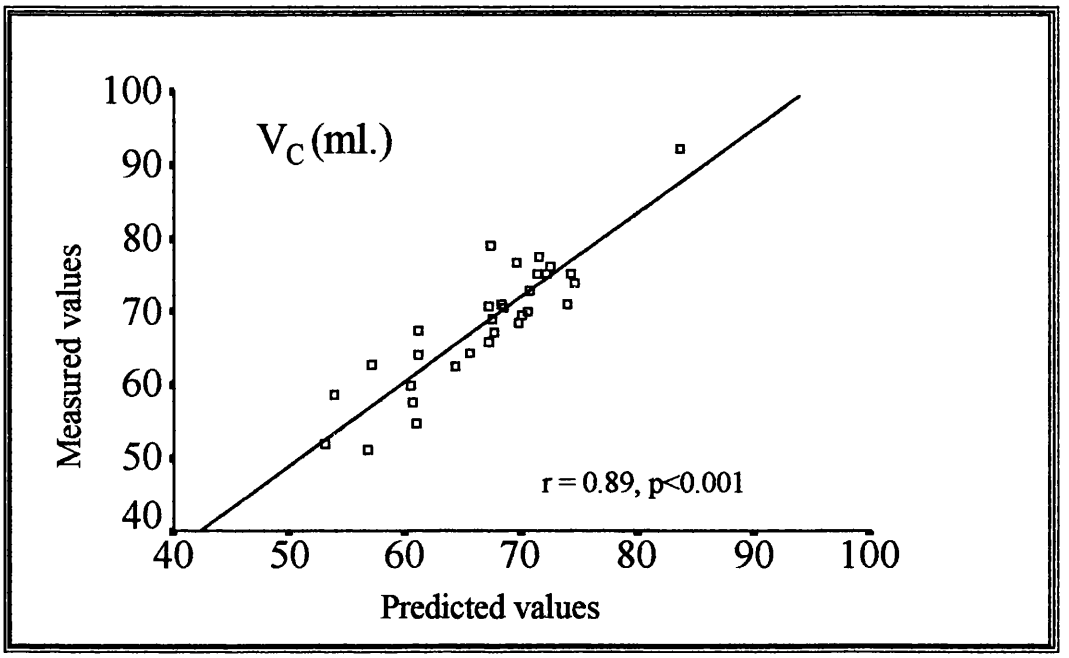


Figure 6.11: Scatterplot comparing of measured values of V_C against their reference values by Cotes.



Comments

The results of D_M and V_C in normal subjects obtained by the method used in this thesis are comparable with established reference values

6.7 Summary and conclusions

The results of studies comprising this chapter shows that:

1. Under identical conditions, The Transflow Model (PK Morgan, Kent, UK) and the Transfer Test Model B (PK Morgan, Kent, UK) yield identical results.

2. The method used to estimate TL_{CO} and its components using the Transflow Model gives reproducible results in both normal subjects and heart transplant patients and these results and the results in normal subjects are comparable with established reference values

3. Under the condition of measurement in these studies, the chosen time intervals between the different stages of measurement give similar results to those obtained using the time intervals recommended by Cotes (2). In addition, the sequence of O_2 mixtures appears to have no influence on the values of TL_{CO} and its components.

The results of these studies allowed the production of a standard protocol for the estimation of TL_{CO} components. The details of this protocol have been described in chapter 5. The short duration of the test (about 30 minutes) is suitable for clinical use without compromising precision or reproducibility. The measurement of TL_{CO} components using this protocol was used as part of the investigation of the mechanisms underlying TL_{CO} decline following heart transplantation.

PART 3

LUNG FUNCTION STUDIES IN HEART TRANSPLANT PATIENTS

CHAPTER 7

LUNG FUNCTION CHANGES FOLLOWING HEART TRANSPLANTATION

7.1 Introduction

As noted previously, heart transplantation has been shown to restore lung volumes and airway function towards normal (14). In contrast, TL_{CO} and K_{CO} have been persistently shown either to deteriorate or remain sub-normal following heart transplantation (15-21,182). Since most of these studies were retrospective and based on cross-sectional analysis of patients assessed at different intervals following transplantation (15,17-21), there are little data on the time course of TL_{CO} changes after heart transplantation (16,182). At the time of starting the present study, there was only one report on longitudinal changes in TL_{CO} following heart transplantation (16). In this study, Greon and associates (16) assessed 34 patients before and at one year after heart transplantation and 27 of them had repeat assessments at 2 years, with a third assessment in 21 patients at 3 years after transplantation. In the first post-operative year, static and dynamic lung volumes (TLC, VC, and FEV_1) tended to normalise. In contrast, K_{CO} decreased by a mean of 12%. In the following 2 years, K_{CO} tended to improve, but remained below its base-line pre-operative value. Most of the potential causes of TL_{CO} decline in the setting of heart transplantation occur during the first post-transplant year (42). These include the

effects of surgery including sternotomy and cardio-pulmonary bypass, pulmonary infections, drug toxicity and cardiac allograft rejection and dysfunction (42,183). It is therefore important to define the longitudinal changes in TL_{CO} during the first post-transplant year. In 1995, while the present study was in progress, Bussieres et al. (182) reported the only available longitudinal study of lung function changes during the first year after heart transplantation. In this study, lung function changes before and at 1, 3 and 12 months after transplantation were reported in 14 patients. Before transplantation, TL_{CO} and lung volumes were reduced, and despite significant improvement of lung volumes and indices of airway function by 3 months after transplantation, TL_{CO} decreased further soon after transplantation and did not improve afterwards. The findings of this small study suggest that the aetiology of TL_{CO} decline occurs within the first few weeks after heart transplantation.

Aim

To determine the longitudinal changes in pulmonary function during the first 3 years after heart transplantation.

7.2 Methods

7.2.1 Study population

Between January 1992 and February 1996, 98 patients underwent orthotopic heart transplantation at the Scottish Cardio-pulmonary Transplantation Unit. The

longitudinal nature of the study resulted in a number of patients with incomplete pulmonary function results. This was due to one of the following reasons:

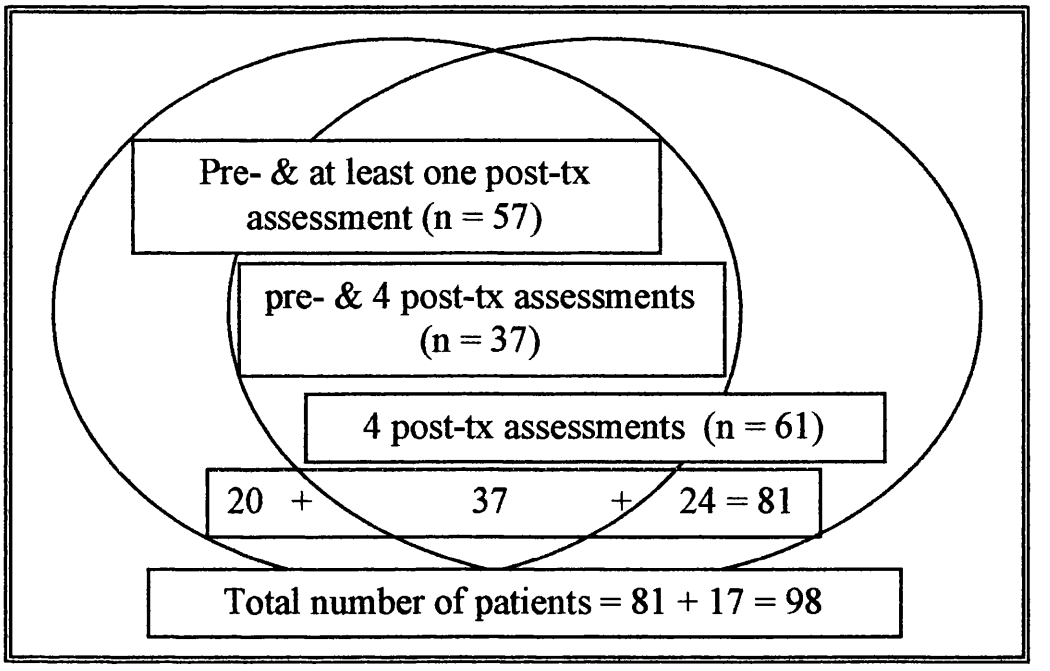
1. Transplantation without referral for pre-transplant pulmonary function assessment.
2. Completion of the follow-up period of the study before recent recipients had full post-transplant assessment.
3. Early post-transplant death.

For the purpose of this study patients were included in the analysis if they fulfilled one or both of the following criteria:

1. Complete pre-transplant pulmonary function assessment and at least one further assessment following transplantation.
2. At least 4 sequential pulmonary function assessments following transplantation.

Figure 7.1 shows that these criteria were met by 81 of the 98 heart transplant recipients and of these: 57 patients had pre-transplant and at least one post-transplant pulmonary function assessment (Group 1), 61 patients had at least 4 post-transplant assessments (Group 2) and 37 patients had both pre-transplant and at least 4 assessments following transplantation (Group 3). The results of these 3 groups were analysed separately.

Figure 7.1: Heart transplant recipients categorised according to the number of repeat pulmonary function assessments performed



The findings in heart transplant patients were compared with data from 28 (23 males) normal subjects recruited as volunteers from the general population in whom there was no evidence of cardio-pulmonary disease. Their mean age was 40.4 years (range, 19-61), and 16 of them were life-long non-smokers, 9 former smokers and 3 were current smokers.

7.2.2 Protocol

The protocol of serial lung function assessment has been described in chapter 4. In brief, patients were assessed before transplantation and at 6 weeks, 3, 6 and 12 months following transplantation (early assessment). Patients who were

transplanted before the start of the study were assessed at 12, 18, 24 and 36 months after transplantation (late assessment).

7.2.3 Pulmonary function tests

Methods of lung function testing were as described in chapter 5. Measured and calculated variables included FEV_1, FVC, FEV_1/FVC ratio, RV, TLC, TL_{CO} and K_{CO}.

7.2.4 Data presentation and analysis

All lung function results were expressed as percentages of predicted (41). Unless stated otherwise, values are expressed as mean +/- one standard error of the mean (SEM). Mean changes in lung function parameters following heart transplantation were calculated as the mean of % predicted values after transplantation minus the mean of % predicted before transplantation. Comparisons between results before and after transplantation were performed using the paired samples Student's t-test and comparisons between heart transplant recipients and normal controls were performed using the independent samples Student's t-test. A p value of <0.05 was considered significant.

7.3 Results

7.3.1 Comparisons between pre- and post-transplant results (Group 1)

1. Clinical characteristics

Table 7.1 shows the clinical characteristics of the 57 heart transplant recipients who had pulmonary function assessment before and at least once after transplantation.

Before transplantation, they all had severe congestive heart failure with mean left ventricular ejection fraction (LVEF) of 13.4%. After transplantation, mean LVEF increased to 47.1% (lower limit of normal = 40%). Thirty two of these patients had their first post-transplant assessment at 6 weeks (early) and the remaining 25 patients at one year following transplantation (late). The mean time between pre-transplant pulmonary function assessment and transplantation was 6.2 months (range; 1 week to 14 months) for the entire group, 7.0 months for the early group and 5.1 months for the late group. There was no significant difference between the 2 sub-groups in any of the listed variables.

Table 7.1: Clinical characteristics of 57 (Group 1) recipients with lung function results before and after heart transplantation

	All	*Early*	*Late*
Number of subjects	57	32	25
Age; mean in years (range)	49.1 (19-61)	48.2 (34-60)	50.4 (39-59)
Sex			
male	46 (81%)	26 (81%)	25 (80%)
female	11 (19%)	6 (19%)	5 (20%)
Smoking status			
non-smokers	13 (23%)	8 (25%)	5 (20%)
ex-smokers	44 (77%)	24 (75%)	20 (80%)
Diagnosis			
ischaemic heart disease	34 (60%)	18 (56%)	16 (64%)
dilated cardiomyopathy	20 (35%)	12 (38%)	8 (32%)
others	3 (5%)	2 (6%)	1 (4%)
Pre-transplant LVEF, mean (SD)	13.4 (5.8)	13.0 (5.7)	13.8 (6.0)
post-transplant LVEF, mean (SD)	47.1 (8.3)	46.3 (9.3)	48.2 (8.0)

2. Lung function before transplantation

Table 7.2 shows lung function in heart transplant patients compared to normal controls. Before transplantation, mean values of FEV_1, FVC, FEV_1/FVC and TLC were slightly reduced. Although above 80% of predicted, they were all significantly lower than the values in normal controls ($P<0.05$). RV was slightly elevated, but this was not significantly different from that of normal controls. The greatest impairment, however, was in TL_{CO} at 72.1% of predicted compared to 98.6% of predicted in normal subjects ($p <0.001$). Although K_{CO} was relatively preserved before transplantation, it was significantly lower than that of controls (90.1% and 105.3% of predicted respectively, $P<0.05$).

Table 7.2: Resting pulmonary function results in 57 heart transplant recipients (Group 1) compared to normal controls

	mean % predicted (SEM)		
	normal	*heart transplant patients*	
	controls	*before*	*after*
FEV_1	107.2 (3.9)*	86.0 (2.0)	88.6 (2.1)
FVC	110.9 (2.1)*	93.3 (2.0)	94.6 (2.3)
FEV_1/FVC	98.6 (1.8)*	91.7 (1.6)	91.0 (1.5)
RV	101.9 (3.3)	104.9 (5.0)	103.2 (3.7)
TLC	100.2 (3.3)*	92.7 (2.2)	93.3 (2.3)
TL_{CO}	98.6 (1.3)*	72.1 (2.1)	54.2 (1.7)**
K_{CO}	105.3 (2.2)*	90.1 (2.7)	64.0(2.0)**

* = significant difference between normal controls and patients before and after transplantation, ** = significant difference between pre- and post-transplant values

3. Effect of heart transplantation

Table 7.2 also shows that there was no significant change in any of the mean values of static and dynamic lung volumes after transplantation. In contrast, mean TL_{CO} declined significantly following transplantation (from 72.1% to 54.2% of predicted, $p < 0.001$), and the decline in K_{CO} was even greater (from 90.1% to 64.0% of predicted, $p < 0.001$).

Figures 7.2 and 7.3 show that the lack of any significant change in the mean values of static and dynamic lung volumes in the entire group following transplantation was a composite of different changes in the 2 sub-groups. At 6 weeks following transplantation, there was a reduction in all lung volumes and flow rates, but these changes were not statistically significant. In the late assessment group (one year), there was evidence of improvement in these parameters (FEV_1, FVC and TLC increased and RV decreased), but again these changes were not significant. The decline in TL_{CO} and K_{CO} were similar in the 2 sub-groups.

Figure 7.2: Means of changes in % predicted values of lung function parameters in 57 patients (Group 1) following heart transplantation

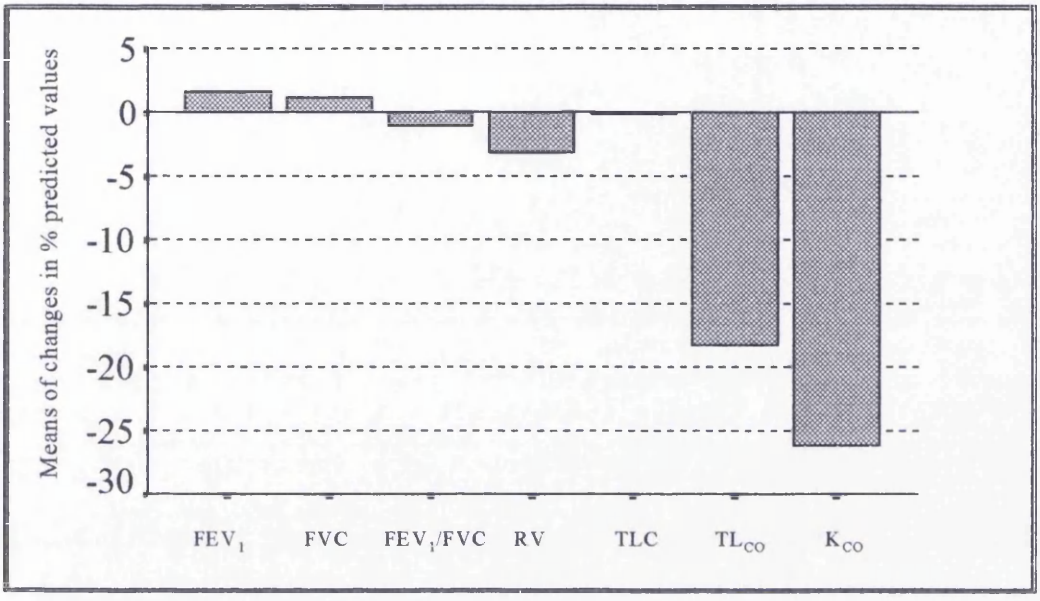
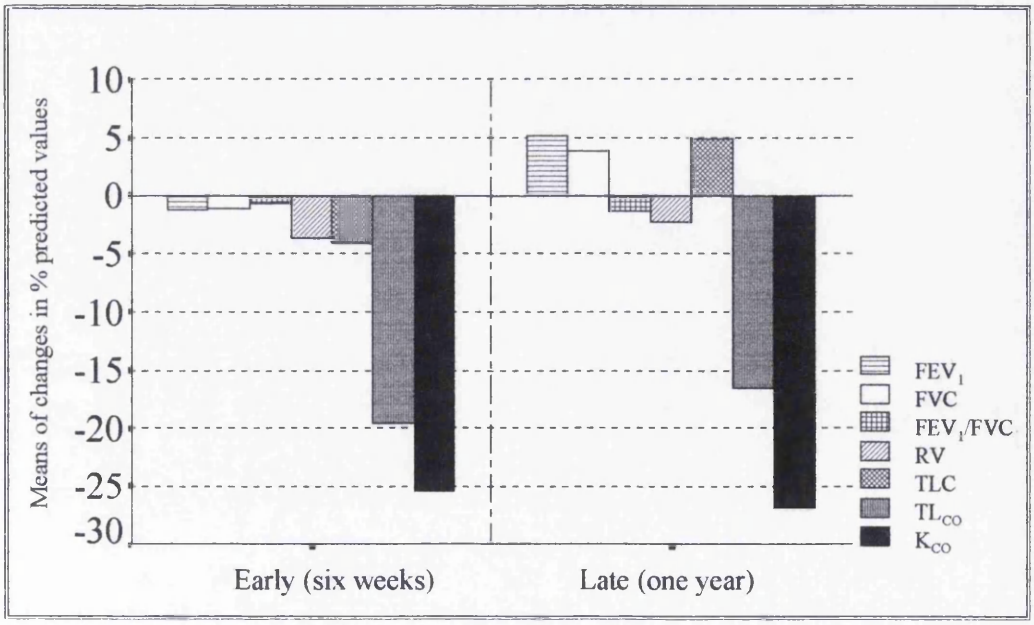


Figure 7.3: Comparison between changes in lung function in 2 sub-groups of heart transplant recipients (Group 1); six weeks vs. one year post-transplantation



Figures 7.4 to 7.8 display the changes in static and dynamic lung volumes following heart transplantation in individual patients. The upper plot of each figure represents the entire group whereas the lower 2 plots show the individual changes in the early and late assessment groups separately. Inspection of these graphs reveals marked individual variability in the changes in these parameters following transplantation. The individual changes in the % predicted values ranged from -38 to 36 for FEV_1, -40 to 39 for FVC, -22 to 18 for FEV_1/FVC, -62 to 65 for RV and from -38 to 32 for TLC. For the entire group, approximately 50% of patients had changes less than 10% of predicted values of FEV_1, FVC and TLC and the remaining 50% who had changes greater than 10% of predicted were equally divided into those who had improvement and those who had deterioration. On the other hand more than 75% of patients had no change (< 10% of predicted) in their FEV_1/FVC or RV with the remaining equally distributed between those who improved and those who deteriorated. Comparisons between early and late assessment sub-groups revealed similar pattern of individual variability for all of these parameters with 2 notable differences:

1. In the early assessment group, the proportion of patients with decline was greater than those with improvement for all static and dynamic lung volumes and the opposite was true in the late group.
2. The magnitude of change in any of these indices was greater in the early compared to the late assessment group.

In contrast, almost all patients had reductions in TL_{CO} and K_{CO} with the majority deteriorating by more than 10% of predicted with no difference between the 2 sub-groups (Figures 7.9 and 7.10).

Figure 7.4: Individual changes in % predicted FEV_1 following heart transplantation (Group 1)

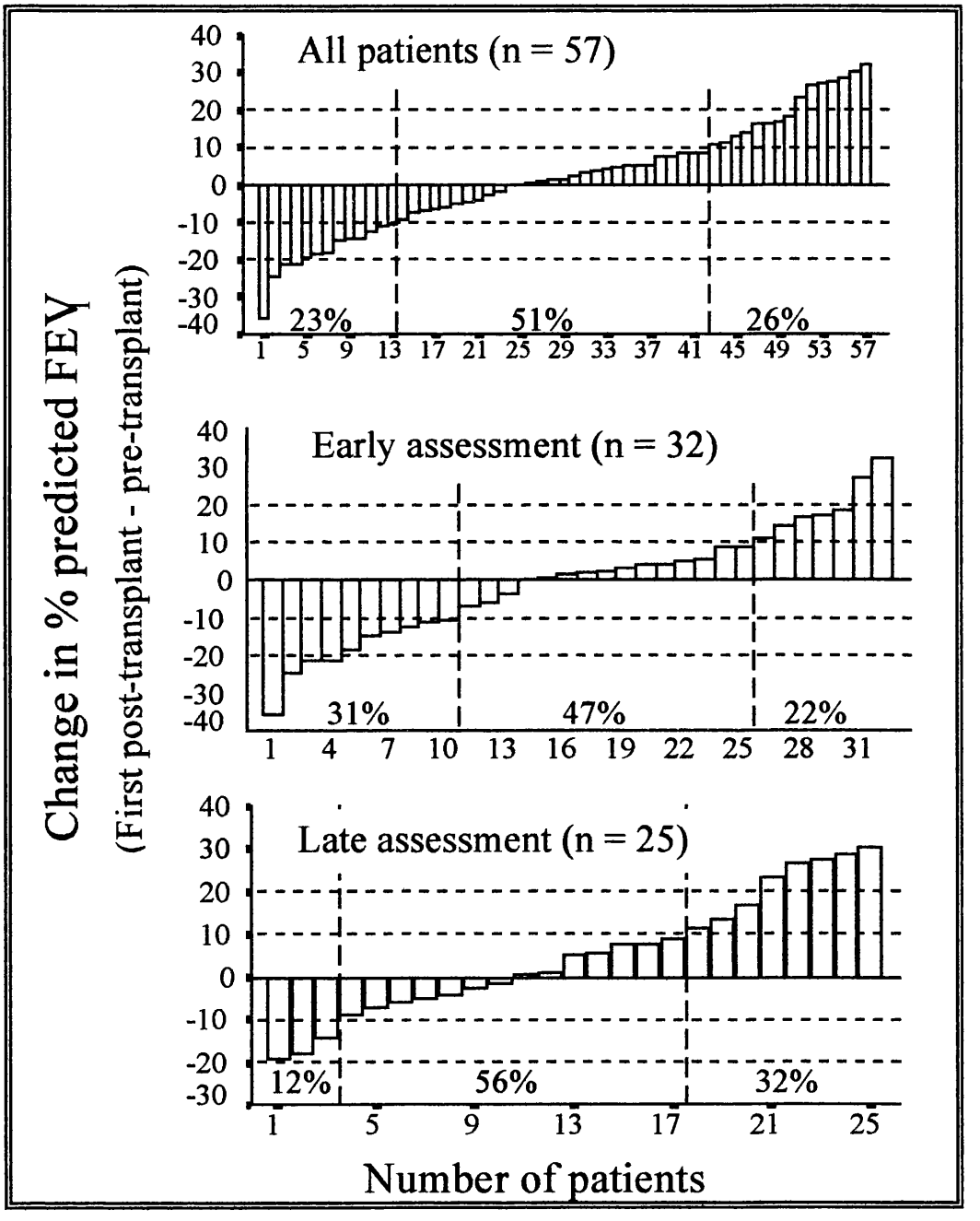


Figure 7.5: Individual changes in % predicted FVC following heart transplantation (Group 1)

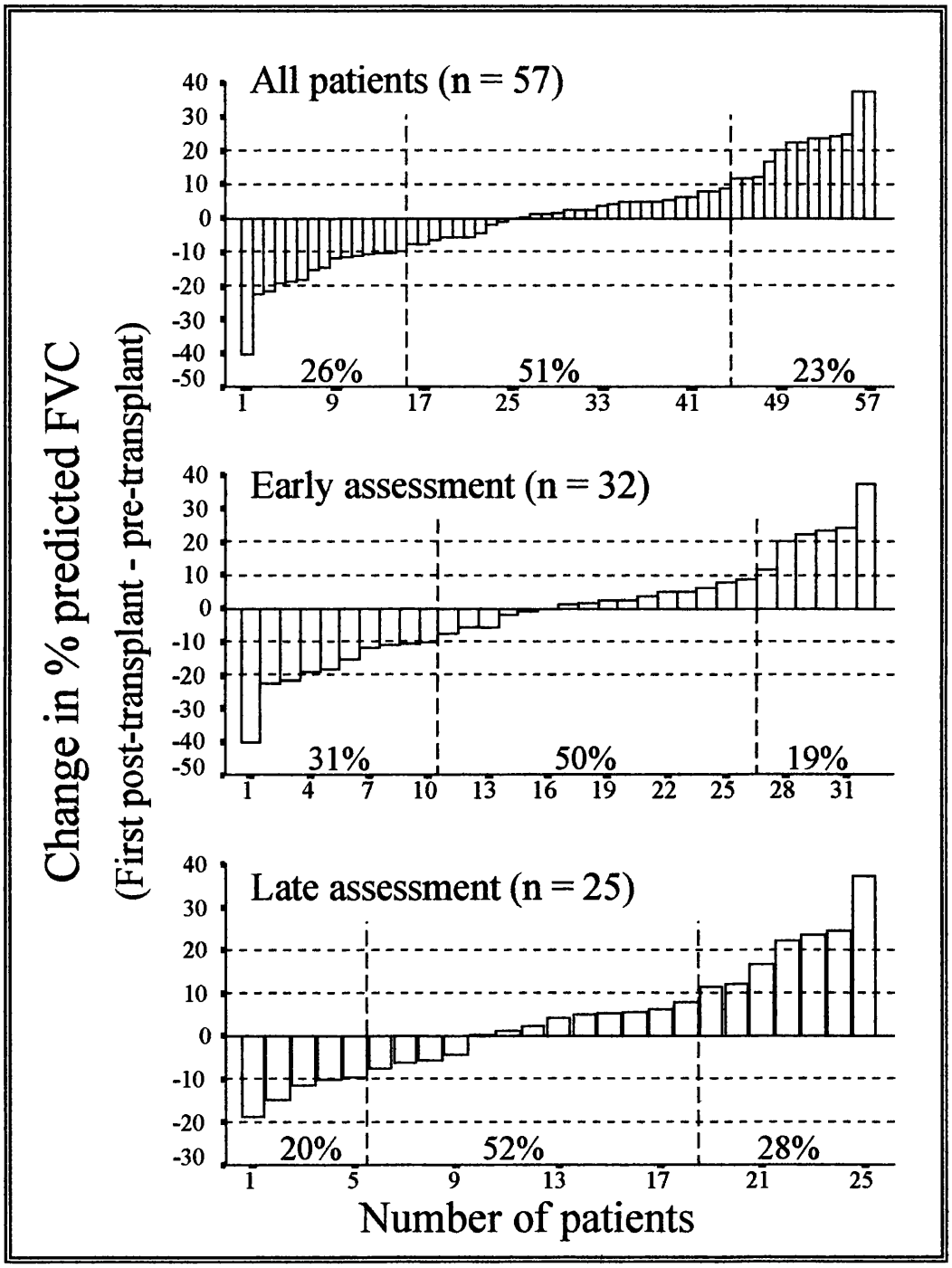


Figure 7.6: Individual changes in % predicted ~~FVC/FEV~~FEV_1/FVC following heart transplantation (Group 1)

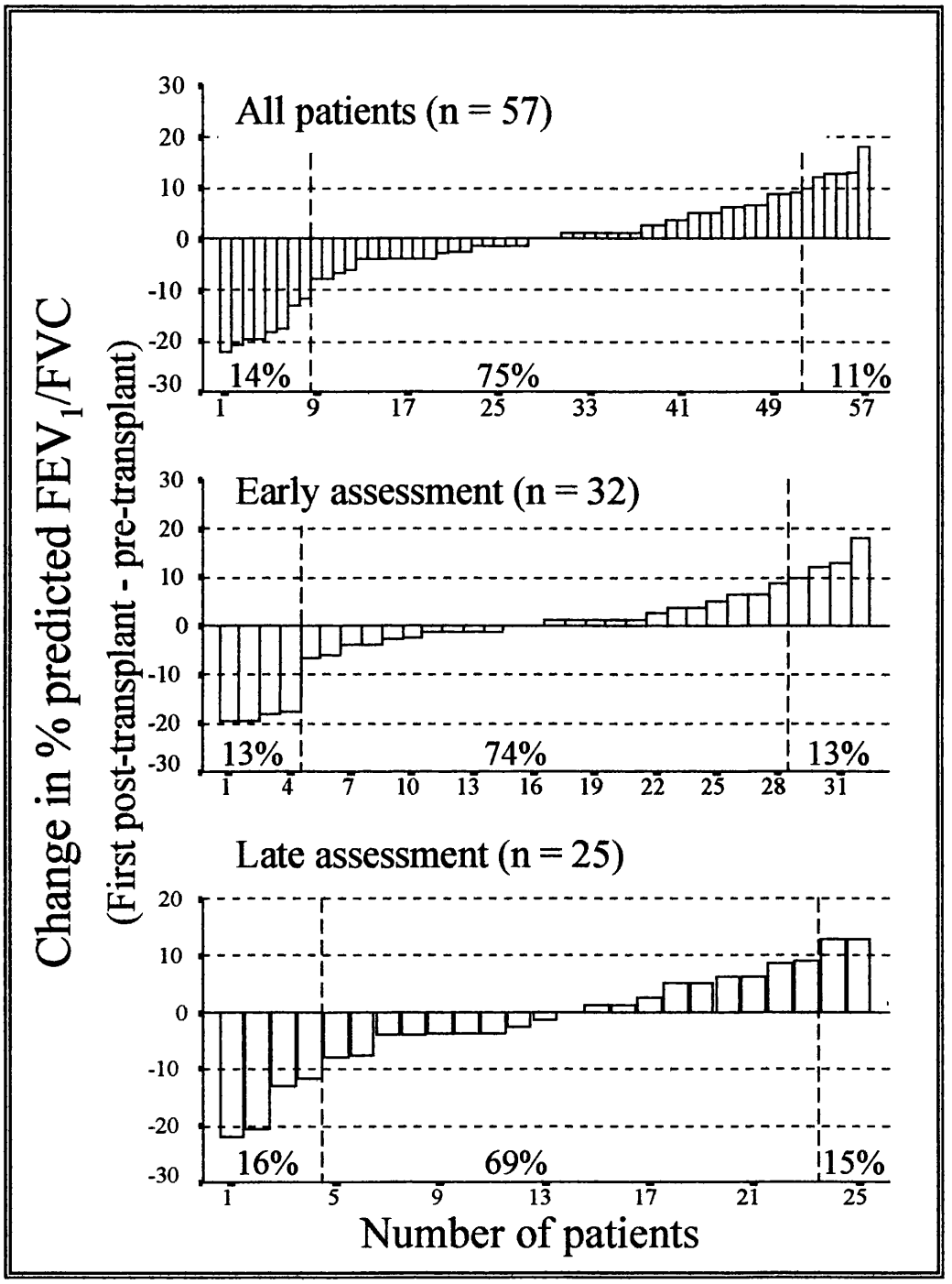


Figure 7.7: Individual changes in % predicted RV following heart transplantation (Group 1)

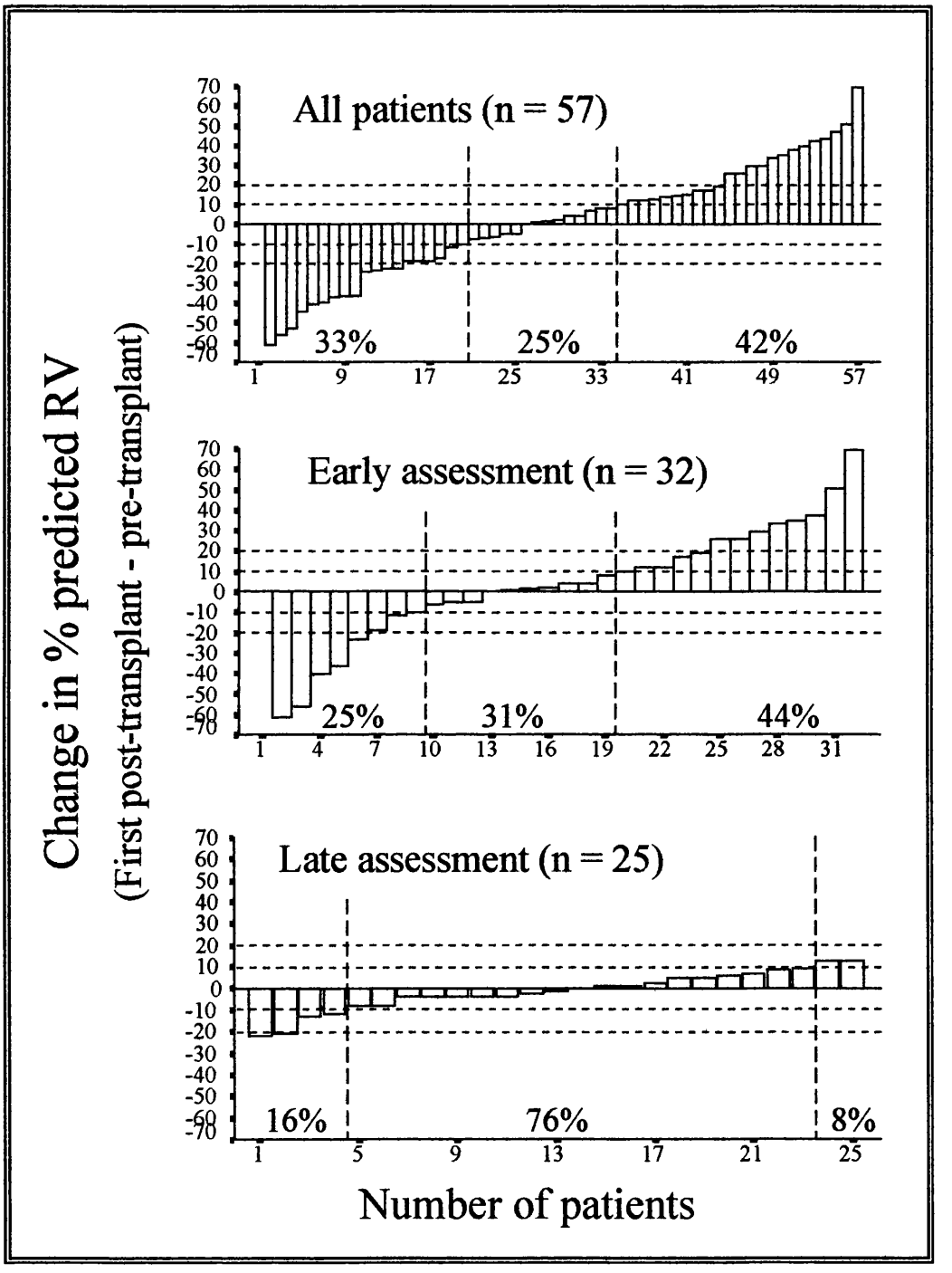


Figure 7.8: Individual changes in % predicted TLC following heart transplantation (Group 1)

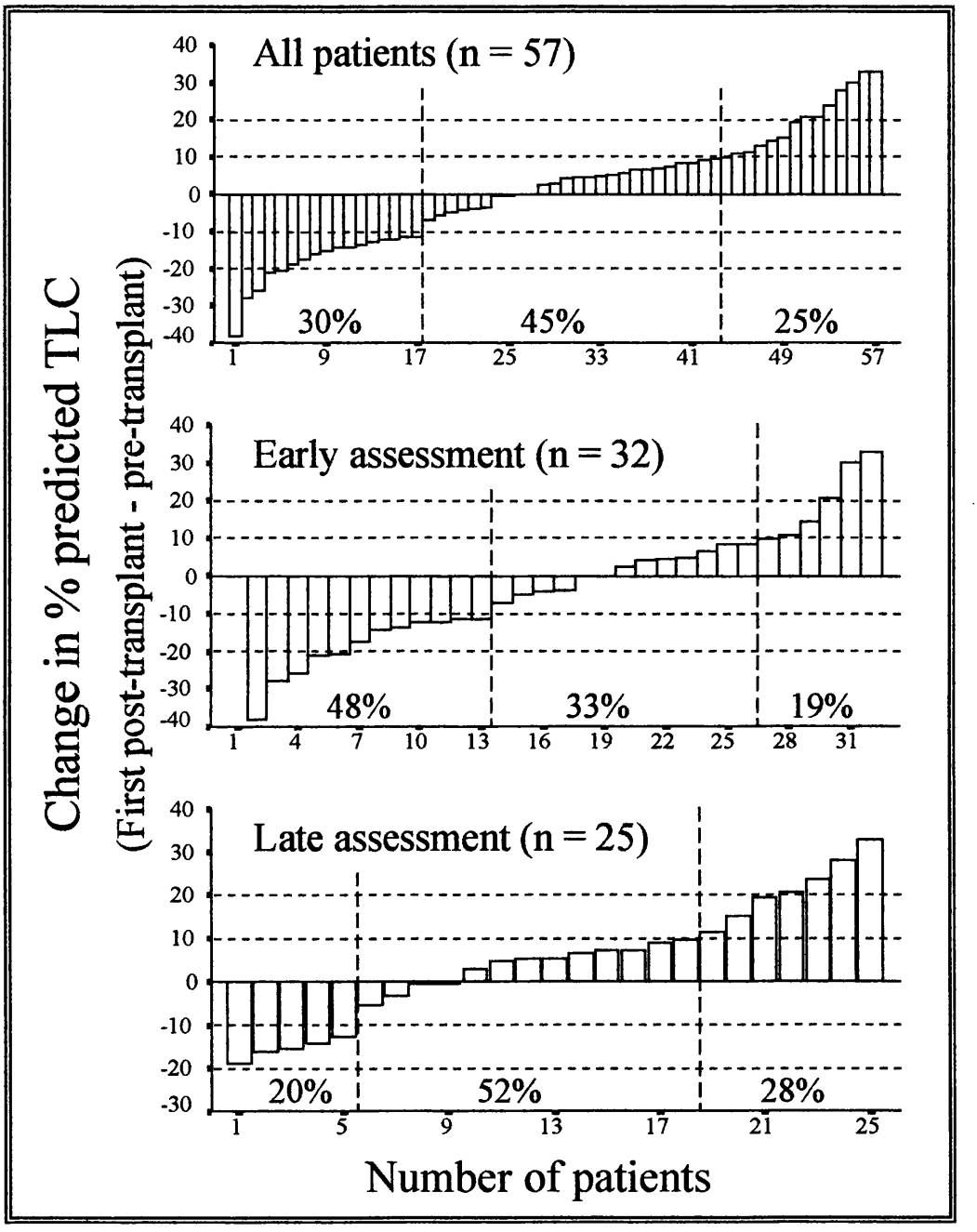


Figure 7.9: Individual changes in % predicted TL_{CO} following heart transplantation (Group 1)

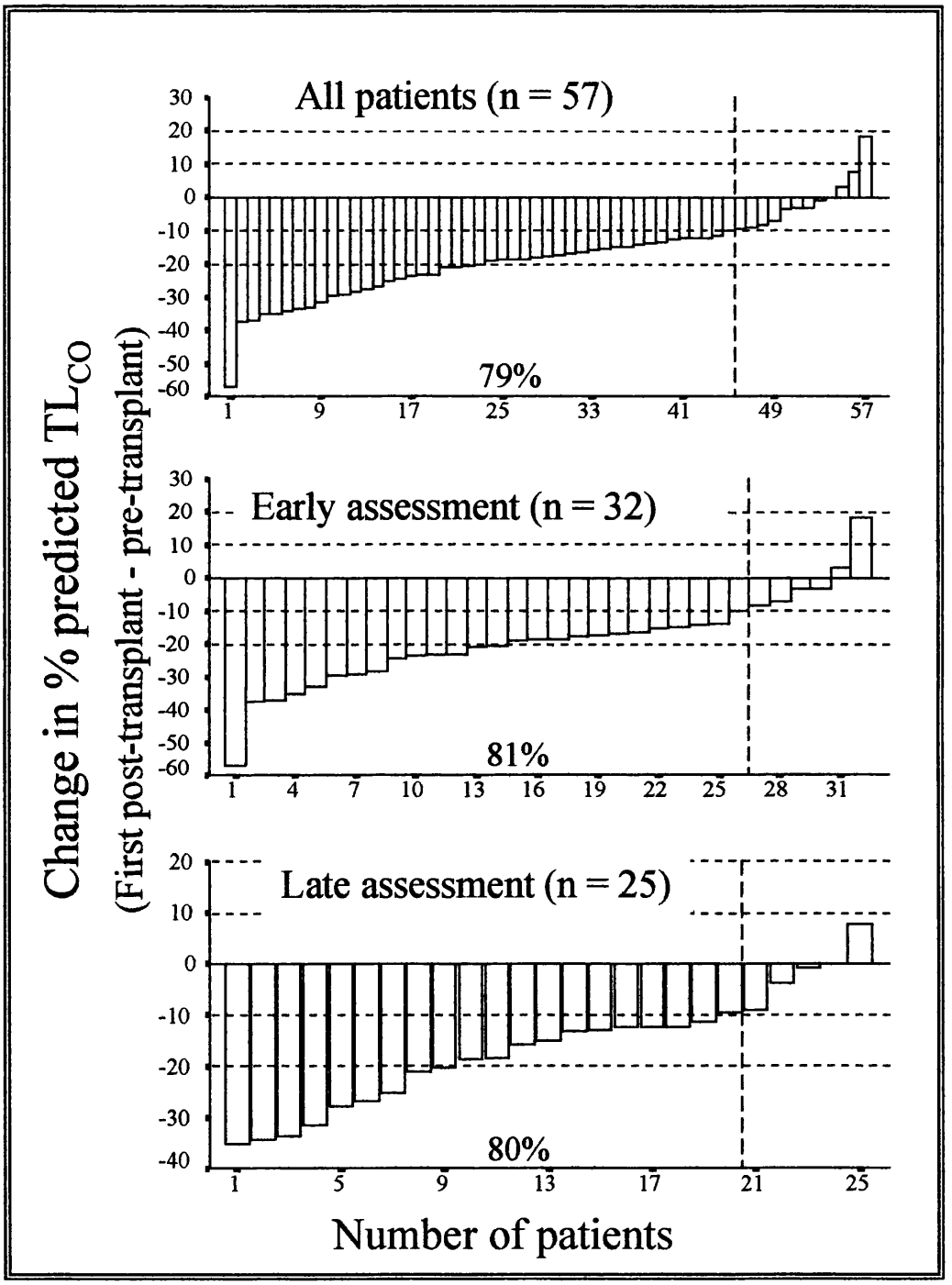
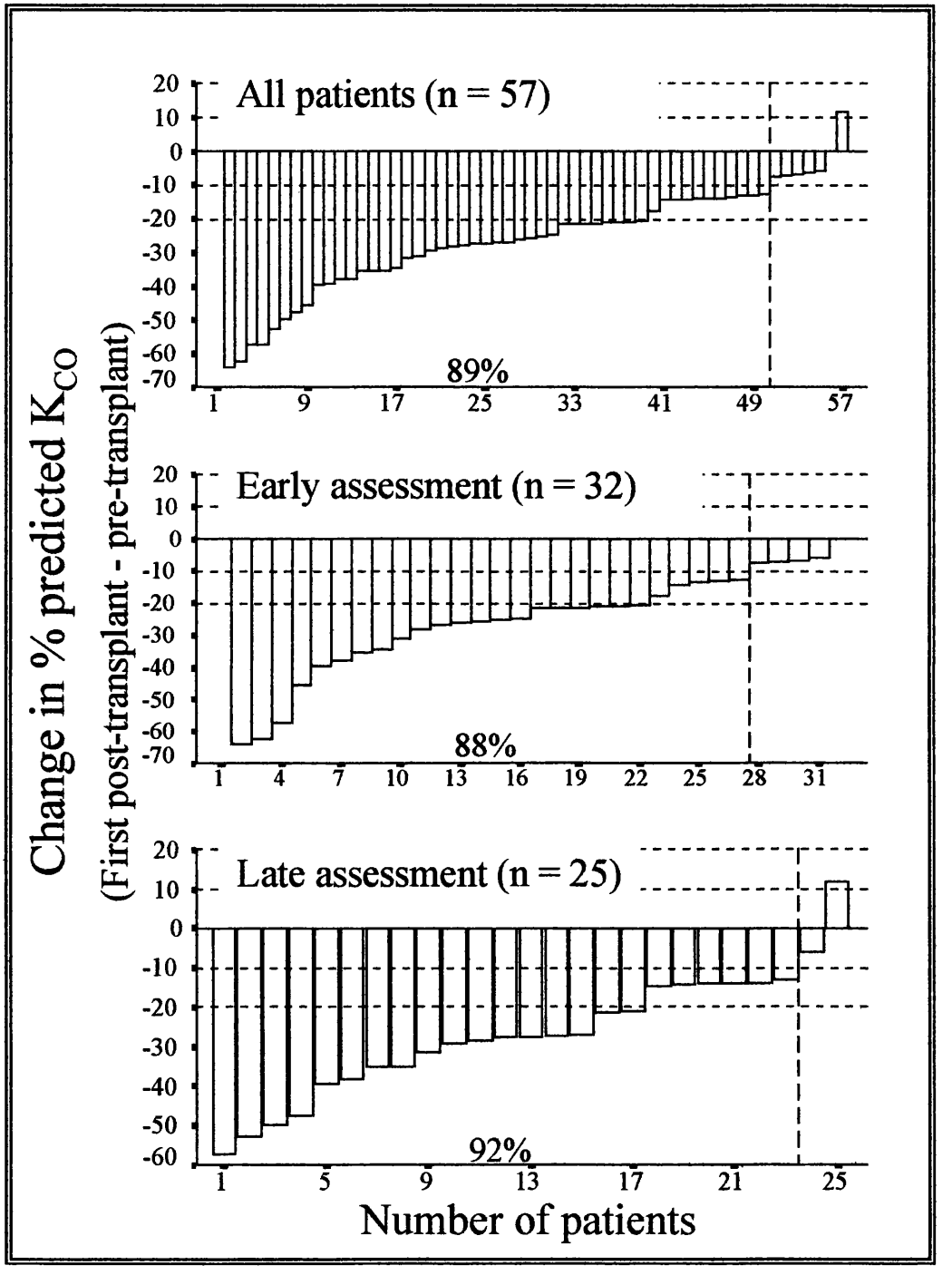


Figure 7.10: Individual changes in % predicted K_{CO} following heart transplantation (Group 1)



7.3.2 Longitudinal changes following heart transplantation (Group 2)

1. Clinical characteristics

Table 7.3 shows the clinical characteristics of the 61 patients who had 4 sequential pulmonary function assessments following transplantation sub-divided into early (34 patients) and late (27 patients) assessment groups. There was no significant difference between the 2 sub-groups in any of the listed parameters.

Table 7.3: Clinical characteristics of 61 recipients (Group 2) with serial lung function results after heart transplantation

	All	*Early*	*Late*
Number of subjects	61	34	27
Age; mean in years (range)	48.2 (19-61)	47.1 (19-61)	49.7 (39-59)
Sex			
male	50 (83%)	28 (82%)	22 (81%)
female	11 (17%)	6 (18%)	5 (19%)
Smoking status			
non-smokers	16 (26%)	8 (24%)	8 (30%)
ex-smokers	45 (74%)	26 (76%)	19 (70%)
Diagnosis			
ischaemic heart disease	37 (61%)	20 (59%)	17 (63%)
dilated cardiomyopathy	20 (33%)	12 (35%)	8 (30%)
others	4 (6%)	2 (6%)	2 (7%)
Pre-transplant LVEF, mean (SD)	14.3 (6.3)	14.1 (6.0)	15.1 (6.2)
post-transplant LVEF, mean (SD)	50.5 (9.2)	49.9 (8.4)	51.3 (10.4)

2. Lung function

Using the first post-transplant pulmonary function assessment as a base-line for subsequent results, figure 7.11 shows that in the entire group FEV_1, FVC and TLC increased slightly in the second assessment with no further changes later. Sub-group plots show that the initial low values for these parameters in the entire group were due to the lower values in the early assessment group (i.e. at 6 weeks after transplantation). In contrast, there was no change in either TL_{CO} or K_{CO} from the first post-transplant assessment (Figure 7.12). Although sub-group plots show a small decline in both parameters during the last 2 measurements in the late group (i.e. at 24 and 36 months following transplantation), this was not statistically significant.

Figure 7.11: Post-transplant longitudinal changes in lung volumes and expiratory flow rate in 61 recipients (Group 2)

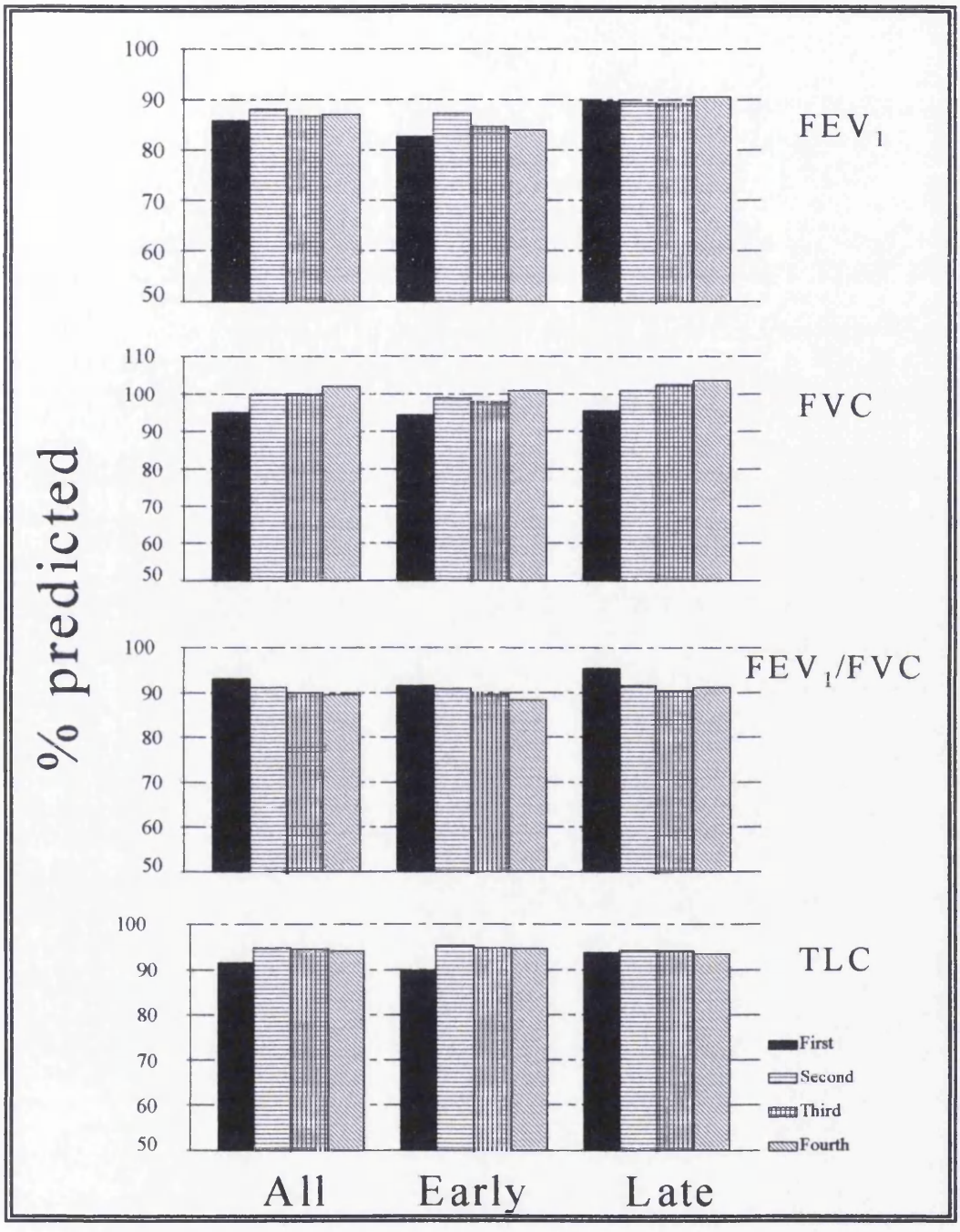
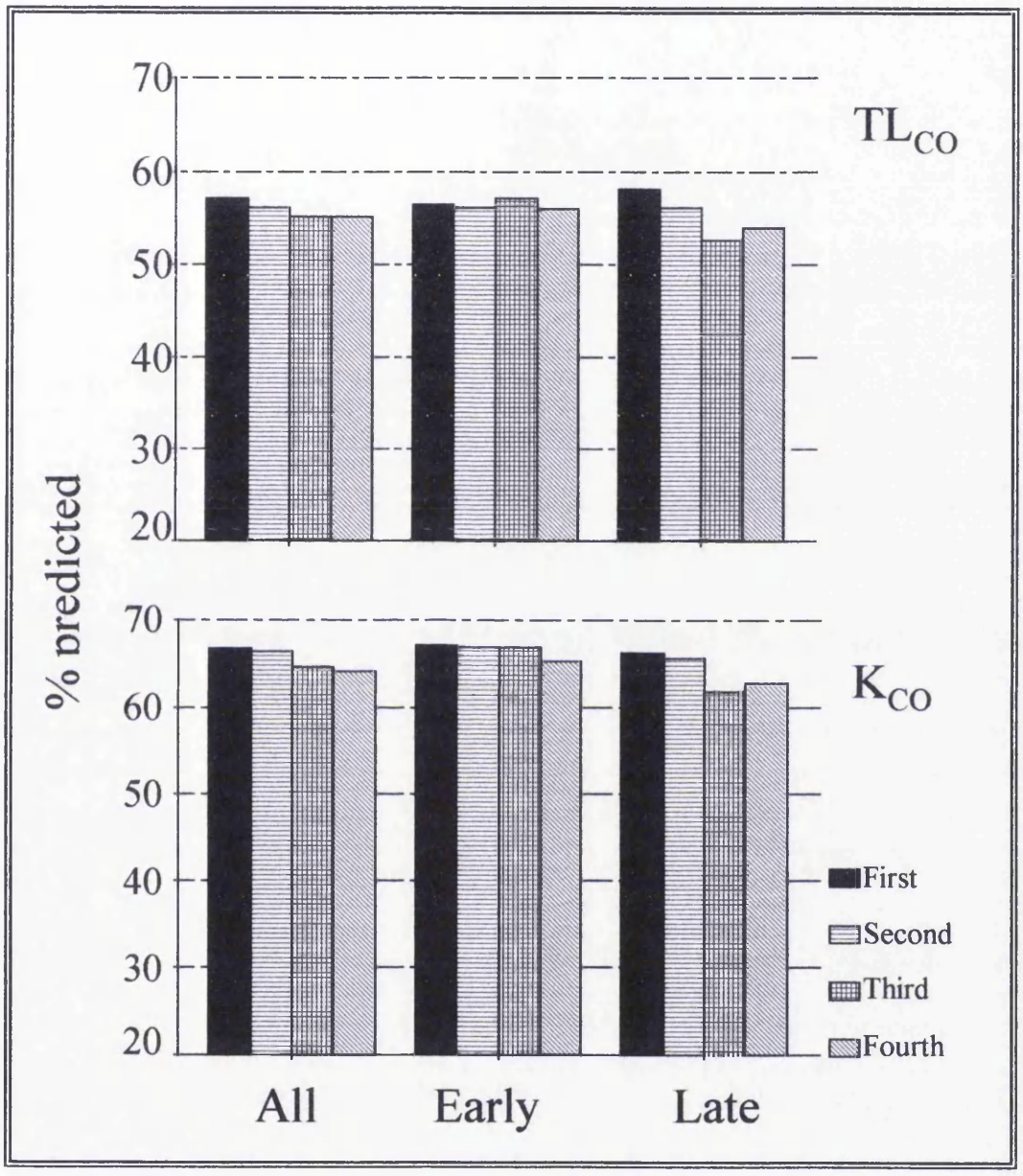


Figure 7.12: Post-transplant longitudinal changes in TL_{CO} and K_{CO} in 61 recipients (Group 2)



Thirty seven of the 81 heart transplant recipients studied, had both full pulmonary function assessment before and serially after transplantation (Group 3). Table 7.4 shows that their clinical characteristics were similar to the entire group of heart transplant recipients (Tables 7.1 and 7.3). The first pulmonary assessment after transplantation was at 6 weeks in 26 patients (early) and at 12 months in 11 patients (late). Apart from the difference in the number of patients in each sub-group, there was no significant difference between them in any of the listed parameters (Table 7.4).

Table 7.4: Clinical characteristics of 37 recipients (Group 3) with lung function results before and serially after transplantation

	All	*Early*	*Late*
Number of subjects	37	26	11
Age; mean in years (range)	49.2 (19-61)	47.8 (19-61)	50.0 (44-58)
Sex			
male	31 (84%)	21 (81%)	10 (91%)
female	6 (16%)	5 (19%)	1 (9%)
Smoking status			
non-smokers	10 (27%)	9 (35%)	1 (9%)
ex-smokers	27 (73%)	17 (65%)	10 (91%)
Diagnosis			
ischaemic heart disease	20 (54%)	14 (54%)	6 (55%)
dilated cardiomyopathy	15 (41%)	10 (38%)	5 (45%)
others	2 (5%)	2 (8%)	0
Pre-transplant LVEF, mean (SD)	13.2 (5.6)	13.3 (5.9)	13.0 (5.0)
post-transplant LVEF, mean (SD)	50.3 (8.3)	49.0 (8.7)	53.7 (6.3)

Figures 7.13 to 7.15 show that the changes in lung function in these patients were similar to what would be expected from the results in the previous 2 groups. Although there was a tendency for FEV_1, FVC and TLC to improve with time, this was not statistically significant. Sub-group plots show that FVC and TLC were lower than their pre-transplant values at 6 weeks after transplantation, but both exceeded their pre-transplant baseline in the subsequent interval. There were no consistent changes in RV or FEV_1/FVC.

Figure 11.15 shows that the decline in TL_{CO} and K_{CO} was evident at 6 weeks after transplantation with no further changes afterwards.

Figure 7.13: Longitudinal changes in FEV_1, FVC and FEV_1/FVC in 37 recipients (Group 3) with full results before and serially after transplantation

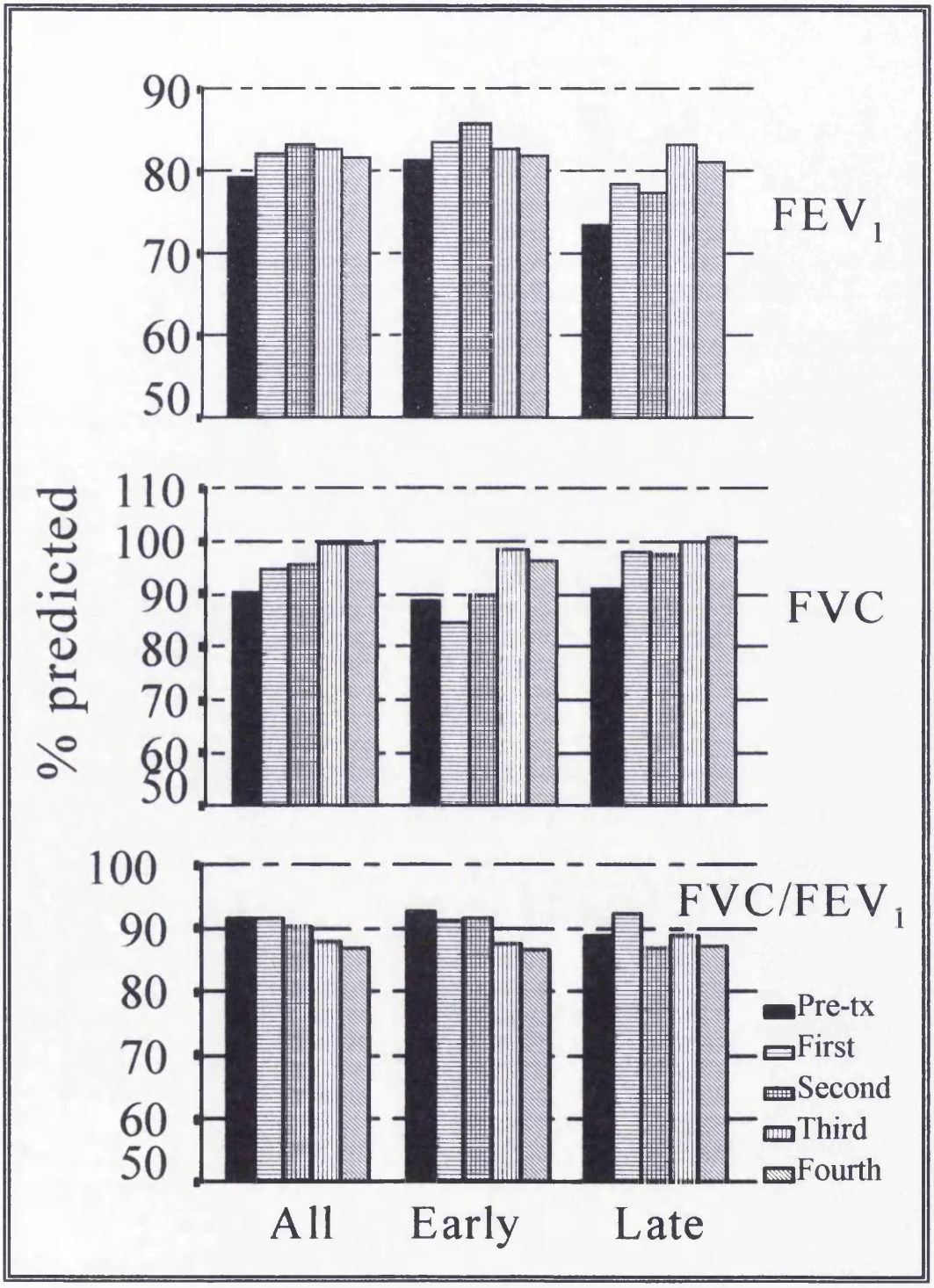


Figure 7.14: Longitudinal changes in RV and TLC in 37 recipients (Group 3) with full results before and after transplantation

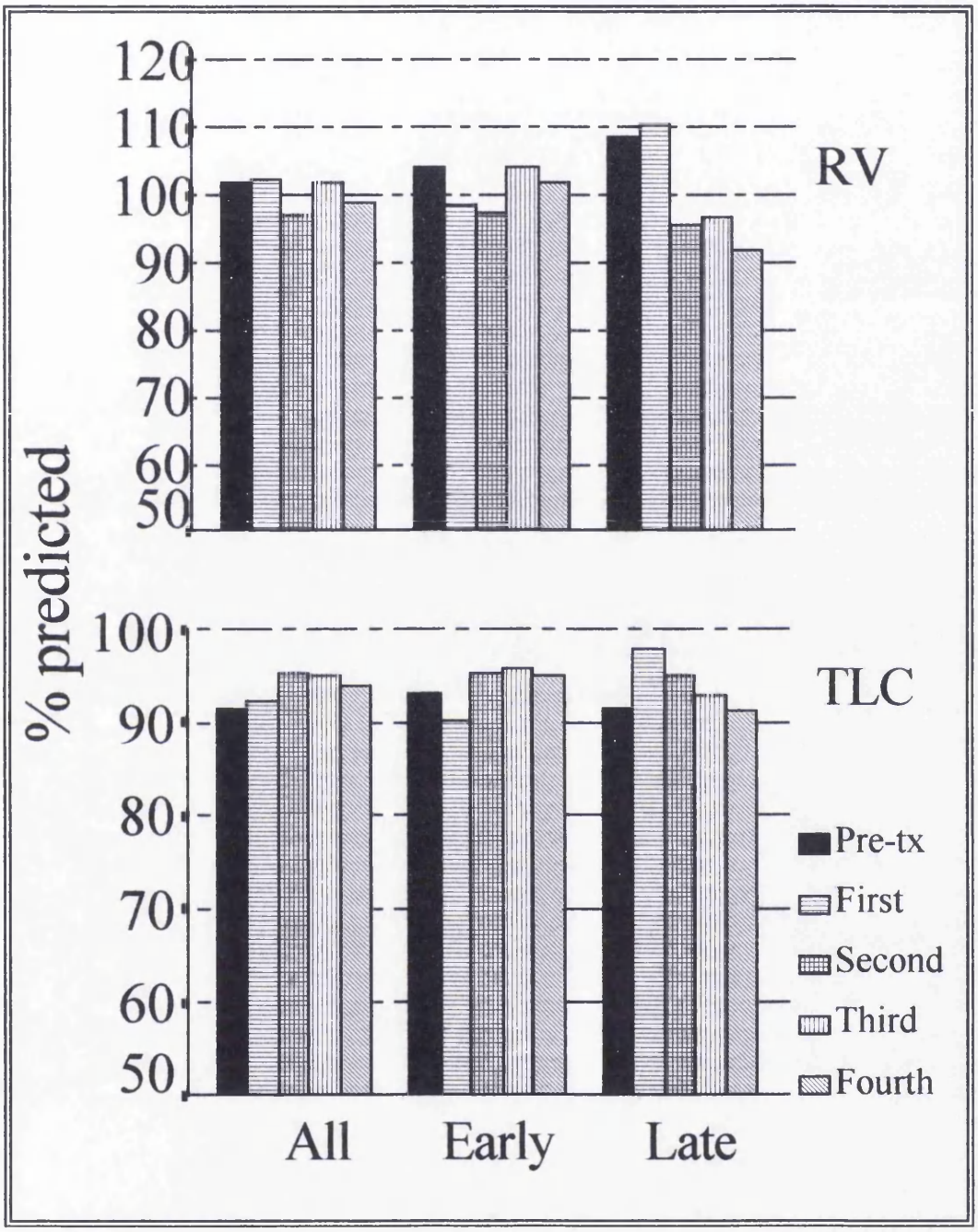
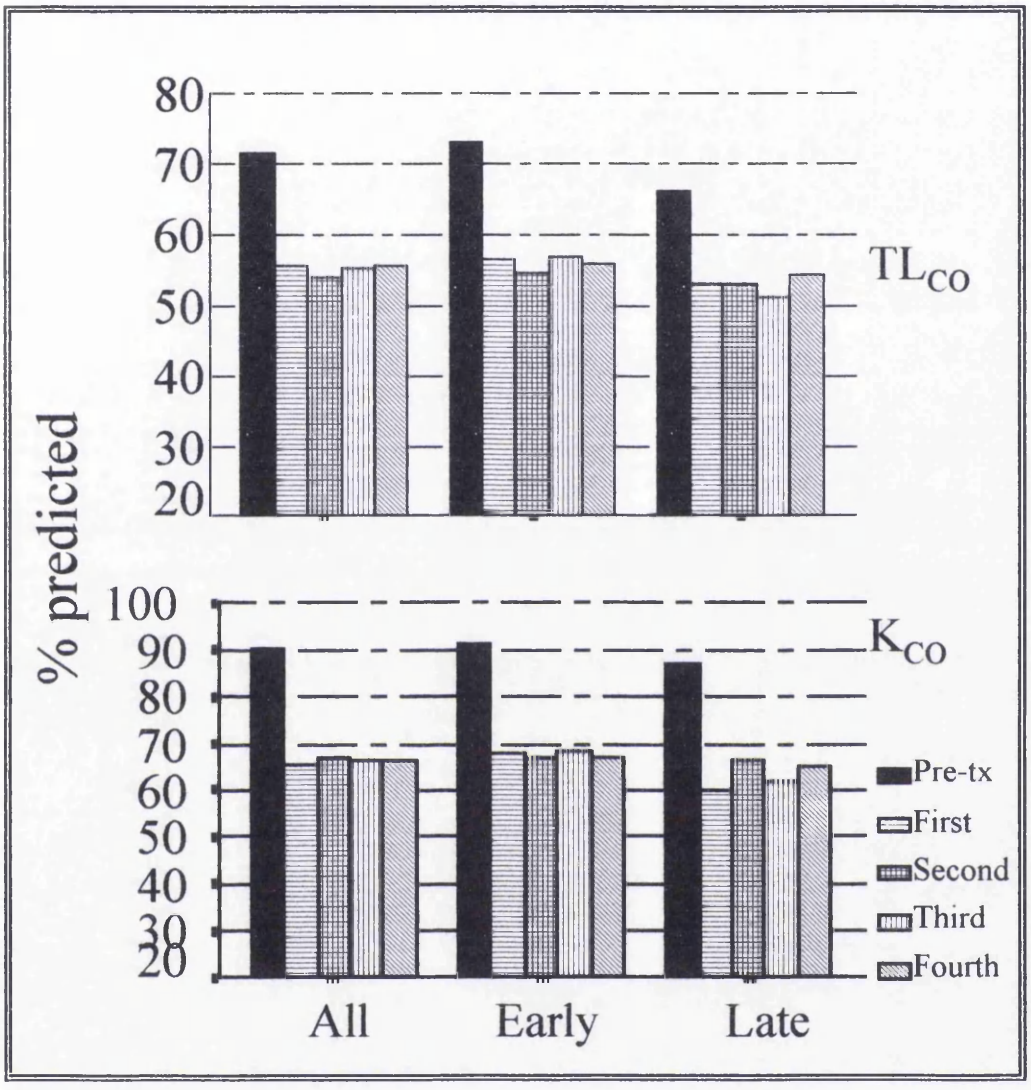


Figure 7.15: Longitudinal changes in TL_{CO} and K_{CO} in 37 recipients (Group 3) with full results before and serially after transplantation



7.4 Discussion

7.4.1 Summary of results

The findings of the studies comprising this chapter may be summarised as follows:

Despite the wide individual variability and the lack of significant change in any of the static and dynamic lung volumes following heart transplantation, this study demonstrated a consistent trend of change in the mean values of these parameters. Before transplantation there was a mild impairment of static and dynamic lung volumes. At 6 weeks after transplantation, there was a further reduction in FEV_1, FVC, RV and TLC, but all of these increased in the subsequent measurements to exceed their pre-transplant values at about one year after transplantation. However, all of these parameters were still significantly lower than in normal controls. TL_{CO} and K_{CO} decline was evident at 6 weeks after transplantation with no changes afterwards. In contrast to the wide variability in static and dynamic lung volumes, TL_{CO} and K_{CO} decline occurred in almost all patients with no significant difference between the early and the late assessment sub-groups.

7.4.2 Static and dynamic lung volumes

The changes in static and dynamic lung volumes demonstrated in this study were in agreement with previous reports (14,16,20). In 17 patients studied before and 15 +/- 10 months (mean +/- SD), Hosenpud and associates reported a significant improvement in FEV_1 and FVC following transplantation, but there was no change observed in FEV_1/FVC. Despite an overall improvement in lung volumes in this

small group of patients, those with smoking history continued to have sub-normal results. Plots of changes in individual patients demonstrated a wide variability similar to that of our study. Most patients had no change and most of the improvement in the mean values in FEV_1 and FVC appeared to be due to marked improvement in 4 patients.

In the longitudinal study of Groen et al. (16), FEV_1, FVC and TLC were all reduced before transplantation and all demonstrated significant improvement at one year after transplantation with no further changes in the subsequent 2 years. In contrast, FEV_1/FVC was normal before transplantation and did not change afterwards. In 100 heart transplant patients studied before and at different intervals (1 month to 10 years) after transplantation, Degre et al. (20) reported persistence of the pre-transplant restrictive ventilatory defect in patients assessed at one month after transplantation, but this was absent in those assessed at one year or thereafter.

To date there have been no reports on the changes in the residual volume (RV) in heart transplant patients. In patients with chronic heart failure, RV is usually preserved and may even be elevated despite decreases in other lung volumes (28,184). In the present study, mean pre-transplant RV was normal (104% of predicted). After transplantation, there was a tendency for RV to decrease, but this was not statistically significant. This may, in part, explain the lack of complete normalisation of TLC following transplantation in our patients.

The early changes in static and dynamic lung volumes reported in the present study are similar to those reported in patients following coronary artery bypass grafting

(CABG) (141,146) and valve replacement surgery (38). The decline in these parameters has been suggested to result from the adverse effects of sternotomy and cardio-pulmonary bypass (CPB) procedure on lung mechanics. In heart transplant recipients, these effects would tend to counteract the expected improvement in lung mechanics resulting from the relief of pulmonary congestion. After CABG, lung volumes and airway function abnormalities have been shown to resolve within 3 to 6 months (141,146). In our patients, the improvement in lung volumes and indices of airway function beyond this time to values higher than their pre-transplant may be due to the resolution of the pulmonary complications of chronic congestive heart failure following transplantation (e.g. pulmonary congestion and interstitial oedema, cardiomegaly and pleural effusions). However, the lack of complete normalisation of these parameters during 3 years of follow-up suggests residual sub-clinical pulmonary dysfunction in heart transplant recipients.

7.4.3 TL_{CO} changes

TL_{CO} changes during the first year after transplantation in the present study are in agreement with those recently reported by Bussieres et al. (182). In addition to confirming the findings of Bussieres and associates in a larger number of patients, the present study showed that the early decline in TL_{CO} and K_{CO} persisted during 3 years of follow-up. In the cross-sectional study of Degre et al. (20), TL_{CO} decline was observed in patients assessed at one month after transplantation and the magnitude of decline was similar to that found in patients assessed as late as 10 years after transplantation. Since the earliest post-transplant assessment in the present study was at 6 weeks, it is possible that the TL_{CO} decline occurred well

before this time, perhaps immediately after transplantation (e.g. intra-operatively or during the first few days). In patients undergoing conventional cardiac surgery including CABG, pulmonary dysfunction including TL_{CO} decline has been reported to be maximal within the first few hours after surgery (123,145). In addition, measurement of lung function before and one week after percutaneous transvenous mitral commissurotomy demonstrated TL_{CO} decline despite improvement of static and dynamic lung volumes (116).

The cause and the underlying mechanisms of TL_{CO} decline following heart transplantation have not been determined and these are the topics of the next 2 chapters of this thesis. The importance of the findings in this chapter lies in that they focus attention on the early post-transplant period as the time during which the potential causes of TL_{CO} decline should be sought. These include sternotomy and CPB, early post-operative pulmonary complications and the changes in pulmonary vascular pressures following transplantation. In CABG patients, sternotomy and CPB have been shown to be associated with early post-operative decline in TL_{CO}, but this usually resolves within 3 to 6 months after surgery (141,146). It is possible that the adverse effects of sternotomy and CPB are greater and or last longer in patients with long standing heart failure as compared to those undergoing CABG who usually have normal lungs.

In patients with congenital and valvular heart disease, TL_{CO} changes have been shown to be related to the changes in the pulmonary vascular pressures (30) and surgery for these disorders has been reported to be associated with reductions in both pulmonary vascular pressures and TL_{CO} (23,115). Cyclosporin pulmonary

toxicity (15,16) and CMV infection (21) have also been suggested as causes for TL_{CO} decline after heart transplantation. The possible roles of all of these factors are investigated in chapter 9.

7.5 Conclusions

From the findings of this study and from data in the literature on TL_{CO} changes following various forms of cardiac surgery, the following points may be made regarding TL_{CO} decline following heart transplantation:

1. The isolated decline in TL_{CO} in the face of improving lung volumes and airway function following transplantation is consistent with pulmonary vascular dysfunction.
2. The early and non-progressive nature of TL_{CO} decline suggest an aetiology occurring early after transplantation.
3. The resulting reduction in pulmonary haemodynamics immediately after transplantation appears to be a plausible cause. Before transplantation, the long-standing pulmonary congestion and the associated chronic pulmonary vascular and parenchymal changes would be expected to lead to TL_{CO} impairment by reducing both of its components (D_M and V_C). The reduction in pulmonary haemodynamics following heart transplantation, would be expected to decrease V_C and, therefore, may contribute to TL_{CO} decline after heart transplantation. This concept may be tested by the measurement of the TL_{CO} components in heart transplant patients (chapter 8).

CHAPTER 8

MECHANISMS OF TL_{CO} REDUCTION IN HEART TRANSPLANT RECIPIENTS

8.1 Introduction

Although TL_{CO} reduction in heart transplant recipients is well documented, the causes and mechanisms of this reduction remain unknown (15-21). It has been suggested that it might be due to a reduction in the diffusing capacity of the alveolar-capillary membrane (D_M) secondary to new insults to the membrane after heart transplantation (16,21). However, TL_{CO} components have not been determined in heart transplant recipients.

As has been noted in chapters 3 and 5, Roughton and Forster showed that the measurement of TL_{CO} at different alveolar oxygen tensions allows the estimation of the diffusing capacity of the alveolar-capillary membrane (D_M) and the instantaneous pulmonary capillary blood volume available for gas transfer (V_C) (5). According to Roughton and Forster, the relationship between TL_{CO} and its components is described by the following equation:

$$1/TL_{CO} = 1/D_M + 1/\theta V_C$$

where $1/TL_{CO}$ is reciprocal of the transfer factor for the entire lung and represents the total resistance of the lung to CO transfer, and by analogy $1/D_M$ represents the resistance of the alveolar-capillary membrane, and θV_C represents the resistance of the total mass of the erythrocytes in the capillary blood (intra-capillary resistance),

where, θ is the standard rate of at which one millilitre of whole blood takes up CO and this is dependent on the prevailing alveolar oxygen tension and haemoglobin concentration. In the conventional calculation of TL_{CO}, haemoglobin concentration is assumed to be normal (14.6 $g.dL^{-1}$) (41). The effect of haemoglobin variability on TL_{CO} values can be determined using a version of the classic Roughton and Forster equation as described by Cotes and recommended by both the European Respiratory Society and the American Thoracic Society (41,88);

$$1/TL_{CO} = 1/D_M + 1/([Hb] \times \theta V_C)$$

where [Hb] is the haemoglobin concentration as a fraction of normal (i.e. actual haemoglobin divided by 14.6). Thus, the application of Roughton and Forster model, permits the determination of the relative contribution of D_M, V_C, and blood haemoglobin to TL_{CO} changes. The findings in chapter 7 showed that TL_{CO} decline occurs early after heart transplantation. It was suggested that this may be due to a reduction in V_C secondary to normalisation of the pulmonary vascular pressures following heart transplantation.

Aim

The aim of the studies comprising this chapter was, therefore, to determine the mechanism of TL_{CO} reduction in heart transplant recipients in terms of the relative contribution of each of its individual components.

8.2 Methods

8.2.1 Study population

TL_{CO} and its components were determined in 75 heart transplant recipients at 6 weeks to 36 months after transplantation. The findings in heart transplant recipients were compared with data from 38 patients with severe chronic heart failure awaiting heart transplantation (candidates) and 26 normal subjects recruited as volunteers from the general population in whom there was no evidence of cardio-pulmonary disease. Table 8.1 shows the clinical characteristics of the 3 study groups.

Table 8.1 Clinical characteristics of heart transplant recipients compared to heart transplant candidates and normal controls

	Recipients	*Candidates*	*Normal*
Number of subjects	75	38	26
Age; mean in years (range)	47.7 (19-61)	50.6 (34-61)	47.3 (27-62)
Sex			
male	61 (81%)	30 (79%)	20 (77%)
female	14 (19%)	8 (21%)	6 (23%)
Smoking status			
non-smokers	17 (23%)	10 (26%)	21 (81%)
ex-smokers	58 (77%)	28 (74%)	5 (19%)
Diagnosis			
ischaemic heart disease	44 (59%)	25 (66%)	
dilated cardiomyopathy	27 (36%)	10 (26%)	
valvular heart disease	3 (5%)	3 (8%)	
Haemoglobin, $g.dL^{-1}$, mean (SD)	12.1 (1.4)	14.0 (1.7)	assumed 14.6
LVEF, mean (SD)	47.2 (10.4)	12.8 (6.3)	

Nine of the 75 heart transplant recipients had TL_{CO} and its components measured before and at six weeks after transplantation. The pre- and post-transplant findings in these patients were compared with results from 7 patients with mitral stenosis studied one day before and 6 weeks after mitral valve replacement (MVR). Table 8.2 show the clinical characteristics of these patients.

Table 8.2 Clinical characteristics of 9 heart transplant recipients with TL_{CO} components measured before and after transplantation compared to 7 mitral valve replacement patients (MVR)

	Transplant	*MVR*
Number of subjects	9	7
Age; mean in years (range)	49.7 (34-61)	53.7 (31-65)
Sex		
male	9 (100%)	3 (43%)
female	0	4 (57%)
Smoking status		
non-smokers	3 (33%)	3 (43%)
ex-smokers	6 (67%)	4 (57%)
Haemoglobin, $g.dL^{-1}$, mean (SD)		
before surgery	13.3 (1.3)	13.7 (0.8)
after surgery	11.6 (1.7)	12.2 (0.7)

8.2.2 Measurement of TL_{CO} and its components

TL_{CO} and its components were measured as described in chapter 5. The influence of haemoglobin concentration was estimated by comparing TL_{CO} results before and after correction for actual haemoglobin. Haemoglobin concentration in patients was

determined on the same day of TL_{CO} measurement using venous blood samples. Normal subjects were assumed to have normal haemoglobin concentration (i.e. 14.6 $g.dL^{-1}$).

8.2.3 Data presentation and analysis

TL_{CO} and its components were expressed as percentages of predicted using the European Community for Steel and Coal equations for TL_{CO} (41) and the reference values of Cotes for D_M and V_C (49). The total resistance to CO transfer ($1/TL_{CO}$) and its components ($1/D_M$ and $1/\theta V_C$) were expressed in absolute values ($Pa.min^{-1}.mmol^{-1}$). Unless stated otherwise, values were presented as mean +/- standard error of the mean (SEM). Comparisons between groups were performed using one-way analysis of variance (ANOVA), whereas comparisons within groups (e.g. TL_{CO} results before and after correction for haemoglobin in heart transplant recipients and candidates, and TL_{CO} and its components before and after surgery in heart transplant and mitral valve replacement groups) were performed using the paired samples Student's t-test. A p value of <0.05 was considered significant.

8.3 Results

8.3.1 TL_{CO} and its components in recipients compared to candidates and normal controls

Figure 8.1 shows TL_{CO} and its components in heart transplant recipients compared to candidates and normal controls. Mean TL_{CO} was significantly reduced in heart transplant recipients compared to heart transplant candidates (56.9 +/- 1.4% and

69.9 +/- 2.0% of predicted respectively, $p<0.001$) and in both it was significantly lower than that of normal controls (97.7 +/- 1.6% of predicted, $p<0.001$). Similarly, V_C was reduced in recipients compared to candidates (52.8 +/- 2.0% vs. 80.2 +/- 4.2% of predicted, $p<0.001$). In contrast, D_M was similar in heart transplant recipients and candidates (77.7 +/- 2.5% and 81.4 +/- 5.4% of predicted respectively, $p = 0.48$) and in both it was significantly lower than that of normal subjects (100.0 +/- 1.3% of predicted, $p<0.001$).

Figure 8.1 TL_{CO} and its components in recipients compared to candidates and normal controls

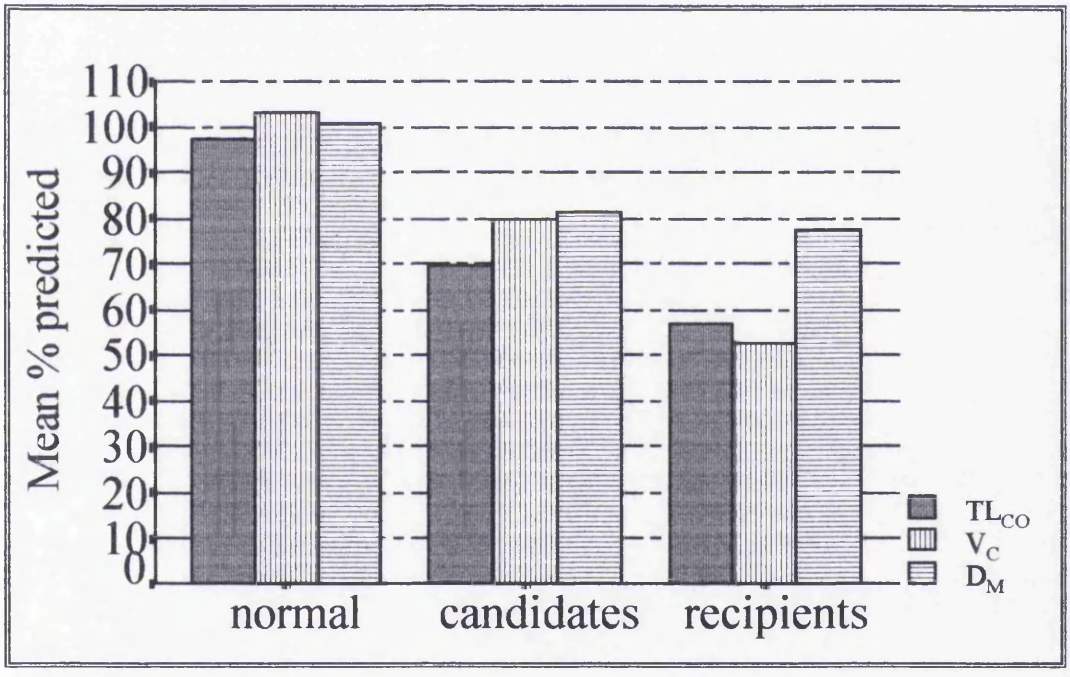


Figure 8.2 shows that the change in the TL_{CO} and its components following heart transplantation is independent of the time between transplantation and assessment.

Figure 8.2 Scatterplots of individual results of TL_{CO} and its components at different intervals after heart transplantation

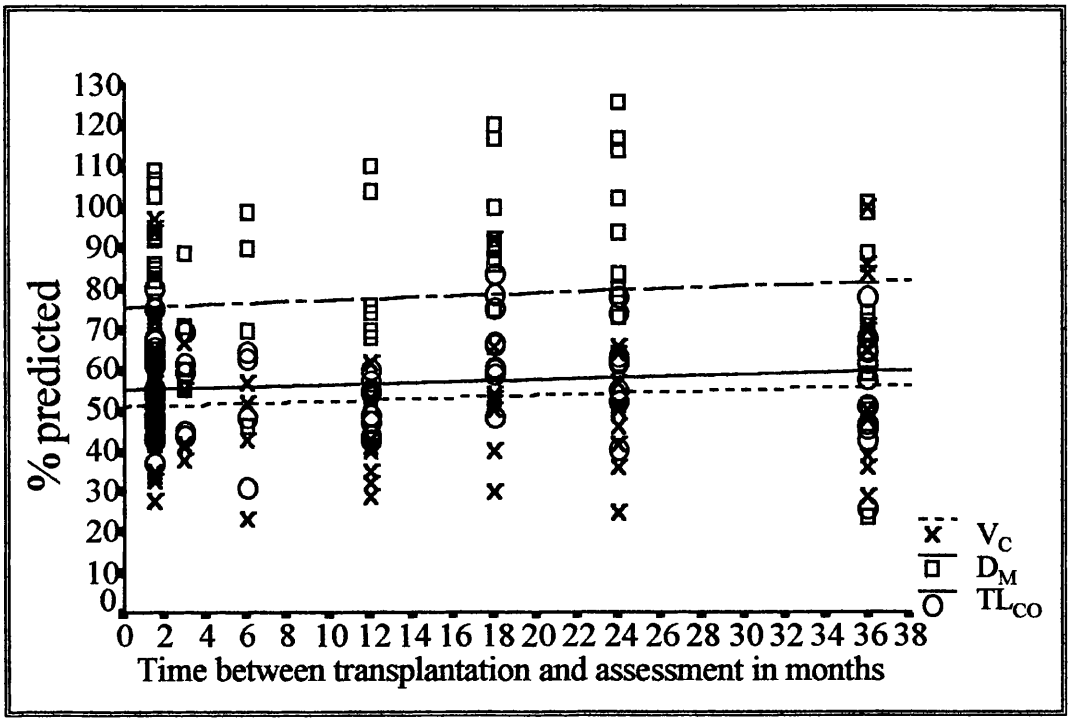


Figure 8.3 displays the diffusion parameters in terms of their reciprocals (i.e. resistance to diffusion) in the 3 study groups. The total resistance to CO transfer ($1/TL_{CO}$) was higher in heart transplant recipients compared to candidates (212.9 +/- 7.8 vs. 176.1 +/- 8.6 $Pa.min^{-1}.mmol^{-1}$., $p<0.001$) and in both it was higher than that of normal controls (113.9 +/- 3.8 $Pa.min^{-1}.mmol^{-1}$., $p<0.001$). The increase in $1/TL_{CO}$ in heart transplant candidates was due to a proportionate increase in both the alveolar-capillary membrane resistance ($1/D_M$) and the intra-capillary resistance

($1/\theta V_C$), being 88.4 +/- 6.3 and 87.7 +/- 5.0 $Pa.min^{-1}.mmol^{-1}$., respectively compared to 59.0 +/- 1.0 and 54.9 +/- 3.8 $Pa.min^{-1}.mmol^{-1}$., in normal subjects. The increase in $1/TL_{CO}$ in heart transplant recipients above that of candidates was entirely due to the marked increase in $1/\theta V_C$ (127.4 +/- 5.3 vs. 87.7 +/-5.0 $Pa.min^{-1}.mmol^{-1}$, $p<0.001$) with $1/D_M$ being similar in both groups (85.5 +/- 4.0 and 88.4 +/- 6.3 $Pa.min^{-1}.mmol^{-1}$, respectively, $p = 0.69$).

Figure 8.3 The total resistance to CO transfer ($1/TL_{CO}$) and its components ($1/D_M$ and $1/\theta V_C$) in recipients compared to candidates and normal controls

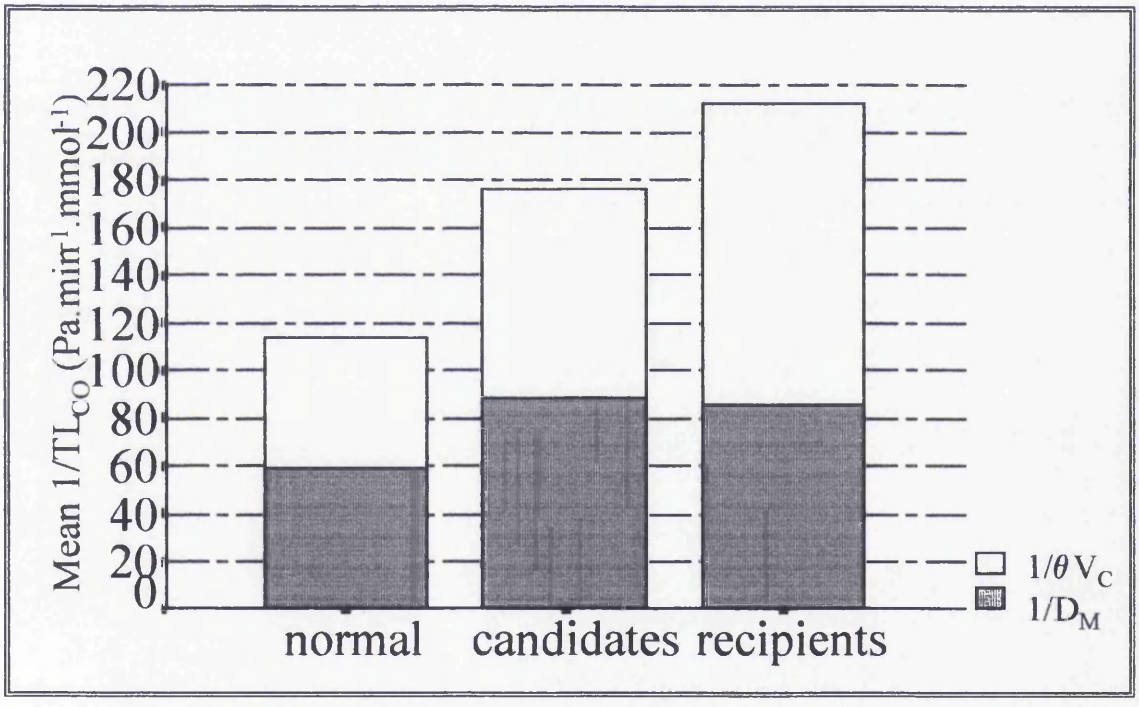
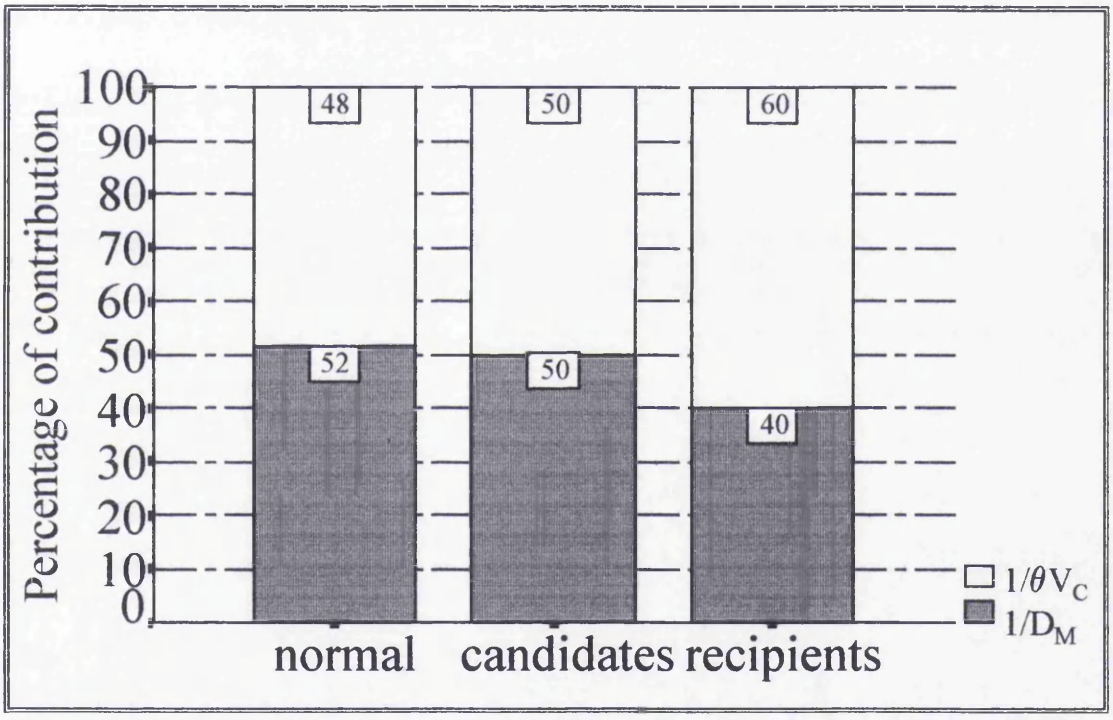


Figure 8.4 is a 100% stacked bar chart showing the relative contribution of the alveolar-capillary membrane resistance ($1/D_M$) and the intra-capillary resistance

($1/\theta V_C$) to the total resistance to CO transfer ($1/TL_{CO}$) in heart transplant recipients compared to candidates and normal controls. $1/D_M$ and $1/\theta V_C$ contributed equally to $1/TL_{CO}$ (approximately 50% each) in both normal subjects and heart transplant candidates. In contrast, $1/\theta V_C$ provided the main resistance to CO transfer in heart transplant recipients (60% of $1/TL_{CO}$).

Figure 8.4 100% stacked bar chart showing the relative contribution of $1/D_M$ and $1/\theta V_C$ to $1/TL_{CO}$ in recipients compared to candidates and normal controls



8.3.2 The influence of haemoglobin concentration

Figure 8.5 displays scatter plots of % predicted TL_{CO} against haemoglobin concentration in heart transplant recipients and candidates. Mean haemoglobin concentration in recipients was reduced compared to candidates (12.1 vs. 14.0 g.dL-1, $p<0.001$). There was a weak but significant correlation between % predicted TL_{CO} and haemoglobin concentration in recipients ($r = 0.27$, $p<0.05$), but there was no significant correlation between the two variables in candidates ($r = 0.16$, $p = 0.33$).

Figure 8.5 Scatter plots of % predicted TL_{CO} against haemoglobin concentration in heart transplant recipients and candidates

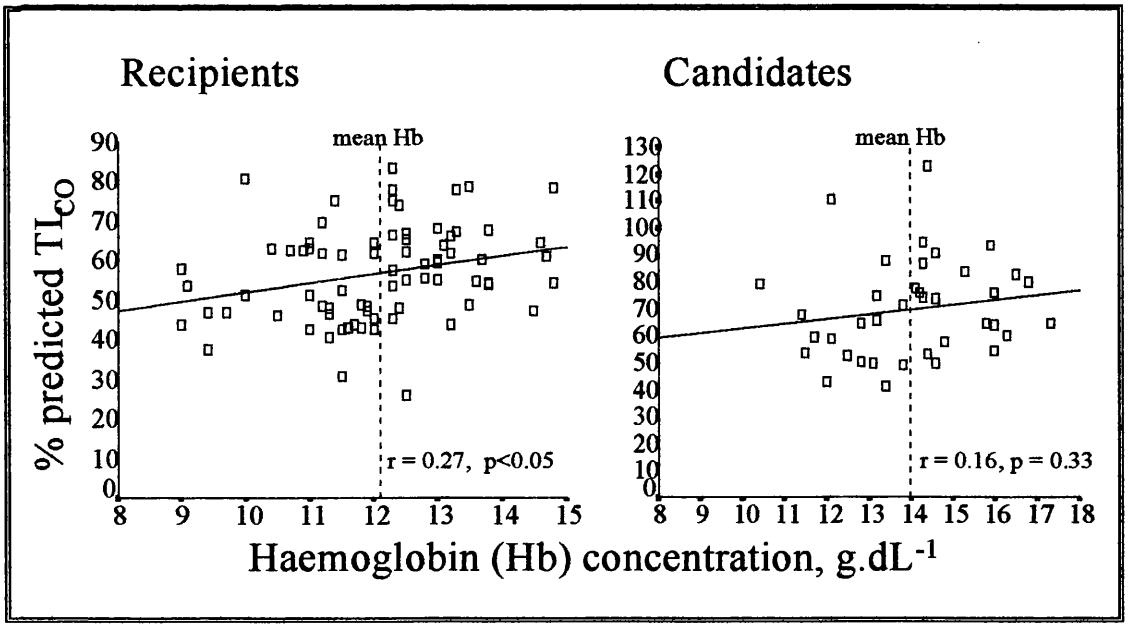
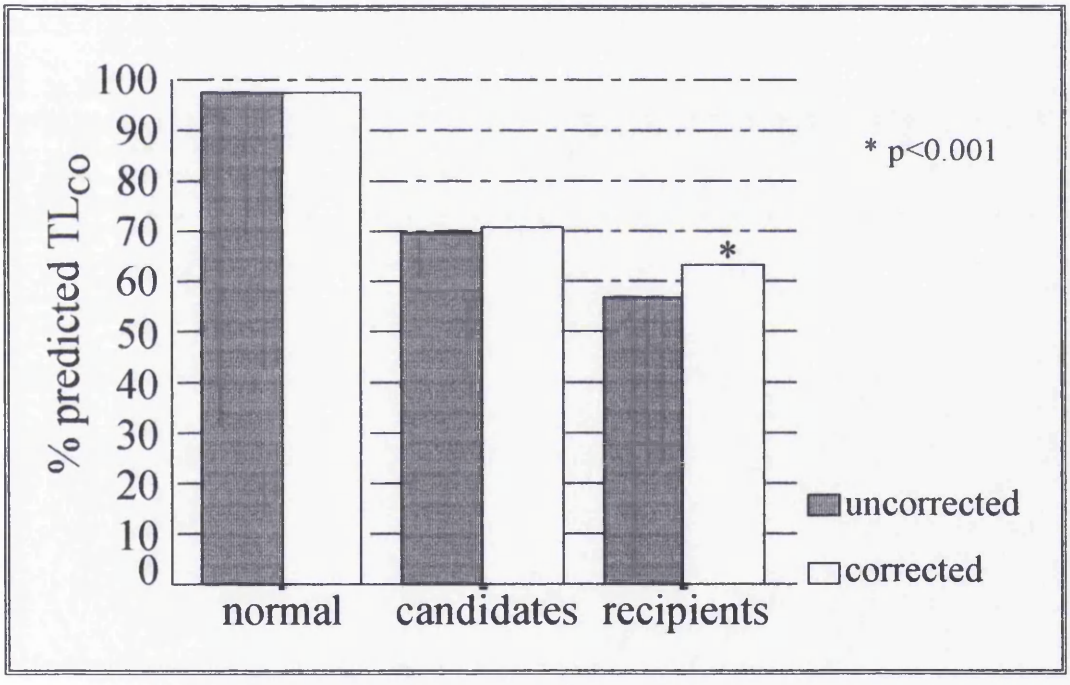


Figure 8.6 compares TL_{CO} as percentage of predicted before and after correction for actual haemoglobin concentration in the study groups. Correction for haemoglobin in heart transplant candidates produced no significant effect (69.9 +/- 3.0% vs. 71.1 +/- 3.1% of predicted, p = 0.09). In contrast, TL_{CO} in heart transplant recipients increased from 56.9 +/-1.4% to 63.5 +/- 1.5% of predicted ($p<0.001$), but this was still lower than that of candidates ($p<0.05$).

Figure 8.6 TL_{CO} in heart transplant recipients and candidates before and after correction for haemoglobin



In terms of resistance to CO transfer, the correction for actual haemoglobin concentration in heart transplant recipients reduced $1/TL_{CO}$ (from 212.9 +/- 7.8 to 190.0 +/- 7.0 $Pa.min.^{-1}mmol^{-1}$., <0.001) by reducing $1/\theta V_C$ (from, 127.4 +/- 5.3 to 104.5 +/- 4.4 $Pa.min.^{-1}mmol^{-1}$, $p<0.001$), but this was still significantly higher than that of heart transplant candidates (84.3 +/- 5.0 $Pa.min^{-1}.mmol^{-1}$, $p<0.01$) and normal subjects (54.9 +/- 3.8 $Pa.min.^{-1}mmol^{-1}$, $p<0.001$), both in absolute (Figure 8.7) and relative terms (Figure 8.8).

Figure 8.7 The total resistance to CO transfer ($1/TL_{CO}$) and its components ($1/D_M$ and $1/\theta V_C$) after correction for actual haemoglobin

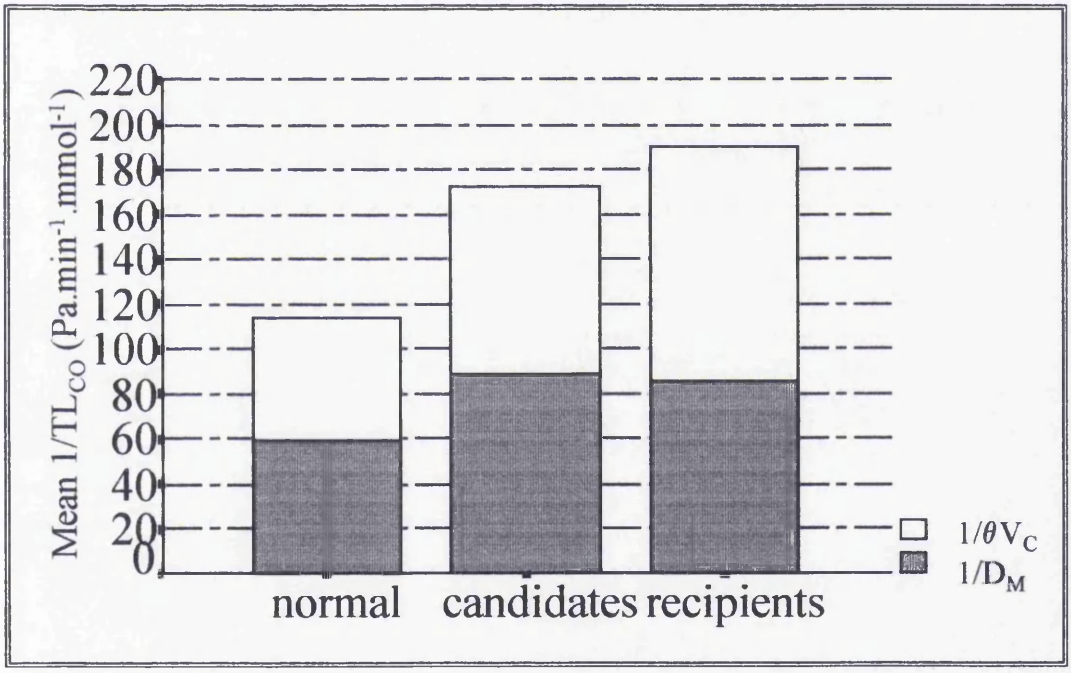
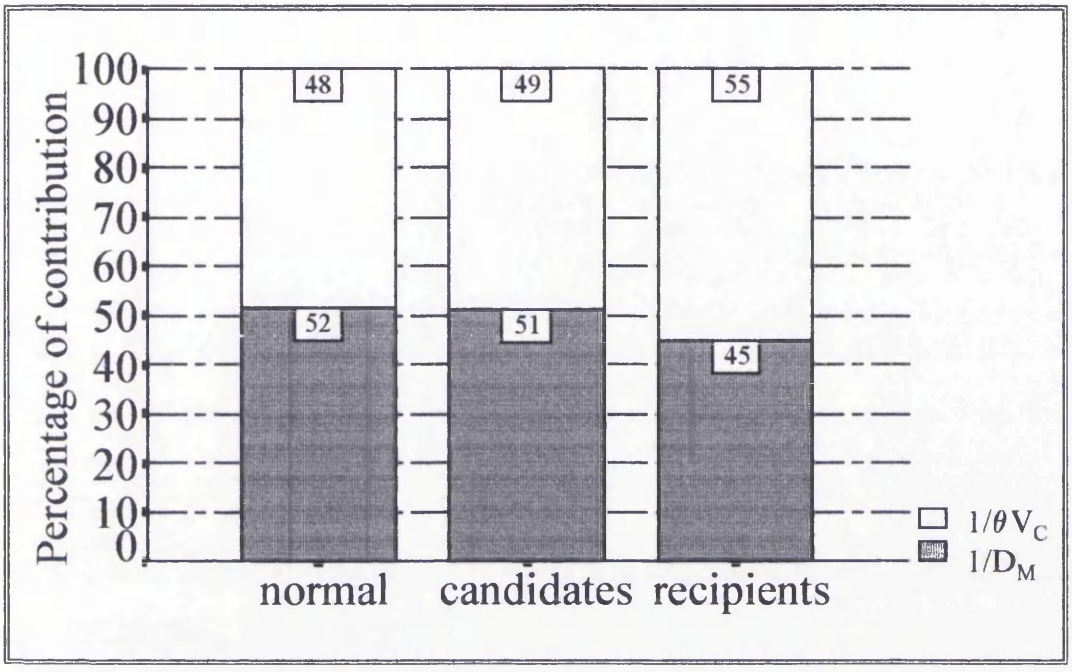


Figure 8.8 100% stacked bar chart showing the relative contributions of $1/D_M$ and $1/\theta V_C$ to $1/TL_{CO}$ in the 3 study groups after correction for haemoglobin



8.3.3 Comparison between heart transplant recipients and mitral valve replacement (MVR)

Table 8.3 shows the mean values of TL_{CO} and its components (% predicted) in 9 heart transplant recipients and 7 MVR patients before and at 6 weeks after surgery. Before surgery, TL_{CO} and D_M were similarly reduced in both groups of patients. V_C was also reduced in both groups, but it was significantly lower in MVR patients compared to heart transplant patients ($p<0.05$). Although, TL_{CO} and its components all declined in both groups after surgery, the decline in D_M was not statistically significant.

Table 8.3: Mean values of TL_{CO} and its components in heart transplant recipients compared to mitral valve replacement patients before and 6 weeks after surgery

	Recipients		*MVR*	
	Before	After	Before	After
TL_{CO}	70.0 (8.6)	53.7 (4.6)*	75.2 (6.6)	64.1(5.8)*
D_M	84.2 (12.1)	75.7 (6.4)	83.0 (5.4)	80.4 (7.3)
V_C	89.2 (9.6)	56.2 (8.7)*	75.3 (6.7)**	58.4 (6.7)*

* = significant difference between results before and after surgery in the same group, ** = significant difference between the two groups

Figure 8.9 shows that changes in diffusion parameters were also similar in the two groups. Although the magnitude of decline in TL_{CO} and V_C was higher in recipients compared to MVR patients, this difference was not statistically significant. The decline in D_M was small in both groups, and it was significantly greater in recipients compared to MVR patients.

Figure 8.9 Mean changes in TL_{CO} and its components in heart transplant recipients and mitral valve replacement patients 6 weeks after surgery

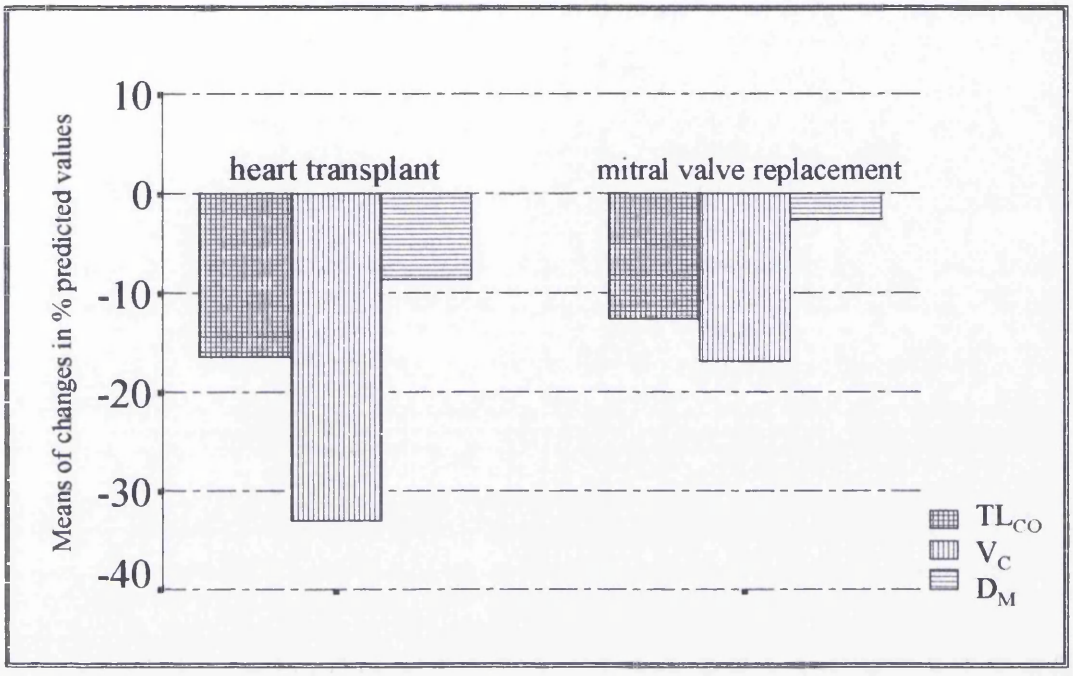
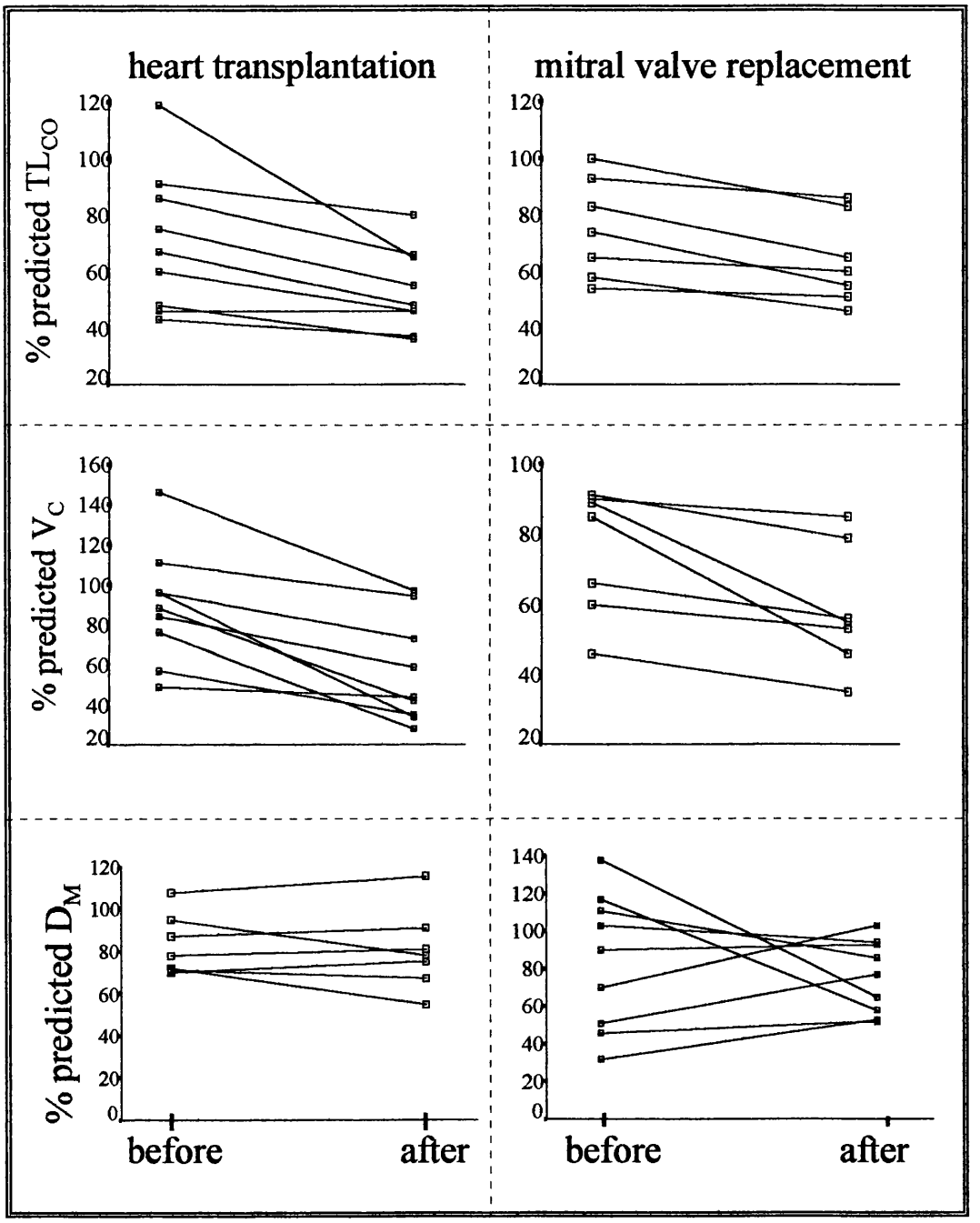


Figure 8.10 displays the results of TL_{CO} and its components in individual patients before and after surgery in the 2 study groups. Despite the wide range of values, all heart transplant recipients and mitral valve replacement patients had declines in TL_{CO} and V_C at 6 weeks after surgery. In contrast, there was no consistent trend in the changes in D_M in either of the 2 groups.

Figure 8.10 Changes in TL_{CO} and its components in individual patients 6 weeks after heart transplantation compared to mitral valve replacement



8.4 Discussion

8.4.1 Summary of results

The main findings of this study may be summarised in 4 points:

1. TL_{CO} reduction in patients with severe chronic heart failure (heart transplant candidates) was found to be due to a proportionate reduction in both of its components (D_M and V_C).
2. TL_{CO} reduction in heart transplant recipients beyond that of heart transplant candidates was due to an increase in the intra-capillary resistance ($1/\theta V_C$) with $1/D_M$ being similarly elevated in both recipients and candidates
3. The increase in $1/\theta V_C$ in heart transplant recipients was shown to be due to a combination of anaemia and reduced V_C.
4. The changes in TL_{CO} and its components after heart transplantation were similar to the changes following mitral valve replacements six weeks after surgery

8.4.2 TL_{CO} and its components in heart transplant candidates

The results of this study represent the first report on the changes in TL_{CO} components in heart transplant candidates. In addition to confirming TL_{CO} reduction in these patients, it was shown that this reduction was due to a proportionate reduction in both of its components (D_M and V_C). The lack of any effect for haemoglobin concentrations on TL_{CO} values is a reflection of their relatively normal haemoglobin concentration. In the only study of TL_{CO} components in patients with chronic congestive heart failure, Puri and associates

(9) reported that TL_{CO} reduction was common in this condition and was primarily due to a reduction in D_M with V_C being normal. The apparent discrepancy between these results and ours is probably due to the difference in severity of heart failure. In the study of Puri et al., patients had mild to moderately severe chronic heart failure with NYHA classes II and III and mean left ventricular ejection fraction (LVEF) of 33% whereas our patients, who were awaiting heart transplantation, had severe long-standing heart failure (mean LVEF = 12.8%). In patients with mitral stenosis D_M has been shown to decline progressively with increasing severity of the disease (32). In contrast, V_C has a biphasic relationship with disease severity (32). In mild to moderately severe mitral stenosis, V_C is usually normal and may even be elevated whereas in severe cases, it is reduced. The reduction in V_C has been suggested to be secondary to fibrosis and obliteration of the pulmonary vascular bed which are common in severe mitral stenosis (30,89). The finding of equally reduced D_M and V_C in our heart transplant candidates is similar to those of patients with severe mitral stenosis suggesting that these patients have significant pulmonary parenchymal and vascular abnormalities.

8.4.3 TL_{CO} and its components in heart transplant recipients

In addition to confirming previous reports of TL_{CO} reduction in heart transplant recipients (15-21), the present study identified the increase in the intra-capillary resistance ($1/\theta V_C$) as the underlying mechanism. The increase in $1/\theta V_C$ was shown to be due to a combination of anaemia and reduced V_C. Contrary to what has been suggested by previous investigators (16), D_M in heart transplant recipients was similar to that of candidates.

The finding of anaemia in heart transplant recipients was not unexpected (185,186). Possible cause include bone marrow suppression caused by immunosuppressive therapy, blood loss during surgery and repeated venous blood sampling. Although the effect of haemoglobin concentration on TL_{CO} measurement is well documented and correction for its effect when outside the normal range is recommended (41), this study is the first report on the relative contribution of anaemia to TL_{CO} reduction in heart transplant recipients. Mean TL_{CO} was lower in heart transplant recipients than heart transplant candidates by 13% of predicted and this difference was reduced to 8% of predicted after correction for haemoglobin. Thus, the difference in haemoglobin levels between recipients and candidates accounted for approximately 40% of the total difference in TL_{CO}. As D_M was similar in the 2 groups and V_C was significantly reduced in recipients compared to candidates, it appears reasonable to assume that the remaining difference (60% of the total difference) in TL_{CO} was due to the reduction in V_C in heart transplant recipients.

8.4.4 The mechanisms of V_C reduction in heart transplant recipients

The volume of the pulmonary capillary blood at any instant (V_C) depends on the number and dimensions of functioning pulmonary capillaries (89). The mechanisms by which the size of the pulmonary capillary bed is increased are not fully understood, but experimental and clinical studies suggest that it is caused by increases in pulmonary blood flow or pulmonary capillary transmural pressure, with the transmural capillary pressure appearing more important than the pulmonary blood flow (59). These haemodynamic factors are believed to increase the size and uniformity of the pulmonary capillary bed by vascular recruitment and distension

uniformity of the pulmonary capillary bed by vascular recruitment and distension (59). Therefore in any condition where pulmonary capillary transmural pressure or the pulmonary blood flow is increased (e.g. exercise, congenital heart disease with left to right shunts and early stages of mitral stenosis and congestive heart failure), V_C would be expected to be higher than normal (89). Under these circumstances, a normal or reduced V_C would suggest derangement of the pulmonary vascular bed (89).

The present study did not investigate the direct relationship between TL_{CO} components and the pulmonary haemodynamics in heart transplant patients. However, previous reports on the relationship between TL_{CO} in general and V_C in particular and pulmonary haemodynamics in patients with congenital and valvular heart diseases suggest an important role for the changes in pulmonary haemodynamics in the decline of V_C and TL_{CO} following heart transplantation. Congenital heart diseases which result in pulmonary vascular congestion and increased pulmonary blood flow without significant pulmonary arterial hypertension are associated with increased values of TL_{CO} and its components with V_C increasing relatively more than D_M (22,23). However, if pulmonary arterial hypertension or pulmonary vascular resistance is severe, these parameters are usually normal or reduced (22). Surgical correction of the congenital defects results in restoration of the pulmonary haemodynamics towards normal and this is associated with reduction in TL_{CO} and its components (23).

TL_{CO} in mitral valve disease has been shown to be related to the severity of pulmonary haemodynamics (30). In moderately severe mitral stenosis, the increase

in pulmonary venous pressure would be expected to increase V_C by expanding and increasing the uniformity of the pulmonary vascular bed. However, V_C is usually normal or even reduced in these cases (32). This was explained by an opposite force which counterbalances the augmenting effect of pulmonary congestion; namely, the obliteration of the pulmonary vascular bed by progressive fibrosis and repeated pulmonary emboli which are common in severe mitral stenosis (30,54). It follows therefore, that the relief of pulmonary congestion without concomitant restoration of the structural changes in the pulmonary vascular bed would be expected to reduce V_C. In keeping with this, mitral valve surgery has been shown to result in further decline in TL_{CO} (116-118) and this was found to be due to reduction in V_C with D_M remaining unchanged (118). The results of our MVR patients were in agreement with these reports.

Heart transplant and mitral valve patients have very similar haemodynamic profiles both before and after surgery (13,29). The fact that the changes in TL_{CO} and its components before and after transplantation were also very similar to those of patients undergoing mitral valve surgery suggests that TL_{CO} reduction has a common mechanism in both conditions. Like patients with mitral valve disease, V_C in patients with severe chronic heart failure awaiting heart transplantation is determined by two factors acting in opposite directions; the increased pulmonary venous pressure tending to increase it and the pulmonary fibrosis and destruction of the pulmonary vascular bed tending to decrease it. The finding of reduced V_C before transplantation indicates that these patients have significant structural pulmonary vascular abnormalities and the lack of any improvement in D_M after

volumes suggests that these structural abnormalities persist after transplantation. Thus, the reduction in the pulmonary vascular pressures without resolution of the pulmonary vascular abnormalities after heart transplantation may be the cause of V_C reduction which in addition to anaemia leads to TL_{CO} decline following heart transplantation.

Although the changes in pulmonary haemodynamics and anaemia appear to explain TL_{CO} reduction in heart transplant recipients, other factors may also have some influence. Cyclosporin pulmonary toxicity (15,16) and CMV infection have been proposed as possible factors (21). Other potential factors include sternotomy and cardio-pulmonary bypass procedure, post-operative pulmonary complications and cardiac allograft rejection.

8.5 Conclusions

The findings of this study suggest that TL_{CO} reduction in heart transplant recipients results from an increase in the intra-capillary resistance to CO transfer which is due to a combination of anaemia and reduction in the pulmonary capillary blood volume (V_C). Although the reduction in pulmonary vascular pressures after transplantation, in the face of persisting pulmonary vascular abnormalities of the pre-transplant severe chronic heart failure appears, a plausible explanation for the decline in V_C and therefore haemoglobin-corrected TL_{CO}, other potential contributory factors need to be evaluated.

CHAPTER 9

DETERMINANTS OF TL_{CO} DECLINE AFTER HEART TRANSPLANTATION

9.1 Introduction

In the preceding chapter, TL_{CO} decline after heart transplantation was shown to be due to a combination of anaemia and reduction in the pulmonary capillary blood volume (V_C). It was suggested that the reduction in V_C and, therefore, TL_{CO} was secondary to the reduction in pulmonary vascular pressures after heart transplantation. However, there are several other factors that potentially can be responsible for TL_{CO} decline after heart transplantation. These include: pre-transplant cardiac and lung function (10,11), cardio-pulmonary bypass (141), post-transplant pulmonary complications (42), CMV infection (21), cardiac allograft rejection (42), and cyclosporin pulmonary toxicity (15).

Aim

The aim of the studies comprising this chapter was to determine the responsible factors for TL_{CO} decline after heart transplantation.

9.2 Subjects and Methods

9.2.1 Subjects

A. Heart transplant patients

The influence of potential factors on TL_{CO} changes after heart transplantation was analysed in 57 patients who had pulmonary function assessment before and after transplantation. Thirty-two patients had their first post-transplant assessment at 6 weeks and the remaining 25 at one year following transplantation. The clinical characteristics of these patients have already been described in chapter 7 (Table 7.1).

B. Coronary artery bypass graft patients

The potential effects of cardio-pulmonary bypass on TL_{CO} were assessed by comparing the results in heart transplant patients with data from 15 (14 male) patients who had lung function tests before and at 6 weeks after coronary artery bypass graft surgery. The inclusion criteria of coronary artery surgery patients were as out-lined in chapter 4 (section 4.3.2). Their mean age was 54.7 years (range; 41-65 years). Three were life-long non-smokers, and 12 were ex-smokers.

9.2.2 TL_{CO} and other lung function tests

Methods of lung function tests were as previously described. Measured TL_{CO} was corrected for actual haemoglobin concentration using the equation of Dinakara and associates (187):

Haemoglobin-corrected TL_{CO} = Observed TL_{CO} / (0.06965 × Hb);

where Hb is the patient's actual haemoglobin and 0.06965 is a correction factor to a standard haemoglobin concentration of 14.4 $g.dL^{-1}$. This equation was derived from TL_{CO} measurement in 50 patients with haemoglobin concentrations ranging from 6.7 to 16.8 $g.dL^{-1}$ and is one of recommended equations by the American Thoracic Society (65). It was chosen in preference to the more commonly used equation of Cotes and associates (41,65);

Haemoglobin-corrected TL_{CO} = Observed TL_{CO} ([10.22 + Hb]/1.7 ×Hb)

This equation is based on the classic Roughton and Forster equation (41) and the assumption of a constant D_M/V_C ratio (D_M/V_C = 0.7 in traditional units). This assumption is not valid in heart transplant recipients due to a disproportionate reduction in V_C (in the 75 heart transplant recipients analysed in chapter 7, D_M/V_C ratio was 1.2).

K_{CO} was corrected for haemoglobin concentration from the following relationship:

corrected TL_{CO} /corrected K_{CO} = observed TL_{CO} /observed K_{CO}, hence:

Hb-corrected K_{CO} = Hb-corrected TL_{CO} × observed K_{CO} /observed TL_{CO}

9.2.3 Potential determinants of TL_{CO} decline after heart transplantation

A. Pre-transplant lung function

All patients had complete pre-transplant lung function tests as previously out-lined at an average of 22 weeks before transplantation (range; 1-54 weeks).

B. Pre-transplant cardiac status

The severity of heart failure was assessed by left ventricular ejection fraction (LVEF) duration of exertional dyspnoea and New York Heart Association (NYHA) functional status. LVEF was measured by radionuclide imaging at the time of lung function assessment. Pulmonary haemodynamics were measured by the referring cardiologists at various centres as part of routine assessment for possible heart transplantation. They were all performed within 3 months (range 1-13 weeks) of pre-transplant lung function assessment and included mean pulmonary artery pressure (mPAP), mean pulmonary capillary wedge pressure (mPCWP) and the transpulmonary gradient (TPG). Data on cardiac output and pulmonary vascular resistance were incomplete and were therefore, not included in this analysis.

C. Post-transplant pulmonary complications

For the purpose of this study, a pulmonary complication after heart transplantation was defined by symptoms and signs occurring during the period between transplantation and lung function assessment, which were directly attributable to lower respiratory tract involvement and required specific respiratory treatment, other than routine post-operative chest physiotherapy. Lower respiratory tract involvement was often confirmed by microbiological studies, imaging, or both. Pneumonia was diagnosed if a specific organism was identified in the sputum or broncho-alveolar lavage fluid analysis in a patient with new pulmonary infiltrates on the chest radiograph. Radiographic infiltrates and respiratory symptoms which were

not associated with a definable organism or any specific histopathology were classified according to the working diagnosis documented in patients' case notes.

D. Cytomegalovirus (CMV) infection

CMV infection was accepted if there was laboratory evidence of viral replication in the surveillance specimens and CMV disease was defined as the combination of CMV infection with a compatible clinical picture and laboratory evidence of organ dysfunction in absence of other causes (188). In addition to data from surveillance specimens, the incidence of asymptomatic pulmonary CMV infection was prospectively evaluated by serial bronchoalveolar lavage (BAL) fluid analysis during the first three months after transplantation. The protocol and procedure of BAL were as out-lined in chapter 5 (section 5.4).

E. Cardiac allograft rejection

The diagnosis, grading and treatment of cardiac allograft rejection in our centre have been described in chapter 4. In this analysis, the number of rejection episodes refers to the number of rejections with histological grades 2 or higher occurring during the period between transplantation and post-transplant lung function measurement.

F. Cyclosporin blood levels

Cyclosporin whole blood trough levels were routinely determined in all heart transplant recipients according to the following protocol:

- 0-4 weeks - twice a week

- 4-6 weeks - weekly
- 6-12 weeks - fortnightly
- 3-6 months - monthly
- 6-12 months - 3 monthly
- 1-2 years - 6 monthly
- > 2 years - yearly

For each patient, the level at the time of lung function assessment and the mean value of all measurements performed between transplantation and lung function assessment were used to determine the influence of cyclosporin on TL_{CO} changes after heart transplantation.

G. Other factors

The influence of the following factors on TL_{CO} changes after heart transplantation were also evaluated: sex, smoking status, cardiac diagnosis (ischaemic versus dilated cardiomyopathy) and cardio-pulmonary bypass time.

9.2.4 Data presentation and analysis

The change in lung function parameters after heart transplantation was calculated by subtracting the % predicted value before transplantation from that after transplantation. As a result, positive values represent increases and negative values represent reductions after transplantation. The relationship between the change in TL_{CO} after heart transplantation and continuous variables (e.g. pre-transplant lung function and pulmonary haemodynamics) was determined for each factor using the Pearson correlation. To determine the relationship between the change in TL_{CO} and categorical variables (e.g. sex, presence and absence of pulmonary complications),

patients were first sub-divided into 2 groups on the basis of the presence and absence of each of the factors under study. The difference between the 2 sub-groups in TL_{CO} change for each category was then assessed using the independent samples Student's t-test. The independent effects of factors showing significant association ($p<0.05$) with the change in TL_{CO} were determined by stepwise multiple regression analysis.

9.3 Results

9.3.1 Comparison between heart transplant recipients and coronary artery bypass graft (CABG) patients

Table 9.1 shows lung function results in 15 patients before and at 6 weeks after CABG. Before surgery, static and dynamic lung volumes were all above 90% of predicted. Six weeks after surgery, there was a significant reduction in FVC, FEV_1 and TLC with no significant change in either RV or FEV_1/FVC. TL_{CO} was slightly reduced before surgery and decreased further after surgery. Pre-operative K_{CO} was normal and did not change after surgery. Mean haemoglobin concentration decreased from 14.9 +/- 0.3 before surgery to 13.0 +/- 0.2 afterwards. Correction of the observed TL_{CO} and K_{CO} for haemoglobin concentrations before and after surgery showed that the apparent decrease in TL_{CO} at 6 weeks after CABG was entirely due to the reduction in haemoglobin (Hb-corrected TL_{CO} increased from 84.3% to 87.9% of predicted). Similarly, Hb-corrected K_{CO} increased from 96.7% to 106.2% of predicted after surgery.

Table 9.1: Lung function results in 15 patients before and 6 weeks after coronary artery bypass graft

	before	*after*	*P value*
FEV_1	93.8 (4.8)	81.8 (4.4)	<0.001
FVC	103.8 (3.7)	90.0 (3.5)	<0.001
FEV_1/FVC	92.4 (2.6)	93.1 (3.1)	ns
RV	92.3 (9.8)	89.2 (5.4)	ns
TLC	93.1 (3.0)	85.9 (4.0)	<0.05
observed TL_{CO}	87.8 (3.3)	79.2 (3.0)	<0.01
Hb-corrected	84.3 (2.7)	87.9 (3.3)	ns
observed K_{CO}	100.8 (4.4)	95.7 (4.8)	ns
Hb-corrected K_{CO}	96.7 (3.7)	106.2 (5.3)	<0.05

Table 9.2 compares lung function results in 32 heart transplant recipients with the 15 CABG patients before and at 6 weeks after surgery. Before surgery, FEV_1, FVC and observed TL_{CO} were significantly reduced in recipients compared to CABG patients. Although Hb-corrected TL_{CO} and K_{CO} (mean pre-transplant haemoglobin concentration was 13.9 +/- 0.2 decreasing to 12.1 +/- 0.2 after transplantation) were lower in recipients compared to CABG patients, the difference was not statistically significant. Six weeks after surgery, the pre-operative difference between the two groups in FEV_1 and FVC disappeared due to a greater reduction in these two parameters after CABG. In contrast, the difference between the 2 groups in TL_{CO} and K_{CO} widened, especially after correction for haemoglobin.

Table 9.2: Pulmonary function results in 32 heart transplant recipients compared to 15 coronary artery bypass graft patients before and 6 weeks after surgery

	Before surgery			*6 weeks after surgery*		
	Recipients	*CABG*	*p value*	*Recipients*	*CABG*	*P value*
FEV_1	80.9 (2.3)	93.8 (4.8)	<0.01	80.4 (2.4)	81.8 (4.4)	ns
FVC	94.0 (2.6)	103.8 (3.7)	<0.05	93.4 (3.0)	90.0 (3.5)	ns
RV	105.2 (8.1)	92.3 (9.8)	ns	101.2 (5.1)	89.2 (5.4)	ns
TLC	95.0 (3.2)	93.1 (3.0)	ns	89.8 (2.6)	85.9 (4.0)	ns
observed TL_{CO}	71.9 (3.2)	87.8 (3.3)	<0.01	52.6 (2.2)	79.2 (3.0)	<0.001
Hb-corrected	76.3 (3.5)	84.3 (2.7)	ns	64.6 (3.0)	87.9 (3.3)	<0.001
observed K_{CO}	88.8 (3.6)	100.8 (4.4)	ns	62.8 (2.6)	95.7 (4.8)	<0.001
Hb-corrected K_{CO}	93.7 (4.0)	96.7 (3.7)	ns	77.0 (3.8)	106.2 (5.3)	<0.001

The changes in lung function parameters in recipients and CABG patients after surgery are shown in Table 9.3. At 6 weeks after surgery, there was a reduction in all measures of lung volume and airway function in both groups. However, the decrease in heart transplant recipients was minimal with almost no change in mean FEV_1 and FVC. The magnitude of reduction in these 2 parameters was significantly greater after CABG. The reduction in TLC was also greater after CABG, but the difference was not statistically significant. The observed TL_{CO} and K_{CO} were also reduced after surgery in both groups, but the decline in these indices was significantly greater after heart transplantation. Correction for haemoglobin concentration reduced the magnitude of change in TL_{CO} and K_{CO} after surgery in both groups. Hb-corrected TL_{CO} and K_{CO} were the only parameters which did not decline 6 weeks after CABG.

Table 9.3: The change (Δ) in lung function parameters (expressed in % of predicted) at 6 weeks after surgery: comparisons between heart transplant recipients and coronary artery bypass graft (CABG) patients

	Recipients (n=32)	*CABG (n=15)*	*P value*
Δ FEV_1	-0.6 (2.7)	-12 (2.4)	<0.01
Δ FVC	-0.7 (2.8)	-13.8 (2.1)	<0.01
Δ RV	-3.3 (8.0)	-2.4 (7.0)	ns
Δ TLC	-4.9 (2.9)	-7.2 (2.6)	ns
Δ observed TL_{CO}	-19.4 (2.4)	-8.7 (2.5)	<0.01
Δ Hb-corrected TL_{CO}	-11.8 (2.9)	+3.6 (2.9)	<0.01
Δ observed K_{CO}	-25.0 (2.8)	-5.1 (3.20	<0.001
Δ Hb-corrected K_{CO}	-16.1 (3.5)	+9.5 (3.3)	<0.001

Cardio-pulmonary bypass time was significantly longer in recipients compared to CABG patients (136.1 +/- 4.7 and 85.8 +/- 6.1 minutes, respectively, p<0.001), but there was no relationship between cardio-pulmonary bypass time and the change in TL_{CO} in any of the 2 groups.

9.3.2 The incidence and types of CMV complications after heart transplantation

Table 9.4 shows the individual results of the BAL-CMV study in 15 consecutive heart transplant recipients during the first 3 months after transplantation. A total of 30 BALs were performed with no complications and all patients were free of respiratory symptoms during the period of study. Two patients were matched negative for CMV (both donor and recipient were negative for CMV). The

the 15 patients, only one patient had laboratory evidence of CMV infection. In this patient, serum IgM was positive at 2 weeks with the other specimens, including the BAL, being negative at this stage. At 6 and 12 weeks after transplantation all specimens from this patient showed evidence of CMV infection, but he remained asymptomatic. Except for serum IgM, the concordance between BAL and conventional surveillance specimen (buffy coats, urine and throat swabs) was 100%.

Table 9.4: Individual results of 30 CMV-BAL studies in 15 heart transplant recipients

patient number	BAL number	CMV status		Interval (weeks)	Results				
		Donor	Recipients		BAL	IgM	Buffy coats	Th. swab	Urine
1	1	+	+	2	-	+	-	-	-
	2			6	+	+	+	+	+
	3			12	+	+	+	+	+
2	1	-	+	2	-	-	-	-	-
	2			6	-	-	-	-	-
	3			12	-	-	-	-	-
3	1	+	+	2	-	-	-	-	-
	2			6	-	-	-	-	-
4	1	+	+	2	-	-	-	-	-
	2			6	-	-	-	-	-
5	1	-	+	2	-	-	-	-	-
	2			6	-	-	-	-	-
	3			12	-	-	-	-	-
6	1	+	+	2	-	-	-	-	-
	2			6	-	-	-	-	-
7	1	+	+	6	-	-	-	-	-
8	1	+	-	2	-	-	-	-	-
9	1	+	+	2	-	-	-	-	-
	2			6	-	-	-	-	-
	3			12	-	-	-	-	-
10	1	+	+	6	-	-	-	-	-
11	1	-	-	2	-	-	-	-	-
	2			6	-	-	-	-	-
	3			12	-	-	-	-	-
12	1	-	+	2	-	-	-	-	-
13	1	-	-	2	-	-	-	-	-
14	1	+	-	2	-	-	-	-	-
15	1	+	+	6	-	-	-	-	-

Table 9.5 shows the incidence and types of CMV complications in heart transplant recipients during the period between heart transplantation and lung function measurement. In the entire group of 57 patients, only 5 patients (9%) developed laboratory evidence of CMV infection during the first 6 weeks (all between 5 to 6 weeks) after transplantation and only one of these was symptomatic. In the 25 patients with one year follow up, 15 patients (60%) developed evidence of CMV infection with 12 of these becoming symptomatic (9 non-pulmonary and 3 pulmonary). All of the 3 CMV pneumonias developed beyond 6 weeks (8, 13, and 18 weeks) after transplantation.

Table 9.5: The incidence and types of CMV complications in the period between heart transplantation and lung function assessment (6 weeks in 32 patients and 12 months in 25 patients)

	6 weeks (n=32)	*12 months (n=25)*	*all (n=57)*
CMV infection	3 (9%)	15 (60%)	18 (32%)
CMV disease (all types)	1 (3%)	12 (48%)	13 (23%)
pulmonary	0	3 (12%)	3 (5%)
non-pulmonary	1 (3%)	9 (36%)	10 (18%)

9.3.3 Pulmonary complications after heart transplantation

Table 9.6 shows the incidence and types of pulmonary complications in the study group during the time between heart transplantation and lung function assessment. In the entire group, 20 patients (35%) developed pulmonary complications as defined previously at an average of 11.4 weeks after transplantation. Four of the 13

complications in the long follow up group were within the first 6 weeks after transplantation and therefore, the total number of patients with a pulmonary complication during this period was 11 patients (19%).

Table 9.6: The incidence and type of pulmonary complications in the period between heart transplantation and lung function assessment(6 weeks in 32 patients and 12 months in 25 patients)

	6 weeks (n=32)	*12 months (n=25)**	*all (n=57)*
Pneumonia	3 (9%)	8 (32%)	11 (19%)
Acute bronchitis	4 (13%)	4 (16%)	8 (14%)
pulmonary embolism	0	1 (4%)	1 (2%)
Total	7 (22%)	13 (52%)	20 (35%)
Mean interval (weeks)	3.3 (1-6)	15.7 (1-44)	11.4 (1-44)

* In the long follow-up group, 4 patients (16%) had pulmonary complications during the first 6 weeks after transplantation.

9.3.4 Determinants of the change in TL_{CO} after heart transplantation

Table 9.7 shows the Pearson correlation coefficients between the change in TL_{CO} (corrected for haemoglobin) and a selected number of pre- and post-transplant factors which could potentially influence TL_{CO} in the setting of heart transplantation. Of the listed factors, only three pre-transplant variables showed a significant correlation with the change in TL_{CO} after heart transplantation. These were the pre-transplant TL_{CO}, K_{CO} and mean PCWP. Figures 9.1-9.3 display the

scatterplots of these factors against the change in TL_{CO} after heart transplantation. For each of these variables, the higher the value, the greater the decline in TL_{CO}.

Table 9.7: The relationship between the change in TL_{CO} after heart transplantation and selected pre- and post-transplant factors

Variables	*Mean (SEM)*	*r* value*	*P value*
Pre-transplant lung function (% of predicted) (57 patients)			
FEV_1	86.0 (2.0)	0.04	ns
FVC	93.3 (2.0)	-0.09	ns
TLC	92.7 (2.2)	-0.07	ns
Hb-corrected TL_{CO}	75.1 (2.4)	-0.55	<0.001
Hb-corrected K_{CO}	93.5 (2.9)	-0.30	<0.05
Pre-transplant pulmonary pressures (mmHg) (55 patients)			
Mean pulmonary artery pressure	28.8 (1.2)	-0.22	ns
Mean pulmonary capillary wedge pressure	19.9 (1.0)	-0.27	<0.05
Transpulmonary gradient	8.8 (0.4)	0.08	ns
Pre-transplant cardiac status (57 patients)			
Duration of dyspnoea (months)	37.7 (4.2)	0.04	ns
NYHA functional class	3.7 (0.4)	-0.08	ns
Left ventricular ejection fraction (LVEF)	13.4 (0.6)	0.25	ns
Cardio-pulmonary bypass time (minutes)	136.1 (4.7)	0.19	ns
Cyclosporin blood level ($mcg.L^{-1}$)			
Absolute level			
At 6 weeks (32 patients)	464.0 (23.4)	-0.05	ns
At 12 months (25 patients	302.7 (20.3)	0.19	ns
Mean of total blood levels			
At 6 weeks (32 patients)	522.2 (19.4)	0.16	ns
At 12 months (25 patients	482.7 (28.9)	-0.06	ns
Number of rejection episodes			
At 6 weeks (32 patients)	1.0 (0.2)	0.23	ns
At 12 months (25 patients)	2.7 (0.3)	0.29	ns
Post-transplant LVEF (57 patients)	45.1 (0.8)	0.05	ns

* r ; denotes Pearson correlation coefficient

Figure 9.1: Scatterplot of the relationship between the change in TL_{CO} after heart transplantation and the pre-transplant TL_{CO}

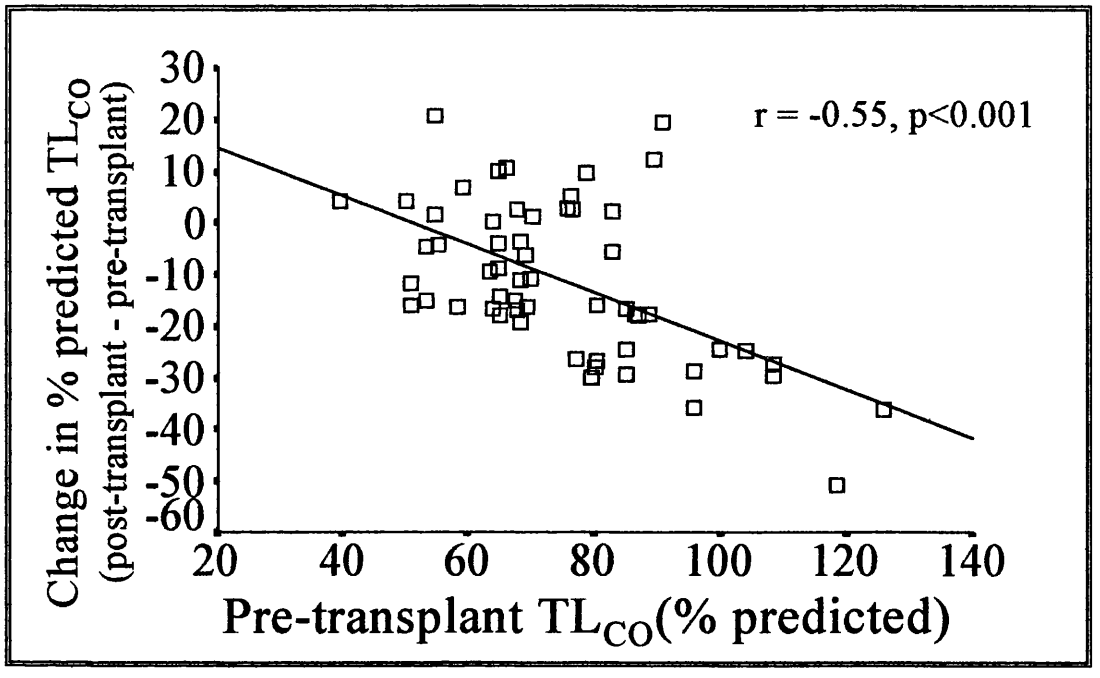


Figure 9.2: Scatterplot of the relationship between the change in TL_{CO} after heart transplantation and the pre-transplant K_{CO}

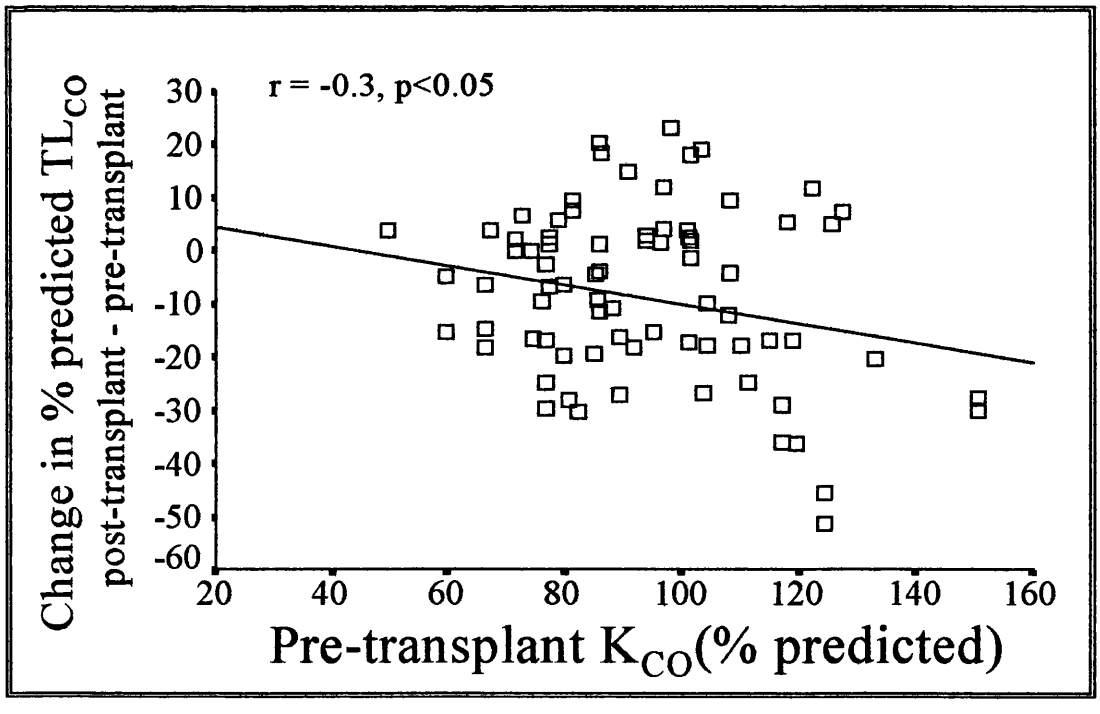


Figure 9.3: Scatterplot of the relationship between the change in TL_{CO} after heart transplantation and the pre-transplant pulmonary capillary wedge pressure

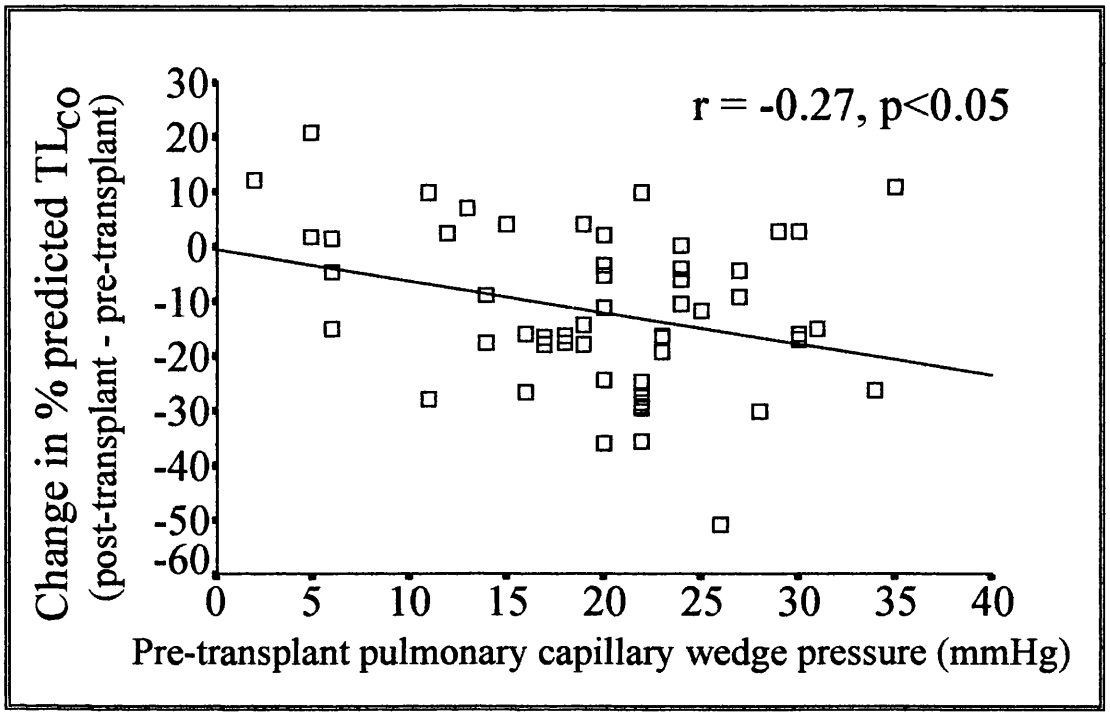


Table 9.8 compares the change in TL_{CO} after heart transplantation in patients sub-divided on the basis of the presence and absence of selected categorical factors which could potentially influence the change in TL_{CO} after heart transplantation. The change in TL_{CO} after heart transplantation appears to be independent of factors such as sex, pre-transplant smoking status, the primary cardiac diagnosis, the time of post-transplant TL_{CO} measurement, and post-transplant problems such as pulmonary complications, CMV infection and episodes of cardiac allograft rejection.

Table 9.8: Comparisons between the changes in TL_{CO} (ΔTL_{CO}) after heart transplantation in patients grouped on the basis of selected pre- and post-transplant categorical factors

	no. of patients	*ΔTL_{CO} Mean (SEM)*	*P value*
Sex			
Male	46 (81%)	-11.0 (2.3)	
Female	11 (19%)	-11.6 (4.1)	ns
Smoking status			
Non-smoker	12 (21%)	-11.7 (4.9)	
Ex-smoker	45 (79%)	-11.2 (2.2)	ns
Pre-transplant cardiac diagnosis			
Ischaemic heart disease	34 (60%)	-11.7 (2.3)	
Dilated cardiomyopathy	20 (40%)	-11.3 (4.0)	ns
Time of post-transplant assessment			
6 weeks	32 (56%)	-11.5 (2.9)	
12 months	25 (44%)	-9.6 (2.6)	ns
Pulmonary complication			
Present	20 (35%)	-12.2 (3.6)	
Absent	37 (65%)	-10.6 (2.4)	ns
CMV infection			
Present	18 (32%)	-11.0 (3.9)	
Absent	39 (68%)	-11.2 (2.3)	ns
CMV disease			
Present	13 (23%)	-8.6 (4.6)	
Absent	44 (77%)	-11.9 (2.2)	ns
CMV pneumonia			
Present	3 (5%)	-8.8 (7.6)	
Absent	54 (95%)	-11.3 (2.1)	ns
Cardiac allograft rejection			
Present	47 (82%)	-10.8 (1.9)	
Absent	10 (18%)	-12.9 (7.2)	ns

9.4 Discussion

9.4.1 Summary

The results of the studies in this chapter show that the reduction in haemoglobin-corrected TL_{CO} after heart transplantation is related to the pre-transplant TL_{CO} and the pre-transplant PCWP. The change in TL_{CO} after heart transplantation was shown to be independent of factors such as pre-transplant static and dynamic lung volumes, pre-transplant cardiac status, cardio-pulmonary bypass, cyclosporin blood levels and post-transplant complications such as pulmonary complications, CMV infection, and episodes of cardiac allograft rejection.

9.4.2 Potential determinants of TL_{CO} decline after heart transplantation

A. Pre-transplant lung function

As has been noted previously, lung function abnormalities in heart transplant candidates are common (10,11). These include restrictive and obstructive ventilatory defects and a significant reduction in TL_{CO}. In conventional cardiac surgery, patients with greater impairment of pre-operative lung function have been shown to be at higher risk of post-operative pulmonary complications (137). However, Bussieres and associates (160) did not find any relationship between pre-transplant lung function and survival in 32 patients during the first year after heart transplantation. The influence of pre-transplant pulmonary function on post-transplant lung function is not well documented. In the study of Groen et al. (16), the percentage reduction in K_{CO} after transplantation was greater in patients with

respiratory crackles compared with those without crackles before transplantation. It was suggested that patients with clinical evidence of pulmonary oedema experience greater reduction in K_{CO} after transplantation. In the same study, the percentage change in K_{CO} was positively correlated with pre-transplant K_{CO} (patients with higher pre-transplant K_{CO} had greater decreases in K_{CO} after transplantation), but the relationship between the post-transplant decline in K_{CO} and other indices of pre-transplant lung function was not reported. In another study of 22 heart transplant patients, Ohar et al. (19) found no relationship between the change in TL_{CO} and the changes in static and dynamic lung volumes after heart transplantation. The results of the present investigation are in agreement with the findings of the above 2 studies. The change in TL_{CO} after transplantation was correlated with the pre-transplant TL_{CO} and K_{CO}, with patients with higher pre-transplant TL_{CO} and K_{CO} having greater decline in TL_{CO} after transplantation. In contrast, there was no correlation between the change in TL_{CO} and other pre-transplant indices of lung function which included FEV_1, FVC and TLC. The lack of any significant correlation between the change in TL_{CO} and the pre-transplant static and dynamic lung volumes may be due to the fact that these were only mildly reduced before transplantation. Patients with severe lung function abnormalities and those with significant co-existing primary lung disease are usually excluded in the selection process of heart transplant candidates. This may also explain the absence of any relationship between pre-transplant lung function and outcome after heart transplantation reported by Bussieres et al. (160). The relationship between pre-transplant TL_{CO} and its change after transplantation may be due to the known

relationship between TL_{CO} and pulmonary haemodynamics in states of pulmonary venous hypertension (31,32). The link between the changes in TL_{CO} and pulmonary vascular pressures in our patients is suggested by the finding that the pre-transplant mean PCWP predicts the magnitude of TL_{CO} decline after heart transplantation.

B. Cardiac function before and after transplantation

By definition, heart transplant candidates have severe end-stage heart disease. The majority of these patients have severe long-standing heart failure with LVEF less than 20% (42). They are also characterised by mild to moderate pulmonary arterial hypertension secondary to chronically elevated end-diastolic left ventricular pressure (161), Previous studies have failed to show any relationship between the decline in TL_{CO} after heart transplantation and any of the indices of pre-transplant cardiac function including LVEF, duration of symptoms and cardiomegaly (16,19,21). The results in this chapter are in agreement with these findings. There was no relationship between the decline in TL_{CO} and any of the measures of pre-transplant cardiac status which included LVEF, duration of dyspnoea, and NYHA functional class. The change in TL_{CO} was also independent of the pre-transplant primary cardiac diagnosis. In addition, the failure to demonstrate any relationship between the change in TL_{CO} and either the post-transplant LVEF or the number of cardiac rejection episodes is consistent with the findings of Egan and associates (21).

C. Sternotomy and cardio-pulmonary bypass

The effects of coronary artery bypass graft (CABG) surgery on lung function are well documented and the relevant investigations have been reviewed in chapter 3 (section 3.3.3). The changes in lung function in our CABG patients were similar to previous reports (141,146). Before surgery, there was a minimal reduction in static and dynamic lung volumes with a greater reduction in TL_{CO}. Six weeks after surgery, there was a further reduction in all indices of lung function including the measured TL_{CO} and K_{CO}. However, when TL_{CO} and K_{CO} were corrected for haemoglobin concentration which declined after surgery, the post-operative values of these 2 parameters exceeded their pre-operative values. Therefore, the reduction in TL_{CO} after CABG appears to be due to the post-operative decline in haemoglobin concentration which was common in our patients. Previous reports did not give any information regarding correction for the effects of haemoglobin on the measured TL_{CO}. In agreement with previous studies, there was no relationship between the length of cardio-pulmonary bypass and the post-operative change in TL_{CO}.

Another interesting finding in this analysis was that the reduction in static and dynamic lung volumes at 6 weeks after surgery was greater in CABG patients than in heart transplant recipients. It has been suggested that the reduction in lung volumes and indices of airway function after CABG may be secondary to the effects of sternotomy and cardio-pulmonary bypass on lung and chest wall mechanics (133). The expected decline in static and dynamic lung volumes from the

effects of sternotomy and cardio-pulmonary bypass in heart transplant recipients was probably counterbalanced by the improvement in these parameters resulting from the resolution of pulmonary congestion and oedema after heart transplantation. Although our CABG patients were not assessed beyond 6 weeks after surgery, previous investigators have shown that the early post-operative decline in lung function gradually improves with almost complete resolution within 4 to 6 months after surgery. The persistence of TL_{CO} decline after heart transplantation together with the lack of any decline in haemoglobin-corrected TL_{CO} after CABG suggests that the decline in TL_{CO} after heart transplantation is unlikely to be due to the effects of sternotomy or cardio-pulmonary bypass.

D. Post-transplant pulmonary complications

Pulmonary complications are common after heart transplantation and are due to both infectious and non-infectious causes (42,159). Non-infectious pulmonary complications include pulmonary atelectasis, pleural effusions and left phrenic nerve paralysis resulting in left hemi-diaphragmatic dysfunction in the immediate post-transplant period (42). These complications are common and similar to those reported in other forms of cardiac surgery (132). They usually resolve within the first few weeks after transplantation in most patients (42). Pulmonary oedema is also common in the immediate post-transplant period and is believed to be related to the pre-operative pulmonary congestion together with the effects of cardio-pulmonary bypass and pre-transplant renal impairment (42). Pulmonary infections are the most common infectious complication after heart transplantation occurring

in 20 to 40% of patients (159,190,191). A wide variety of common and opportunistic infections have been reported and the majority of these infections occur within the first 4 months after transplantation (159,190,191). Post-transplant pulmonary complications are therefore a potential cause for the reported decline in TL_{CO} after heart transplantation. Although data on pulmonary complications were based on a retrospective review of the clinical notes, the incidence of infectious complications found in this limited analysis was comparable to previous reports (159,190). Post-transplant pulmonary complications as defined in this study appear to have no relationship to the decline in TL_{CO} after heart transplantation.

E. Cytomegalovirus infection

Cytomegalovirus (CMV), a member of the herpesvirus group, is the most common opportunistic pathogen in transplant patients (188). It is estimated that 50 to 80% of adults carry this virus, but it is virtually always asymptomatic in the immunocompetent host. When the host becomes immunocompromised, either by disease or immunosuppressive therapy as in transplant recipients, CMV infection can become a serious problem (188). The clinical manifestations of symptomatic CMV infection (i.e., CMV disease) are very variable and range from a self-limiting febrile illness to severe life-threatening multi-organ disease (188).

Pneumonia is the most serious pulmonary complication of CMV infection in heart transplant recipients and has been reported in 15% of patients, and associated with high mortality (42,159). The use of ganciclovir has reduced the mortality rate of CMV pneumonia in heart transplant patients from 46 - 75% to 15 - 20% (42). In

bone marrow transplant patients, where CMV pneumonia is much more common and serious, sub-clinical CMV infection of the lungs, where the virus is isolated from the bronchoalveolar lavage in absence of any respiratory symptoms, has been shown to be a major risk factor for the subsequent development of CMV pneumonia (42).

Asymptomatic CMV infection has been implicated in the pathogenesis of a variety of complications in heart transplant recipients, including increased incidence of cardiac allograft rejection and atherosclerosis, increased risk of other infections and TL_{CO} reduction (21,42). The incidence of sub-clinical pulmonary CMV infection in heart transplant recipients has not been determined. The association between asymptomatic CMV infection and TL_{CO} decline after heart transplantation was suggested by Egan et al. (21). Thirteen of their 22 patients had serological evidence of CMV infection and despite absence of respiratory symptoms, the mean percentage reduction in TL_{CO} in this sub-group of patients was greater (31%) than in patients who had no evidence of CMV infection (16%), but this difference was not statistically significant ($p = 0.06$). The decline in TL_{CO} did not show any correlation with cardiac allograft rejection, cyclosporin levels, or cardio-pulmonary haemodynamics. It was suggested that the decline in TL_{CO} following heart transplantation may represent an additional marker of CMV infection and may reflect an infective or immune injury to the lung in these patients. In an earlier study, van Son and associates investigated the relationship between CMV infection and K_{CO} in 24 renal transplant patients who had normal chest radiographs, normal arterial blood gas analysis, and no respiratory symptoms (192). TL_{CO} was measured

using the rebreathing method starting in the second week after transplantation and once a week thereafter for three months. During the study period, 12 patients developed evidence of CMV infection and in these patients mean K_{CO} was significantly lower than that of the remaining patients (0.83 and 1.28 mmol.min.$^{-1}$ kPa^{-1}, respectively). It was concluded that CMV infection in renal transplant patients was the cause of the observed pulmonary dysfunction.

Although the findings of these two studies suggest an association between CMV infection and the decline of TL_{CO} and K_{CO} in heart and renal transplant recipients, this does not necessarily establish a causal relationship. Both studies had a small number of patients and the evidence of CMV infection was derived from non-pulmonary specimens. In addition, the study of Egan et al. was retrospective, and TL_{CO} was measured at different post-transplant intervals which would preclude the establishment of any temporal association between CMV infection and TL_{CO} decline after heart transplantation. It is possible that TL_{CO} had declined for other reasons well before the development of CMV infection.

In the present analysis, the BAL-CMV study represents the first report on the incidence of asymptomatic pulmonary CMV infection during the first 3 months after heart transplantation. It showed that asymptomatic pulmonary CMV infection was uncommon during this period (only one of 15 patients). Perhaps of more importance was the finding that when the BAL fluid was positive for evidence of CMV infection, all the conventional specimens (urine, buffy coats and throat swabs) were also positive. This finding is in keeping with the hypothesis that during an episode of CMV viraemia, the virus reaches various organs where it replicates

causing sub-clinical systemic infection manifested by virus shedding into various body secretions (e.g. urine and BAL fluid) (193,194). Because of the frequent isolation of CMV in asymptomatic patients, the diagnosis of CMV disease requires evidence of organ damage or dysfunction in addition to laboratory evidence of virus replication (188).

The overall incidence of CMV infection in our heart transplant recipients is similar to previous reports (42). The finding in our patients that a previous history of CMV pneumonia was not associated with greater decline in TL_{CO} after heart transplantation is in agreement with results from Ettinger and associates (195) in double-lung transplant patients. In their study, the longitudinal changes (before and at 1, 3, 6 and 12 months after transplantation) in lung function in 10 patients with documented CMV pneumonia were compared with those of 7 patients who never had any evidence of CMV infection or pneumonia (both donors and recipients were CMV-negative). There was no significant difference between the 2 groups in the changes of lung function parameters including TL_{CO} during the period of follow-up. The authors concluded that documented CMV pneumonia after double lung transplantation was not associated with any adverse effects on lung function.

The findings in the studies of this thesis provide evidence against the implication of CMV infection in the aetiology of TL_{CO} decline after heart transplantation. First, CMV infection was uncommon during the first 6 weeks after transplantation. On the other hand, TL_{CO} decline was almost universal and evident at 6 weeks after transplantation with no further changes afterwards. Second, there was no difference in TL_{CO} changes in patients with and without evidence of CMV infection and the

changes in TL_{CO} in patients who recovered from documented CMV pneumonia were similar to those who did not have this complication. Finally, the similarity between heart transplant recipients and patients undergoing mitral valve replacement, who are not at any increased risk for CMV infection, is further evidence against the implication of CMV infection as a cause of TL_{CO} decline after heart transplantation.

F. Cyclosporin

Cyclosporin is a lipophilic cyclic peptide of fungal origin (196). It is a strong immunosuppressant and represents a major advance in immunosuppressive therapy in organ transplantation. However, it is associated with many potentially serious side-effects including systemic hypertension, dose-dependent nephrotoxicity, neurotoxicity and gastrointestinal upset (196,197). The mechanisms by which cyclosporin induces systemic hypertension and renal dysfunction are complex and appear to be related to disturbance of vasomotor tone with abnormal reactions to physiological vaso-active agents (196). Cyclosporin has been found to cause disturbance of many vascular regulatory systems including activation of the sympathetic system, change in relative activity of prostacycline and thromboxane A_2 in the vascular wall, local platelet aggregation with microemboli formation and stimulation of contraction and hyperplasia of the small renal arteries (198,199).

In contrast to other systems, cyclosporin pulmonary toxicity appears to be rare with only few case reports in the literature linking cyclosporin toxicity to an acute respiratory distress similar to adult respiratory distress syndrome (196,197,200).

However, cyclosporin pulmonary toxicity has been suggested as a possible cause for TL_{CO} decline after heart transplantation (15,16), but this has not been confirmed by subsequent investigators (21,201). In 10 patients studied before and after heart transplantation, Casan and associates (15) reported a significant correlation between the post-transplant TL_{CO} and cyclosporin whole blood levels determined within a few days of lung function assessment. In addition to the small number of patients and the use of a single cyclosporin blood level to reflect exposure, the analysis in this study was made using the post-transplant TL_{CO} rather than the change in TL_{CO} after transplantation. Furthermore, it was stated that one of the 10 patients experienced repeated episodes of severe rejection requiring augmented immunosuppression complicated by the development of *pneumocystis carinii* pneumonia. This patient had the highest cyclosporin level and the lowest post-transplant TL_{CO}. The findings of Casan et al. have been reinforced by Greon and co-workers (16) who carried out a longitudinal study of 34 heart transplant patients. They claimed that the percentage change in K_{CO} in the first post-transplant year was significantly related to the cyclosporin blood level measured nearest to the time of K_{CO} measurement. However, this relationship disappeared in the subsequent 2 years and there was no correlation between cyclosporin levels and the post-transplant pulmonary vascular resistance. Based on the known adverse effects of cyclosporin on the systemic and renal vasculature, it was suggested that cyclosporin may reduce K_{CO} by causing fibrotic thickening of the alveolar-capillary membrane.

Contrary to the findings of these 2 studies, Egan and associates (21) found in 22 patients that cyclosporin blood levels (both the absolute level collected at the time

of lung function measurement and the total mean level for each patient from transplantation to the time of lung function measurement) have no relationship to the change in TL_{CO} after heart transplantation. In our 57 patients, the results on the relationship between cyclosporin and the change in TL_{CO} after heart transplantation were in agreement with those of Egan and associates. The discrepancy between the results of these studies may be due to the differences in numbers of patients and the methods of analysis.

Studies from other organ transplants do not support the association between cyclosporin and lung dysfunction (201,202). In a study of 95 liver transplant patients receiving cyclosporin-based immunosuppression, mean TL_{CO} was reduced before transplantation (78.0% of predicted) and was unchanged at 9 to 12 months after transplantation (78.5% of predicted) (202). Morales and associates (201) also showed no relationship between cyclosporin levels and lung function in renal transplant recipients. In this longitudinal prospective study, lung function was assessed in 21 patients before and at 3, 6 and 12 months after renal transplantation. TL_{CO} and K_{CO} corrected for haemoglobin remained unchanged throughout the follow-up period. Based on these findings, the authors concluded that cyclosporin is not associated with TL_{CO} decline in renal transplant recipients.

G. Cardio-pulmonary haemodynamics

As stated earlier, heart transplant candidates are characterised by mild to moderate pulmonary arterial hypertension with raised pulmonary vascular resistance (167). Although many investigators have consistently reported a rapid reduction of pulmonary vascular pressures after heart transplantation, they remain at the upper limit of normal (13,163,164). In addition pulmonary haemodynamic responses to exercise after heart transplantation have been shown to be abnormal and resemble those of patients with mild to moderate chronic heart failure (165-167). These haemodynamic studies suggest a significant but incomplete resolution of pulmonary vascular bed abnormalities. The relationship between the changes in pulmonary haemodynamics and the changes in TL_{CO} and its components in heart disease is well documented and has been out-lined in chapter 3. In the present study, the finding of a significant relationship between the pre-transplant PCWP and the change in TL_{CO} was in keeping with findings of Greon et. al (16).

An important limitation of studies in this thesis is the absence of any reliable pulmonary haemodynamic data. The pre-transplant haemodynamic data were retrospective and not standardised and there were no post-transplant haemodynamic data. The measurement of post-transplant cardio-pulmonary haemodynamics was not part of the routine care of our patients because of the invasive procedures required. Therefore such data are not available for analysis in this thesis. Although the relationship between changes in pulmonary haemodynamics and changes in TL_{CO} after heart transplantation has not been

determined, the results of the various studies in the thesis supported by evidence from patients with congenital heart disease and mitral valve disease undergoing surgery, suggest that the reduction in haemoglobin-corrected TL_{CO} after heart transplantation is due to a reduction in pulmonary vascular pressures.

9.5 Conclusion

In conclusion the studies comprising this chapter identified pre-transplant TL_{CO} and PCWP as the only predictors of TL_{CO} change after heart transplantation. The fact that these 2 factors tend to reflect the degree of pre-transplant pulmonary vascular engorgement and the failure to identify any relationship between the change in TL_{CO} and the various other factors analysed supports the hypothesis that TL_{CO} decline after heart transplantation is probably due the associated decline in pulmonary vascular pressure after heart transplantation. The reduction in TL_{CO} together with the incomplete normalisation of pulmonary haemodynamics after heart transplantation suggests that the structural abnormalities of the pulmonary vascular bed in severe chronic heart failure are only partially reversed by successful heart transplantation.

CHAPTER 10

THE CLINICAL SIGNIFICANCE OF TL_{CO} REDUCTION IN HEART TRANSPLANT RECIPIENTS

10.1 Introduction

Heart transplantation is an established treatment for end-stage heart failure (203). In addition to increased life expectancy, heart transplant recipients report a remarkable improvement in symptoms and functional capacity (157). However, exercise performance following heart transplantation remains impaired, even in the absence of exertional symptoms (167). The maximum symptom-limited oxygen uptake and the ventilatory anaerobic threshold are in the range of 50% to 70% of predicted (204). These values are comparable to those of patients with severe heart failure (left ventricular ejection fraction less than 20%) managed with tailored medical therapy (205). The cause of exercise intolerance in heart transplant recipients is not clear, but there is increasing evidence that it is multifactorial and is related to cardiac, neurohormonal, vascular, muscle and pulmonary changes (167,183). The denervated heart has a reduced heart rate reserve due to the elevated resting heart rate and its blunted response to exercise (183). Heart compliance is also reduced resulting in left ventricular diastolic dysfunction with relatively preserved systolic function (167). Several factors have been identified as contributing to myocardial stiffness following heart transplantation including: myocardial ischaemia due to prolonged donor heart ischaemic time and ischaemia

sustained during the operation, cyclosporin-induced systemic hypertension, cyclosporin myocardial toxicity and recurrent minor episodes of rejection (167,183). Persistent peripheral abnormalities which could contribute to exercise limitation in these patients include abnormal neuro-hormonal responses to exercise (206), deconditioning (207) and peripheral circulatory dysfunction (208).

Efficient pulmonary gas exchange is an essential part of the complex process of exercise (209). Pulmonary dysfunction following heart transplantation is therefore a potential cause of exercise intolerance in heart transplant recipients (183). Although central haemodynamic and peripheral circulatory changes have been extensively evaluated in heart transplant recipients (167), there is little information on the possible effects of lung dysfunction on exercise performance (210-212).

TL_{CO} impairment has been suggested as a possible contributory factor to exercise limitation following heart transplantation (210,211). In a study of 11 patients evaluated before and after transplantation, arterial blood gases and pH were significantly lower in recipients with TL_{CO} below 70% of predicted compared to those with higher TL_{CO} at similar work loads (210). In another post-transplant study, TL_{CO} per unit alveolar volume (K_{CO}) was found to be significantly correlated with heart rate, oxygenation and lactate levels at maximum exercise (211). However, both studies had important limitations; the first was limited by the small number of patients, and the second by the lack of pre-transplant data. In addition, the maximum symptom-limited oxygen uptake and it relationship to TL_{CO} were not reported.

Aim

The aim of this chapter was to determine the impact of TL_{CO} reduction on exercise capacity in heart transplant recipients.

10.2 Methods

10.2.1 Study population

The study population consisted of three groups: heart transplant recipients, heart transplant candidates and normal volunteers.

A. Heart transplant recipients

Between January 1992 and January 1995, 67 patients underwent orthotopic heart transplantation at the Scottish Cardio-pulmonary Transplantation Unit. As part of routine post-transplantation assessment, 53 of these patients performed resting pulmonary function tests and cardio-pulmonary exercise test at 6 to 12 months following transplantation. Twenty six of the 53 patients had also performed these tests as part of their pre-transplant assessment. The remaining 27 patients were transplanted before the combined pulmonary function tests and cardio-pulmonary exercise test became part of routine assessment for possible heart transplantation. Assessment was performed in stable patients who had not suffered from any respiratory illness during the preceding 2 weeks. Patients who received treatment for rejection or systemic infection were not tested until at least 2 weeks after completing treatment. All patients were on standard triple immunosupression (cyclosporin, azathioprine and prednisolone).

B. Heart transplant candidates

This group consisted of 53 heart transplant candidates selected randomly from patients who had complete pulmonary function and cardio-pulmonary exercise during assessment for possible heart transplantation. They all complained of breathlessness on exertion due to left ventricular dysfunction. Anti-failure medication consisted of diuretics (all patients), ACE inhibitors (41 patients), digoxin (32 patients) and other vasodilators (21 patients). All patients were stable at the time of assessment and none had a history of primary lung disease.

C. Normal subjects

The findings in heart transplant patients were compared with data from 28 normal subjects recruited as volunteers from the general population in whom there was no evidence of cardio-pulmonary disease.

10.2.2 Pulmonary function and cardio-pulmonary exercise tests

Methods of lung function tests and cardio-pulmonary exercise testing were as described in chapter 5. Measured TL_{CO} was corrected for actual haemoglobin concentration using the equation of Dinakara and associates (187).

10.2.3 Data presentation and analysis

Unless stated otherwise, values are expressed as mean +/- one standard error of the mean (SEM). Lung function and cardio-pulmonary exercise data in heart transplant recipients were compared to those of candidates and normal subjects using the one way analysis of variance (ANOVA). Comparisons within the heart transplant

recipients group were performed using Student's t-test (the paired samples t-test for comparing data from the 26 recipients with pre and post-transplant results and the independent samples t-test for comparing recipients with normal and abnormal resting lung function). The relationship between haemoglobin-corrected TL_{CO} and exercise parameters was assessed using the Pearson correlation and linear regression analysis. A p value of <0.05 was considered significant.

10.3 Results

10.3.1 Subject characteristics

Subject characteristics are summarised in table 10.1. All groups had similar age and sex distribution. All patients were either life-long non-smokers or former smokers with no significant difference in the smoking status between recipients and candidates. There was no difference in the underlying cardiac diagnosis in the 2 groups. Before transplantation, LVEF and NYHA functional class in recipients were similar to those of the candidates. After transplantation, there was a significant improvement in both LVEF and the functional status. Mean haemoglobin concentration was significantly reduced in recipients compared with candidates.

Table 10.1: Characteristics of the study groups

	Recipients	*Candidates*	*Normal*
Number of subjects	53	53	28
Age; mean in years (range)	48.1 (19-61)	49.4 (34-60)	40.4 (19-61)
Sex			
Male	42 (79%)	42 (79%)	23 (82%)
Female	11 (21%)	11 (21%)	5 (18%)
Smoking status			
Non-smokers	13 (25%)	8 (15%)	16 (57%)
Ex-smokers	40 (75%)	45 (85%)	9 (32%)
Current smokers	0	0	3 (11%)
Diagnosis			
Ischaemic heart disease	31 (58%)	31 (58%)	-
Dilated cardiomyopathy	19 (36%)	20 (38%	-
Others	3 (6%)	2 (4%)	-
Pre-transplant LVEF, mean (SD)	13.4 (5.4)	12.3 (4.5)	-
Post-transplant LVEF, mean (SD)	44.3 (11.2)	-	-
Pre-transplant transpulmonary Mean (SD)	9.2 (4.8)	8.7 (3.4)	-
Pre-transplant functional class			
NYHA III	17 (32%)	20 (38%)	-
NYHA IV	36 (68%)	33 (62%)	-
Mean (SD)	3.7 (0.5)	3.6 (0.5)	-
Post-transplant functional class			
NYHA I	17 (32%)	-	-
NYHA II	35 (66%)	-	-
NYHA III	1 (2%)	-	-
Mean (SD)	1.7 (0.3)	-	-
Haemoglobin, $g.dL^{-1}$; mean (SD)	12.2 (1.2)	14.1 (1.5)	assumed 14.6

10.3.2 Resting pulmonary function

Table 10.2 compares the resting pulmonary function results in the study groups. TLC, FEV_1 and FEV_1/FVC were slightly reduced in heart transplant recipients and candidates compared to normal subjects. Although, TLC was greater in recipients compared to candidates, this did not reach statistical significance. In contrast TL_{CO} and K_{CO} (before and after correction for haemoglobin) were significantly lower in recipients compared to candidates in whom these parameters were significantly lower than normal subjects.

Table 10.2: Resting pulmonary function results (as percentages of predicted) in recipients compared to candidates and normal controls

	Recipients	*Candidates*	*Normal*
FEV_1	90.1 (2.3)	88.0 (2.2)	107.2 (3.9)*
FEV_1/FVC	93.1 (1.4)	91.1 (1.4)**	98.6 (1.8)**
TLC	96.3 (2.3)	90.4 (1.9)**	100.2 (3.3)**
TL_{CO}	57.5 (2.0)#	71.7 (2.1)	98.6 (1.3)
TL_{CO} (Hb-corrected)	62.3 (2.1)	72.9 (2.0)	-
K_{CO}	65.9 (2.4)#	82.7 (2.7)	105.3 (2.2)
K_{CO} (Hb-corrected)	71.4 (2.5)**	84.1 (2.7)**	-

* Significant difference between the marked group and each of the other 2 groups

** Significant difference between the 2 marked groups

\# Significant difference between all groups

10.3.3 Cardio-pulmonary responses to exercise

Table 10.3 shows the results of resting cardio-respiratory data in heart transplant recipients compared to heart transplant candidates and normal subjects. There was no significant difference in resting oxygen uptake across the study groups. Resting heart rate was significantly higher in recipients than in candidates and normal subjects. The pulmonary dead space to tidal volume ratio (V_D/V_T) at rest was similar in heart transplant recipients and candidates, and in both it was significantly higher than in normal controls. The alveolar-arterial oxygen pressure gradient ($P_{(A-a)},O_2$) was significantly higher in candidates compared with recipients and normal subjects. Although $P_{(A-a)},O_2$ was higher in recipients than in normal subjects, the difference was not significant.

Table 10.3: Resting cardio-respiratory data in the study groups

	Recipients	*Candidates*	*Normal*
$\dot{V}O_2$ L.min^{-1}	0.26	0.25	0.25
HR beats.min^{-1}	91.0 (2.0)**	83.0 (2)	79.0 (2.0)**
V_D/V_T	0.40 (0.01)	0.44 (0.01)	0.30 (0.01)*
$P_{(A-a)}$, O_2 kPa	1.9 (0.1)	2.9 (0.1)*	1.6 (0.1)

* Significant difference between the marked group and each of the other 2 groups

** Significant difference between the 2 marked groups

Table 10.4 displays the cardio-respiratory responses to symptom-limited exercise in heart transplant recipients compared with heart transplant candidates and normal controls. Maximum symptom-limited oxygen uptake ($\dot{V}O_2$) as a percentage of predicted was significantly higher in recipients than in ~~controls~~ candidates (46.0% vs. 39.8% of predicted, $p<0.05$), and both were substantially lower than normal controls (92.9% of predicted, $p<0.001$). Although the ventilatory anaerobic threshold was higher in recipients compared to candidates, the difference was not statistically significant and in both groups it was markedly reduced compared to controls. The ventilatory and gas exchange responses to exercise ($\dot{V}_E/\dot{V}_{CO2}$, V_D/V_T and $P_{(A-a)}O_2$) were significantly lower in recipients than in candidates, but they were all higher than in normal controls. The heart rate response was reduced in recipients compared with normal controls, but it was markedly elevated in candidates. The oxygen pulse at maximum symptom-limited exercise was higher in recipients than in candidates and in both it was significantly lower than in normal subjects.

Table 10.4: Cardio-respiratory responses to symptom-limited exercise in the study groups

	Recipients	*Candidates*	*Normal*
$\dot{V}O_2$ % pred.	46.0 (1.9)#	39.8 (1.5)	92.9 (2.5)
$\dot{V}O_2$, AT % ††	32.6 (1.1)	30.4 (0.9)	52.6 (1.9)*
$\dot{V}_E$ L.min^{-1}	46.4 (1.6)	45.6 (2.0)	72.4 (4.2)*
$\dot{V}_E$ % pred.	45.6 (1.8)	43.9 (1.8)	57.9 (2.1)
$\dot{V}_E$ /$\dot{V}_{CO2}$	35.6 (1.0)#	46.6 (2.8)	24.3 (0.06)
V_D/V_T	0.30 (0.01)#	0.36 (0.01)	0.19 (0.01)
$P_{(A-a)}$, O_2 kPa	2.4 (0.2)	3.5 (0.2)*	1.8 (0.1)
Heart rate (HR) % pred.	67.9 (1.4)#	91.3 (1.7)	86.4 (1.9)
HR response beats.L^{-1}	26.8 (2.0)	70.6 (4.5)*	35.7 (2.0)
O_2 pulse, ml.beats^{-1}	10.6 (0.6)#	7.4 (0.3)	16.5 (0.6)

†† The ventilatory anaerobic threshold as a percentage of predicted maximal $\dot{V}O_2$

* Significant difference between the marked group and each of the other 2 groups,

** Significant difference between the 2 marked groups,

\# Significant difference between all groups

10.3.4 The relationship between haemoglobin-corrected TL_{CO} and exercise parameters

Since measured TL_{CO} is directly influenced by haemoglobin concentration, analysis of the relationship between TL_{CO} and exercise parameters was made on haemoglobin-corrected TL_{CO}. Figure 10.1 shows regression plots of % predicted TL_{CO} at rest against % predicted maximal $\dot{V}O_2$ in the three study groups. There was a significant positive correlation between TL_{CO} and $\dot{V}O_2$ in heart transplant recipients ($r = 0.62$, $p<0.001$). In contrast, there was no correlation between the 2 variables in either heart transplant candidates or normal controls. The relationship between TL_{CO} and $\dot{V}O_2$ in recipients persisted even after correction for lung volumes (the correlation between K_{CO} and $\dot{V}O_2$ was 0.38, $p<0.001$). There was no relationship between % predicted $\dot{V}O_2$ and any of the other indices of lung function in any of the studied groups. TL_{CO} was also positively correlated with the anaerobic threshold in recipients, but not in candidates or normal subjects (Table 10.5). TL_{CO} was also inversely related to the ventilatory response ($\dot{V}_E/\dot{V}_{CO2}$), V_D/V_T and $P_{(A\text{-}a)}O_2$ in heart transplant recipients, but there was no relationship between TL_{CO} and any of these parameters in the other 2 groups.

Figure 10.1 - Regression plots of % predicted TL_{CO} (haemoglobin-corrected) against % predicted maximum symptom-limited $\dot{V}O_2$ in heart transplant recipients compared with heart transplant candidates and normal controls.

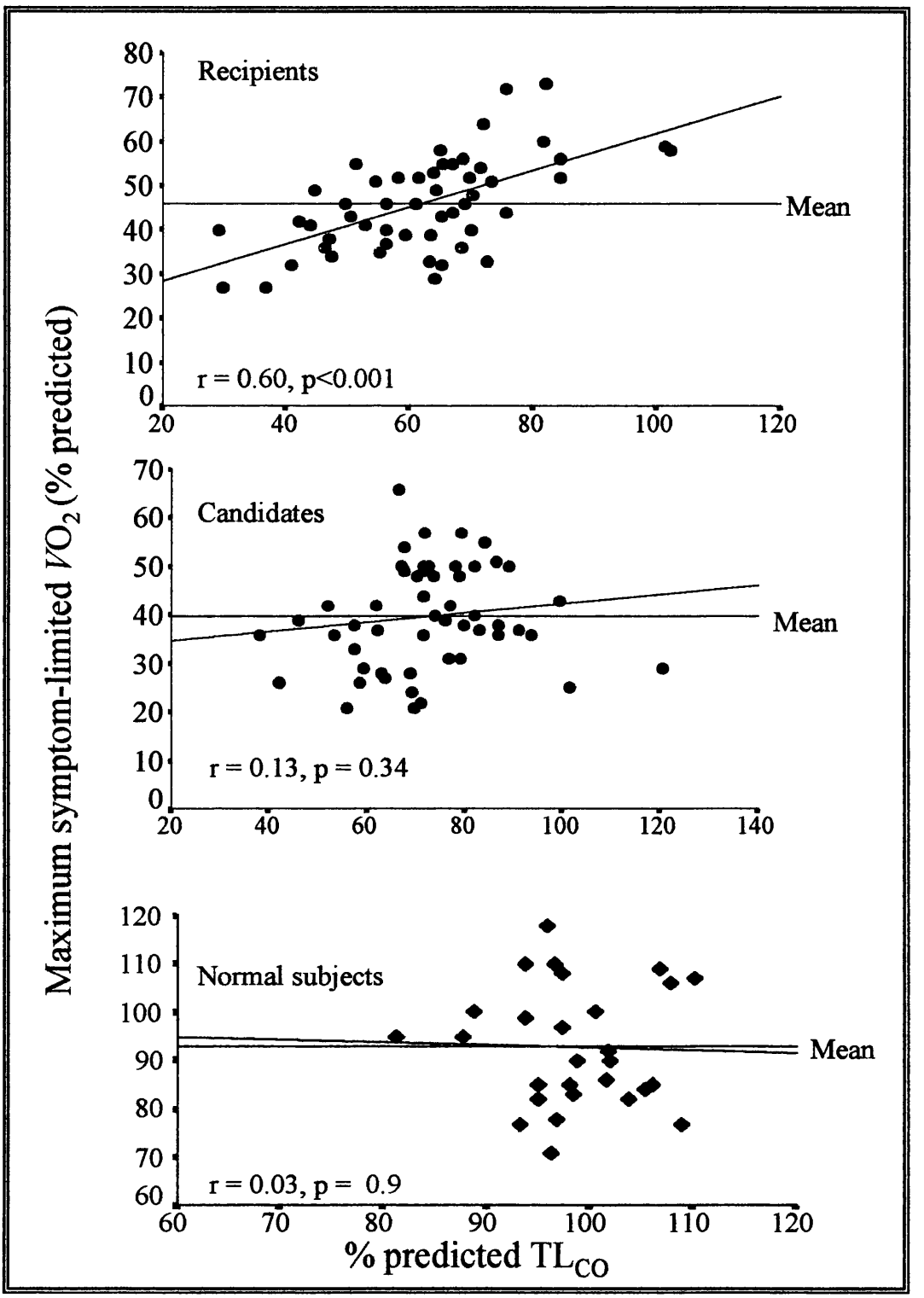


Table 10.5: The relationship between % predicted TL_{CO} (haemoglobin-corrected) and maximum symptom-limited exercise variables

	Correlation with % predicted TL_{CO}					
	Recipients		*Candidates*		*Normal*	
	r*	p	r	p	r	p
$\dot{V}O_2$ % pred.	0.60	<0.001	0.13	0.34	0.03	0.90
$\dot{V}O_2$, AT %	0.54	<0.001	0.04	0.79	0.27	0.17
$\dot{V}_E / \dot{V}_{CO2}$	- 0.43	<0.001	- 0.06	0.67	- 0.10	0.60
V_D/V_T	- 0.29	<0.05	- 0.27	0.05	- 0.14	0.48
$P_{(A-a)}$, O_2 kPa	- 0.38	<0.01	- 0.24	0.07	0.23	0.14

* Pearson correlation coefficient

Mean haemoglobin concentration was significantly lower in recipients than in candidates (12.2 and 14.1 $g.dL^{-1}$ respectively, $p<0.001$), but there was no correlation between haemoglobin and % predicted maximum symptom-limited $\dot{V}O_2$ in either groups.

Forty of the 53 heart transplant recipients had haemoglobin-corrected TL_{CO} below 70% of predicted, and in these maximum symptom-limited $\dot{V}O_2$ was significantly reduced compared with the remaining 13 patients with higher TL_{CO} (42.9% vs. 55.7% of predicted, $p<0.001$).

The possible influence of static and dynamic lung volumes on the cardio-respiratory responses to exercise in heart transplant recipients was evaluated by dividing them into 2 groups based on the results of static and dynamic lung volumes. The lower

limit of normality for all indices of lung function was defined as the predicted value minus 1.64 standard deviations of the reference regression equation (41). Using this definition, 32 recipients had normal results whereas 21 had at least one abnormal results. Table 10.6 shows that there was no significant difference between these 2 sub-groups in any of the cardio-respiratory responses to exercise.

Table 10.6: Maximum symptom-limited exercise responses in heart transplant recipients with normal and abnormal spirometric results

	Normal (32)	*Abnormal (21)*	*P value*
$\dot{V}O_2$ % pred.	47.5 (2.0)	43.8 (1.9)	0.21
$\dot{V}O_2$, AT %	32.2 (1.7)	33.2 (1.1)	0.65
$\dot{V}_E / \dot{V}_{CO2}$	35.0 (1.4)	36.5 (1.6)	0.47
V_D/V_T	0.29 (0.01)	0.32 (0.02)	0.29
$P_{(A-a),}O_2$ kPa	2.3 (0.3)	2.5 (0.3	0.55
HR % pred.	68.5 (2.1)	66.9 (1.6)	0.58
HR response beats.L^{-1}	29.2 (2.7)	22.9 (2.8)	0.13
O_2 pulse, ml.beats^{-1}	11.2 (1.0)	9.7 (0.5)	0.24

Twenty six of the heart transplant recipients had complete lung function and cardio-pulmonary exercise data before and after transplantation. Table 10.7 shows that the clinical characteristics of these patients are similar to those who had no pre-transplant data. They also had similar resting pulmonary function and cardio-pulmonary responses to exercise following transplantation (Table 10.8).

Table 10.7: Clinical characteristics and resting cardio-respiratory parameters in heart transplant recipients: patients with data before and after transplantation (Group 1) compared to those with post-transplant data only (Group 2)

	Group 1	*Group 2*
Number of subjects	26	27
Age; mean in years (range)	48.6 (19-59)	47.6 (32-61)
Sex		
Male	21 (81%)	23 (79%)
Female	5 (19%)	6 (21%)
Diagnosis		
Ischaemic heart disease	16 (61%)	17 (59%)
Dilated cardiomyopathy	9 (23%)	10 (34%)
Valvular heart disease	1 (4%)	2 (7%)
Pre-transplant LVEF, mean (SD)	13.8 (5.4)	13.6 (6.6)
Post-transplant LVEF, mean (SD)	42.7 (11.1)	45.2 (11.2)

Table 10.8: Resting pulmonary function and maximum symptom-limited exercise results in heart transplant recipients with data before and after transplantation (Group 1) compared to those who had post-transplant data only (Group 2)

	Group 1	*Group 2*	*p value*
FEV_1*	88.7 (3.0)	91.1 (3.5)	0.61
FEV_1/FVC*	90.7 (2.0)	92.4 (1.8)	0.50
TLC*	96.6 (2.7)	92.3 (2.4)	0.24
TL_{CO}* (Hb-corrected)	61.5 (2.3)	63.7 (2.5)	0.44
K_{CO} * (Hb-corrected)	70.9 (3.0)	72.2 (3.1)	0.69
$\dot{V}O_2$*	48.6 (1.7)	45.0 (2.0)	0.15
$\dot{V}O_2$, AT %	34.6 (1.2)	32.3 (1.1)	0.27
$\dot{V}_E/\dot{V}_{CO2}$	34.1 (1.3)	37.6 (2.1)	0.16
V_D/V_T	0.31 (0.01)	0.30 (0.01)	0.78
$P_{(A\text{-}a)_2}$ O_2 kPa	2.4 (0.3)	2.4 (0.3)	0.84
Heart rate (HR)*	70.5 (1.7)	67.1 (2.3)	0.25
HR response, $beats.L^{-1}$	24.2 (2.5)	27.8 (2.9)	0.35
O_2 pulse, $ml.beats^{-1}$	10.4 (0.6)	10.0 (0.5)	0.64

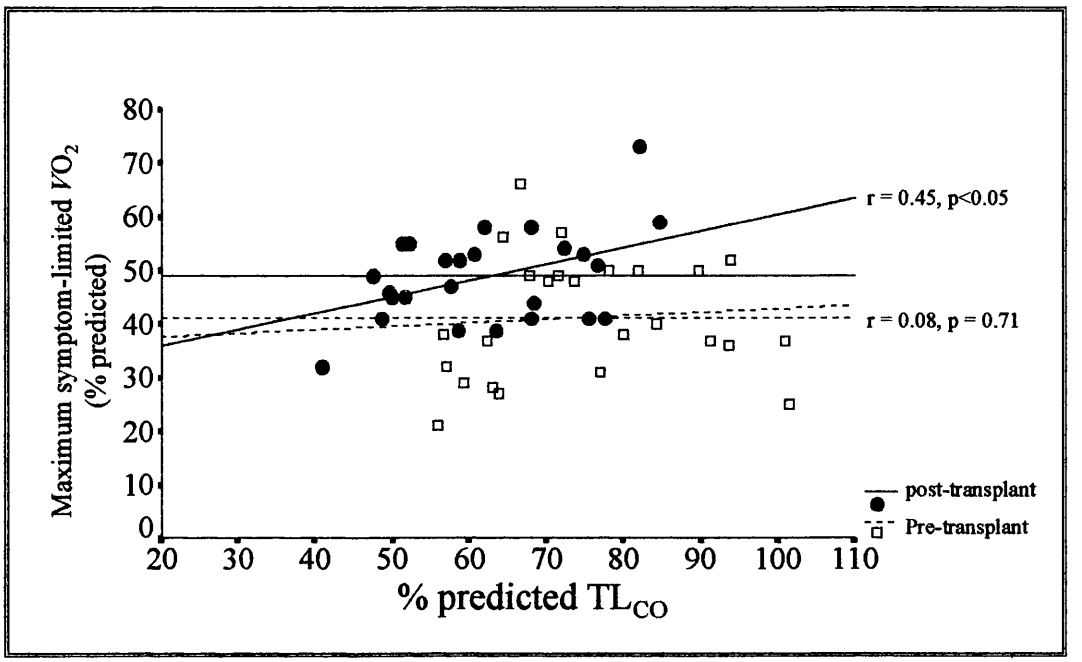
* Results expressed as percentage of predicted

Table 10.9 shows maximum symptom-limited exercise responses in 26 heart transplant recipients before and after transplantation and figure 10.2 compares the relationship between % predicted TL_{CO} and % predicted $\dot{V}O_2$ in these patients. The trend and magnitude of changes in all parameters are similar to those obtained by the cross-sectional comparisons between heart transplant recipients and candidates (Table 10.4 and Figure 10.1).

Table 10.9: Maximum symptom-limited exercise responses in 26 heart transplant recipients before and after transplantation

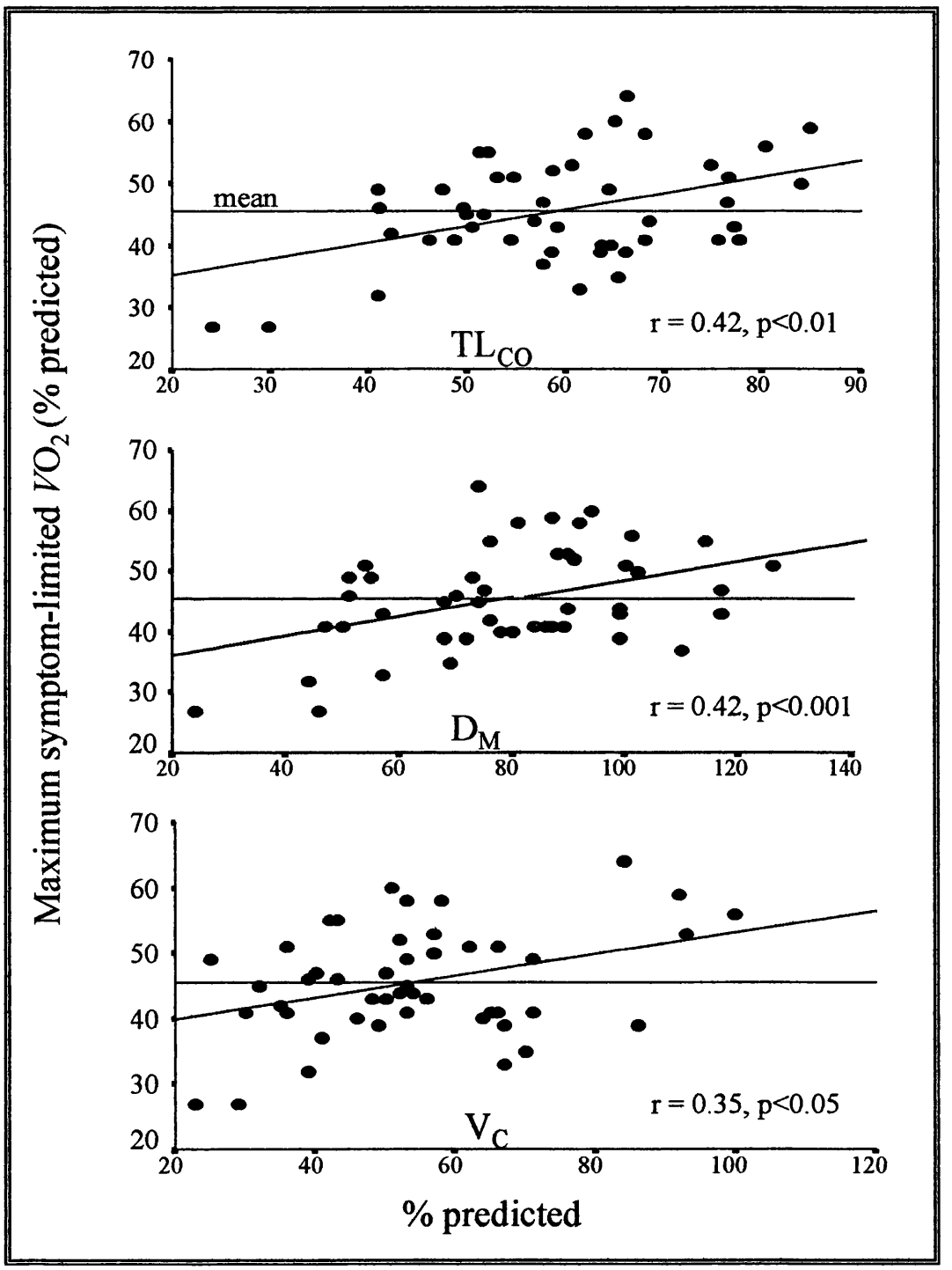
	pre-transplant	*post-transplant*	*P value*
$\dot{V}O_2$ % pred.	41.3 (2.2)	48.6 (1.7)	<0.01
$\dot{V}O_2$, AT %	31.5 (1.1)	35.6 (1.0)	<0.05
$\dot{V}_E/\dot{V}_{CO2}$	45.6 (2.5)	34.1 (1.3)	<0.001
V_D/V_T	0.35 (0.02)	0.31 (0.01)	<0.05
$P_{(A-a)}O_2$ kPa	3.4 (0.3)	2.4 (0.3)	<0.01
Heart rate (HR) % pred.	82.2 (2.6)	70.5 (1.7)	<0.001
HR response, beats.L^{-1}	72.1 (6.5)	24.2 (2.5)	<0.001
O_2 pulse, ml.beats^{-1}	7.7 (0.4)	10.4 (0.4)	<0.001

Figure 10.2: The relationship between % predicted TL_{CO} (haemoglobin-corrected) and maximum symptom-limited $\dot{V}O_2$ (% predicted) in 26 patients before and after transplantation



TL_{CO} components were measured in 47 of the 53 heart transplant recipients. As in the entire group, TL_{CO} was positively correlated with maximal $\dot{V}O_2$ ($r = 0.42$, $p<0.01$). Figure 10.3 shows that $\dot{V}O_2$ was also positively correlated with both V_C and D_M.

Figure 10.3: Regression plots of % predicted $\dot{V}O_2$ against each of the diffusion parameters in 48 heart transplant recipients



10.4 Discussion

10.4.1 Summary of main results

In addition to confirming previous reports on exercise intolerance in heart transplant recipients, this study demonstrated three new findings. First, these data demonstrated that haemoglobin-corrected TL_{CO} is significantly correlated with maximum symptom-limited oxygen uptake in heart transplant recipients, so that even in presence of normal arterial oxygenation, patients with the lowest TL_{CO} had the lowest exercise capacity. Second, TL_{CO} was also inversely related to the ventilatory and pulmonary gas exchange responses to exercise following transplantation (V_E/V_{CO2}, V_D/V_T and $P_{(A-a)},O_2$). Third, the effect of TL_{CO} impairment on exercise performance in heart transplant recipients was independent of resting lung function tests which were relatively normal and had no influence on exercise capacity in these patients.

10.4.2 Resting pulmonary function

The results of resting pulmonary function tests in this study are in agreement with previous reports (15-18,20,21), and have been described in the previous chapters of this thesis.

10.4.3 Cardio-pulmonary responses to exercise

In agreement with previous reports (165), the results of this study show that despite substantial improvement of subjective functional capacity, heart transplant recipients continue to have limited exercise performance as assessed by incremental cardio-pulmonary exercise testing. The ventilatory response to exercise in our

patients was similar to that reported by Marzo and associates (213). Before transplantation, $\dot{V}_E/\dot{V}_{CO2}$ was elevated and decreased significantly following transplantation, but remained higher than normal. In addition, the present study showed that despite significant improvement in V_D/V_T after transplantation, it remained higher than normal. It is not known why ventilatory and gas exchange abnormalities on exercise fail to resolve completely after heart transplantation. One possible explanation is that long-standing pre-transplant heart failure leads to irreversible structural lung damage. Alternatively, these abnormal pulmonary responses may be functional in origin, resulting from a sub-optimal cardiac output response to exercise. Heart failure is characterised by excessive ventilatory response to exercise (214). Patients with chronic heart failure also have increased "wasted ventilation" as assessed by V_D/V_T (214). We and others have previously shown that the ventilatory response and V_D/V_T in heart failure are positively correlated and suggested that they be causally linked (170,215). It was postulated that failure to increase cardiac output to match ventilation during exercise increases the proportion of lung units with high ventilation-perfusion ratio thereby increasing the V_D/V_T and consequently leading to an excessive ventilatory response to exercise (170,215). Although cardiac output is markedly improved after heart transplantation, its response to exercise remains sub-normal (216) and this may explain the residual abnormalities of ventilatory and gas exchange responses to exercise following transplantation.

10.4.4 TL_{CO} and exercise capacity

Exercise intolerance in heart transplant recipients is well documented (167). Although the underlying pathophysiology not fully understood, the complex nature of exercise process favours multiple causes (167). Factors identified as contributory to exercise intolerance following transplantation include: inotropic and chronotropic incompetence of the denervated heart (165,216), abnormal peripheral circulation (208), abnormal neuro-hormonal responses (206), persistent skeletal muscle abnormalities (207) and pulmonary dysfunction (210,211).

In this study, we showed that TL_{CO} impairment which was very common in heart transplant recipients was associated with exercise intolerance. The correlation between haemoglobin-corrected TL_{CO} and exercise performance was independent of lung volumes and airway function both of which were normal in most patients and had no influence on exercise capacity. The lack of any correlation between TL_{CO} and exercise capacity in heart transplant candidates and normal subjects suggests that TL_{CO} becomes a limiting factor in exercise performance when impairment is severe. In patients with chronic obstructive pulmonary disease, TL_{CO} below 70% of predicted is associated with frequent gas exchange abnormalities on exercise, whereas above 70% of predicted, these abnormalities are uncommon (44). In addition TL_{CO} impairment has been shown to predict pulmonary gas exchange abnormalities and exercise intolerance in patients with mild to moderately severe heart failure (153,217,218). In a recent study, Puri et al. (153) reported a positive correlation between TL_{CO} and exercise capacity in patients with mild to moderately

severe congestive heart failure (NYHA, grades II and III) and showed that it was primarily due to impairment of the diffusing capacity of the alveolar-capillary membrane (D_M). Unlike these reports, there was no relationship between $\dot{V}O_2$ and any of the diffusion parameters in heart transplant candidates. The difference between our findings and the others may be due to the severity of heart failure in heart transplant candidates. Heart transplant candidates may stop exercising because of severe cardiac insufficiency before any TL_{CO} impairment becomes important.

The mechanism by which TL_{CO} impairment causes exercise intolerance is not clear. Gas exchange in the lungs depends on several interdependent processes. These include ventilation, perfusion, ventilation-perfusion matching and diffusion (28). The measured TL_{CO} is affected by disturbance in any of these processes and therefore, can be considered as a composite index of the integrity of the pulmonary gas exchanging unit, rather than being specifically determined by the process of diffusion (43). In addition, isolated impairment of diffusion is very rare and because of the large physiological reserve, diffusion impairment is not considered an important limiting factor in the transfer of oxygen to the arterial blood even in patients with severe lung disease (43). The relationship between TL_{CO} and exercise performance in heart transplant recipients is therefore likely to represent a general dysfunction of pulmonary gas exchange rather than an isolated diffusion defect. This is supported by the lack of complete resolution of the ventilatory and pulmonary gas exchange responses ($\dot{V}_E/\dot{V}_{CO2}$, V_D/V_T and $P_{(A-a)}O_2$) to exercise, and by the significant inverse relationship between TL_{CO} and each of these parameters.

10.4.5 Study limitations

This study is limited by the fact that measurements of resting pulmonary function were related to variability in the cardio-respiratory responses to exercise. Measurement of TL_{CO} during exercise may have a stronger relationship to exercise capacity. In addition, other potential contributory factors to exercise limitation such as cardio-pulmonary haemodynamics, skeletal muscle function and peripheral circulatory responses were not assessed. These factors have been previously evaluated (165,206,207,216) and the aim of this chapter was primarily to evaluate the effect of TL_{CO} impairment on exercise capacity. It is also important to emphasise that the correlation between TL_{CO} and the various responses to exercise noted above represent associations and do not necessarily imply causative relationship.

10.5 Conclusion

TL_{CO} impairment in heart transplant recipients is associated with abnormal ventilatory and pulmonary gas exchange responses to exercise. Pulmonary dysfunction as reflected by TL_{CO} impairment appears to contribute to exercise intolerance after heart transplantation.

CHAPTER 11

CONCLUSIONS

Heart transplantation is now an established therapeutic option in the management of end-stage heart disease (156). Severe chronic heart failure, the primary indication for heart transplantation is characterised by chronic pulmonary congestion and associated with a variety of pulmonary function abnormalities (10). The transplanted heart would be expected to improve lung function abnormalities specifically related to heart failure. However, the procedure of heart transplantation and its associated complications may lead to new pulmonary function abnormalities. It has been suggested that the decline in TL_{CO} after heart transplantation is secondary to the adverse effects of heart transplantation on the lungs (15,21). The studies comprising this thesis were carried out to answer specific questions about the reported reduction in TL_{CO} after heart transplantation. This chapter summarises and brings together the results of the various studies presented in the previous chapters. On the basis of these findings and evidence from the literature, a theory is proposed to explain TL_{CO} decline after heart transplantation.

11.1 Summary of results

The main findings of the studies comprising this thesis were as follows:

1. lung function in heart transplant candidates is characterised by a mild restrictive ventilatory defect with a greater reduction in TL_{CO} and the latter occurs because of a proportionate reduction in both of its components (D_M and V_C).

2. Heart transplantation is associated with a further reduction in the mean values of static and dynamic lung volumes at 6 weeks after transplantation with gradual improvement in the subsequent measurements, exceeding their pre-transplant values at one year after transplantation. There was marked variability in the individual responses.

3. TL_{CO} and K_{CO} declined significantly in almost all patients at 6 weeks after transplantation with no further changes up to 3 years after transplantation. TL_{CO} reduction in heart transplant recipients was due to an increase in the intra-capillary resistance ($1/\theta V_C$) caused by a combination of post-transplant anaemia and reduced V_C. Correction of TL_{CO} for the effect of anaemia reduced the magnitude of TL_{CO} decline after heart transplantation by about 50%. D_M was unchanged by transplantation being similarly reduced in both candidates and recipients.

4. The reduction in haemoglobin-corrected TL_{CO} after heart transplantation is related to the pre-transplant TL_{CO} and PCWP. The change in TL_{CO} after heart transplantation was shown to be independent of factors such as pre-transplant static and dynamic lung volumes, pre-transplant cardiac status, cardio-pulmonary bypass, cyclosporin blood levels and post-transplant problems including pulmonary complications, CMV infection and episodes of cardiac allograft rejection.

5. TL_{CO} in heart transplant recipients was found to be positively correlated with maximum symptom-limited oxygen uptake, so that patients with lowest TL_{CO}

had the lowest exercise capacity. TL_{CO} was also inversely related to the ventilatory and pulmonary gas exchange responses to exercise following transplantation (V_E/V_{CO2}, V_D/V_T and $P_{(A-a)}O_2$). These associations were independent of the other resting lung function parameters which were relatively normal and had no influence on exercise capacity.

6. In addition these studies have demonstrated that:

a) The changes in TL_{CO} and its components after heart transplantation were similar to the changes following mitral valve replacement six weeks after surgery.

b) The reduction in TL_{CO} after coronary artery bypass graft surgery was due to the reduction in haemoglobin after surgery.

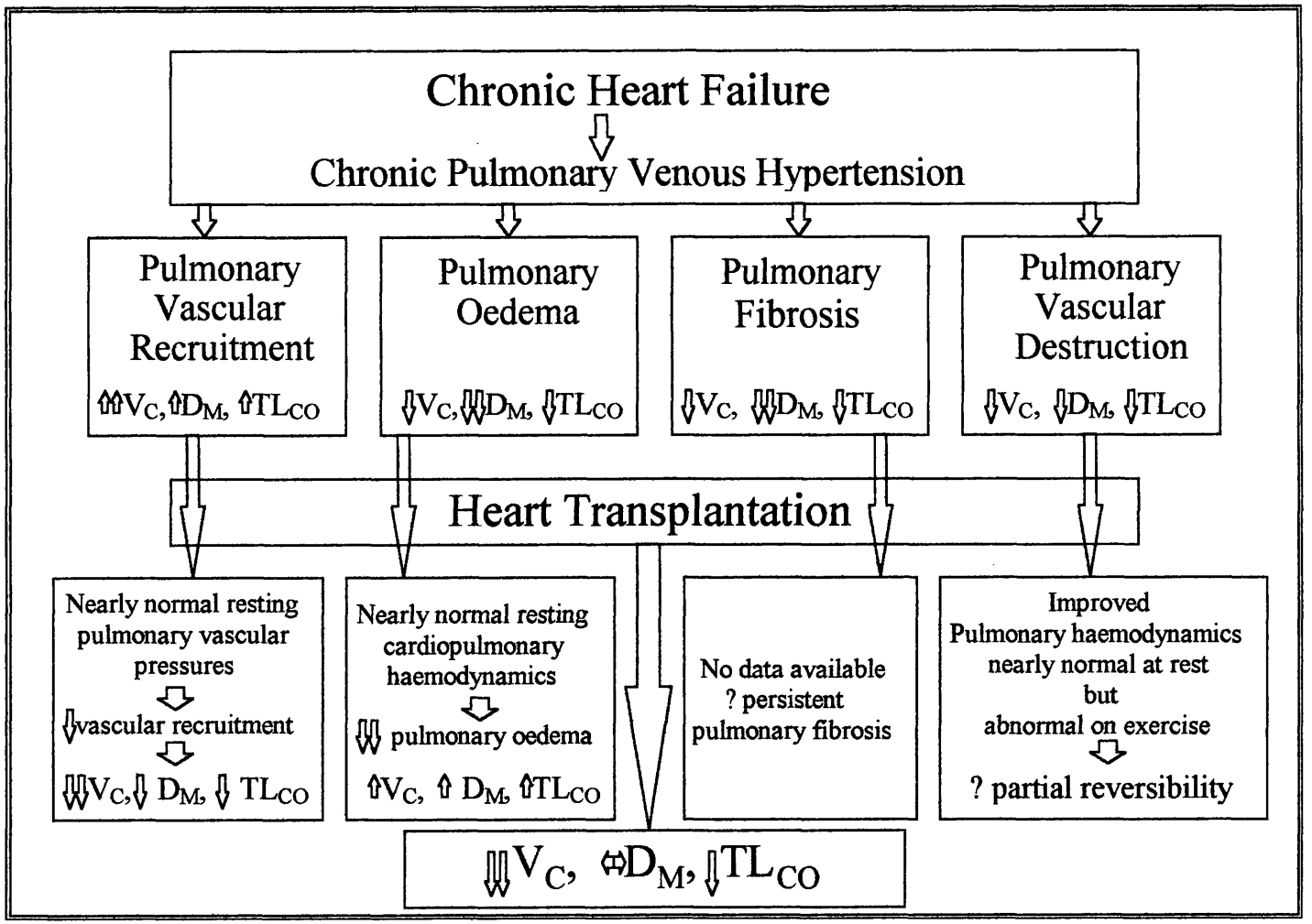
7. A protocol was also described for the estimation of TL_{CO} components using the Roughton and Forster method. The validation studies presented in this thesis showed that the described procedure yields reproducible estimation of TL_{CO} components comparable with previous reports (49).

11.2 The pulmonary consequences of chronic pulmonary venous hypertension with particular reference to TL_{CO} decline after heart transplantation

The interpretation of the above results and their relationship to previous work have been discussed in the previous chapters. The following account is an attempt to explain the mechanism of TL_{CO} reduction after heart transplantation using mitral stenosis as a model for the pulmonary consequences of chronic pulmonary venous hypertension.

Figure 11.1 illustrates the pulmonary consequences of chronic pulmonary venous hypertension and the impact of heart transplantation on these changes in terms of the changes in pulmonary haemodynamics, TL_{CO} and its components. Any cause of chronic heart failure including coronary artery disease, valvular heart disease and the various forms of cardiomyopathy can potentially cause pulmonary arterial hypertension with structural vascular and parenchymal changes in the lungs (12). Mildly elevated pulmonary venous pressure is associated with a corresponding increase in pulmonary artery pressure to maintain a normal gradient across the pulmonary capillary bed (94). The increase in pulmonary vascular pressures in absence of significant structural changes in the pulmonary vasculature leads to an increase in the functioning pulmonary capillaries by opening collapsed vessels (vascular recruitment) and distending open ones (vascular distension) (27). These functional changes act to maintain a normal pulmonary vascular resistance (27). In addition, they result in an increase in both the total pulmonary blood volume and the pulmonary capillary blood volume (V_C) (32). TL_{CO} at this stage may be greater than predicted, mainly due to the increase in V_C, but D_M is also increased because of the increase in the effective surface area available for gas exchange resulting from improved matching between ventilation and perfusion (31,60). However, the augmenting effects of vascular recruitment and distension on TL_{CO} and its components may be offset by co-existing pulmonary interstitial oedema (7).

Figure 11.1: The pulmonary consequences of chronic pulmonary venous hypertension and the impact of heart transplantation on these changes in terms of the changes in pulmonary haemodynamics, TL_{CO} and its components



Progression of the disease process leads to structural vascular and parenchymal changes in the lungs. In severe mitral stenosis, these include intimal and medial hyperplasia of the small pulmonary arteries and veins together with pulmonary interstitial fibrosis resulting in thickened alveolar-capillary membranes and destruction of the pulmonary vascular bed (27,107). Pathophysiologically, these structural changes are reflected by disproportionate increase in pulmonary arterial pressure in relation to the increase in pulmonary venous pressure indicating the development of established pulmonary arterial hypertension with increased pulmonary vascular resistance. TL_{CO} is also reduced due to reductions in both of its components.

There are no data on the pulmonary structural abnormalities in heart transplant candidates. However, the finding of moderate reduction in TL_{CO} with proportional reduction in D_M and V_C in heart transplant candidates in the studies of this thesis suggests that these patients have significant pulmonary parenchymal and vascular abnormalities. This is also suggested by the well documented pulmonary haemodynamic profile of these patients (161). Heart transplantation has been shown to relieve the pulmonary congestion and oedema of heart failure and to restore resting pulmonary haemodynamics towards normal (13), but there are no data on its effects on the pulmonary structural abnormalities. The reduction in pulmonary vascular pressures would be expected to eliminate the augmenting effects of vascular recruitment and distension on TL_{CO} and its components. If this was not accompanied by improvement in the pulmonary structural changes of heart failure, TL_{CO} and its components would be expected to decline after heart

transplantation. The finding of TL_{CO} and V_C decline after heart transplantation suggests that the pulmonary structural abnormalities are not reversed by heart transplantation. The lack of any change in D_M after heart transplantation may be due to opposing effects of the relief of pulmonary oedema and the elimination of pulmonary vascular recruitment. The persistence of pulmonary vascular dysfunction after heart transplantation is also suggested by the incomplete normalisation of resting pulmonary haemodynamics (13,163,164) and by the abnormal pulmonary haemodynamic responses to exercise, resembling that of mild to moderate heart failure (167). The relationship between TL_{CO} and gas exchange abnormalities on exercise in heart transplant recipients demonstrated in this thesis is in keeping with a contribution from pulmonary parenchymal and vascular dysfunction to exercise limitation. At the time of concluding this thesis, Egan and associates (219) described the bronchoalveolar lavage (BAL) and transbronchial lung biopsy findings in 20 heart transplant recipients studied at a mean of 152 days (range 62-331 days) after heart transplantation. Four of these patients had histological evidence of CMV pneumonitis and 3 had evidence of *Pneumocystis carinii* pneumonia. Light microscopy demonstrated focal pulmonary fibrosis in 12 patients and of these only one had evidence of CMV pneumonitis. The BAL differential cell counts were normal, but 11 of the 18 patients had high haemosiderin scores in BAL macrophages. It was suggested that these changes may underlie the fall in TL_{CO} after heart transplantation. Although the authors suggested CMV infection or cyclosporin toxicity as possible causes, the described changes are consistent with those found in patients with mitral stenosis (27).

In summary, based on the results of the studies in this thesis and previous knowledge on the pulmonary consequences of chronic pulmonary venous hypertension, the decline in TL_{CO} after heart transplantation appears to be secondary to reduction in the pulmonary vascular pressures in the absence of any significant reversibility in the structural abnormalities of the pulmonary bed. Thus, the fall in TL_{CO} after heart transplantation is probably a marker of persistent pulmonary dysfunction associated with previous heart failure rather than being due to new damage to the lungs caused by heart transplantation or its complications.

The evidence adduced to implicate changes in pulmonary haemodynamics as a cause of TL_{CO} decline after heart transplantation was indirect and further research is required to define the precise relationship between changes in TL_{CO} and its components with pulmonary haemodynamics both before and after transplantation. This will require simultaneous measurement of these indices under standardised conditions before and serially after transplantation. Definite proof of persisting pulmonary vascular and parenchymal structural abnormalities resulting from pre-transplant heart failure would require demonstration of similar histopathology in the pulmonary vascular bed before and after heart transplantation. This would entail the performance of small lung biopsies at the time of heart transplantation and serially, perhaps by the transbronchial route, after transplantation. Although taking small lung biopsies at the time of operation is feasible and has been performed during mitral valve replacement without any added risks (30), the performance of serial post-transplant transbronchial lung biopsies would be difficult to justify because of the risk of significant complications (haemorrhage and pneumothorax) and the

possibility of sampling errors due to the patchy nature of pulmonary abnormalities in pulmonary venous hypertension (30).

Finally, it is important to emphasise some points of clinical relevance in the use of TL_{CO} measurement in heart transplant patients. First, TL_{CO} may be normal despite definite abnormalities of the pulmonary vascular bed resulting in abnormal, but opposite changes in TL_{CO} components. Second, the longitudinal studies of lung function in this thesis have shown that lung function including TL_{CO} does not decline after the first post-transplantation assessment in asymptomatic patients. Therefore, a significant decline in lung function from the first post-transplant values would suggest a new pulmonary complication. Third, in conditions associated with significant changes in haemoglobin (e.g. cardiac surgery and heart transplantation) it is important to correct for the effect of haemoglobin concentration on the measured TL_{CO}.

Presentations and Publications Arising

Presentations

1. **Breathlessness in patients with cardiac failure awaiting heart transplantation.**
   O A Al-Rawas, R Carter, D Richens, A Tweedle, R D Stevenson, S K Naik and DJ Wheatley. J Heart Lung Transplant. 1994; 13:S86 (The International Society For Heart and Lung Transplantation 14TH Annual Meeting and Scientific Sessions; March 24-26,1994, Venice, Italy

2. **The role of excessive ventilatory response to exercise in patients with chronic heart failure awaiting heart transplantation.**
   OA Al-Rawas, R Carter, D Richens, RD Stevenson, S K Naik and DJ Wheatley. Scottish Thoracic Society Meeting; Perth, 23-24 June 1994

3. **Breathlessness and ventilatory abnormalities in heart transplant candidates.**
   OA Al-Rawas, R Carter, D Richens, RD Stevenson, S K Naik and DJ Wheatley. Association of Respiratory Technicians and Physiologist Winter Meeting; Sterling, 25-26 November 1994

4. **Effects of heart transplantation on the dead space ventilation and ventilatory response to exercise.**
   OA Al-Rawas, R Carter, D Richens, RD Stevenson, and DJ Wheatley. Scottish Thoracic Society; Glasgow, 24 November 1995

5. **The diffusion characteristics of the lung following heart transplantation.**
   OA Al-Rawas, R Carter, D Richens, RD Stevenson, and DJ Wheatley. Thorax 1995;50 (suppl 2):A68 (British Thoracic Society Winter Meeting; London, 11-13 December 1995)

6. **The role of pulmonary transfer factor (TL_{CO}) impairment in exercise intolerance in heart transplant recipients.**
   OA Al-Rawas, R Carter, RD Stevenson, SK Naik and DJ Wheatley. Eur Respir J 1996;9 (suppl 23):S388 (ERS Annual Congress; Stockholm, Sweden, September 7-11,1996)

7. **Changes in pulmonary transfer factor following heart transplantation.**
   OA Al-Rawas, R Carter, RD Stevenson, SK Naik and DJ Wheatley. The 10^{TH} Annual Meeting of The European Association for Cardio-Thoracic Surgery; Prague, Czech Republic, 6-9 October 1996

8. **The alveolar-capillary membrane diffusing capacity and the pulmonary capillary blood volume in heart transplant candidates.**
   OA Al-Rawas, R Carter, RD Stevenson, SK Naik and DJ Wheatley. Submitted for presentation at The American Thoracic Society International Conference, San Francisco, USA, May 1997

Publications

OA Al-Rawas , R Carter, D Richens, A Tweedle, RD Stevenson, S K Naik and DJ Wheatley. Ventilatory and gas exchange abnormalities on exercise in chronic heart failure. Eur Respir J 1995;8:2022-28

Submitted for publication

1. OA Al-Rawas, R Carter, RD Stevenson, SK Naik and DJ Wheatley. The time course of pulmonary transfer factor changes following heart transplantation. (provisionally accepted for publication in Eur J Cardio-Thorac Surg)

2. R Carter, OA Al-Rawas, RD Stevenson, SK Naik and DJ Wheatley. The measurement of the Single Breath Transfer Factor for Carbon Monoxide and its components using the Morgan Transflow System (provisionally accepted for publication in Eur Respir J).

In preparation

1. OA Al-Rawas, R Carter, RD Stevenson, SK Naik and DJ Wheatley. Exercise intolerance following heart transplantation: The role of pulmonary transfer factor impairment.

2. OA Al-Rawas, R Carter, RD Stevenson, SK Naik and DJ Wheatley. Mechanisms of pulmonary transfer factor reduction in heart transplant recipients.

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