Respiratory Medicine (2007) **101**, 989–994

ELSEVIER

respiratoryMEDICINE

DLCO versus DLCO/VA as predictors of pulmonary gas exchange ☆

David A. Kaminsky[a,*], Todd Whitman[b], Peter W. Callas[c]

[a]*Pulmonary Disease and Critical Care Medicine, University of Vermont College of Medicine, Given D-302, 89 Beaumont Avenue, Burlington, VT 05405, USA*
[b]*Fletcher Allen Health Care, University of Vermont College of Medicine, 111 Colchester Avenue, Burlington, VT 05401, USA*
[c]*University of Vermont, 25H Hills Agricultural Sciences Building, Burlington, VT 05401, USA*

Received 28 March 2006; accepted 1 September 2006
Available online 18 October 2006

KEYWORDS
Diffusing capacity;
Transfer factor;
Transfer coefficient;
Alveolar volume;
Gas exchange

Summary
Background: The diffusing capacity of the lung is usually reported as both the diffusing capacity (DLCO) and the diffusing capacity divided by the alveolar volume (DLCO/VA). However, it is unclear which measure to use when interpreting pulmonary gas exchange. We therefore conducted this study to determine whether the DLCO or the DLCO/VA is a better predictor of oxygen desaturation with exercise.
Methods: We retrospectively analyzed the pulmonary function records of all patients who had measurement of their diffusing capacity and 6-min walk oximetry in our university pulmonary function laboratory over a 2-year period.
Results: There were data available on 97 patients, most of who had interstitial lung disease and/or lung volume restriction. The median DLCO was 51% predicted and the median DLCO/VA was 64% predicted. The prevalence of exercise desaturation was 43%. The overall sensitivity and specificity as determined by the area under the receiver operator characteristic (ROC) curve was higher for DLCO than DLCO/VA, with an optimal cut-off of normal of 55% predicted. The positive predictive values were equally low for both measures, ranging from 50% to 70%. After adjustment for VA, there were no differences between the ROC curves or predictive values for DLCO and DLCO/VA.
Conclusion: After adjusting for VA, neither the DLCO nor the DLCO/VA was better at predicting oxygen desaturation with exercise. The optimal cut-off of normal was 55% predicted.

Abbreviations: DLCO, diffusing capacity of the lung for carbon monoxide; DLCO/VA, diffusing capacity of the lung for carbon monoxide divided by alveolar volume; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; KCO, transfer coefficient of carbon monoxide (also known as DLCO/VA); NPV, negative predictive value; PFT, pulmonary function test; PPV, positive predictive value; ROC, receiver operator characteristic; TL, transfer factor of the lung (also known as DLCO); TLC, total lung capacity; VA, alveolar volume

☆Financial Disclosure: Supported by the Vermont Lung Center, University of Vermont.

*Corresponding author. Tel.: +802 656 8819; fax: +802 847 8194.
E-mail address: david.kaminsky@uvm.edu (D.A. Kaminsky).

0954-6111/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2006.09.003

Introduction

The diffusing capacity of the lung for carbon monoxide (DLCO), also known as the transfer factor (TL), is commonly recognized as an indicator of the gas exchange function of the lungs. The DLCO is useful in a variety of clinical settings, including distinguishing emphysema from chronic bronchitis and asthma; evaluating diseases of the pulmonary vasculature and interstitium; screening for tolerability of lung resection surgery; and establishing criteria for disability benefits. The DLCO has also been used to predict exercise desaturation in patients with COPD[1,2] and interstitial lung disease,[3,4] and in unselected patients.[5–8]

Like any clinical test, the DLCO has its limitations and must be interpreted carefully. One of the most important considerations is the effect of lung volume on the DLCO.[9] The DLCO is usually reported both as an absolute number (DLCO) as well as a value that has been divided by alveolar volume (VA), the DLCO/VA, also known as the transfer coefficient (KCO). Since the DLCO/VA appears to account for differences in lung size, it might be thought to represent a more accurate expression of the intrinsic gas exchange function of the lung. However, in normal subjects, the DLCO increases and the DLCO/VA decreases with VA,[9] so clearly the expression does not correct or standardize for lung volume. In fact, the DLCO/VA is the transfer coefficient for the diffusion of CO into the blood, and is an essential part of the equation used to calculate the DLCO.[10] The global diffusing capacity is calculated as the DLCO/VA multiplied by the overall lung volume, VA. Thus, the two parameters are fundamental to calculating the diffusing capacity of the lung.

In disease states, the DLCO and DLCO/VA are often discordant. For example, in patients with low lung volumes, including interstitial lung disease and patients with extraparenchymal restrictive disease (e.g. kyphoscoliosis), the DLCO/VA often exceeds the DLCO when expressed as percentage of predicted values. While some authors interpret these findings as relating to underlying lung disease states,[11] others believe the discrepancies can be largely explained by the inadequacies of the predicted equations.[12,13] These predicted equations are usually improved by incorporating lung size into the predicted formula.[12–14]

Because both the DLCO and DLCO/VA are reported along with the VA in most pulmonary function laboratories, the clinician is faced with the dilemma of deciding which value to use in interpreting the diffusing capacity of the lung. Since one of the most common reasons for measuring the diffusing capacity is to ascertain information about the gas exchange function of the lung, we asked whether the DLCO or the DLCO/VA would be more accurate in predicting oxygen desaturation with exercise. To answer this question, we conducted a retrospective chart review of patients who had concurrent measurement of both diffusing capacity and exercise oximetry. From this information, we assessed the diagnostic utility of the DLCO and DLCO/VA for exercise desaturation by comparing their respective receiver operator characteristic (ROC) curves.

Methods

We analyzed the pulmonary function test (PFT) records of all patients over a 2-year period that had testing that included both diffusing capacity and 6-min walk with oximetry. We recorded demographic data including age, sex, height, weight and diagnosis, as well as lung function measurements including FEV_1, FVC, DLCO, DLCO/VA, 6-min walk distance and oximetry, and, when available, TLC. All tests were done at our PFT laboratory by certified technologists using commercial equipment (Medical Graphics Corporation, Minneapolis, MN). Spirometry was measured by pneumotachometer, lung volumes by body plethysmography, and diffusing capacity by the single breath method using neon as a tracer gas and incorporating a breath-hold time as defined by Jones and Meade. All spirometry and diffusing capacity measurements met quality guidelines as established by the American Thoracic Society.[15,16] Oximetry during the 6-min walk test was recorded with a pulse oximeter (Nellcor, Pleasanton, CA). The study was approved by the Institutional Review Board of the University of Vermont and was granted a waiver of informed consent.

Definitions of lung function abnormalities included "Normal", if the $FEV_1 \geqslant 80\%$ predicted value,[17] $FVC \geqslant 80\%$ predicted and $FEV_1/FVC >$ lower limit of normal (LLN = 5th percentile); "Obstruction", if $FVC \geqslant 80\%$ predicted and $FEV_1/FVC <$ LLN; and "Restriction", if $TLC < 80\%$ predicted, or, if the TLC was not available, if $VA < 75\%$ predicted and $FEV_1/FVC >$ LLN.[18]

We calculated the predicted DLCO based on the equations of Miller,[19] and then[14] calculated the predicted DLCO/VA by dividing the predicted DLCO by the predicted VA. The predicted VA was calculated as the predicted TLC (from the equations of Goldman)[20] minus the predicted deadspace (calculated from $V_d = 24 \times \text{height (cm)} \times \text{height (cm)}/4545$).[21] Because only a few patients had hemoglobin levels available, we did not correct the DLCO for hemoglobin. However, we did adjust the DLCO and DLCO/VA for each subject's own VA using the equations of Johnson[13] in order to determine whether adjustment for VA influenced the findings.

We defined oxygen desaturation as a $\geqslant 4\%$ absolute fall in saturation by pulse oximetry during a 6-min walk test.[5,6] The 6-min walk distance was referenced according to the predicted values of Enright and Sherrill.[22]

Statistical analysis

All data are summarized using descriptive statistics based on data distribution using JMP 3.1 (SAS Institute Inc., Cary, NC). We calculated the correlation between exercise desaturation and the DLCO and DLCO/VA using Spearman rank correlation. We calculated sensitivity, specificity and positive predictive value (PPV) and negative predictive values (NPV) for DLCO and DLCO/VA relative to abnormal gas exchange (desaturation) for cut-off values of normal from 50% to 80% predicted, in increments of 5. Comparisons of sensitivity and specificity between DLCO and DLCO/VA were made using the exact binomial test,[23] and of PPV and NPV between DLCO and DLCO/VA using score statistics.[24] We derived optimum cut-offs for DLCO and DLCO/VA based on ROC curves and compared the two tests for their utility based on a comparison of the area under the curve (AUC) of the ROC data.[25] Two-sided P-values $\leqslant 0.05$ were considered statistically significant.

To determine the sample size, a previous study found that an abnormal DLCO (defined as <80% predicted) had a sensitivity of 0.22 for abnormal gas exchange.[8] Assuming DLCO/VA would be the same, we required 61 subjects to reveal a sensitivity of 22% with a 95% confidence interval of ± 0.10. This range of sensitivity seems appropriate as it was seen in that study for cutoff values of normal DLCO from 70% to 90% predicted.[8] Based on a survey of the tests done in our laboratory, we estimated that we needed to review the PFT records of all patients that had DLCO and 6-min walk testing over a 2-year period in order to achieve the target sample size.

Results

A total of 104 individual subjects who had both DLCO and 6 min walk testing were identified. After discarding data from 7 subjects that were technically invalid (based on VA<90% best VC in all cases),[15] we included data from 97 patients. Their demographic data are shown in Table 1. There were a nearly equal number of men and women, of average age 66 years. More than half had a history of smoking cigarettes. The most prevalent diagnosis was interstitial lung disease ($n = 44$) or restriction on lung function testing ($n = 28$, 14 of whom were also classified as interstitial lung disease). We had a small number with emphysema ($n = 8$), asthma or chronic bronchitis ($n = 5$), mixed obstruction and restriction ($n = 1$) and pulmonary vascular disease ($n = 8$). Some patients did not clearly have a diagnosis other than dyspnea ($n = 17$). When classified purely by lung function testing, 21 subjects had airflow limitation as previously defined. This group included 7 subjects with emphysema, 5 with asthma or chronic bronchitis, 5 with concomitant interstitial disease or restriction, 3 with unknown disease, and 1 with pulmonary vascular disease.

Table 1 Characteristics of the study population.

	Absolute*	% Predicted
Age (years)	66 ± 11	
Sex	51M, 46F	
Ever smokers (%)	65	
Pack-years	20 (0–41)	
Prevalence of exercise desaturation (%)	43	
FEV_1 (L)	2.66 (2.13–3.37)	78 (67–89)
FEV_1/FVC	0.75 (0.69–0.81)	98 (93–106)
DLCO (ml/min/mmHg)	11.4 (8.5–16.1)	51 (38–66)
DLCO/VA (ml/min/mmHg/L)	2.8 (2.1–3.6)	64 (48–81)
VA (L)	4.1 (3.5–5.2)	84 (74–93)
Resting oxygen saturation (%)	98 (96–100)	
Maximal change in oxygen saturation with exercise (absolute %)	−3 (−2 to −6)	
6-min walk distance (m)	306 (200–424)	61 (47–77)

*Data given as mean ± SD or median (25–75 interquartile range).

Table 2 Sensitivity, specificity, positive predictive value and negative predictive value of DLCO and DLCO/VA related to oxygen desaturation at various % predicted cut-off values.

Cut-off	Sensitivity	Specificity	PPV	NPV
50%				
DLCO	0.76*	0.73	0.68	0.80*
DLCO/VA	0.45	0.85*	0.70	0.67
55%				
DLCO	0.86*	0.67	0.67	0.86*
DLCO/VA	0.55	0.75	0.62	0.68
60%				
DLCO	0.88*	0.60	0.63	0.87*
DLCO/VA	0.67	0.71	0.64	0.74
65%				
DLCO	0.95*	0.47	0.58	0.93*
DLCO/VA	0.71	0.65*	0.61	0.75
70%				
DLCO	0.95*	0.35	0.53	0.90*
DLCO/VA	0.81	0.53*	0.57	0.78
75%				
DLCO	0.95	0.29	0.51	0.89
DLCO/VA	0.88	0.49*	0.57*	0.84
80%				
DLCO	0.98	0.25	0.50	0.93
DLCO/VA	0.88	0.36	0.51	0.80

*Indicates DLCO and DLCO/VA differ significantly ($P \leqslant 0.05$) within cut-off value.

Pulmonary function data are also shown in Table 1. On average, subjects had a reduced FEV_1 and FVC but no obstruction. The median DLCO and DLCO/VA were reduced 50–60% predicted, and 43% demonstrated desaturation by pulse oximetry during 6-min walk testing. The DLCO was highly correlated with the DLCO/VA (rho = 0.88, $P<0.0001$). Changes in oxygen saturation correlated poorly but significantly with both the DLCO and the DLCO/VA (rho = −0.51, $P<0.0001$; −0.40, $P<0.0001$, respectively).

The sensitivity, specificity, PPV and NPV for DLCO and DLCO/VA vs. oxygen desaturation are shown in Tables 2 and 3. In general, the PPVs were quite low, ranging from 0.50 to 0.70 for various cut-off values of DLCO and DLCO/VA between 50% and 80% predicted. The NPVs were somewhat better, ranging from 0.67 to 0.93.

Comparing the DLCO and DLCO/VA, the sensitivity of DLCO was greater than that of DLCO/VA for all cut-off values = 50–70%, and the area under the ROC curve was greater for DLCO than for DLCO/VA (0.81 vs. 0.74, $P = 0.01$), Fig. 1. The optimal cut-off for maximizing sensitivity and specificity was 55%. There were no differences in the ROC

Table 3 Sensitivity, specificity, positive predictive value and negative predictive value of DLCO and DLCO/VA corrected for VA related to oxygen desaturation at various % predicted cut-off values.

Cut-off	Sensitivity	Specificity	PPV	NPV
50%				
DLCO	0.62	0.80	0.70	0.73
DLCO/VA	0.62	0.78	0.68	0.73
55%				
DLCO	0.71	0.73	0.67	0.77
DLCO/VA	0.71	0.73	0.67	0.77
60%				
DLCO	0.86	0.60	0.62	0.85
DLCO/VA	0.83	0.64	0.64	0.83
65%				
DLCO	0.90	0.53	0.59	0.88
DLCO/VA	0.90	0.56	0.61	0.89
70%				
DLCO	0.95	0.45	0.57	0.93
DLCO/VA	0.93	0.49	0.58	0.90
75%				
DLCO	0.95	0.33	0.52	0.90
DLCO/VA	0.95	0.35	0.53	0.90
80%				
DLCO	0.95	0.25	0.49	0.88
DLCO/VA	0.95	0.27	0.50	0.88

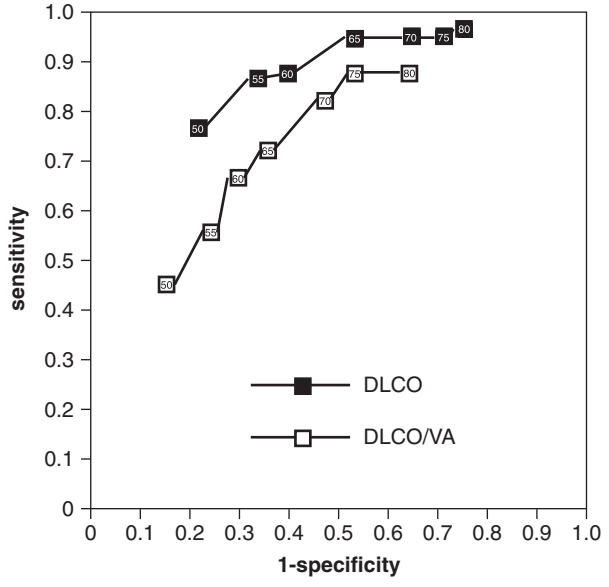

Figure 1 Receiver operator characteristic curves for DLCO and DLCO/VA in relation to oxygen desaturation during 6-min walk test. The symbols represent the cut-off values of 50–55–60–65–70–75–80% predicted.

curves or predicative values of DLCO and DLCO/VA when corrected for VA (AUC = 0.80 vs. 0.79, $P = 0.80$).

Because we thought that the DLCO/VA might be better than DLCO in relating to gas exchange in patients with restrictive lung disease, we analyzed patients with restriction separately. In this group of 28 subjects, the prevalence of oxygen desaturation was 64%, and the PPV for each measure increased slightly, as expected, compared to the PPV for these measures in relation to the whole study population (e.g., at the 55% cut-off, the PPV of DLCO increased from 0.67 to 0.77, and the PPV of DLCO/VA increased from 0.62 to 0.80). The PPV for DLCO/VA was slightly higher than that for DLCO at the 70% and 75% cut-offs (0.81 and 0.79 vs. 0.64 and 0.64, $P = 0.04$ and 0.02, respectively). The areas under the ROC curves for the restricted patients only remained similar (DLCO = 0.84 vs. DLCO/VA = 0.80, $P = 0.37$). After adjusting the DLCO and DLCO/VA for each patient's VA, there were no longer any differences between the DLCO and DLCO/VA for the restricted patients only.

Because gas maldistribution may affect the DLCO test, we reanalyzed the data for the entire cohort excluding the patients with airflow limitation ($n = 76$). Similar to the whole cohort, we found that the area under the ROC curve for DLCO was greater than that for DLCO/VA (0.79 vs. 0.73, $P = 0.03$), with the DLCO having better sensitivity and NPV and the DLCO/VA having better specificity. After adjusting the DLCO and DLCO/VA for each patient's VA, there were no longer any differences between the measures for this cohort.

Discussion

The results of this study show that the DLCO had a slightly better ability to predict oxygen desaturation than the DLCO/VA, with a cut-off of normal being 55% predicted. However, when the DLCO and DLCO/VA were adjusted for VA, neither measure outperformed the other in terms of predicting oxygen desaturation, with both measures having equally poor PPVs in the 50–70% range. The NPVs were somewhat higher, depending on the definition of the cut-off point for normal. Thus, a normal DLCO or DLCO/VA was better at excluding exercise desaturation than an abnormal value was at predicting exercise desaturation, which is in line with previous studies.[1,2,6–8] The advantage of DLCO over DLCO/VA was not apparent for the restricted only patients, but this was a very small sample from which to draw firm conclusions. The results did not appear to be influenced by the presence of airflow limitation, since they were similar whether or not patients with airflow limitation were included in the analysis.

To our knowledge, only one other study has directly compared DLCO vs. DLCO/VA as an indicator of gas exchange.[3] This study found that DLCO/VA correlated more closely with gas exchange than DLCO in patients with idiopathic pulmonary fibrosis, although the exact magnitude of the difference was not stated. In another study of unselected subjects,[8] the DLCO alone was found to be a specific but relatively insensitive predictor of gas exchange. In that study, a cut-off of 70% predicted appeared to best discriminate gas exchange abnormalities. Other studies of

patients with COPD[1,2] and interstitial lung disease[4] found that quite low DLCO percent predicted values (55–70%) were required to achieve adequate specificity in predicting oxygen desaturation. Similarly low DLCO percent predicted (50–60%) was associated with high sensitivity and specificity in the study of unselected patients by Kelley and coworkers.[6]

It is important to recognize that the DLCO and the DLCO/VA are rather poor predictors of oxygen desaturation, with PPVs in only the 50–70% range. The primary reason for this is likely that the DLCO and DLCO/VA are static measures of oxygen diffusing capacity. Yet, the exchange of oxygen under conditions of exercise is very complex, involving alterations in pulmonary blood flow, lung volume and metabolism. The reason that the DLCO was a better predictor of gas exchange than the DLCO/VA may be because the DLCO is a more global measure of diffusing capacity, which takes into account not only the intrinsic gas exchange ability of the lung (the DLCO/VA), but also the overall lung size and distribution of ventilation (VA).

The fact that the slight advantage of the DLCO over the DLCO/VA as a predictor of gas exchange was no longer apparent after adjusting both for VA illustrates an important physiological characteristic of the diffusing capacity. The DLCO increases with VA, but the DLCO/VA decreases with VA,[9] so adjustment for VA is critical in interpreting the diffusing capacity. While the clinical significance of these effects is unclear,[21] the adjusted values appear to be more comparable as they behave similarly in their ability to predict oxygen desaturation. Our study did not provide insight into any distinct advantage of using the DLCO or the DLCO/VA as an indicator of gas exchange. However, analyzing the DLCO in terms of DLCO/VA and VA (its two components) may aid in understanding the mechanism of a low diffusing capacity.[10–13,26]

Our findings must be considered in the context of the limitations of this study. First, the data only apply to the population tested, with its unique distribution of lung disease and prevalence of exercise desaturation. Other populations would yield different predictive values based on disease prevalence. Since this study was a retrospective review, all diagnoses were obtained from the PFT report, so there may be some diagnostic inaccuracy involved. Also, since not all patients had lung volumes measured, we relied on classifying disease by FEV_1, FVC and VA, which may have resulted in further diagnostic inaccuracy. However, the fact that most of our patients had interstitial lung disease or restriction is consistent with the type of patients for whom pulmonary physicians would order both DLCO and 6-min walk testing. Second, we did not correct the DLCO values for hemoglobin concentration, as this information was not available on all subjects. While this certainly may have changed the DLCO and DLCO/VA values, it should have changed both equally, and thus not affected the primary purpose of our study, which was to compare the two values in relation to exercise desaturation. Third, the equations we used to adjust the DLCO and DLCO/VA for VA were derived from healthy subjects, not patients with lung disease, and thus may not be accurate for the type of patients involved in this study.[21] Finally, we relied on pulse oximetry to define gas exchange abnormalities, which may not be sensitive to small changes in oxygen tension, and may register falsely low saturation in patients with poor peripheral perfusion. We did not measure arterial blood gases in this study. However, we believe that exercise oximetry is a very clinically relevant test, since it is easily performed in the office setting and is generally accurate for important levels of desaturation.[14]

In summary, while the DLCO and DLCO/VA yield complimentary information about the nature of any diffusing capacity defect that may exist, when adjusted for VA, neither is better than the other at predicting gas exchange abnormalities, and both are relatively poor predictors. In our study population, the best sensitivity and specificity of the DLCO or DLCO/VA for exercise desaturation occurred at a cut-off of 55% predicted.

References

1. Owens G, Rogers R, Pennock B, Levin D. The diffusing capacity as a predictor of arterial oxygen desaturation during exercise in patients with chronic obstructive disease. *New Engl J Med* 1984;**310**:1218–21.
2. Ries A, Farrow J, Clausen J. Pulmonary function tests cannot predict exercise-induced hypoxemia in chronic obstructive pulmonary disease. *Chest* 1988;**93**:454–9.
3. Agusti A, Roca J, Gea J, Wagner P, Xaubet A, Rodriguez-Roisin R. Mechanisms of gas-exchange impairment in idiopathic pulmonary fibrosis. *Am Rev Respir Dis* 1991;**143**:219–25.
4. Risk C, Epler G, Gaensler E. Exercise alveolar-arterial oxygen pressure difference in interstitial lung disease. *Chest* 1984;**85**:69–74.
5. Hadeli K, Siegel E, Sherrill D, Beck K, Enright P. Predictors of oxygen desaturation during submaximal exercise in 8,000 patients. *Chest* 2001;**120**:88–92.
6. Kelley M, Panettieri R, Krupinski A. Resting single-breath diffusing capacity as a screening test of exercise-induced hypoxemia. *Am J Med* 1986;**80**:807–12.
7. Mohsenifar Z, Collier J, Belman M, Koerner S. Isolated reduction in single-breath diffusing capacity in the evaluation of exertional dyspnea. *Chest* 1992;**101**:965–9.
8. Sue D, Oren A, Hansen J, Wasserman K. Diffusing capacity for carbon monoxide as a predictor of gas exchange during exercise. *New Engl J Med* 1987;**316**:1301–6.
9. Stam H, Kreuzer F, Versprille A. Effect of lung volume and positional changes on pulmonary diffusing capacity and its components. *J Appl Physiol* 1991;**71**:1477–88.
10. Hughes J, Pride N. In defence of the carbon monoxide transfer coefficient KCO (TL/VA). *Eur Respir J* 2001;**17**:168–74.
11. Ayers L, Ginsberg M, Fein J, Wasserman K. Diffusing capacity, specific diffusing capacity and interpretation of diffusion defects. *West J Med* 1975;**123**:255–64.
12. Frans A, Nemery B, Veriter C, Lacquet L, Francis C. Effect of alveolar volume on the interpretation of single breath CO. *Respir Med* 1997;**91**:263–73.
13. Johnson D. Importance of adjusting carbon monoxide diffusing capacity (DLCO) and carbon monoxide transfer coefficient (KCO) for alveolar volume. *Respir Med* 2000;**94**:28–37.
14. Gaensler E, Smith A. Attachment for automated single breath diffusing capacity measurement. *Chest* 1973;**63**:136–45.
15. American Thoracic Society. Single-breath carbon monoxide diffusing capacity. *Am J Respir Crit Care Med* 1995;**152**:2185–98.
16. American Thoracic Society. Standardization of Spirometry. 1994 Update. *Am J Respir Crit Care Med* 1995;**152**:1107–36.
17. Hankinson JL, Odencrantz J, Fedan KB. Spirometric reference values from a sample of the general US population. *Am J Respir Crit Care Med* 1999;**159**:179–87.

18. Punjabi N, Shade D, Wise R. Correction of single-breath helium lung volumes in patients with airflow obstruction. *Chest* 1998;**114**:907–18.
19. Miller A, Thornton J, Warshaw R, Anderson H, Teirstein A, Selikoff I. Single breath diffusing capacity in a representative sample of the population of Michigan, a large industrial state. *Am Rev Respir Dis* 1983;**127**:270–7.
20. Goldman H, Becklake M. Respiratory function tests. *Am Rev Tuberc* 1959;**79**:457–67.
21. MacIntyre N, Crapo R, Viegi G, Johnson D, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005;**26**:720–35.
22. Enright P, Sherrill D. Reference equations for the six-minute walk in healthy adults. *Am J Respir Crit Care Med* 1998;**158**:1384–7.
23. Zar J. *Biostatistical analysis*, fourth ed. Prentice-Hall: Upper Saddle River, NJ; 1999.
24. Leisenring W, Alonzo T, Pepe M. Comparisons of predictive values of binary medical diagnostic tests for paired designs. *Biometrics* 2000;**56**:345–51.
25. DeLong E, DeLong D, Clarke-Pearson D. Comparing the areas under two or more correlated receiver operator characteristic curves: a nonparametric approach. *Biometrics* 1988;**44**:837–45.
26. Fitting J. Transfer factor for carbon monoxide: a glance behind the scene. *Swiss Med Wkly* 2004;**134**:413–8.