Intra-session and inter-session variability of nitric oxide pulmonary diffusing capacity in adults with cystic fibrosis

Thomas Radtke\textsuperscript{a,b,⁎}, Christian Benden\textsuperscript{b}, Marion Maggi-Beba\textsuperscript{c}, Susi Kriemler\textsuperscript{b}, Ivo van der Lee\textsuperscript{d}, Holger Dressel\textsuperscript{b}

\textsuperscript{a} Epidemiology, Biostatistics and Prevention Institute (EBPI), University of Zurich, Zurich, Switzerland
\textsuperscript{b} Division of Pulmonology, University Hospital of Zurich, Zurich, Switzerland
\textsuperscript{c} Division of Occupational and Environmental Medicine, University of Zurich and University Hospital Zurich, Zurich, Switzerland
\textsuperscript{d} Spauwe Hospital, Department of Pulmonary Diseases, Hoofddorp, The Netherlands

A R T I C L E   I N F O

Keywords:
Lung disease
Reliability
Diffusing capacity for nitric oxide
Single-breath
Reproducibility

A B S T R A C T

We evaluated the intra-session and inter-session variability of the diffusing capacity of nitric oxide (DLNO), carbon monoxide (DLCO), alveolar-capillary membrane diffusing capacity for carbon monoxide (DMCO) and pulmonary capillary blood volume (Vc) in patients with cystic fibrosis (CF). Patients performed single-breath diffusing capacity measurements during all of 3 consecutive study visits. Precision of gas diffusing parameters was quantified by within-subject standard deviation (SD\textsubscript{ws}) and coefficient of variation (CV). Intra-session and inter-session reproducibility was determined by SD\textsubscript{ws}^2\textsuperscript{2.77}. 15 clinically stable patients were included. The intra-session precision of gas diffusing parameters improved over the study visits. The inter-session SD\textsubscript{ws} for DLNO, DLCO, DMCO, and Vc was 4.8, 1.3, 2.4, and 4.3, respectively. Reproducibility was 13.3, 3.8, 6.7 and 12.0 mLmin\textsuperscript{-1} mmHg\textsuperscript{-1}; CV was 4.4, 4.7, 4.4 and 5.8%, respectively. The intra-session variability of DLNO, DLCO, DMCO and Vc improves with breath-hold maneuver training in test-naïve patients with CF, indicating a learning effect. Inter-session reproducibility data are lower than those previously reported in healthy subjects.

1. Introduction

Three decades ago, pulmonary diffusing capacity for nitric oxide (DLNO, also known as the transfer factor for nitric oxide, TlNO) was introduced (Borland and Higenbottam, 1989; Guenard et al., 1987b) and has raised growing interest among researchers and clinicians working with patients with various pulmonary diseases including cystic fibrosis (CF) (Dressel et al., 2008; Dressel et al., 2009; Wheatley et al., 2011). An important step towards the standardized application of DLNO has been achieved by the recent publication of a European Respiratory Society Task Force statement (Zavorsky et al., 2017). Knowledge on the technical (intra-session) and biological (inter-session) variability is a prerequisite for the correct interpretation of diffusing capacity measurements and/or the progression of lung disease over time. Previous studies on the intra-session and inter-session variability of DLNO and its components included only healthy subjects (Murius and Zavorsky, 2007; Zavorsky and Murias, 2006), but it is not clear whether these data are transferable to patients with pulmonary parenchymal and vascular pathology. Importantly, differences in measurement variability between healthy subjects and those with chronic airflow obstruction have been reported for different pulmonary function tests such as spirometry (Pennock et al., 1981; Rozas and Goldman, 1982) and inert tracer gas washout tests (Aurora et al., 2005; Horsley et al., 2008; Husemann et al., 2014). The reasons are likely to be multifactorial and may include ventilation-perfusion inequalities (Soni et al., 2008) and airway obstruction. Furthermore, the effect of learning on the intra-session variability of DLNO and its components over time, i.e., when subjects are familiar with the single-breath maneuvers has not been investigated previously.

The aim of this study was: i) to perform an in-depth evaluation of the intra-session and inter-session variability of DLNO, diffusing capacity of carbon monoxide (DLCO), alveolar-capillary membrane diffusing capacity for carbon monoxide (DMCO) and pulmonary capillary blood volume (Vc) in patients with CF and ii) to compare variability data of our patients with CF to those reported previously in healthy adults.

⁎ Corresponding author at: University of Zurich Epidemiology, Biostatistics and Prevention Institute (EBPI) Hirschengraben 84 8001 Zurich, Switzerland.
E-mail address: thomas.radtke@uzh.ch (T. Radtke).

http://dx.doi.org/10.1016/j.resp.2017.08.002
Received 25 April 2017; Received in revised form 30 July 2017; Accepted 1 August 2017
Available online 03 August 2017
1569-9048/ © 2017 Elsevier B.V. All rights reserved.
2. Methods

2.1. Study design

This study is a substudy of a randomized crossover trial investigating acute effects of a combined exercise and oscillatory positive expiratory pressure therapy in patients with CF (registered with Clinicaltrials.gov, NCT02750722). Herein, we report on the intra-session and inter-session variability of pulmonary function measurements that were performed at the start of each of three consecutive study visits.

2.2. Patients

Patients with CF were recruited from the Adult CF Center at the University Hospital Zurich, Switzerland between June 2016 and January 2017. Inclusion criteria relevant for this substudy were: i) a confirmed diagnosis of CF based on either two CF-causing mutations and/or a sweat chloride concentration during two tests of >60 mmol/l and ii) age ≥18 years. Exclusion criteria were: i) listing for lung transplantation or status post lung transplantation, ii) infection with Burkholderia cepacia complex, iii) unstable clinical condition (i.e. major hemoptysis or pneumothorax within the last 3 months, acute pulmonary exacerbation, intravenous antibiotic treatment during the last 4 weeks, change in pulmonary medication during the study period), iv) active hemoptysis or pneumothorax within the last 3 months, acute pulmonary exacerbation, intravenous antibiotic treatment during the last 4 weeks, change in pulmonary medication during the study period (Maccintyre et al., 2005). As our Pulmonary Function Laboratory is about 400 m above sea level, atmospheric pressure adjustments were made (Graham et al., 2017). The transfer coefficients for nitric oxide (DLNO/V_\text{A}_{\text{LNO}} also known as KNO) and carbon monoxide (DLCO/V_\text{A}_{\text{LCO}} also known as KCO) were obtained by dividing DLNO and DLCO through the corresponding V_\text{A}, DMCO was calculated as DLNO divided by 1.97 (Aguilaniu et al., 2008; Zavorsky et al., 2017) and Vc was derived as previously described (Guerard et al., 1987a).

DLCO measurements were corrected for the patient’s hemoglobin concentration (Zavorsky et al., 2017). We calculated percent-predicted values for DLNO, DLCO, Vc and DMCO according to reference equations published by Zavorsky et al. (2017). At our Laboratory, quality control testing (biological control, healthy, non-smoking person) is performed on a weekly to bi-weekly basis. During the study period, the difference in DLNO and DLCO over time in our biological control was lower than 3.5 and 0.6 mL/min−1 mmHg−1, respectively.

2.3.3. Patient-reported health status

Patient-reported health status was assessed with the Feeling Thermometer (EuroQol, 1990). Details can be found in the online supplements.

2.3.4. Statistical analysis

All statistical analyses were performed with the statistical software package SPSS version 23 (IBM Corp. Armon, NY, USA). Data are presented as median (interquartile range, IQR), mean ± standard deviation (SD), mean (95% confidence intervals, CI) or N (%). We tested data for normal distribution using the Kolmogorov-Smirnov test. Correlations between outcome variables were analyzed using Pearson’s Correlation (r, correlation coefficient). Correlation strength of 0-0.19 was regarded very weak, 0.20-0.39 weak, 0.40-0.59 moderate, 0.60-0.79 strong and 0.80-1.0 as very strong (Swinscow and Campbell, 2002).

To test the intra-session variability of DLNO, DLCO, DLNO/DLCO ratio, DMCO and Vc, we compared all three single-breaths tests that were performed during each of the three study visits. Precision of gas measurements was quantified using the within-subject standard deviations (SD_{\text{within}} = root mean square error) calculated by the root-mean-square (RMS) method and the coefficient of variation (CV, SD_{\text{within}}/overall mean) (Gluers et al., 1995). The intra-session repeatability was determined by multiplying the SD_{\text{within}} with 2.77 (95% level of confidence) (Bland and Altman, 1996; Zavorsky et al., 2017). For the analysis of the inter-session variability we also calculated the measurement error by the RMS method, CV’s as well as the inter-session reproducibility (i.e., SD_{\text{within}} × 2.77). For the latter analysis, we compared the average value of the first two single-breaths tests between the three study visits (reproducibility analysis), when intra-session acceptability criteria were fulfilled (Zavorsky et al., 2017). If the criteria were not fulfilled, the third single-breath test was considered and the average of the two highest test results was used. We used a one-way repeated measures ANOVA (Tukey’s post hoc tests for between test comparisons) or the non-parametric Friedman Test (Dunn-Bonferroni post hoc tests for between test comparisons) to test for differences in DLNO, DLCO, DLNO/DLCO ratio, DMCO and Vc between the three study visits. In addition, we calculated intraclass correlation coefficients (ICCs) for the selected gas diffusing variables to estimate the inter-session reliability.
using a two-way random model.

For three-point measurements at least 14 participants (42 exams) were needed to obtain valid precision errors using the RMS method (Glaeser et al., 1995). Assuming a 10% dropout rate, we decided to recruit 16 patients to achieve the required sample size.

3. Results

Baseline characteristics for 16 patients are shown in Table 1. Between the first and second study visit, one female patient required oral antibiotic therapy for treatment of a pulmonary exacerbation and was therefore excluded from the final analyses. All other patients completed all assessments without experiencing any adverse events. Our patients had a moderate to severe CF lung disease with a median (IQR) FEV1 and DLNO of 52 (43, 72) and 59 (51, 73) percent predicted, respectively. Alterations of DLCO were less pronounced with a median of 82% predicted (Table 1). We observed strong and very strong correlations between FEV1 percent predicted and DLCO (r = 0.69) and DLNO (r = 0.90), respectively. Accordingly, very strong correlations were also observed between percent-predicted FEV1 and DMCO (r = 0.90), however not for FEV1 and Vc (r = −0.09). Pulmonary function data and patient-reported health status of all three-study visits are summarized in Table 2. Further pulmonary function data are provided in the online supplements (e-Table 1 and 2).

3.1. Intra-session variability

Intra-session variability characteristics are shown in Table 3. At study visits 1, 2 and 3 all patients fulfilled the recommended intra-session repeatability criteria for DLNO and DLCO (17.0 and 3.2 mL min⁻¹ mmHg⁻¹) (Zavorsky et al., 2017). The measurement error (SDm), repeatability and CV’s for DLNO, DLCO, DLNO/DLCO ratio, DMCO, Vc and inspiratory volume (Vin) improved over the three study visits (Table 3).

3.2. Inter-session variability

The inter-session variability characteristics are summarized in Table 4. Individual data and group means (95% CI’s) for DLNO, DLCO, DMCO and Vc for each study visit are shown in Fig. 1. No differences were observed in any pulmonary diffusing parameters between the three different study visits (Table 4).

4. Discussion

This study investigates the intra-session and inter-session variability of pulmonary gas diffusing measurements in patients with CF. We demonstrate that patients with chronic lung disease are able to achieve intra-session and inter-session variability values previously reported for healthy individuals (Murius and Zavorsky, 2007; Zavorsky et al., 2017; Zavorsky and Murius, 2006). Moreover, maneuver experience resulted in a substantially lower intra-session variability of gas diffusing parameters, even if those patients were otherwise spirometry-trained.

In our study, the intra-session variability (i.e., measurement error, repeatability and CV) of pulmonary gas diffusing variables improved consistently over the three study visits, indicating a learning effect. The observed improvement is likely due to an improvement in the quality and consistency of the breathing maneuver over time. Interestingly, with maneuver experience, we found lower measurement errors and CV’s for Vin that may contribute to the overall lower variability of gas diffusing parameters (see Table 3). To the best of our knowledge only one previous study investigated the intra-session variability of DLNO (Zavorsky and Murius, 2006). Zavorsky and Murius (2006) studied the intra-session variability of DLNO, DLCO and its components in 31 healthy subjects during five consecutive single-breath maneuvers. Their intra-session measurement error and repeatability values for DLNO, DLCO, DMCO and Vc (using the same statistical approach) were comparable to values observed in our study population, but only when we considered our first study visit. The measurement error and repeatability values in our CF patients were substantially lower compared to data from Zavorsky and Murius (2006) when considering our third study visit, i.e. when patients had considerable maneuver experience. This is indicative of a learning effect and suggests that diffusing measurement naïve patient’s can considerably lower the measurement variability after they have learned the breathing maneuver. However, the learning effect had no impact on the mean gas diffusing values from each visit (see Fig. 1). Of note, our patients took part in an acute exercise study and all of them performed another five to six breathing maneuvers at each study visit (i.e., immediately after exercise and 45 min post-exercise; data not shown). Therefore, each patient had two
In our patients, the diDLNO measured in SI units (mmol min$^{-1}$ kPa$^{-1}$) by multiplying with 2.987. DMCO = DLNO/1.97. * The measurement error (or within-subject standard deviation, SD$_{ww}$) was calculated by the root-mean-square (RMS) method. 

$b$ Reproducibility values were calculated as the SD$_{ws}$ over three study visits multiplied by 2.77 (95% level of confidence). 

$\text{VIN (L)} = 0.14 \pm 0.39 \pm 3.88 \pm 0.10 \pm 0.29 \pm 3.36 \pm 0.09 \pm 0.24 \pm 2.77$

Intra-session variability of three consecutive single-breath diffusing capacity measurements during three different study visits.

| Variables | Study visit 1 | | Study visit 2 | | Study visit 3 | |
|-----------|--------------|---|---|---|---|
| DLNO      | Measurement error$^a$ | Repeatability$^b$ | CV (%) | Measurement error | Repeatability | CV (%) | Measurement error | Repeatability | CV (%) |
| ml min$^{-1}$ mmol$^{-1}$ kPa$^{-1}$ | 5.34 | 14.80 | 5.99 | 4.61 | 12.78 | 4.78 | 3.22 | 8.93 | 3.85 |
| mmol min$^{-1}$ kPa$^{-1}$ | 1.79 | 4.96 | 1.55 | 4.28 | 1.08 | 2.99 |
| DLCO      | 1.08 | 3.00 | 4.16 | 0.90 | 2.49 | 3.60 | 0.88 | 2.44 | 3.14 |
| mmol min$^{-1}$ kPa$^{-1}$ | 0.36 | 1.00 | 0.30 | 0.83 | 0.30 | 0.82 |
| DLNO/DLCO | 0.21 | 0.57 | 5.15 | 0.13 | 0.36 | 3.29 | 0.10 | 0.25 | 2.52 |
| DMCO      | 2.72 | 7.52 | 6.00 | 2.34 | 6.48 | 4.78 | 1.63 | 4.51 | 3.80 |
| mmol min$^{-1}$ kPa$^{-1}$ | 0.91 | 2.52 | 0.78 | 2.17 | 0.55 | 1.51 |
| Vc (ml)   | 5.09 | 14.09 | 7.54 | 3.93 | 10.90 | 5.01 | 2.98 | 8.26 | 3.70 |
| Vc (mL)   | 0.14 | 0.39 | 3.88 | 0.10 | 0.29 | 3.36 | 0.09 | 0.24 | 2.77 |

Table 3 Inter-session variability of single-breath diffusing capacity measurements from three different study visits.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Measurement error$^a$</th>
<th>Reproducibility$^b$</th>
<th>ICC (95% CI)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLNO</td>
<td>4.8</td>
<td>13.3</td>
<td>0.994 (0.986-0.998)</td>
<td>4.41</td>
</tr>
<tr>
<td>mmol min$^{-1}$ kPa$^{-1}$</td>
<td>1.6</td>
<td>4.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLCO</td>
<td>1.3</td>
<td>3.8</td>
<td>0.989 (0.974-0.996)</td>
<td>4.72</td>
</tr>
<tr>
<td>mmol min$^{-1}$ kPa$^{-1}$</td>
<td>0.4</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLNO/DLCO</td>
<td>0.1</td>
<td>0.25</td>
<td>0.983 (0.960-0.994)</td>
<td>2.40</td>
</tr>
<tr>
<td>DMCO</td>
<td>2.4</td>
<td>6.7</td>
<td>0.994 (0.986-0.998)</td>
<td>4.41</td>
</tr>
<tr>
<td>mmol min$^{-1}$ kPa$^{-1}$</td>
<td>0.8</td>
<td>2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vc (ml)</td>
<td>4.3</td>
<td>12.0</td>
<td>0.975 (0.941-0.991)</td>
<td>5.84</td>
</tr>
</tbody>
</table>

Table 4 Intra-session variability of single-breath diffusing capacity measurements from three different study visits.

Data were measured using a target breath-hold time of 8 s. CV, coefficient of variation; DLCO, pulmonary diffusing capacity for carbon monoxide; DLNO, pulmonary diffusing capacity for nitric oxide; DMCO, alveolar-capillary membrane diffusing capacity for carbon monoxide; Vc, pulmonary capillary blood volume; VIN, inspired volume. Data were measured in SI units (mmol min$^{-1}$ kPa$^{-1}$) and converted to traditional units (ml min$^{-1}$ mmol$^{-1}$) by multiplying with 2.987. DMCO = DLNO/1.97. * The measurement error (or within-subject standard deviation, SD$_{ww}$) was calculated by the root-mean-square (RMS) method. 

$b$ Reproducibility values were calculated as the SD$_{ws}$ over three study visits multiplied by 2.77 (95% level of confidence).

additional measurements between the visits, which would have also contributed to the postulated learning effect.

In addition, we studied the inter-session variability of pulmonary gas diffusing measurements over a period of 8 ± 2 days. The reproducibility values of DLNO, DLCO, DMCO and Vc were 33%, 24%, 32% and 17% lower than those previously reported in twelve healthy subjects (Muirias and Zavorsky, 2007) using the same statistical analyses approach. The differences in the variation between our observations and those reported by Murias and Zavorsky (2007) are likely the result of a much longer observation period in their study (eight testing sessions during 2 months) and consequently a higher chance for greater week-to-week variation (i.e., week-to-week variation was suggested to be affected by mild pathophysiological changes over time) (Muirias and Zavorsky, 2007).

Our data support previous observations demonstrating that DLCO is not considerably affected until late stages of CF lung disease, (Dressel et al., 2009; Espiritu et al., 2003; Merkus et al., 2004) most likely due to less alterations of Vc in our population. Despite a fall of FEV$_1$, DLCO remained stable over time in children with CF (Merkus et al., 2004) and consequently the clinical value of DLCO in early CF lung disease has been questioned. In line with previous studies (Dressel et al., 2009) mean DLNO percent predicted was about 20% lower in our study population than DLCO percent predicted and correlations with FEV$_1$ were much stronger than with DLCO. In COPD, DMCO and Vc decrease with increasing disease severity (GOLD stages), but the decrease in Vc is more pronounced (Schulz et al., 2014) and possibly due to a loss of pulmonary capillaries. In our patients, DMCO was markedly reduced but Vc was normal in line with previous investigations in adults with CF (Wheatley et al., 2011). This suggests that the limitation in the alveolar-capillary membrane conductance is more specific to CF pulmonary disease. On theoretical grounds, DLNO is a better representative of the function of the alveolar-capillary membrane than DLCO (Zavorsky et al., 2017). Whether DLNO and DMCO could serve as markers of early lung disease in CF and for monitoring the disease needs to be further investigated.

We studied a heterogeneous group of patients with a broad range of CF pulmonary disease severity (FEV$_1$ 24–94%predicted) to depict the entire spectrum of CF pulmonary disease. Our experiments were
conducted under controlled laboratory conditions and potential confounders affecting gas diffusing measurements such as diurnal variation (Cinkotai and Thomson, 1966), bronchodilator- and airway clearance therapy, nutrition and exercise (Wheatley et al., 2015) were minimized. One would expect a higher variation in pulmonary diffusing measurements when patients are tested in less-controlled settings, for example during routine clinical visits. Moreover, our data were collected within a short time period (< 2 weeks), which limits the generalizability of our variability estimates to longer time periods.

In general, information on the technical and biological variability is crucial for the accurate interpretation of diffusing capacity measurements and is a prerequisite for statistical power calculations. We extend the current knowledge on the intra-session and inter-session variability of DLNO and its components to patients with pulmonary pathophysiology. The variability values reported herein may guide clinicians, technicians and researchers to discriminate between measurement error and clinically meaningful change of gas diffusing measurements.

5. Conclusion

In conclusion, clinically stable patients with CF are able to achieve intra-session and inter-session variability criteria for DLNO, DLCO and its components previously reported for healthy subjects. Single-breath maneuver experience results in substantially lower intra-session variation of gas diffusing parameters.

Author contributions

TR takes responsibility for the overall content as guarantor. TR, SK and HD contributed to the study design. TR and MMB conducted all experiments. TR performed the data analysis. CB, HD, IVDL, SK, and TR contributed to the data interpretation and the writing of the manuscript. TR wrote the first draft of this manuscript and all authors approved the final version.

Funding

The Swiss Cystic Fibrosis Society (CFCH) funded the study. The sponsor had no role in the design of the study, data collection, analysis and interpretation, or the content of the manuscript.

Competing interests

None.

Conflicts of interest

None declared.

Acknowledgments

We acknowledge and kindly thank André Königs from the University Hospital Zurich for his assistance in patients’ recruitment.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.resp.2017.08.002.

References

Aguilaniu, B., Maitre, J., Glenet, S., Gegout-Petit, A., Guenard, H., 2008. European...