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EDITORIAL

A place for $T_{L,NO}$ with $T_{L,CO}$?

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In 1915, KROGH [1] first measured the diffusion of carbon monoxide to demonstrate that oxygen passively diffused from the alveolus to pulmonary capillary blood. The history review by HUGHES and BATES [2] discusses this excellently. Subsequently, in 1957, ROUGHTON and FORSTER [3] demonstrated that CO diffusion measured by the diffusing capacity of the lung for CO ($D_{L,CO}$) reflected both alveolar capillary membrane diffusion and reaction with pulmonary capillary blood. The overall resistance is:

$$1/D_{L,CO} = 1/D_m + 1/\Theta V_c \quad (1)$$

where D_m is the membrane diffusing capacity, Θ the specific transfer conductance of the blood (measured by CO reaction with red cells using a rapid reaction apparatus) and V_c the pulmonary capillary blood volume [3]. In healthy young volunteers, $D_{L,CO}$ is 30 mL·min^{-1} torr^{-1}, D_m is 57 mL·min^{-1} torr^{-1} and V_c is 80 mL. To acknowledge that the overall process involved more than just diffusion, J. Cotes coined the term transfer factor of the lung for CO ($T_{L,CO}$).

In 1982, T. Higenbottam and I were studying lung uptake and toxicity of NO in cigarette smokers; A. Chamberlain (our then research assistant) suggested that we measure this as transfer factor of the lung for NO ($T_{L,NO}$). Quite independently, ~10 yrs earlier, the late D. Bargeton and H. Guénard had speculated that if another gas could be found that reacted with haemoglobin, by inhaling it simultaneously with CO, the equation of ROUGHTON and FORSTER [3] could be solved for D_m and V_c in a single breath rather than by measuring $T_{L,CO}$ at two or more oxygen concentrations and cardiac outputs. NO reacts, in effect, instantly with haemoglobin. The resulting single-breath studies from our respective two groups [4, 5] generated much interest, including an editorial in the *European Respiratory Journal* (*ERJ*) [6]. However, after 25 yrs, combined $T_{L,NO}$ and $T_{L,CO}$ is measured by only a few enthusiasts worldwide. In contrast, 25 yrs after the study by ROUGHTON and FORSTER [3], $T_{L,CO}$ had become a standard lung function test in every clinical respiratory laboratory [7]. Why this difference?

For CO, the technique has been standardised by the European Respiratory Society and the American Thoracic Society. Unfortunately for single breath $T_{L,NO}$ and $T_{L,CO}$, there is no such standardisation; therefore, differing inspired NO concentrations and breath-hold times have been used and D_m and V_c have been calculated very differently. We chose 40 ppm NO originally as we calculated this as the alveolar NO concentration after a smoker inhales from a popular UK cigarette brand! Unlike CO, NO is oxidised to the toxic NO_2 in air so the inhaled mixture has to be made immediately prior to inhalation. For NO there is disagreement about the ideal breath-hold time. The original description used 7.5 s rather than 10 s for $T_{L,CO}$. We pragmatically chose this because >40 ppm NO in air is toxic and oxidised too rapidly, and the standard 10-s $T_{L,CO}$ breath-hold gave insufficient exhaled NO for our analyser to detect. Newer analysers are more sensitive, allowing inhaled concentrations as low as 4 ppm to be used with longer breath-holds; however, there is then concern about contamination by endogenous nasal and alveolar NO.

Clinicians and manufacturers have rightly questioned whether combining $T_{L,CO}$ with $T_{L,NO}$ justifies the practical difficulties, potential toxicity and expense. There is also disagreement about what is measured. Whilst nobody disagrees that the equation of ROUGHTON and FORSTER [3] can be solved for the two gases yielding D_m and V_c, there has been disagreement regarding whether $T_{L,NO}$ is equal to or less than the membrane diffusing capacity of NO ($D_{m,NO}$). GUENARD *et al.* [5] reasoned that because the reaction of NO with haemoglobin was instantaneous, Θ_{NO} was, therefore, infinity so that rearrangement of the equation of ROUGHTON and FORSTER [3] yielded:

$$1/V_c = \Theta_{CO}\,(1/T_{L,CO} + a\,/T_{L,NO}) \quad (2)$$

and

$$T_{L,NO} = D_{m,NO} = a\,D_{m,CO} \quad (3)$$

The constant a equals the ratio of diffusivity of:

$$NO/CO = \text{water solubility}/\sqrt{\text{molecular weight}} = 1.97 \quad (4)$$

The results of GUENARD *et al.* [5] for diffusing capacity of CO ($D_{m,CO}$) and V_c were very close to those obtained by the equation of ROUGHTON and FORSTER [3] using $T_{L,CO}$. Others have taken the pragmatic approach further. In 1957, in a combined group of healthy subjects and patients with sarcoidosis, a was found to be 2.42 [8]. Hence some groups have taken a as 2.42 rather than 1.97.

There are a number of scientific concerns about this pragmatic approach. First, laboratory estimates of Θ_{NO} are substantially less than infinity, ~4.5 mL·min^{-1} torr^{-1}. Using these estimates for Θ_{NO} gives higher values for D_m and lower values for V_c [9]. Secondly, in 1987, FORSTER [10] recalculated Θ_{CO} at a physiological pH of 7.4 and obtained a different value, which he thought was the correct one. Using the value from 1987 and $T_{L,CO}$ at differing oxygen tensions also gives lower values for V_c and higher values for D_m, but one group have obtained negative values for $1/D_m$ making the values from 1987

STATEMENT OF INTEREST: A statement of Interest for C. Borland can be found at www.erj.ersjournals.com/misc/statements.html

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unusable for the traditional two-step $T_{L,CO}$ approach in their sample [11]. Thirdly, if a really is 2.42 as a result of chemical interaction with, say nitrosothiols in the alveolar capillary membrane, then arguably the assumption by ROUGHTON and FORSTER [3] of diffusion but not chemical reaction in the membrane is violated. Irrespective of these concerns if the numbers generated for D_m and V_c are reproducible and help clinicians in the diagnosis and management of patients then they are worth using even if some scientists are uncertain of their exact physical and chemical basis.

For CO, large populations of healthy people were tested, giving reference equations based on height, age, sex and smoking status so that individuals with known or suspected lung disease could be tested and a ''% predicted'' or reference to a ''normal range'' (generally 1 SD) quoted. Until 2007, no such data existed for $T_{L,NO}$. Within the last year three such papers have been published; one appears in the current issue of the *ERJ* [11]. AGUILANIU *et al.* [11] have obtained statistically significant associations between $T_{L,NO}$ and age, height and sex. Current smokers were excluded. This growing body of reference data will assist those using $T_{L,NO}$ and $T_{L,CO}$ as a routine clinical respiratory function test. We commend them and the Montreal group [12] on consistency of breath-hold time (5.5 s), inspired NO concentration (40 ppm) and quoting results for Dm and V_c using both values for constant a. The other recent population reference data on $T_{L,NO}$ used an inhaled concentration of 7–9 ppm and a breath-hold time of 10 s consistent with standard $T_{L,CO}$ practice and did not calculate $D_{m,CO}$ or V_c [13]. AGUILANIU *et al.* [11] make a reasonable case for a short breath-hold time; apart from considerations of NO oxidation and detection, breathless patients may have difficulty holding their breath for 10 s.

AGUILANIU *et al.* [11] have also noted a difference in $T_{L,NO}$ between geographic locations and have speculated that this is due to pollution. There are good recent longitudinal data linking particulate matter and airway disease [14] but less that link gas transfer. Clearly, proof of causality would be difficult as dose–response data and robust longitudinal studies are needed. A more likely reason for differences is interlaboratory variation in technique and gas analysis, even if laboratories use identical equipment and algorithms. A study of a single individual tested in five laboratories in London, UK, showed that $T_{L,CO}$ varied from 10.5–20.4 $mL \cdot min^{-1} \cdot torr^{-1}$ [15]. In this context, the 8.5% difference in $T_{L,CO}$ is not unexpected. It was a pity that a subgroup of individuals could not be tested in both laboratories on several occasions over time in the study by AGUILANIU *et al.* [11].

Two other noteworthy developments in the last few years have occurred in $T_{L,NO}$ and $T_{L,CO}$ research. First, the Bordeaux group [16] have taken the mathematical analysis further, considering the pulmonary membrane and capillary as two rectangular boxes sharing a side of identical surface area. $T_{L,NO}/T_{L,CO}$ can then be shown to be inversely proportional to the product of membrane and capillary blood layer thickness [16]. $T_{L,NO}/T_{L,CO}$ thus becomes an index of lung function irrespective of which value for a or Θ_{CO} is used. Secondly, a prototype commercial instrument has now been produced with $T_{L,NO}$, $T_{L,CO}$, D_m and V_c capability (Masterscreen PFT; Viasys-Jaeger, Höchberg Germany). Undoubtedly, part of the success of the single-breath $T_{L,CO}$ test was that robust and practical measuring equipment that gave quick, painless and reproducible results was developed.

Will all these developments mean that combined transfer factor of the lung for NO and transfer factor of the lung for CO will become an essential test in all clinical respiratory function testing laboratories? Time will tell.

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