Clin Physiol Funct Imaging (2017) 37, pp357–365 doi: 10.1111/cpf.12316

REVIEW ARTICLE

Nitrogen monoxide and carbon monoxide transfer interpretation: state of the art

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Summary

Just a few clinicians routinely measure the subcomponents of the lung diffusing capacity for Carbone monoxide (DL_{CO}). This is because the measurement of membrane and blood conductances for CO (Dm_{CO} and $Db_{CO} = \theta_{CO} \times V_c$, respectively) by the classic Roughton and Forster method is complicated and time consuming. In addition, it mistakenly assumes a close relationship between alveolar oxygen partial pressure (PAO_2) and mean intracapillary oxygen partial pressure ($PcapO_2$) which is the true determinant of specific conductance of haemoglobin for CO (θ_{CO}). Besides that, the critical multistep oxygenation method along with different linear equations relating $1/\theta_{CO}$ to $PcapO_2$ gave highly scattered Dm_{CO} and V_c values. The Dm and V_c can also be derived from a simultaneous measurement of DL_{NO} and DL_{CO} with the blood resistance for NO assumed to be negligible. However, recent *in vitro* and *in vivo* experiments point towards a finite value of θ_{NO} (about 4·5 $ml_{NO} \times ml_{blood}^{-1} \times min^{-1} \times mmHg^{-1}$). Putting together the arguments and our clinical data allows us to report here the state of the art in partitioning the CO diffusing capacity into its constitutive components, with the goal to encourage further studies examining the sensitivity of Dm_{CO} and V_c to alterations observed in parenchymal diseases.

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Accepted for publication
Received 12 January 2015;
accepted 5 October 2015

Key words
interstitial lung disease; nitric oxide; pulmonary fibrosis; respiratory function tests

Introduction

Considering that the detection of diseases involving lung parenchyma, for example, idiopathic pulmonary fibrosis (IPF) and pulmonary arterial hypertension (PAH) in preclinical and early stages will improve outcomes and quality of life, prolong survival and, possibly, cure the disease, there is a need for new sensitive physiological tests. Such an effort is critically relevant and would inform issues such as staging of diseases and identification of additional surrogate endpoints for clinical trials.

Following Roughton and Forster (Roughton & Forster, 1957), the transfer of CO from air to blood consists of two conductances in series (Fig. 1). The first is membrane conductance, related to the diffusing capacity of alveolar–capillary membrane. This conductance is the result of passive diffusion through a more or less thin tissue barrier (1 μm), consisting of the alveolar epithelial type 1 cell, a basement membrane and a capillary endothelial cell. Additionally, there is an intracapillary component consisting of a plasma layer of variable thickness between the endothelium and the red cell membrane. The second is lung capillary blood conductance (Db_{CO}) which depends on both the reactivity of CO with Hb and the mass of Hb in the lung capillaries which in turn depends on lung capillary blood volume (V_c): $Db_{CO} = \theta_{CO} \times V_c$.

Diseases that damage the alveolar–capillary membrane could reduce both the membrane conductance (Dm) and the blood conductance (Db) for the gas in test because the alveolar epithelium and pulmonary capillaries are anatomically in such close proximity. Consequently measuring the subcomponents of DL_{CO}, the membrane conductance for CO and the blood conductance is usually believed to add little useful clinical information. However, no further evidence came to support this assumption and this might be incorrect.

IPF is a good example of the putative interest of the calculation of Dm and V_c. Time variations in lung function variables are predictors of mortality in IPF. Pulmonary function testing provides the most standardized approach to objective monitoring and quantification of disease progression. A decline in forced vital capacity (FVC) of 5–10% over 6 or 12 months

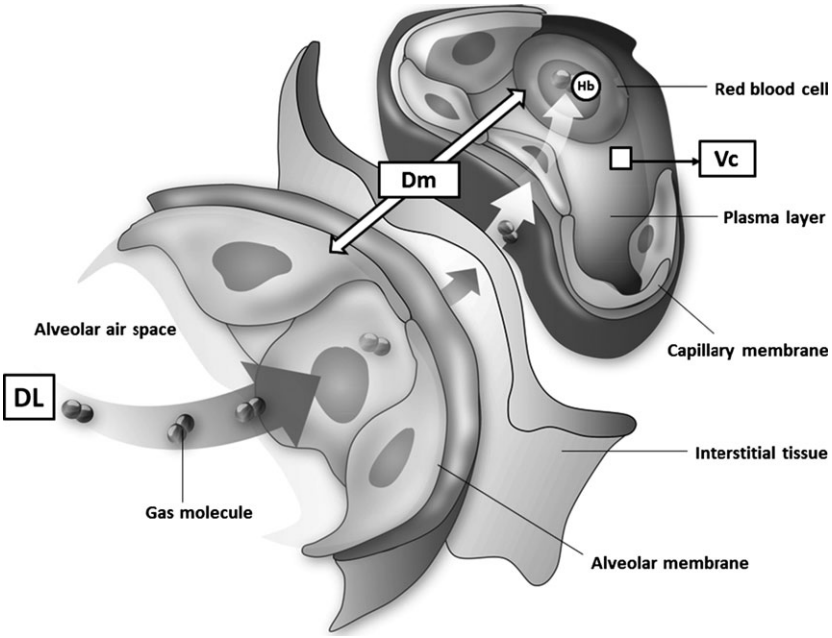

Figure 1 Structure of the alveolar–capillary membrane: DL is defined as the flow rate of a given gas X passing through the structure for a unit of partial pressure difference between alveolar and capillary blood. Dm refers to the total conductance of the tissue layers including: alveolar epithelium, basal membrane, interstitium, capillary membrane, plasma layer and red blood cell membrane. V_c refers to the capillary blood volume.

has been reliably associated with decreased survival (Flaherty *et al.*, 2003). Recent data indicate that in IPF, decline in DL_{CO} has also been associated with decreased survival, although less consistently than FVC (Collard *et al.*, 2003). A decline in absolute DL_{CO} value in the absence of an alternative explanation is consistent with progressive disease altering the membrane structure, although such a decline may also reflect changes in the pulmonary vasculature and coexistent pulmonary hypertension (Nadrous *et al.*, 2005). However, less than 10% change in DL_{CO} should be interpreted with caution. Changes in this range are more likely to overlap with the intrinsic variability of the test (Zavorsky *et al.*, 2008).

The final objective of the article was to discuss the potential added value of the partitioning of DL_{CO} into Dm and Db by providing physiological arguments issued from the use of Roughton–Forster (RF) equation for NO and CO. The conditions to fulfil this objective are as follows: (i) to strengthen the methodology of the measurements and (ii) to disseminate the knowledge of the new trends in the field by all educative means.

Reconsidering the 'classic' Roughton–Forster (RF) equation

The RF model was a major conceptual step forward in understanding alveolar–capillary diffusion for CO, a surrogate for O_2, but it does depend upon a value for the specific conductance of CO with haemoglobin (Hb), θ_{CO}, which is the flow of CO due to the chemical reaction with Hb. θ_{CO} was measured *in vitro* under conditions remote from the rheological situation of red cells flowing through pulmonary capillaries. So, measurements of θ_{CO} *in vitro* may or may not represent the condition *in vivo*.

Impact of the choice of oxygen pressure (PO_2) value on $1/\theta_{CO}$ calculation

The overall conductance for CO transfer is related to its components following RF equation:

$$\frac{1}{DL_{CO}} = \frac{1}{Dm_{CO}} + \frac{1}{\theta_{CO} \times V_c}$$

V_c is corrected taking into account Hb when multiplied by the ratio Hb standard/Hb measured with Hb standard = 14·6 g/dl in male and 13·4 g/dl in female.

In this equation, $1/\theta_{CO}$ depends linearly on PO_2: $1/\theta_{CO} = a + bx\ PO_2$ because O_2 competes with CO for the same Hb-binding sites, causing θ_{CO} to decrease with increasing mean capillary oxygen pressure ($PcapO_2$). As a consequence, DL_{CO} decreases while PO_2 increases. Many equations linking $1/\theta_{CO}$ and PO_2 have been proposed (Hughes & Bates, 2003). An added complexity concerns the red cell membrane permeability (λ) initially introduced by Roughton and Forster which looks now useless as, owing to its thickness and structure, the red cell membrane cannot oppose a significant resistance to gas diffusion as already written by Forster (Forster, 1987).

Dm_{CO} and V_c are traditionally derived from at least two measurements of DL_{CO} at different alveolar PO_2 (PAO_2). One measurement is taken in normoxia and at least another one in hyperoxic condition. Measurements are usually taken using the single breath method. However, other methods can be used provided that reference values with these methods have been published. A plot of $1/DL_{CO}$ (y axis) versus $1/\theta co$ (x axis) yields a straight line where the y-intercept is $1/Dm_{CO}$ and the slope is $1/V_c$ (1) (Fig. 2).

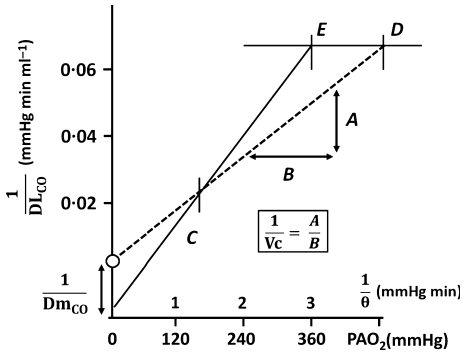

Figure 2 Two-step method to determine Dm and V_c by plotting $1/DL_{CO}$ against $1/\theta_{CO}$. This requires at least two data points which are measured at normal (point C) and high (point D) fraction of inspired O_2. The bias of this method is due to the equivalence made between alveolar PO_2 and mean capillary PO_2, which is the true determinant of $1/\theta_{CO}$. The error is much greater at high PO_2 leading to an overestimation of $1/\theta_{CO}$. Using the right value for PO_2 (point E) would increase the slope of the relationship decreasing then the $1/Dm$ value and increasing the $1/V_c$ value. Therefore, Dm could be grossly underestimated by the method as V_c would be overestimated.

The values of Dm_{CO} and V_c depend critically on the chosen $1/\theta_{CO}$ equation. Various equations (Roughton & Forster, 1957; Holland, 1969; Forster, 1987) led to a wide scatter of data for V_c and Dm_{CO} which render the results given by the method uncertain and poorly used. In this study, we used the 1987 version of RF equation for determining Dm_{CO} and V_c. A recent study aiming to find the right $1/\theta_{CO}$ equation *in vivo* in humans led to the conclusion that Forster and Holland equations were each not optimal as an equation mixing their slopes and ordinates gave better results (Guenard *et al.*, 2013). A future scientifically based consensus on $1/\theta_{CO}$ equation will therefore strengthen the whole method.

The PO_2 value introduced in the $1/\theta_{CO}$ equation is the PAO_2 as, for the sake by coherency, it should be the $PcapO_2$ which is in healthy at rest is slightly different from PAO_2 (−3 mmHg). In patients, the difference between PAO_2 and $PcapO_2$ is greater owing in most case to great VA/Q heterogeneities. As the calculation of $PcapO_2$ in these patients may be unreliable, a compromise would be to take PaO_2 in place of $PcapO_2$ which would slightly overestimate $PcapO_2$, however, less than PAO_2. The difference (PAO_2–PaO_2) increases of 5–7 mmHg for every 10% increase in FiO_2 and the assumed distribution of the ventilation/perfusion ratio (West, 1969). For example, the use of PAO_2, in place of $PcapO_2$, might overestimate the oxygen capillary pressure by 40 mmHg for a PAO_2 value of 450 mmHg obtained with increased FiO_2 which would lead to an underestimation of Dm_{CO} by approximately 150 ml min^{-1} $mmHg^{-1}$ using the Forster $1/\theta co$ equation (Forster, 1987). Thus, the DL_{CO} multiple oxygen fraction method, as used currently, is an unreliable method for the calculations of Dm and V_c. As an accurate measurement of $PcapO_2$ is hardly feasible in hyperoxic condition, this method looks not promising. Data already published with this method (Overbeek *et al.*, 2008) should be considered with care.

Towards a new consensus about DL_{NO} and its subcomponents

$$\frac{1}{DL_{NO}} = \frac{1}{Dm_{NO}} + \frac{1}{\theta_{NO} \times V_c}$$

The DL_{NO} is similar in many ways to the more established DL_{CO}. It differs from the DL_{CO} in being independent of PO_2 and Hb concentration in the normal range. Van der Lee *et al.* investigated the effect of Hb concentration on the DL_{NO} by measuring DL_{NO} (in anaemic patients) and DL_{CO} before and shortly after red cell transfusion. The authors showed that unlike DL_{CO}, DL_{NO} did not change by the increase of Hb concentration (Van der Lee *et al.*, 2005). The DL_{NO} was considered as depending mostly on membrane conductance. This assessment was supported by the rapid reaction of NO with free Hb (hundreds time faster than CO) leading to a very high θ_{NO} value and a negligible $1/(\theta_{NO} \times V_c)$ value. This assumption allowed to simplify the above RF equation to $1/DL_{NO} = 1/Dm_{NO}$. In this condition, DL_{NO} looks to be a surrogate for Dm. Dm_{NO} and Dm_{CO} are proportional, depending on a physical coefficient (α) which has a fixed physical value according to the solubilities and the molecular weights of both gases and is about 2 (Guenard *et al.*, 1987; Borland & Cox, 1991). Any alteration in this coefficient should receive scientifically based evidence.

With Hb in red cell, the reaction rate of NO is much lower owing to the high concentration of molecules of Hb packed in the cell. *In vitro*, the rate of reaction of NO with Hb in vesicles depends on both the concentration of Hb and the size of the vesicles (Sakai *et al.*, 2008). Early *in vitro* measurement of θ_{NO} in red cells (Carlsen & Comroe, 1958) gave a finite value of 4·5 ml NO (min × mmHg)$^{-1}$ per unit of blood volume. This figure was confirmed in dogs (Borland *et al.*, 2010) and in humans (Guenard *et al.*, 2013). Therefore, it appears that the assumption $1/DL_{NO} = 1/Dm_{NO}$ is not correct and that both Dm_{CO} and V_c do contribute to DL_{NO}.

Revised method for calculating Dm and V_c by double NO/CO transfer measurement

The simultaneous measurements of DL_{CO} and DL_{NO} allow calculating Dm_{CO} and V_c; however, the θ_{NO} value used to solve the RF equation is critical. It has been suggested that there is no practical advantage to choosing a finite value for θ_{NO} compared to the initially described method which assumed infinity for θ_{NO} (Hughes, 2013). This point of view was supported by the fact that NO transfer is poorly sensitive to changes in blood Hb concentration unless this concentration becomes very low. However, this reasoning was made as if NO

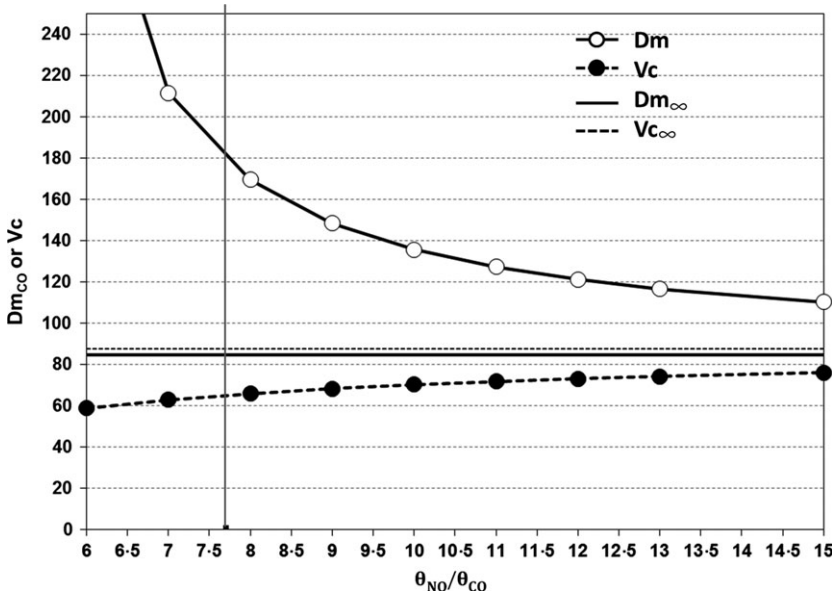

Figure 3 Plot of Dm and V_c values as function of the ratio of NO- and CO-specific conductance with Hb (k). The horizontal lines, continuous and doted, correspond, respectively, to V_c and Dm with the hypothesis of an infinite k ratio for a given set of DL_{CO} and DL_{NO} values (52 and 160 ml min^{-1} mmHg, respectively). The upper curve is Dm as function of k between 6 and 15. Note the curvilinear form of the relation which increases steeply when k decreases. By contrast, the decrease in V_c with k looks small and progressive. The vertical line indicates the recommended k value in normoxia.

behaved as CO. The fact that the reactivity of Hb with NO implies three molecules of NO for one of Hb while one molecule of CO reacts with one molecule of Hb supports the notion that the red blood cell resistance to NO uptake is independent of the Hb concentration at least in the physiological range.

How could we derive Dm and Db from a simultaneous measurement of both the CO and the NO diffusing capacities?

Using the following set of RF equations:

$$\frac{1}{DL_{NO}} = \frac{1}{\alpha \times Dm_{CO}} + \frac{1}{\theta_{NO} \times V_c}$$

with $1/(\alpha \times Dm_{CO}) = 1/Dm_{NO}$ and $\alpha = 1{\cdot}97$.

$$\frac{1}{DL_{CO}} = \frac{1}{Dm_{CO}} + \frac{1}{\theta_{CO} \times V_c}$$

Dm and Db can be calculated if the right set of values for θ_{CO} and θ_{NO} is introduced (Fig. 3).

On theoretical basis, θ_{NO}/θ_{CO} ratio (k) cannot be lower than the DL_{NO}/DL_{CO} ratio which maximal published value in healthy human is 6·5 (Martinot and Guénard, 2014). θ_{CO} recommended value in normoxia is about 0·58 (Forster, 1987) as the recent consensus on θ_{NO} led to a value of 4·5 (both values in ml gas × min^{-1} × $mmHg^{-1}ml^{-1}$ blood).

Thus, the normal value of θ_{NO}/θ_{CO} ratio (k) is 4·5/0·58 = 7·7 above the maximal experimental DL_{NO}/DL_{CO} value observed in healthy subjects. Both the numerator and the denominator in the ratio could change. θ_{NO} could increase in the presence of small concentration of free Hb as observed in several diseases including pulmonary hypertension (Brittain *et al.*, 2014). Our group observed increases in DL_{NO} in such patients, leading to calculated Dm values out of the range of healthy and of patients without free Hb (Martinot *et al.*, 2014). A relative lower DL_{CO} might significantly increase DL_{NO}/DL_{CO} ratio, as shown in experimental massive pulmonary embolism with autologous clog (Harris *et al.*, 2004). The presence of free Hb in this condition, however, not documented is likely.

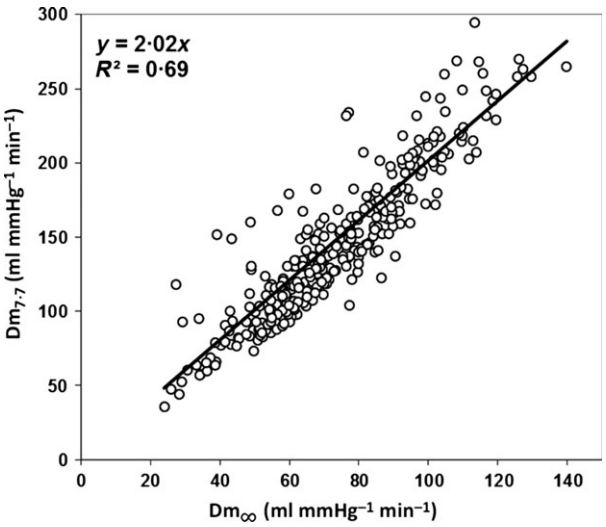

Figure 4 The graph shows the linear, however, slightly scattered relationship between membrane conductances calculated with finite ($Dm_{7\cdot7}$) and infinite (Dm_∞) θ_{NO} in 307 healthy subjects (R^2 = 0·69). Of note, the approximately two times greater value of $Dm_{7\cdot7}$ compared to Dm_∞. The scatter may explain the disagreement in results using Dm7·7 or Dm_∞ in studies comparing the effects of a therapy. The characteristics of the subjects are reported in the supplemental material.

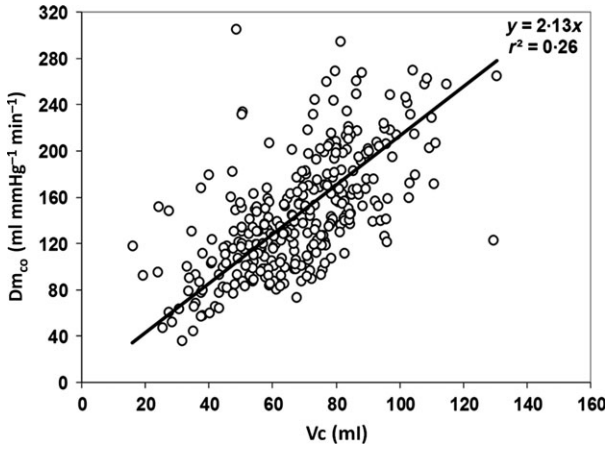

Figure 5 Poor correlation ($r^2 = 0{\cdot}26$) between the membrane conductance (Dm_{CO}) and the capillary volume (V_c) obtained in 307 healthy subjects suggesting a great variability of the structure of the alveolocapillary unit. The characteristics of the subjects are reported in the supplemental material.

θ_{CO} could increase in hypoxic condition whatever the cause and decrease in hyperoxic condition. Moreover, we have to keep in mind the effect of Hb concentration which increases θ_{CO} value in polycythemic patients and decreases it in anaemic patients.

The impact of the use of two values of θ_{NO} one infinite and the other finite is given here, for example. Dm has been calculated using the data of a reference value cohort of healthy subjects (Aguilaniu *et al.*, 2008; Martinot *et al.*, 2014) (Fig. 4) using these two values. The plots of Dm with the finite θ_{NO} option ($Dm_{7.7}$) versus Dm with the infinite option (Dm_{∞}) illustrate three points:

1 $Dm_{7.7}$ is as a mean two times greater that Dm_{∞};

2 for one given value of Dm_{∞}, several values of $Dm_{7.7}$ are possible making the prediction of $Dm_{7.7}$ value from Dm_{∞} inaccurate;

3 the highest values of $Dm_{7.7}$ reached the values given by morphometry (Weibel *et al.*, 1993), providing an indirect proof of the validity of the finite assumption.

Moreover, the relationship between Dm and V_c even in healthy subjects is not tight, when applying the finite θ_{NO} option (Fig. 5). This suggests that in the presence of a local pathological process, Dm_{CO} and V_c could bring different information.

The respective sensitivity of DL_{NO} and DL_{CO} to Dm and V_c values

With the results given by the multistep oxygen method, DL_{CO} was considered to depend on both Dm and V_c in a nearly equal proportion. Thus, patients with either membrane or capillary alterations should have been identified. Currently, using the NO/CO method with $k = 7{\cdot}7$, it can be demonstrated that DL_{CO} is mainly dependent on V_c in most conditions except if Dm is severely reduced (Fig. 6). DL_{NO} is in most conditions dependent equally from Dm and V_c hence DL_{NO} would give alone a better estimation of the function of the alveolocapillary structure. These characteristics could explain the high correlation found between DL_{NO} at rest and the maximal aerobic capacity ($V'O_2max$) (Dridi *et al.*, 2006; Zavorsky *et al.*, 2010). In patients, DL_{NO} would be more sensitive to membrane alteration than DL_{CO}, as DL_{CO} would be more sensitive to microvascular alteration.

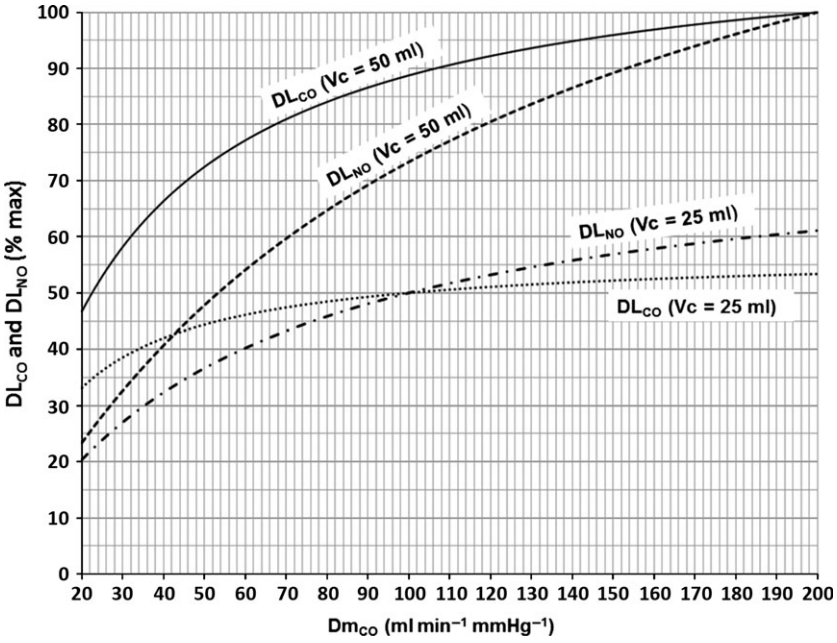

Figure 6 Plot of DL_{CO} and DL_{NO} values as function of Dm_{CO} for two values of V_c, 50 ml for the two upper traces and 25 ml for the two lower traces. Note that DL_{CO} is only sensitive to Dm_{CO} value in the low range below 60 ml (min × mmHg) $^{-1}$ as DL_{NO} remains sensitive to Dm change in the whole range. A decrease of V_c from 50 to 25 ml reduces DL_{CO} by about 50% even when Dm_{CO} increases. By contrast, DL_{NO} increases continuously with the increase in Dm_{CO}.

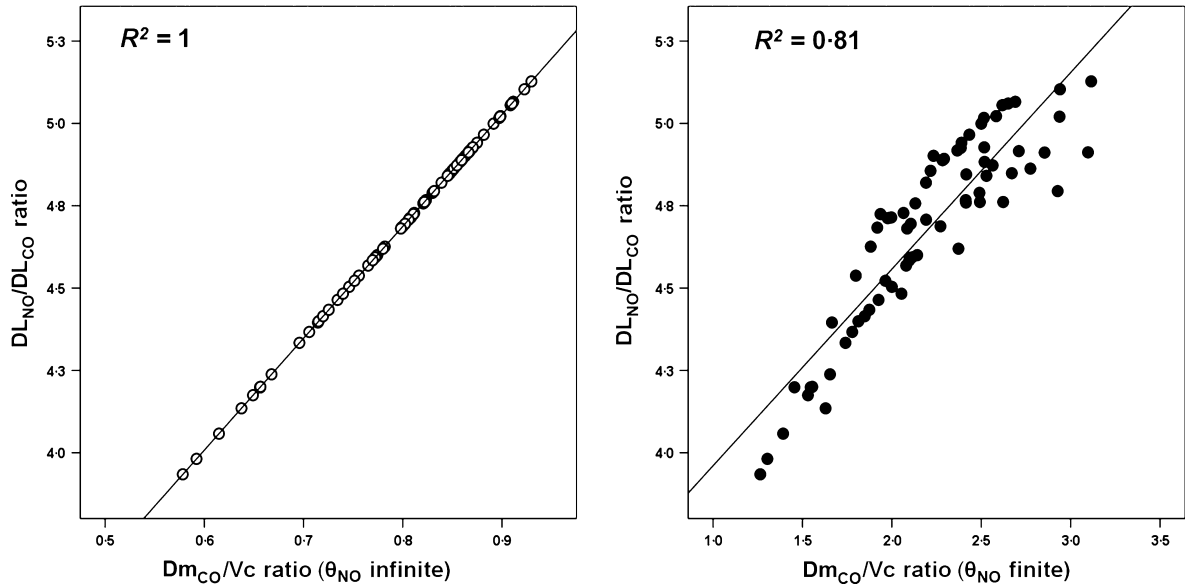

Figure 7 Plots of DL_{NO}/DL_{CO} versus Dm_{CO}/V_c ratio using either an infinite (left) or a finite (right) value for θ_{NO} in a series of healthy subjects. The introduction of a finite value alters the linearity of the relationship and increases by three times the ratio Dm/V_c. The characteristics of the subjects studied are reported in the supplemental material.

In patients with haematological malignancies, lung diffusing capacity might be compromised either before or after the haematopoietic stem cell transplantation (HSCT) procedure. Based on the Dm and V_c values derived from a simultaneous measurement of DL_{CO} and DL_{NO}, Barisione *et al.* have showed that the reduction in DL_{CO} before HSCT is mainly due to a membrane conductance defect and this may worsen after HSCT without lung capillary involvement (Barisione *et al.*, 2014).

DL_{NO}/DL_{CO} ratio

The use of DL_{NO}/DL_{CO} ratio would avoid the uncertainties and assumptions concerning the values of θ_{NO} and θ_{CO}. The normal ratio value lies between 4·3 and 4·9 (Hughes & van der Lee, 2013). DL_{NO}/DL_{CO} ratio is increased in interstitial lung disease mainly in more end-stage diseases and fibrosis. In heavy smokers, it is also increased, but it is not yet known if it will predict the onset of emphysema.

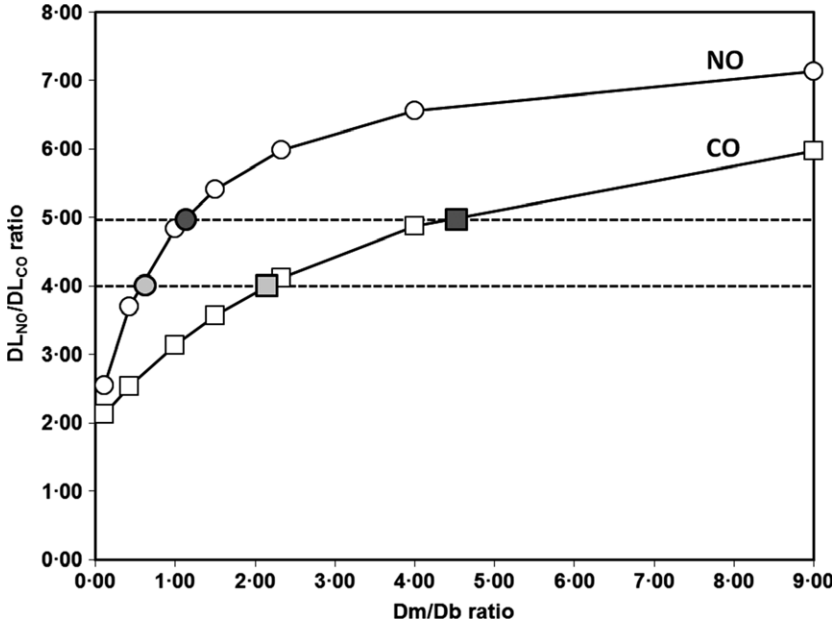

Figure 8 Plots of DL_{NO}/DL_{CO} ratio versus Dm/Db, that is the ratio of membrane and blood conductance for both NO (upper curve, circles) and CO (lower curve, squares): The continuous curves are fitted by eye. The horizontal lines and their intersections with the curves illustrate the changes in Dm/Db for both gases for a difference of 1 in the DL_{NO}/DL_{CO} ratio in the physiological range (4-5). Both Dm/Db ratios double with an increase of DL_{NO}/DL_{CO} of 4 to 5; however, Dm/Db remains around 1 for NO (grey circles) showing that DL_{NO} remained dependent of both Dm and V_c as for CO the Dm/Db ratio shifts from 2·2 to 4·5 (grey squares) showing the greater influence of the vascular component limiting the blood transfer of CO. Therefore, NO transfer looks a more global indicator of the function of the deep lung as CO looks more specifically limited by the blood transfer.

In patients with heart failure, DL_{NO} and DL_{CO} have been measured in various conditions (Guazzi, 2008); however, the reported Dm and V_c data are scarce and looks not, for some of them, reliable for the technical reasons evoked above.

The DL_{NO}/DL_{CO} ratio has been considered to provide an alternative way of investigating the blood gas barrier and alveolar–capillary pathology. The DL_{NO}/DL_{CO} ratio is related to the Dm_{CO}/V_c ratio and α. If an assumption of infinity for θ_{NO} is made, the equation relating DL_{NO}/DL_{CO} and Dm_{CO}/V_c is simple and linear, and the interpretation of the DL_{NO}/DL_{CO} ratio is straightforward, whereas this relationship becomes complex if θ_{NO} has a finite value (Fig. 7). For this reason, looking at DL_{NO}/DL_{CO} ratio to predict what would be Dm and Db is speculative and poorly useful for the clinician. For example, pulmonary hypertension patients have, as a mean, higher DL_{NO}/DL_{CO} than healthy controls. However, a normal DL_{NO}/DL_{CO} ratio does not exclude a pathophysiological state, because both the DL_{NO} and DL_{CO} can be lowered in the same proportions (Van der Lee *et al.*, 2009). Finally, small changes in DL_{NO}/DL_{CO} in the range of 3·5–5·0 may reflect large changes in Dm_{CO}/Db ratio making impossible a correct clinical appreciation of the alterations of Dm and V_c (Fig. 8).

Quality control and technical requirements

The method is exquisitely sensitive to errors in the measurements of concentrations of gases whatever they are. The zero concentration should be controlled carefully before each measurement. Linearity of the analysers is initially checked by the manufacturer; however, this linearity should be rechecked at regular interval of at least 2 years. A straightforward approach is either to measure known serial dilutions of the test gas or to measure the concentration of a separate high-precision test gas having a certificate of analysis. At least two concentrations should be measured along with the zero. Defects in linearity would generally need to replace the electrochemical cell. Manufacturers should be encouraged to automate this linearization procedure. The analysers could respond to several gases, and this cross-sensitivity must be described in the documents provided as well as the way chosen to handle the problem. Detailed guidelines concerning the measurement of DL_{CO} have been published (Macintyre *et al.*, 2005).

Concerning DL_{NO}, a consensus has to be made, the main point of scatter between procedures is the breath-holding time which cannot be as long as the one proposed for CO as the NO transfer in the lungs is about five times faster than for CO. Thus, the concentration of NO at the end of the apnoea is relatively low compared to CO. Only highly sensitive apparatus, too expensive to be widely distributed, could detect the remaining concentration of NO after a 10-s apnoea. Electrochemical cells are cheap and could give reliable results with a 5-s apnoea which could be proposed during a consensus meeting.

As V_c is sensitive to cardiac blood flow the measurement should be made after a period of rest in the seated position. Cardiac frequency could be checked and noted before the measurement. Measurements can be taken in lying patients; however, no reference values seem available. This position induces a shift of blood from the lower body to the thorax increasing then the capillary lung pressure and the capillary blood volume. This increase depends both on the capacity of the lung to recruit capillaries and to their distension. Measurements can also be taken easily during exercise (Martinot *et al.*, 2013), one person should hold the head of the apparatus and help by the voice at the correct performance of the manoeuver.

Conclusion

Several progresses have been made in the application of the RF model to calculate Dm and V_c. The subcomponents of DL_{CO} should provide more information on alteration in lung structure than DL_{CO} alone and could be useful in a near future. Consensus can be reached as (i) to the values of θ for both CO and NO which is a main point (ii) to the value of the breath-holding time around 5 s and (iii) to the meaningless interest of λ in the RF model.

The lung function community has also to agree to the proposition made in this text to substitute $PcapO_2$ to PAO_2 in the equation giving $1/\theta_{CO}$ value. This point is not a major one apart in severely hypoxemic patients.

Future clinical investigations taking into account these bases should provide more accurate information on the pathophysiology of lung diseases. The sensitivity of the method to detect Dm changes should be better and would improve the analysis of clinical trials aiming at testing drugs for IPF, for example. Alterations in V_c following vascular diseases should be better followed by DL_{CO} measurements; however, many diseases induce intricated alterations in both components of the alveolocapillary structure making the combination of NO and CO transfer measurements suitable. We are not at the end of the road, but it seems we have made a step. Thanks to all participants to the walk.

Author contributions

MJB, GH, GiH and DC designed the research. MJB, GH, GiH and DC performed the research. MJB, GH, DXAT and DC analysed the data. MJB and GH wrote the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Glossary

Abbreviation	Definition and comments
Db_X	Lung capillary blood conductance for a given gas. Conductance (D) is a flow of gas in standardized conditions. Db_X includes two parameters: (i) the mass of haemoglobin which can react with gas X. This mass is proportional to the capillary lung volume (V_c) and the concentration of haemoglobin in the blood. (ii) the specific conductance of the gas with haemoglobin (θ_X): flow of gas X taken up by the blood per unit of partial pressure and for a unit of mass of haemoglobin (either in g ml^{-1} or in mmole ml^{-1}). Reference values are given for a normal haemoglobin concentration which is 146 and 134 g l^{-1} for men and women, respectively (21). Patients with anaemia have underestimated V_c values which must be corrected. Conversely patients with polycythemia have overestimated values.
DL_X	Total lung conductance for a given gas (CO, NO and O_2). Flow of the gas X for a unit of partial pressure difference between alveolar and capillary blood. Units are either traditional $ml \times min^{-1} \times mmHg^{-1}$ or international (SI) in mmol $\times$ s^{-1} $\times$ kPa^{-1} (divide by 179 to convert in traditional units), some authors use a mixed unit: mmol $\times$ min^{-1} $\times$ kPa^{-1}(divide by 3 to convert in traditional units). As far as ventilation and volume are not expressed in SI units, it looks wise to use traditional units.
Dm_X	Alveolocapillary conductance or membrane conductance for a given gas X. This conductance includes, apart from the three layers of the membrane, the sheet of plasma between the endothelium and the red cell surface. Dm_X is proportional to lung surface and inversely proportional to the membrane thickness. Dm_X is a mean value including thin structure (around 1 μm) and thick structures which did not participate significantly to gas exchange. Therefore, the effective thickness is not the arithmetic mean of the thickness of entire lung but a pondered mean or, in mathematical term, harmonic mean taking into accounts mainly the thickness of the thin part of the membrane (Weibel *et al.*, 1993). Units are same as DL_X.
$Dm_{7.7}$	Membrane conductance based on a finite value of NO specific conductance (4·5 ml NO $\times$ $mmHg^{-1}$ $\times$ min^{-1} per ml blood)
Dm_∞	Membrane conductance based on an Infinite value for θ_{NO}
FVC	Forced vital capacity
Hb	Haemoglobin
IPF	Idiopathic pulmonary fibrosis
	Ratio of NO- and CO-specific conductance with Hb. θ_{NO}/θ_{CO} = k = 7·7 for θ_{NO} = 4·5 ml NO/(mmHg $\times$ min) per ml blood and $1/\theta_{CO}$ = 1·71 or θ_{CO} = 0·58 ml CO/(mmHg $\times$ min) per ml. The above figures illustrate the main difference between CO and NO. As the blood conductance for CO is much lower than that of NO, CO transfer is more sensitive than NO to a blood transport limitation.
PAO_2	Alveolar partial pressure of Oxygen Should be measured in patients during the diffusion procedure. About 110 mmHg in healthy at rest in normoxia.
PaO_2	Arterial partial pressure of Oxygen Slightly dependent of age till 60 years then independent of age in elderly (Guénard & Marthan, 1996). Above 70 mmHg in normal conditions whatever the age. About 80 mmHg in healthy adults.
$PcapO_2$	Oxygen capillary pressure. Low at the venous entry about 45 mmHg, increases very rapidly to reach the arterial value in the first third of the capillary. The mean capillary oxygen pressure Pcap O_2 (100 mmHg) should be considered as the right PO_2 value to introduce in the calculation of $1/\theta_{CO}$. According to Forster, $1/\theta_{CO}$ = 1·3 + 0·0041 $\times$ Pcap O_2. Yet a consensus has not been found on the equation to use. Instead $PcapO_2$, PAO_2 is often used to calculate the specific conductance of CO. The induced error is not negligible in hypoxemic patients and major if a high fraction of oxygen is inhaled.
PAH	Pulmonary arterial hypertension
RF eq.	Roughton and Forster equation.
V_c	Capillary lung volume
α	Dm_{NO}/Dm_{CO} ratio = 1·97 or roughly 2. This ratio is a constant, unless firm physical evidences are given this value cannot be changed.
θ_X	Specific conductance of gas X with haemoglobin is a critical parameter for the calculation of Dm and V_c. Its value has been determined *in vitro* by many with a considerable scatter for CO. This explains the wide range of Dm and V_c reference values reported in the literature. θ_{NO} considered for a time as infinite is now considered as finite, however, much higher than for CO (about eight times). For oxygen, the conductance depends in part on the oxygen capillary pressure ($PcapO_2$). The θ_X decreases as $PcapO_2$ increases. This complex dependency led to avoid in practice the measurement of Dm and V_c using the oxygen diffusion
λ	Membrane permeability. This parameter was introduced by Roughton group in their model to take into account the possibility of a limitation of diffusion by the red cell membrane. λ was the ratio of membrane permeability to red cell medium permeability close to the membrane. Values between infinity and 1·5 were considered. As the red cell membrane is extremely thin (about 0·05 μm) and is made of compounds having normal solubilties compared to other tissues the introduction of this parameter in the model of diffusion seems now useless.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:
Data S1. Supplemental data.