Original Articles

The measurement of the single-breath transfer factor for carbon monoxide and its components using the Morgan Transflow system


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In contrast to the standard single-breath transfer factor for carbon monoxide (TlCO), there are no specific guidelines or recommendations for the measurement of its components, the pulmonary capillary blood volume (Vc) and membrane component (DM), by the Roughton and Forster method.

Ten randomly selected heart transplant patients (three life-long non-smokers, seven ex-smokers >1 yr, age range 24-55 years) were assessed on two occasions using either the standard or high-oxygen mixture as the first inspired gas in random order. Ten normal subjects (all non-smokers, age range 23-54 years) were assessed on two occasions using either a long protocol (30 min waiting time between repeat measurements in an individual set) or a short protocol (5 min waiting time). Two technically acceptable results of TLC0 were used to derive a mean value for DM and Vc for each set of measurements (Transflow, P. K. Morgan, Kent, U.K.). The different sequences of gas mixtures produced no significant differences between the values obtained in ten heart transplant patients for mean LWO (mm01 min -1 kPa -1 ) (standard first 5.13 ± 1.15, high-oxygen first 5.14 ± 1.12; limits of agreement -0.57 to 0.59 for DM or for Vc. The long or short protocol produced no significant differences between the means of TLC0 (mm01 min -1 kPa -1 ) (long 8.0 ± 1.9, short 8.0 ± 1.9; limits of agreement -0.5 to 0.5), DM or Vc. This allows the development of a standard test protocol of short duration (about 40 min) making it practical for clinical use without compromising the precision or reproducibility of the results obtained.

Introduction

In contrast to the standard single-breath transfer factor for carbon monoxide (TlCO), there are no specific recommendations on the measurement of its components; the pulmonary capillary blood volume, Vc, and membrane component, DM (1,2). Although many aspects of the measurement of TlCO components are covered by these recommendations and guidelines, several technical questions remain to be answered. These include the effect of carbon monoxide (CO) back-pressure at high alveolar oxygen tensions, the sequence of oxygen mixtures used and time interval between repeated measurements at each of the inspired oxygen concentrations. Repeated measurements of TlCO can increase the level of carboxyhaemoglobin (HBO2), but this is usually not significant if the number of TlCO measurements are limited to a maximum of four per session (2,3). In order to minimize the effects of CO back-pressure and HBO2 during the measurement of TlCO components, Cotes recommended starting with the high-oxygen mixture and then to allow at least 30 min between each measurement (4). Since at least four measurements (two at each of the inspired oxygen mixtures) are required for reproducible results, a single test would take at least 2 h. This recommendation is based on theoretical considerations and has not been evaluated against shorter intervals. In addition, investigations applying the measurement of TlCO components rarely state the sequence of measurement or time interval between repeat measurements. In the few studies which have provided information on this aspect of methodology, measurement was started with the standard oxygen mixture, and the time interval between measurements was 5-10 min (5-7).

A new system developed by P. K. Morgan, the Transflow is a compact computerized system which enables the measurement of the single-breath transfer factor and its
components using two different oxygen mixtures by the method of Roughton and Forster (8).

The purpose of the present study was to investigate the effects of the sequence of gas mixtures, time interval between measurements and reproducibility of TLCO and its components in normal subjects and patients with particular reference to patients awaiting or having undergone heart transplantation who were to be assessed as part of a wider study of transfer factor impairment in heart transplant candidates and recipients.

Methods

MEASUREMENT OF TLCO AND ITS COMPONENTS

TLCO was measured using the single-breath method (Transflow; P. K. Morgan Ltd, Kent, U.K.) according to the recommendations by the European Respiratory Society (ERS). Quality control and procedures of testing were also performed according to formal guidelines established by the ERS (2). The means of two technically acceptable and repeatable TLCO values were reported as the subject’s TLCO. Duplicate measurements of single-breath TLCO were made using two different test gas concentrations. The standard oxygen gas mixture consisted of carbon monoxide (CO) 0.28%, helium (He) 14%, oxygen O₂ 18% with the remainder nitrogen (4). 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. TLCO at standard oxygen concentration was measured in duplicate. The subject was then allowed 5 min of room air breathing followed by another 5 min of pure oxygen breathing while wearing a nose clip. The single-breath TLCO at high oxygen concentration was then measured using the high-oxygen mixture as the inspired gas. This procedure was repeated to give duplicate results. The means of each of the duplicate values of TLCO with their corresponding θ values obtained at standard and high alveolar oxygen tensions were used to derive the diffusing capacity of the alveolar-capillary membrane (Dₐᵥ) and the pulmonary capillary blood volume (Vₐ) using the classic Roughton and Forster equation

\[ 1/TLCO = 1/Dₐᵥ + 1/θVₐ \]

where:

- \( θVₐ \) is the ‘diffusing capacity’ of the total mass of the erythrocytes in the pulmonary capillary blood available for gas exchange at any instant, and
- \( θ \) is the standard rate at which 1 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 (8,9).

The Roughton and Forster equation was solved graphically (Fig. 1) where the intercept of the plotted line with 1/TLCO as the ordinate equals 1/Dₐᵥ and its slope equals 1/Vₐ.

Ten randomly selected heart transplant recipients (age range 23–55 years; Table 1) were studied to investigate the sequence of administration of the two inspired gas mixtures. Three patients were life-long non-smokers and seven were ex-smokers (stopped smoking for more than 1 yr). All patients performed two sets of measurements of TLCO and its components. Each set of measurements consisted of two technically acceptable standard measurements of transfer factor and two measurements at high oxygen level producing four results for Dₐᵥ and Vₐ. These duplicate results were meant to give a representative value of the transfer factor and components for each set of measurements. For each patient the two sets of measurements were separated by at least 1 h and were performed in random order. One set was started with the standard inspired oxygen concentration and the other with the high-oxygen gas mixture. A 5 min waiting period elapsed between each duplicate measurement.

Ten normal volunteers (all life-long non-smokers; age range 24–54 years; Table 1) performed TLCO measurements at high and standard oxygen concentrations using two different timing protocols, in random order. In the short protocol, the time intervals between measurements within the same session were:
TABLE 2. Mean values of TlCO and its components obtained by the two sequences of inspired gas mixtures and by the short and long protocols

<table>
<thead>
<tr>
<th></th>
<th>Mean (sd)</th>
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<tr>
<td></td>
<td>TlCO</td>
<td>D_M</td>
<td>V_c</td>
</tr>
<tr>
<td>High oxygen first</td>
<td>5.14 (1.12)</td>
<td>13.02 (3.99)</td>
<td>42.62 (16.89)</td>
</tr>
<tr>
<td>Standard oxygen first</td>
<td>5.13 (1.15)</td>
<td>13.18 (1.72)</td>
<td>42.22 (16.65)</td>
</tr>
<tr>
<td>Difference</td>
<td>0.01 (0.29)</td>
<td>-0.16 (0.59)</td>
<td>0.40 (1.47)</td>
</tr>
<tr>
<td>Short protocol</td>
<td>8.08 (1.92)</td>
<td>18.45 (3.25)</td>
<td>61.86 (7.14)</td>
</tr>
<tr>
<td>Long protocol</td>
<td>8.02 (1.97)</td>
<td>18.42 (3.21)</td>
<td>61.71 (7.38)</td>
</tr>
<tr>
<td>Difference</td>
<td>0.06 (0.19)</td>
<td>0.03 (0.37)</td>
<td>0.15 (0.59)</td>
</tr>
</tbody>
</table>

TlCO (mmol min \(^{-1}\) kPa \(^{-1}\)), \(D_M\) (mmol min \(^{-1}\) kPa \(^{-1}\)), \(V_c\) (ml).

- 5 min between each of the measurements at standard oxygen,
- 10 min between the sets of measurements at standard and high oxygen, and
- 10 min between each measurement at high oxygen (5 min waiting time and 5 min oxygen breathing).

The corresponding time intervals for the long protocol were 5 min, 30 min and 30 min. The total duration of a session was about 40 min for the short protocol and about 90 min for the long protocol.

The reproducibility of TlCO and its components as measured by the short protocol was assessed in repeat measurements on two consecutive days of TlCO, \(V_c\) and \(D_M\) in 14 subjects (nine heart transplant recipients and five normal volunteers). Variability in each absolute value of the transfer factor and its components was assessed in percentage terms as the coefficient of variation where

\[
CV = 100 \times \frac{\text{sd of the difference}}{\text{mean of repeat set of measurements}}
\]

STATISTICAL ANALYSIS

The degree of agreement between results obtained by the different sequence of gas mixtures or the two timing protocols was assessed using Bland and Altman statistical analysis (10).

Results

Table 2 shows that the two sequences of inspired gases yield results with virtually identical means. Figure 2 displays plots of the difference between repeated measurements of TlCO and its components obtained by the different sequence of inspired gases against their means. Using Bland and Altman analysis, the limits of agreement between duplicate results were -0.57 to 0.59 mmol min \(^{-1}\) kPa \(^{-1}\) for TlCO, -1.34 to 1.02 mmol min \(^{-1}\) kPa \(^{-1}\) for \(D_M\) and -2.54 to 3.34 ml for \(V_c\). Inspection of individual results on the graphs shows that the means of difference between the values for the two sequences of inspired gases are nearly zero with similar scatter above and below the mean for all parameters.

Table 2 shows summary statistics of TlCO and its components obtained by the short and long protocols. The
Fig. 3. Plots of the difference between repeat measurements of (a) TLCO (mmol min⁻¹ kPa⁻¹) and its components (b) $D_M$ (mmol min⁻¹ kPa⁻¹) and (c) $V_C$ (ml) obtained by the long and short protocols against their means.

Table 3 shows summary statistics of the duplicate measurements performed in 14 subjects. The coefficients of variation were 5.6%, 8.7% and 8.9% for TLCO, $D_M$ and $V_C$, respectively.

**Discussion**

The Transflow system is a new piece of equipment for the measurement of the single-breath transfer factor for carbon monoxide. The system contains integral gas analysers and a fixed pneumotachograph for flow and volume measurement. In addition, solenoid-operated valves within the mouthpiece assembly are used for switching between the inspired bag and expired bag after a preset dead-space wash-out. The mouthpiece assembly also contains a port for voiding exhaled gas to atmosphere or for 100% oxygen breathing, from wall-mounted or cylinder oxygen through a valve box and anaesthetic bag reservoir. The system allows easy switching between standard and high-oxygen gas mixtures for the measurement of the transfer factor and its components based on the Roughton and Forster relationship.

The measurement of the components of the transfer factor is to be used to investigate changes in transfer factor prior to and following heart transplantation at the Scottish Cardiopulmonary Transplant Unit. It has been suggested that the use of high inspired oxygen concentration shortly after exposure to carbon monoxide in the standard mixture can potentially increase both CO tension in the pulmonary capillaries and the concentration of $Hb_{CO}$ in blood. As both CO back-pressure and $Hb_{CO}$ are assumed to be negligible in the calculations of TLCO and its components, a significant increase in these parameters can underestimate the values of TLCO, $D_M$ and $V_C$ (2.3). However, using the protocol suggested by Cotes (4), with 30 min waiting time between each measurement, means that the measurement of the constituent components of the transfer factor would take about 2 h. The results of the present study show that good agreement is found between repeat sets of measurements of $D_M$ and $V_C$, regardless of the order of breathing high or standard mixtures or of using a shorter waiting period of 5 min between repeat measurements in heart mean values of measurements obtained by the short or long protocol were identical for TLCO and $D_M$ and nearly identical for $V_C$. Figure 3 shows plots of Bland and Altman analysis. There was good agreement between the values obtained by the two protocols for all parameters. The limits of agreement between the mean values obtained by the short or long protocol were as follows: -0.5 to 0.5 mmol min⁻¹ kPa⁻¹ for TLCO; -0.5 to 0.5 mmol min⁻¹ kPa⁻¹ for $D_M$; 3.2 to 3.5 ml for $V_C$. There was no systematic difference between results obtained using the short or long protocol in individual subjects (equal points above and below the line of mean difference which was zero for TLCO and $D_M$ and 0.1 ml for $V_C$).

Table 3 shows summary statistics of the duplicate measurements performed in 14 subjects. The coefficients of variation were 5.6%, 8.7% and 8.9% for TLCO, $D_M$ and $V_C$, respectively.

<table>
<thead>
<tr>
<th></th>
<th>First</th>
<th>Second</th>
<th>Both</th>
<th>Difference</th>
<th>SD of difference</th>
<th>Coefficient of variation</th>
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<tbody>
<tr>
<td>TLCO</td>
<td>6.48</td>
<td>6.46</td>
<td>6.47</td>
<td>0.02</td>
<td>0.36</td>
<td>5.6%</td>
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<tr>
<td>$KCO$</td>
<td>1.21</td>
<td>1.21</td>
<td>1.21</td>
<td>0.00</td>
<td>0.08</td>
<td>6.6%</td>
</tr>
<tr>
<td>$D_M$</td>
<td>15.73</td>
<td>15.20</td>
<td>15.47</td>
<td>0.53</td>
<td>1.35</td>
<td>8.7%</td>
</tr>
<tr>
<td>$V_C$</td>
<td>46.46</td>
<td>46.18</td>
<td>46.32</td>
<td>0.28</td>
<td>4.07</td>
<td>8.8%</td>
</tr>
</tbody>
</table>
transplant patients and normal subjects. The lack of any significant difference between the results of these measurements independent of order or waiting time indicates that there is no differential effect of the sequence of oxygen mixtures used on CO back-pressure in this group of patients who, it has to be noted, were non-smokers or ex-smokers. This may not hold true for current heavy smokers where a significant level of \( \text{HB}_{\text{CO}} \) is already present. In addition, the lack of difference between the long and short protocols in this particular group of patients shows that the protocol with 5 min waiting time between repeat measurements is practical and provides results similar to those obtained when longer waiting periods were used, bringing the measurement of the components of the transfer factor into an acceptable time period for routine assessment of all our heart transplant patients. The reproducibility of the results of the measurement of \( T_{1,\text{CO}} \), \( V_C \) and \( D_M \) in normal subjects and in heart transplant patients using the short protocol is good and similar to that of published results (11,12). In 18 patients receiving bleomycin treatment for testicular cancers, the coefficients of variation of \( D_M \) and \( V_C \) from duplicate measurements within one session were 7.1% and 8.9%, respectively (12).

References