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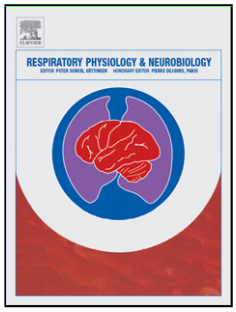

Variability in pulmonary diffusing capacity in heart failure

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Variability in pulmonary diffusing capacity in heart failure

Running head: Reproducibility of DLCO and DLNO in heart failure

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Highlights

- We evaluated diffusion capacity in HF, with DLCO (10 sec breath hold and 4 sec breath hold) and DLNO.
- We measured in 3 different days DLCO and DLNO.
- Day to day fluctuation in DLNO is less than DLCO in patients with HF.

Abstract

Background: As pulmonary diffusing capacity is related to mortality risk and prognosis in patients with heart failure (HF), it is measured frequently. As such, it would be essential to know the week-to-week variability (reproducibility) of pulmonary diffusing capacity for carbon monoxide (DLCO) and nitric oxide (DLNO). This variability would let clinicians understand what a clinically measurable change in DLCO and DLNO would be in these patients.

Methods: On three different days spanning over ten weeks, 40 HF patients underwent testing for DLCO and DLNO. DLCO was determined after a 4s and 10s breath-hold maneuver, while DLNO was determined after a 4s breath-hold maneuver.

Results: Forty heart failure patients (66 ± 10 years; BMI = 28.4 ± 4.6 $kg \cdot m^{-2}$; 28 males), that were referred to our clinic were able to complete the protocol. DLCO (4 s breath-hold) and DLNO (4 s breath-hold) were 79 ± 19% and 59 ± 14% predicted, respectively. Fifty percent of patients (n = 20) were below the lower limit of normal (LLN, below the 5^{th} percentile) for predicted DLCO (4s), while 78% of patients (n = 31) were below the LLN for predicted DLNO. All 16 patients that were below the LLN for DLCO were also below the LLN for DLNO. Over a ten week period, the reproducibility of DLNO (4s) DLCO (4s) and DLCO (10s) was 18.9, 8.2, and 5.9 $mL \cdot min \cdot mmHg^{-1}$, respectively.

Conclusions: The week-to-week fluctuation in DLNO (4 s), as a percentage, is less than DLCO (4 s) in patients with HF. The reproducibility of DLNO in patients with HF is like that of healthy subjects.

Keywords: Lung function; DLCO; DLNO; reproducibility; heart failure.

Introduction

Lung mechanics and pulmonary diffusing capacity are compromised in those with heart failure (HF) (Agostoni et al., 2006; Magini et al., 2015; Zavorsky and Borland, 2015). The former is characterized by lung restriction, while pulmonary diffusion capacity for carbon monoxide (DLCO) is impaired due to a reduction in both alveolar-capillary membrane diffusing capacity for CO (DMCO) (Puri et al., 1995) and pulmonary capillary volume (Vcap) (Magini et al., 2015; Zavorsky and Borland, 2015). Historically, DM is calculated from DLCO through the Roughton and Forster multi-step technique where $1/DLCO = 1/DM + 1/\theta_{CO} \cdot VCap$), where θco is the specific conductance in the blood for CO (Roughton and Forster, 1957). The traditional Roughton and Forster multi-step method has been used as the gold-standard to estimate DM and Vcap. However, the Roughton and Forster method implies patient collaboration and skill, since at several respiratory maneuvers that include 10-second breath-hold time under varying levels of alveolar oxygen pressure are needed (Graham et al., 2017)

Pulmonary diffusing capacity for nitric oxide (DLNO) is another way to estimate pulmonary diffusing capacity, but the transfer gas used is NO and not CO (Guénard et al., 1987). Unlike DLCO, the DLNO measurement is more natural to perform as it requires one short breath-hold of about 4 s and unlike DLCO, DLNO is minimally affected by hemoglobin (Hb) concentration (van der Lee et al., 2005) carboxyhemoglobin concentration (Zavorsky, 2013) or alveolar oxygen pressure (Borland and Cox, 1991). The main barrier to NO uptake NO lies mainly between alveolar and red blood cell membranes while the chief barrier to CO uptake is within the red cell (Zavorsky et al., 2017). Recent studies have demonstrated that DLNO and DLCO are reduced similarly to 40-60% of predicted

(Apostolo et al., 2018; Magini et al., 2015), suggesting that DMCO and Vcap could be similarly impaired.

As pulmonary diffusing capacity is related to mortality risk and prognosis in patients with heart failure (Deis et al., 2019; Hoeper et al., 2016; Nakamura et al., 2019), it is measured frequently. As such, it would be essential to know the week-to-week variability (reproducibility) of pulmonary diffusing capacity for carbon monoxide (DLCO) and nitric oxide (DLNO). This variability would let clinicians understand what would constitute a meaningful change in DLCO and DLNO in these patients.

The literature on studies on the week-to week variability in DLNO is scarce. From what is known to date, the reproducibility of DLNO is about ~19-20 $mL \cdot min \cdot mmHg^{-1}$ in healthy subjects (Desjardin et al., 2020; Lavin et al., 2015; Murias and Zavorsky, 2007) and about 13 $mL \cdot min \cdot mmHg^{-1}$ in those with cystic fibrosis (Radtke et al., 2017). This is equivalent to about a 13-14% week-to-week coefficient of variation in healthy individuals and in those with cystic fibrosis. The reproducibility of DLCO is about ~4-5 $mL \cdot min \cdot mmHg^{-1}$ in both healthy subjects, those with cystic fibrosis and emphysema (Desjardin et al., 2020; Lavin et al., 2015; Murias and Zavorsky, 2007; Radtke et al., 2017; Robson and Innes, 2001). This is equivalent to about a ~10% week-to-week coefficient of variation in healthy individuals and about ~17 to ~35% in those with cystic fibrosis and emphysema, respectively.

Since there is limited knowledge as to the week-to-week variability (reproducibility) in DLCO and DLNO in patients with heart failure, the purpose of the study was to evaluate the week-to-week-variability in DLCO, DLNO, in older patients with heart failure over a ten-week period. As the DLNO measured would be more reflective of DMCO, and the DLCO measured would be more reflective of Vcap (Zavorsky et al., 2017), there was no need to estimate DMCO and Vcap and the measurement of DLNO and DLCO, only, would suffice.

Methods

This study was approved by the Centro Cardiologico Monzino IRCCS scientific and ethics committee (CCM88 16/07/14). Subjects provided informed consent prior to study commencement. The present analysis was performed from data obtained in a more complex study dedicated to the effects on alveolar-capillary diffusion with a β-stimulating inhaled agent (Contini et al. 2020). In this current study, only patients that were not under the influence on any β-stimulating inhaled agents were used for the analyses of week-to-week variability.

Study inclusion criteria were the following: low ejection fraction (≤ 40%), stable clinical conditions, and absence of relevant comorbidities such as pulmonary embolism, primary valvular heart disease, pericardial disease, significant peripheral vascular disease, anemia, severe chronic obstructive pulmonary disease (COPD), renal failure, long QT syndrome, diabetes type I or uncontrolled type 2 and exercise induced angina, ST changes, arrhythmias. Each research step was performed in the morning during which patients underwent full clinical, laboratory (including hemoglobin and brain natriuretic peptide), standard spirometry and cardiopulmonary exercise test evaluations. The latter was performed and analyzed following a standard methodology (Agostoni and Dumitrescu, 2019). These measurements were completed in a ten-week period.

Standard spirometry was performed following ATS/ERS document (Miller et al., 2005) using the V-max metabolic cart (SensorMedics, Yorba Linda, CA, USA). Percent predicted of forced expired volume in on second (FEV_1) and forced vital capacity (FVC) were calculated using 2012 Global Lung Function Initiative (GLI) spirometry reference equations (Quanjer et al., 2012). DLCO measurements were performed using two different pulmonary function systems: (a) the V-max metabolic cart (Vmax 229D; Sensormedics), and (b) the MS-PFT system (Jaeger Masterscreen, Vyaire Medical, Höechberg, Germany). The SensorMedics system was used to measure DLCO using a 10 s breath-hold (10 s). The 2017 European Respiratory Society (ERS) and American Thoracic Society (ATS) standards were followed for the 10 s DLCO measurement (Graham et al., 2017)

Gas mixtures were: 0.3 % CO, 0.30% methane (CH_4), 21% oxygen (O_2) balanced with nitrogen (N_2) for the SensorMedics system. All DLCO values were reported after Hb correction (Graham et al., 2017). Percent predicted values for DLCO (10 s breath-hold) were obtained from the 2017 GLI reference equations (Stanojevic et al., 2017).

The MS-PFT system was used for simultaneous measurement of DLNO and DLCO using a ~4 s breath-hold (4 s). About 40 ppm NO balanced with N_2 for DLNO, and 0.28% CO, 21% O_2, 9.0% helium balanced with N_2 for DLCO was inhaled per 4 s breath-hold maneuver. The 2017 ERS technical standards were followed when performing the 4 s breath-hold maneuver (Zavorsky et al., 2017). The percentage of normal values for DLNO and DLCO (4 s breath-hold) were also calculated according to formula reported by the 2017 ERS technical standards document for DLNO (Zavorsky et al., 2017).

The lower limit of normal (LLN) was defined as any value below the 5th percentile. As such the following reference equations already presented the LLN as the 5th percentile (Quanjer et al., 2012; Stanojevic et al., 2017) or were adjusted for the 5th percentile (Zavorsky et al., 2017).

Statistical analyses

The calculation of the reproducibility was obtained from the on-line supplementary material of a recent ERS task force report on the standardization and application of the single-breath determination of nitric oxide uptake in the lung (Zavorsky et al., 2017). As the variability of DLNO and DLCO can be independent of the magnitude of the measurement, using a percentage value to describe inter-session (or intra-session) variability may not be appropriate. Using a percentage may lead to underestimation of variability in low values and overestimation for high values. Other studies have also suggested using an absolute value rather than a percentage (Punjabi et al., 2003; Robson and Innes, 2001;l Zavorsky and Murias, 2006) since the diffusing capacity was also independent of the magnitude of the measurement. As such, we report intersession variability values for the 4 s breath-hold maneuver for DLNO and DLCO in absolute numbers, but percentages are also provided for an easier interpretation

of the variability. The measurement error (otherwise known as the typical error) is the square root of the mean square error obtained from a repeated measures analysis of variance (ANOVA). In this study, spirometry and diffusing capacity testing was repeated over three different occasions in a ten-week time period. From the repeated measures ANOVA, the square root of the mean square error was defined as the common within-subject week-to-week standard deviation (SD_w). The reproducibility is then reported as $2.77 \cdot SD_W$ (Bland and Altman, 1996). Thus, the difference between the DLNO or DLCO value obtained on different weeks or days for the same subject is expected to be less than 2.77 times the within-subject standard deviation for 95% of pairs of observations (Bland and Altman, 1996). The smallest meaningful change is half the reproducibility and thus less stringent than the reproducibility (Hopkins, 2000). Any week-to-week change in any diffusing capacity parameter that is equal to the smallest meaningful change has an approximate 20% chance that it is not a real change, and an approximate 80% chance that the change is real. It is up to the physician, researcher, or technologist to decide how stringent the week-to-week or month-to-month changes in diffusing capacity need to be before it is considered a "meaningful change."

The lower limit of normal (LLN) was defined as any value at the 5th percentile. As such the following reference equations already presented the LLN as the 5th percentile (Quanjer et al., 2012; Stanojevic et al., 2017) or were adjusted for the 5th percentile (Zavorsky et al., 2017). Data were analyzed using the IBM SPSS Statistics (version 26.0) statistical package (Armonk, NY).

Results

Forty heart failure patients (66 ± 10 years; BMI = 28.4 ± 4.6 kg·m^{-2}; 28 males), that were referred to our clinic were able to complete the full protocol. These patients were prospectively included in a research protocol registered as CCM 89 and ClinicalTrials.gov (NCT02598505), a double-blind, placebo controlled, cross-over research protocol aimed at evaluating the efficacy of stimulating respiratory β_2 receptors on top of the standard heart failure treatment. Twenty patients had idiopathic heart failure and 20 patients had ischemic heart failure. All patients had a left ventricular systolic

dysfunction; they were in stable clinical conditions and on optimized chronic therapy including β-blockers in all cases, angiotensin-converting enzyme ACE inhibitors or AT1-receptor blockers in 36 cases, diuretics in 33 cases, anti-aldosteronic in 31 cases, digoxin in 3 cases. Data of all 40 consecutive ambulatory heart failure patients were analyzed. At enrollment, all patients had moderate heart failure as shown by New York Heart Association (NYHA) class II in all cases, BNP = 279 ± 246 $pg \cdot mL^{-1}$**,** Hemoglobin was 13.8 ± 1.4 $g \cdot dL^{-1}$ and peak VO_2 = 1.2 ± 0.4 $L \cdot min^{-1}$ or 63 ± 14 % of the predicted value (Hansen et al., 1984). Left ventricular ejection fraction, determined via echocardiography, was reduced (34 ± 6%) with no signs of right ventricular failure. All patients remained clinically stable throughout the research protocol as confirmed by clinical evaluation, cardiopulmonary exercise test parameters, and BNP values.

Pulmonary function testing results at baseline are presented in **Table 1**. Five patients demonstrated a clinically significant obstructive air pattern defined by the FEV_1/FVC ratio below the lower limit of normal (LLN, below the 5th percentile). Of those five patients, four were classified as moderate obstructive pattern (FEV_1 60-69% predicted) and one has mild obstruction (FEV_1 > 70% predicted). Nine patients had a clinically significant restriction as their FVC was below the LLN. As well, 18% of the patients (n = 7) were below the LLN for VA from the 10 s breath-hold maneuver, while 75% of the patients (n = 30) were below the LLN for VA with the 4 s breath-hold maneuver. The reproducibility of FVC and FEV_1 measurements was 0.59 L and 0.47 L, respectively, with the smallest measurable change at 0.30 L for FVC and 0.24 L for FEV_1. The reproducibility of the ratio FEV_1/FVC was 0.11 with the smallest measurable change at 0.05. The reproducibility of VA from a 4 s breath-hold maneuver was 0.72 L with the smallest measurable change at 0.36 L. The reproducibility of VA from the 10s breath-hold maneuver was 1.28 L with the smallest measurable change at 0.64 L.

Ten patients (25%) were below the LLN for DLCO when using the 10 s breath-hold maneuver (86 ± 19% of predicted). The DLCO (4 s breath-hold maneuver) and DLNO (4 s breath-hold maneuver) were 79 ± 19% and 59 ± 14% predicted, respectively. Fifty percent of patients (n = 20)

were below the LLN for predicted DLCO (4 s), while 78% of patients (n = 31) were below the LLN for predicted DLNO according to the prediction equation from the 2017 ERS technical standards (Zavorsky et al., 2017) with the LLN adjusted to the 5th percentile. All 20 patients that were below the LLN for DLCO (4 s) were also below the LLN for DLNO (4 s). Over a six-week period, the reproducibility of DLNO (4 s) DLCO (4 s) and DLCO (10 s) was 18.9, 8.2, and 6.4 mL·min·mmHg^{-1}, respectively (**Table 2**). The week to week coefficient of variation was less for DLNO (4 s) compared to DLCO (4 s or 10 s).

About 45% of the variance DLCO was accounted for by DLNO (**Figure 1**, baseline). The percentage of shared variance increased to 52 and 55%, respectively, at the second and third session, respectively. About 64% of the variance in DLCO (4s) was shared by DLCO (10s), (**Figure 2,** baseline), resulting in a 13% coefficient of variation between the two different breath-hold times. The shared variance was similar for the second and third session, too. In addition, about 74% of the variance in VA (10 s) was shared with VA (4 s), resulting in a 12% coefficient of variation between the two breath-hold times (**Figure 3**, baseline). The percentage of shared variance was 83% and 76% for the second and third session, respectively.

Discussion

The purpose of this study was to determine the week-to-week variability (reproducibility) of DLNO (4 s) and DLCO (4s and 10 s) in a group of heart failure patients. In this group of heart failure patients, the reproducibility was about 19 mL·min·mmHg^{-1} for DLNO, which is similar to healthy subjects (Desjardin et al., 2020; Lavin et al., 2015; Murias and Zavorsky, 2007). As such, the smallest measurable change in DLNO, using less stringent criteria would be about 10 mL·min·mmHg^{-1} in heart failure patients, which is similar to the smallest measurable change of DLNO in the 2017 ERS technical standards paper (Zavorsky et al., 2017). As for DLCO, the reproducibility was between ~6 and ~8 mL·min·mmHg^{-1}, significantly higher (worse) than the ~ 5 mL·min·mmHg^{-1} reported in the ERS technical standards (Zavorsky et al., 2017) or elsewhere (Desjardin et al., 2020; Lavin et al., 2015;

Murias and Zavorsky, 2007; Radtke et al., 2017; Robson and Innes, 2001). For FVC and FEV_1, the reproducibility was higher (worse) for the heart failure patients compared to healthy subjects. The reproducibility in FVC and FEV_1 is 0.47 L and 0.35 L, respectively (Lavin et al., 2015) in healthy subjects and for heart failure patients, it is 0.59 L (FVC) and 0.47 L (FEV_1).

There is a higher mortality risk and poor prognosis in patients with heart failure that have low diffusing capacities (Deis et al., 2019; Hoeper et al., 2016; Nakamura et al., 2019). Thus, pulmonary function testing can sometimes occur frequently to help with patient management. As the reproducibility of both DLCO and DLNO are ~8 and ~19 $mL \cdot min^{-1} \cdot mmHg^{-1}$, respectively, clinicians can use these numbers to benchmark significant declines or improvement in a patient's lung function status. Any changes in DLCO and DLNO within ~8 and ~19 $mL \cdot min^{-1} \cdot mmHg^{-1}$ that occur week-to-week, are thus likely not meaningful. Or, if less stringent criteria are used (i.e. the smallest measurable change), any changes in DLCO and DLNO within ~4 and ~10 $mL \cdot min^{-1} \cdot mmHg^{-1}$ that occur week-to-week, are thus likely not meaningful.

In these class II heart failure patients, the coefficient of variation for DLNO over a six-week period was 10%, which was less than the 11-15% coefficient of variation for DLCO. There could be many reasons for a more stable DLNO over time compared to DLCO. One reason for DLNO being more stable over time compared to DLCO is that unlike DLCO, DLNO is minimally affected by hemoglobin concentration (van der Lee et al., 2005). As hemoglobin concentration fluctuates by about 3% week to week (Coskun et al., 2018), DLCO is more sensitive to within subject changes in hemoglobin than DLNO (van der Lee et al., 2005).

The variability in the DLNO/DLCO ratio we observed was much larger than reported in normal subjects (Zavorsky et al., 2017). We observed a week-to-week variability in DLCO/DLNO ratio of ~20% in heart failure patients, which was significantly higher than the week-to-week variability in the same ratio in healthy subjects (~5%) (Zavorsky et al., 2017). The large variability in the ratio in heart failure patients is difficult to interpret especially in the presence of lung disease but may reflect

fluctuations in cardiac output, lung volume, and other dynamic factors that affect alveolar microvascular recruitment, a significant determinant of the ability of the lung to adapt to perturbations while maintaining alveolar diffusive gas exchange. Nonetheless, the large week to week variability in the DLNO/DLCO ratio in these patients do not detract from the conclusion that when DLNO and DLCO are measured simultaneously they complementary insight into the pathophysiology of heart failure.

It is also interesting to note that when the breath-hold time for the DLCO maneuver was prolonged to 10 s (from 4 s), the reproducibility became tighter. A 10 s breath-hold is better than a 4 s breath-hold maneuver in patients with cardiopulmonary disease since there is more penetration of inspired gas and a more homogeneous distribution of gases during a 10 s breath-hold maneuver. A 10 s breath-hold maneuver allows for better gas penetration and gas mixing within the lungs (Zavorsky et al., 2017). This is evident with mean VA from the 4s breath-hold maneuver being approximately 0.5 L lower than the mean VA from the 10s breath-hold maneuver (**Figure 3**). However, a 10 s breath-hold will significantly reduce the exhaled NO concentration, which may reduce the accuracy of DLNO measurement as the electrochemical NO analyzers do not have a resolution in the ppb range.

The slope between DLNO and DLCO was found to be lower in patients with heart failure. DLNO was 3.2 times larger than the DLCO (**Figure 1**), which is lower than the slope of 4.8 in healthy subjects (Zavorsky et al., 2017). This demonstrates that the DLNO in patients with heart failure is affected to a larger extent than the DLCO. That is, the DLNO is more sensitive to alveolar under expansion compared to DLCO (Hughes and Pride, 2012; Zavorsky et al., 2017), and patients with heart failure seem to have alveolar under expansion (reduced lung volumes). But even when examining the rate of change of NO from alveolar gas, per unit pressure of NO (i.e. KNO), the reduction persisted, which would imply an intrinsic abnormality of the alveolar-capillary membrane (alveolar-capillary damage) apart from the reduction in lung volume. Reduced lung volumes and alveolar-capillary membrane abnormalities are commonly found in patients with heart failure (Guazzi et al., 2002; Puri et al., 1995). In this study, ~75% of patients were below the LLN for DLNO, and 50% of the same patients were below the LLN for DLCO. Inasmuch as heart failure diminishes diffusion

between alveolar and red blood cell membranes (a reduction in the alveolar-membrane diffusion capacity), which would point to a lung restrictive process, the relatively normal DLCO in half of the heart failure patients indicate that pulmonary capillary blood volume and/or hemoglobin concentration are normal.

In conclusion, the reproducibility of DLNO (4 s breath-hold maneuver) was ~19 and mL·min·mmHg^{-1} in class II heart failure patients, similar to healthy subjects. For DLCO (4 s breath-hold maneuver) the reproducibility was ~8 mL·min·mmHg^{-1} or ~6 mL·min·mmHg^{-1} (10 s breath-hold maneuver). However, the week-to-week variability in the DLNO/DLCO ratio was large, and thus not very useful in observing changes over time. Clinicians can now use these numbers to benchmark significant declines or improvement in a patient's lung function status.

Conflict of interests: none

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Figure 1. The association between DLNO and DLCO (4s breath-hold maneuver) in 40 patients with class II heart failure. DLNO = 3.23·(DLCO) + 9.72, R^2 = 0.45, residual standard deviation = 17.1 mL·min^{-1}·mmHg^{-1}, p < 0.001. The dashed blue lines represent the 95% confidence interval, the solid red lines represent the 95% prediction interval and the thick black line is the regression line of best fit.

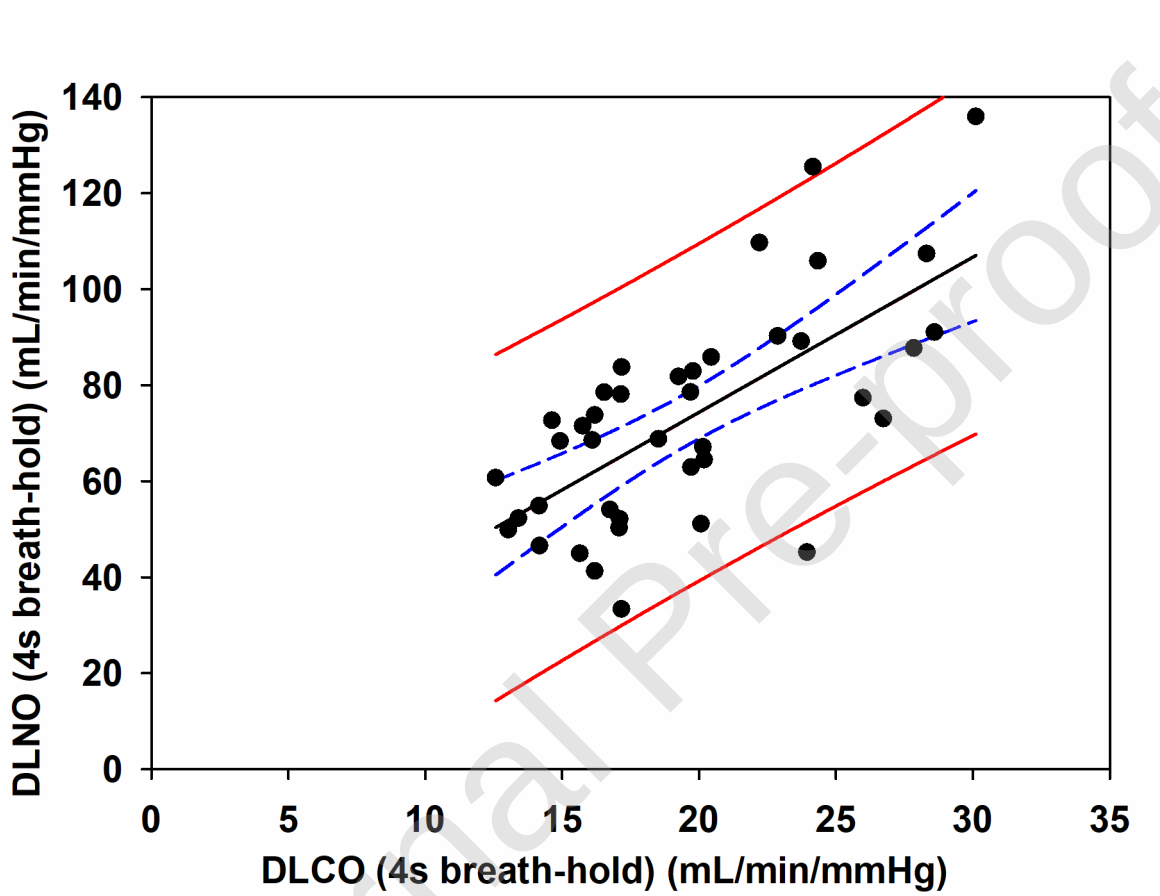

Figure 2. The association between DLCO (10s) and DLCO (4s breath-hold maneuver) in 40 patients with class II heart failure. DLCO (10s) = 1.058·(DLCO 4s) – 0.44, R^2 = 0.66, residual standard deviation = 3.66 mL·min^{-1}·mmHg^{-1}, p < 0.001. The dashed blue lines represent the 95% confidence interval, the solid red lines represent the 95% prediction interval, and the thick black line is the regression line of best fit. The thin black line that extends to the origin of x- and y-axes is used as an indicator of bias. One can see, that the DLCO (10s) provides a ~0.7 mL·min^{-1}·mmHg^{-1} (~6%) higher value compared to DLCO (4s).

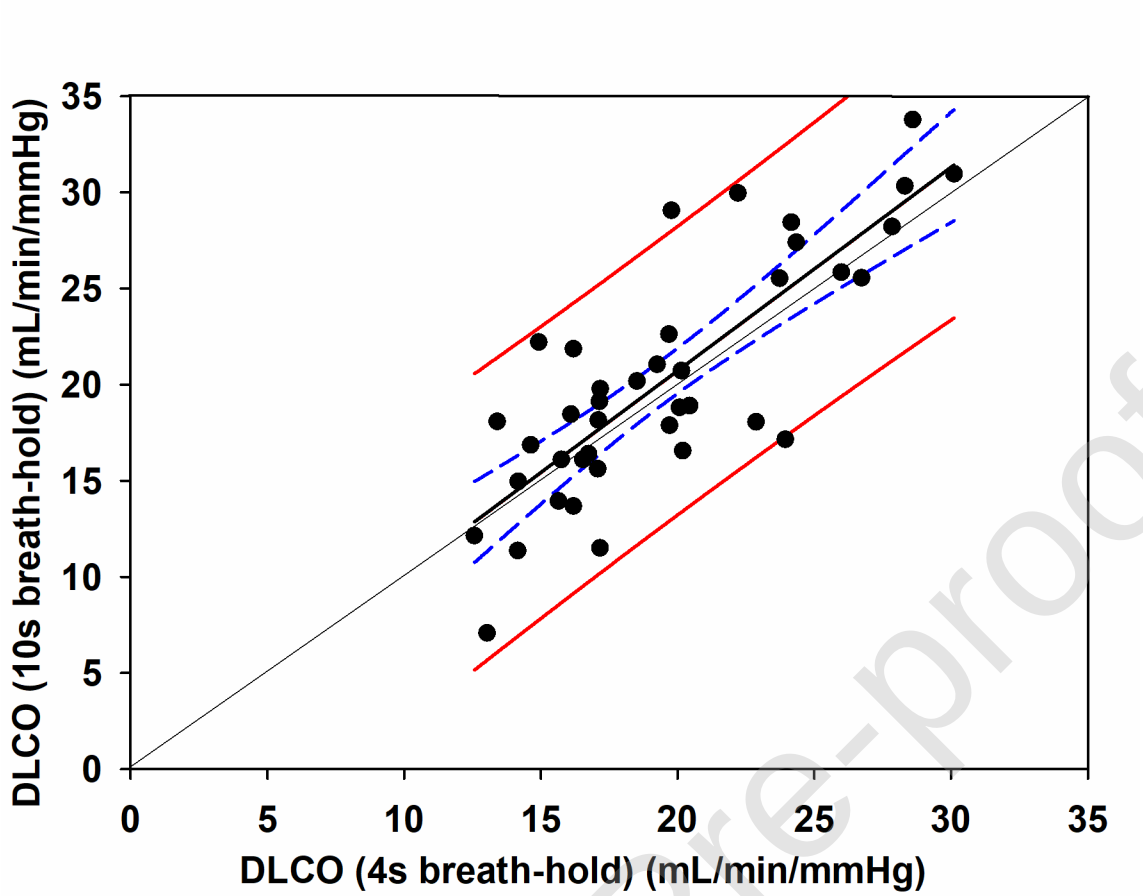

Figure 3. The association between alveolar volume (10s) and alveolar volume (4s breath-hold maneuver) in 40 patients with class II heart failure. VA (10s) = 1.14·(DLCO 4s) – 0.15, R^2 = 0.74, residual standard deviation = 0.62 L, $p < 0.001$. The dashed blue lines represent the 95% confidence interval, the solid red lines represent the 95% prediction interval, and the thick black line is the regression line of best fit. The thin black line that extends to the origin of x- and y-axes is used as an indicator of bias. One can see that the VA (10s) provides a ~0.48 L (~14%) higher value compared to VA (4s).

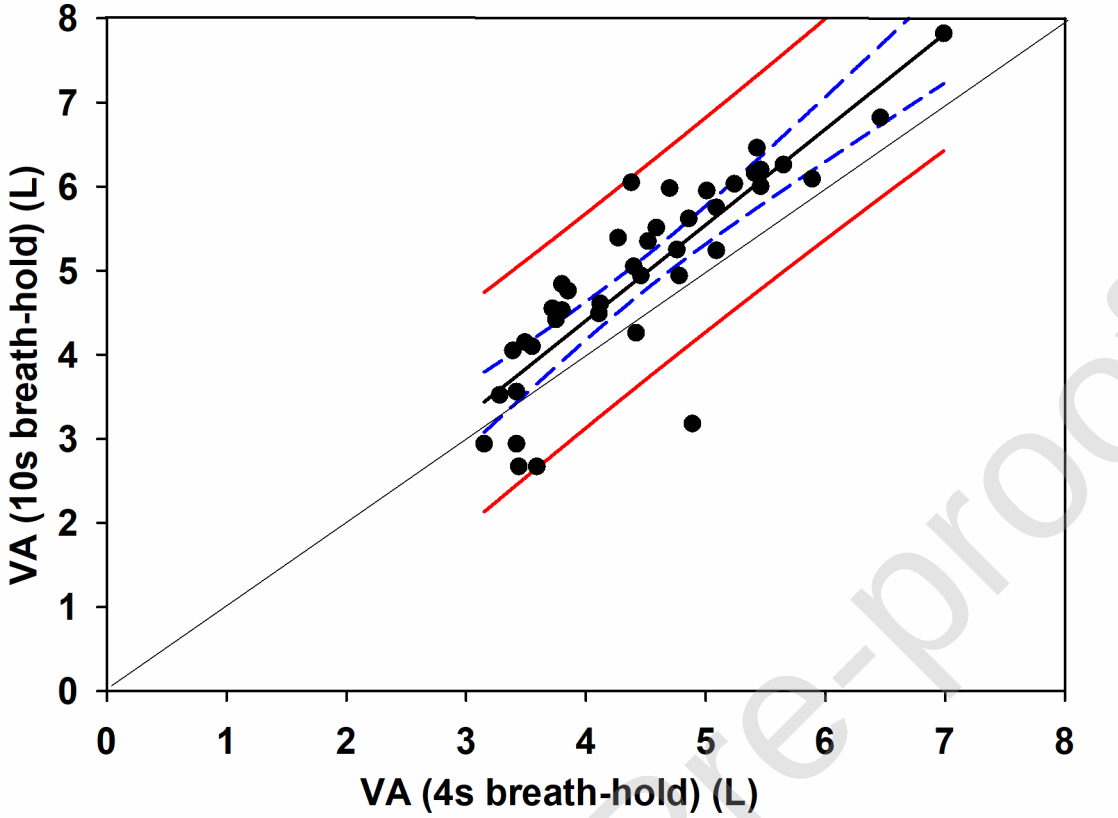

Table 1. Pulmonary Function data at baseline in patients with New York Heart Association Class II heart failure.

N = 40	**Mean (SD)**	**Range**
FVC (L)	3.19 (0.97)	1.72 to 5.06
% predicted FVC	88 (16)%	55% to 123%
FEV_1 (L)	2.29 (0.72)	1.18 to 3.79
% predicted FEV_1	81 (16)%	55% to 123%
FEV_1/FVC	0.72 (0.07)	0.51 to 0.84
% predicted FEV_1/FVC	93 (9)%	67% to 106%
DLCO (10 s) ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	20.3 (6.2)	7.1 to 33.8
DLCO (4 s) ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	19.6 (4.7)	12.6 to 30.1
% predicted DLCO (10 s)	86 (19)%	37% to 129%
% predicted DLCO (4 s)	79 (18)%	54% to 137%
DLNO (4 s) ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	73 (23)	33 to 136
% predicted DLNO (4 s)	59 (14)%	24% to 89%
VA (10s) (L)	5.0 (1.2)	2.7 to 7.8
VA (4 s) (L)	4.5 (0.9)	3.2 to 7.0
% Predicted VA (10 s)	89 (15)%	48% to 113%
% Predicted VA (4s)	74 (9)%	51% to 93%
KCO (10 s) ($mL \cdot min^{-1} \cdot mmHg^{-1} \cdot L^{-1}$)	4.0 (0.9)	2.2 to 6.2
KCO (4s) ($mL \cdot min^{-1} \cdot mmHg^{-1} \cdot L^{-1}$)	4.4 (0.7)	2.7 to 6.2
% predicted KCO (10 s)	97 (21)%	53% to 145%
% predicted KCO (4s)	102 (18)%	66 to 160%
KNO (4s) ($mL \cdot min^{-1} \cdot mmHg^{-1} \cdot L^{-1}$)	16.1 (3.2)	10.2 to 24.0
% predicted KNO (4s)	62 (12%)	39 to 92%
DLNO/DLCO ratio	3.76 (0.85)	1.89 to 5.19

Table 2. Inter-session variability of single-breath measurements of DLNO and DLCO (4 s and 10 s breath hold) at rest in heart failure patients over a ten-week period.

Variable	Standard Deviation	Coefficient of Variation (%)	Reproducibility [5%chance that the week to week differences equal to or more than presented below are not real]	Smallest Measurable Change [20% chance that the week to week differences equal to or more than presented below are not real]
	Over 6 weeks	Over 6 weeks	(More stringent)	(Less stringent)
DLNO (4 s)				
mL·min^{-1}·mmHg^{-1}	6.8	10%	18.9	9.5
mmol·min^{-1}·kPa^{-1}	2.3	10%	6.4	3.2
(n = 40)				
DLCO (4 s)				
mL·min^{-1}·mmHg^{-1}	3.0	15%	8.2	4.1
mmol·min^{-1}·kPa^{-1}				
(n = 40)	1.0	15%	2.8	1.4
DLCO (10 s)				
mL·min^{-1}·mmHg^{-1}	2.1	11%	5.9	3.0
mmol·min^{-1}·kPa^{-1}	0.7	11%	2.1	1.0
(n = 40)				
DLNO/DLCO	0.71	19%	1.97	0.98
(4 s) ratio (n = 40)				

Average ± SD values over the three sessions were 73 ± 23 mL·min^{-1}·mmHg^{-1} (range = 33 to 136) for DLNO (4 s); 19.6 ± 4.7 mL·min^{-1}·mmHg^{-1} (range = 12.6 to 30.1) for DLCO (4 s); 20.3 ± 6.2 mL·min^{-1}·mmHg^{-1} (range = 7.1 to 33.8) for DLCO (10 s); 3.76 ± 0.85 (range 1.89 to 5.19) for the DLNO/DLCO ratio.