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Below is an overview of the single article that appeared in this issue of VOLUME:

Questionnaire Based Study of Inter-Laboratory Variability of the Single Breath TLCO Test: Instrumentation, Technique, Calculation, Quality Control and Predicted Values. (David P. Johns, Peter D. Rochford, Hennig Imberger).

[This study was conducted many years before widely accepted consensus guidelines for the measurement (and interpretation) of single breath TLCO (and VA) were published independently by the Europeans (European Respiratory Society, 1993) and North Americans (American Thoracic Society, 1987). In 1985, fully computerised systems were not in common use and almost all calculations were performed manually using tools ranging from handheld electronic (and even mechanical) calculators, log tables and slide rules. There was heavy reliance on information, including equations, provided in text books and the operating manuals supplied by the manufacturers of the equipment. It was not surprising, therefore, that this study identified large and important differences between laboratories in methodology, TLCO and VA working equations, and in the choice of reference values. However, it was surprising that the effect of these differences on the calculated TLCO and VA were not reflected in the choice of reference values or the applied lower limits of normal.]

Our Society played an important role in facilitating discussion between members to identify and reduce these inter-laboratory differences which culminated in the development and adoption by the Society of a TLCO position statement in 1990 (“Minimum guidelines for the measurement of single breath carbon monoxide transfer factor and alveolar volume”) which supplemented the original ATS guidelines published in 1987.

Essential Reading: In 2005 the ERS and ATS Task Force published the following joint consensus statements to standardise the measurement and calculation of single breath TLCO and VA, and to provide guidelines on the selection of reference values and the interpretation of lung function tests (included TLCO):

- a) Standardisation of the single breath determination of carbon monoxide uptake in the lung. European Respiratory Journal. 2005; 26: 720–735.*
- b) Interpretive strategies for lung function tests. European Respiratory Journal. 2005; 26: 948-968.]*

This study by Johns, Rochford and Imberger reports the results of a TLCO and VA survey (questionnaire published in VOLUME September, 1984) designed to provide information on the range of instruments, methods, quality assurance procedures and predicted values used by laboratories in Australia and New Zealand. The survey was done to identify and quantify factors responsible for the well documented inter-laboratory and inter-system variation in these measurements. The questionnaire also included several sets of raw data from which respondents were asked to calculate VA and TLCO as there was evidence that computational errors or the use of inappropriate equations were a major source of inter-laboratory variability.

The study not only reported responses to each survey question but (where applicable) also discussed the theoretical impact the identified variation in laboratory practices could have on the measurement and interpretation of TLCO and VA.

A summary of the 1985 study findings is given below together with a number of comments, which may be of interest. For 'completeness' and to avoid possible confusion with present day practices I have separately included a number of the recommendations included in the current (2005) ERS/ATS statement. However, I caution readers to refer directly to the published standard for definitive information:

- i. **Response to Survey:** The responses from 25 laboratories (NSW =7; Queensland = 6; Victoria = 5; NZ = 3; Tasmania = 2; WA = 1; SA = 1). Three completed questionnaires were excluded because they used the steady state method. Responses from 22 laboratories were included in the survey. However, not all questions were answered by each laboratory - the total number of responses to a given question is denoted below as the denominator.
- ii. **Type of Testing System:** Eighteen laboratories used a semi-automated testing system and four used a manual system.
- iii. **Number of TLCO Tests per Week:** The mean number of TLCO tests performed per week was 20 (range 5-40).
- iv. **Number of Tests and Repeatability:** Most laboratories (n=18/20) aimed to obtain two technically acceptable results and reported the mean (n=10/22) or the best result (n=9/22). There was little agreement between laboratories with regard to acceptable repeatability between measurements (range 1-2% to 5 ml CO/min/mmHg).

[The current ERS/ATS statement recommends that there should be at least two acceptable tests that meet repeatability criteria (i.e. repeat tests should agree to within 3 ml CO/min/mmHg or 10% of highest value) and the mean should be reported. It is also recommended that to avoid a significant increase in COHb, no more than five tests be performed – five consecutive tests has been shown to increase COHb by about 3.5% i.e. 0.7% per test.]

- v. **Average Test Time:** The average test time to obtain duplicate measurements, including time needed to calculate results (usually manually) and generate a report with predicted values, was 16.5 minutes (5-40).
- vi. **Interval Between Repeat Tests:** Eleven of 22 respondents did not wait a specific time between repeat tests. The remaining laboratories waited between 2 and 20 minutes.

[I am aware of two studies presented by members at the ANZSRS ASM which provide persuasive evidence that repeat tests can be performed without waiting 4 minutes as recommended in the latest ATS/ERS statement. The requirement to wait 4 minutes was included in the 1987 ATS guidelines, and was based on a study using a manual TLCO system with which it was not possible to obtain repeat tests within a period of 4 minutes. I would urge these members to publish their data as full papers.]

- vii. **Inspired Gas Mixture:** Laboratories were evenly divided between those who used an inspired CO/inert gas mixture containing 18-19% oxygen (n=11/22) and those using 21% (n=11/22).

[This finding probably reflected different inspired mixtures commonly used in the UK (18-19% O₂) and USA (21% O₂ at sea level). The 18-19% oxygen mixture was cheaper to manufacture as it was prepared by diluting the two components (10% He and 0.3% CO) with air. The mixture containing close to 21% oxygen was more expensive as it is a blend of four gases (i.e. 10% He + 0.3% CO + 21% O₂, balance nitrogen).

Standardising the inspired oxygen concentration is important because TLCO varies with pulmonary capillary blood PO₂. This is because the specific rate of uptake of CO into pulmonary capillary blood (i.e. 'θ' in the equation given in (xiii) below) is proportional to PO₂. Theoretically, the measured TLCO will be slightly higher using the inspired mixture containing the lower oxygen concentration. Quantitatively, TLCO increases by about 0.35% for each mmHg decrease in PAO₂ (or about 0.3% for each mmHg decrease in PIO₂). Note that it is blood oxygen partial pressure that is important. Thus, for standardisation purposes laboratories located at altitude often compensate for the low ambient pressure by using an inspired gas mixture containing a higher oxygen concentration so that the resultant inspired PO₂ is close to that at sea level.

The current ERS/ATS statement recommends that laboratories use an inspired mixture containing the same oxygen concentration used in the reference set, or apply an appropriate adjustment to the reference or measured TLCO. Appropriate adjustments based on altitude (assumes a PIO₂ of 150 mmHg at sea level) is given in the document. Also included is a TLCO adjustment for patients receiving oxygen therapy in which the PAO₂ is higher than room air and is based on the assumption that the normal PAO₂ at sea level is 100 mmHg.]

- viii. **Maximum IVC:** Most laboratories aimed to achieve a maximum IVC during inspiration (n=19/22) with the remaining laboratories aiming for at least 90% VCI (n=2/22), or IVC minus 200 ml (n=1/22). The minimum IVC for performing the test was reported as 1,324 ml (700-2,000).

[It is well known, at least in healthy people, that a low IVC due to failure to inspire to TLC during the manoeuvre can result in an underestimation of TLCO (usually a relatively small decrease) and an overestimation of TLCO/VA (the change can be large due to disproportionately low VA relative to pulmonary capillary blood volume). However, if the low IVC is due to failure to fully expire to RV (but TLC is achieved) then the effect on TLCO and VA and TLCO/VA is small – again this is at least true in healthy people. Thus, it is important that the patient achieve as close to his/her maximal IVC as possible. It is also important that inspiration of the test gas be completed as quickly as possible (this was not asked in the survey) because a fundamental assumption is that inspiration (and expiration) is instantaneous: the same assumption generally applies when measuring TLCO using the rebreathing method i.e. gas mixing between the rebreathing bag and lung is assumed to be instantaneous.

The current ERS/ATS statement recommends that the IVC should be as close to the known VC as possible, preferably at least 85% of the largest known VC, and that inspiration be achieved as quickly as possible (85% of IVC should be inspired in <4 seconds).]

- ix. **Washout and Sample Volumes:** The mean (n=21/21) “customary” washout volume was 835 ml (500-1000); mean sample volume 845 ml (500-1000). In addition, one laboratory used two-thirds of the IVC as the washout volume and another collected the entire expired breath, after discarding 500 ml, as the sample volume! The minimum washout (n=21/21) and sample (n=19/19) volumes were 515 ml (300-800) and 563 ml (300-800), respectively.

[The adequacy of analyser response time in relation to sample volume had only been assessed in 9 of the 22 laboratories. This was important because (from memory) all systems used He as the inert gas and the He analysis was performed using a slow response gas analyser based on thermoconductivity. Current CO and inert gas analysers are generally faster and the sampling flow is lower, therefore, a smaller sample volume is required in most systems today. Nevertheless, the sample volume has to be large enough to ensure that the collected sample is representative of alveolar gas. Some systems use gas analysers which are fast enough to display ‘end-tidal’ gas concentrations during expiration from which it is possible to ensure not only that a representative alveolar sample is measured but also that the applied washout volume was adequate to clear the subjects dead space.]

The current ERS/ATS statement recommends a washout volume of 0.75-1.0 L (but can be reduced to 0.5 L if IVC <2.0 L), and a sample volume of 0.5-1.0 L (but can be <0.5 L if VC <1.0 L provided VD is cleared). The maximum dead space (apparatus + barrier filter) should be <0.350 ml (ERS/ATS Interpretive strategies for lung function tests. European Respiratory Journal, 2005]

- x. **Breath-hold Time:** The mean (n=22/22) “customary” breath-holding time was 9.6 seconds (7-12). The minimum (n=20/20) breath-hold time was 7.4 seconds (5-10) with one laboratory reporting that they observed no lower limit. Variable methods (data not given) for calculating breath-hold were reported, but most did not give details with respondents accepting the value displayed electronically.

[The reported variation in the method of obtaining breath-hold time may introduce inter-laboratory variability in measured TLCO, especially in patients with airflow limitation. The dependence of TLCO on expiratory flow has been shown by Jones and Meade (Q.J.Exp.Physiol. 46: 131-143, 1961) to be significantly reduced if a small sample volume is taken and the breath-hold time calculated from 0.3 of the inspiratory time to mid-sample collection i.e. to take into account the effects of the finite inspiratory and expiratory times.]

The current ERS/ATS statement recommends the Jones and Meade method and that breath-hold time is within 8-12 seconds. It also recommends that the accuracy of breath-hold should be documented every three months if the equipment obtains this automatically.]

- xi. **Bronchodilator:** Most laboratories performed the test before the administration of a bronchodilator (n=13/22). All remaining laboratories routinely measured TLCO after administering the bronchodilator.

[There are complex theoretical reasons both for and against the measurement of TLCO after administering a bronchodilator in patients with airflow limitation. Several studies have investigated this but more are needed.]

The current ERS/ATS statement does not make a firm recommendation regarding whether or not TLCO should be measured before or after the

administration of a bronchodilator. However, they do recommend that the use of a bronchodilator should be noted in the interpretation.]

- xii. **Conditions of Measurement:** A variety of responses were received regarding units, conditions (e.g. BTPS) and working equations to calculate TLCO and VA. VA was reported at BTPS (n=15/21), at STPD (n=5/21), and ATPS in one laboratory. The correction factor used to express VA at BTPS (n=19/19) ranged from 1.09 to 1.120 at 20°C, with three using the correct theoretical value of 1.051. Laboratories that applied the incorrect factor, 1.09 (n=6/19), did so because this was the conventional ATPS to BTPS factor used for spirometry.

[The current ERS/TAS statement provides working equations for calculating VA, including correction (STPD and BTPS) for barometric pressure, ambient water vapour pressure, partial pressure of CO₂ and temperature.]

- xiii. **Correction to a Standard Haemoglobin Concentration:** Correction of TLCO to a standard haemoglobin concentration was performed by 13 of the 22 laboratories. Most used the theoretical equation given based on the Roughton and Forster classical model ($1/\text{TLCO} = 1/\text{DM} + 1/\theta \cdot \text{Vc}$). However, the constants used in the equation varied between laboratories, presumably because different assumptions were made regarding factors such as DM/Vc ratio and PO₂ (affects θ). One laboratory used the experimentally derived equation from Dinakara et al (i.e. Hb corrected TLCO = TLCO measured x $1/\{0.06956 \times \text{Hb}\}$).

When asked to calculate the Hb correction factor appropriate for 8 g/dL, the mean correction reported (n=12) based on the Roughton and Forster equation was 1.40, but the range was large, 1.34-1.80.

[An abnormal Hb concentration can significantly alter the measured TLCO independently of any alveolar-capillary diffusion defect present. Quantitatively, the effect on TLCO of a low Hb concentration (anaemia) is greater than the effect of a high Hb (polycythemia)! That is, the relationship between the Hb correction factor and Hb concentration is not linear - it may be instructive to consider why that is true. The reported wide variation in the Hb correction factor led the publication of a derivation equation based on the Roughton and Forster model. This appeared in the March 1986 issue of VOLUME, which I will review next month.

The current ERS/ATS statement recommends that the Hb correction be based on Roughton and Forster model ($1/\text{TLCO} = 1/\text{DM} + 1/\theta \cdot \text{Vc}$) with an assumed DM/Vc ratio of 0.7, standard Hb 14.6 g/dL (and presumably a PO₂ of 110 mmHg) in males and adolescence, and 13.4 g/dL in females and children <15 years. Thus, two different Hb correction equations apply.]

- xiv. **Correction for COHb:** Correction for COHb was not routinely applied. One laboratory always corrected for COHb when DM and Vc were measured.

[Elevated COHb (i.e. >2%) reduces TLCO because it causes an effective anaemia (i.e. CO binds to Hb) and the development of significant CO back-pressure (reduces CO driving pressure). Quantitatively, the combined effect of an elevated COHb is to reduce TLCO by 1% for each 1% increase in COHb.

The current ERS/ATS statement recommends that an adjustment be applied in people who have significant COHb (>2%). No correction is required if COHb

levels are <2% because reference values, which are usually based on non-smokers, already takes into account.]

- xv. **Calculation Tools:** Seventeen respondents used a programmed calculator to calculate TLCO and VA. The remainder used a non-programmable calculator.

[In 1985 I was also aware of several scientists who used a slide rule and log tables. A scientist who worked with me at the Austin Hospital, Mrs Vera Marek, used a cylindrical slide-rule and could accurately complete the TLCO and VA calculations within one minute!]

- xvi. **Calculating TLCO and VA from Raw Data:** Respondents were asked to calculate VA and TLCO from four sets of raw data. Two of the four sets included correction for abnormal Hb concentration. Twenty responses were received but the results from two laboratories were excluded as it was not certain whether or not correction to a standard Hb concentration had been applied.

The results indicated large inter-laboratory computational differences for both VA and TLCO. The mean variability for VA was 17% (14-22) and for TLCO, 29% (13-78)! The authors suggested that this cast doubts as to the adequacy of available literature providing complete VA and TLCO working equations.

[This was a major finding and led the authors to develop VA and TLCO (and correction for Hb – see above) working equations, including their derivations and assumptions. As mentioned this paper was published in the March 1986 issue of VOLUME, and I will review this next month.]

- xviii. **Quality Control:** Most laboratories assessed the accuracy of their volume (n=18/22) and time (n=14/22) signals, and assessed for leaks (n=18/22). However, the frequency with which these were performed varied widely from “once only” to ‘every test’. Gas analyser linearity checks were performed by 12 laboratories (not checked by 12 laboratories) using either a Wosthoff gas mixing pump (n=2/12) or volumetric/syringe dilution (n=10/12). Most laboratories (n=19/22) used a healthy laboratory subject to assess the overall performance of their testing system. The frequency of testing varied from weekly to occasionally.

[Although calibration of the CO and inert gas analysers to set the ‘gain’ is not critically important in the measurement of TLCO and VA, it is important that they have a linear response, correctly adjusted ‘zero’, and that they remain stable during the test. The authors calculated the potential errors in VA and TLCO due to various combinations of non-linear CO and inert gas analysers by assuming that the calibration error curve assumed a parabolic function ($Y = AX^2 + BX$) passing through the zero and full scale points. Their data showed that large errors can occur, particularly when the CO analyser is non-linear. For example, errors in TLCO as high as $\pm 5.3\%$ can occur when the CO analyser alone is $\pm 1\%$ non-linear. [In 1988 the authors repeated this analysis using raw TLCO data from 50 patients. For this population they found that if the CO and inert gas analysers were $\pm 1\%$ non-linear in opposite directions (worse case scenario) then the absolute error in TLCO can be as high as 19% (mean 6.5%), thus producing a potential inter-laboratory error as high as 38% (mean 12.9%!)]

The current ERS/ATS statement recommends an analyser linearity (from zero to full scale) of $\pm 0.5\%$ of full scale and that the drift over the duration of the test be $< \pm 0.5\%$. Analyser linearity should be checked every three months. The recommendation for volume accuracy is the same as that given in the ERS/ATS statement and should be checked daily i.e. $\pm 3.5\%$ when assessed using a syringe with an accuracy of $\pm 0.5\%$ (note: accuracy specification = $\pm 3\%$ + accuracy of calibration syringe). The accuracy specification of the time signal is $\pm 1.0\%$ and should be checked every three months.

- xix. **Reference Values:** The survey showed that the most commonly (n=11) used reference values were those published by Cotes (Lung Function: assessment and application in medicine. 4th Ed. 1979). Three laboratories used equations by Gaensler and Wright (Arch. Environ. Health 12: 146-189, 1966) and three used McGrath and Thomson (J. Physiol. (London) 146: 572-582, 1959). Individual laboratories used Crapo and Morris (1981), Sinclair et al (1980) or equations they derived themselves.

For a male aged 40 years and height 170 cm these equations predicted a mean TLCO between 27 to 36 ml CO STPD/min/mmHg. Analysis showed that there was no relationship between each laboratories choice of reference values and the equations they applied to calculation TLCO from the raw data (see xvii).

The normal range used by the respondents as reported by 10 laboratories varied widely: \pm SD (n=2), $\pm 10\%$ (n=2), $\pm 20\%$ (n=3), $\pm 25\%$ (n=2) and $\pm 1.96 \times$ SD (n=1).

[The current ERS/ATS statement recommends that the lower 5th percentile of the reference population should be used as the lower limit of normal for TLCO and TLCO/VA.]

The authors concluded:

“The measurement of alveolar volume and single breath CO transfer factor, often considered a straightforward routine measurement of lung function, requires continual attention and investigation in order to reconcile the large variety of individual approaches demonstrated in this paper”

This relatively early study clearly demonstrated that large differences exist between laboratories across Australia and New Zealand in the measurement and, in particular, the working equations used to calculate VA and TLCO and correct for abnormal Hb concentrations.

Please contact me if you are interested in a copy of this or any other issue of VOLUME.

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