ERJ Express. Published on January 17, 2019 as doi: 10.1183/13993003.02109-2018

EUROPEAN RESPIRATORY *journal*
FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Research letter

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Please cite this article as: Radtke T, Hua-Huy T, Dressel H, *et al*. Of the need to reconcile discrepancies between two different reference equations on combined single-breath *D*LNO-*D*LCO in systemic sclerosis. *Eur Respir J* 2019; in press (https://doi.org/10.1183/13993003.02109-2018).

Of the need to reconcile discrepancies between two different reference equations on combined single-breath *D*LNO-*D*LCO in systemic sclerosis

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Conflict of interest: None declared

Funding: None

Key words: Diffusing capacity, Nitric oxide, Reference values, Systemic sclerosis

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To the editor,

Systemic sclerosis (SSc) is a chronic disease of the connective tissue with an estimated prevalence of 5.8 per 100,000 in a multi-ethnic population living in Europe [1]. The disease mechanisms are highly complex, affecting multiple organs including the pulmonary system. Severe pulmonary complications include systemic sclerosis associated interstitial lung disease (SSc-ILD) and pulmonary hypertension (SSc-PH), which are also associated with premature mortality [2, 3]. Early diagnosis and monitoring of pulmonary complications by spirometry, bodyplethysmography, and pulmonary diffusing capacity are critical to lung health in SSc patients [4, 5]. Pulmonary diffusing capacity for carbon monoxide (*D*LCO) is a well-established and frequently applied test in SSc and is used as a surrogate of SSc-ILD and SSc-PH progression [4, 6]. Recently, nitric oxide pulmonary diffusing capacity (*D*LNO) has received growing attention in the diagnostic setup of SSc patients [7, 8]; yet, its clinical value with respect to disease management and prognosis has to be shown. In this respect, accurate interpretation of test results using robust reference equations [5] is imperative to both clinicians and researchers. While *D*LNO is a promising method, uncertainties in the accuracy of available references equations limit its clinical implementation on a larger scale.

The measurement of *D*LNO has recently been standardised by a European Respiratory Society (ERS) Task Force [9]. The Task Force provides the largest dataset (n=490, age range 18-93 years) on normal values for combined single-breath *D*LNO-*D*LCO tests for white, European and Northern American adults by pooling datasets from previously published studies [10-12]. Despite some technical and methodological inconsistencies between the different underlying studies [10-12], these reference equations are an important step forward towards the interpretation of *D*LNO tests in clinical research and practice. Recently, new reference equations for the combined single-breath *D*LNO-*D*LCO were published for

European adults [13], using ERS technical standards methodology [9] and rigorous quality control in a relatively large population (n=282) covering a broad age range of 18-97 years. To date, the impact of different reference equations on the interpretation of combined single-breath *D*LNO-*D*LCO values in patient populations with pulmonary involvement is unknown. We therefore sought to compare the two most recently published reference equations for *D*LNO-*D*LCO [9, 13] using data from a large cohort of SSc patients with a broad range of disease severity. Those two equations were chosen as the Zavorsky et al. [9] equation is the official reference equation of the ERS Task Force and the Munkholm et al. [13] equation is the first equation that was built upon *D*LNO measurements following ERS Task Force standard methodology [9].

We retrospectively analysed data from an unselected cohort of clinically stable SSc patients assessed at Hôpital Cochin, Université Paris Descartes, Paris, France between February 2007 and October 2016. Three hundred and thirty-seven patients (84% females) with a mean (SD) age of 54.4 (13.6) years, a body mass index of 23.7 (4.4) $kg.m^{-2,}$ a forced expiratory volume in 1s of 86 (18) % and a forced vital capacity of 85 (18) % were included. Of those, 133 (39%) and 19 (6%) had a diagnosis of ILD and pulmonary hypertension (PH), respectively, and 14 (4%) had both ILD and PH. Combined single-breath *D*LNO-*D*LCO tests were performed on a commercially available device (HypAir, Medisoft, Dinant, Belgium) using a breath-hold time of 6s. The mean of two technically satisfactory tests fulfilling inter-test reliability criteria [9] was used for statistical analysis. To compare *D*LNO and *D*LCO values, we calculated z-scores values using Zavorsky et al. [9] and Munkholm et al. [13] equations.

The study complied with our institutional rules and the World Medical Association Declaration of Helsinki that deemed the study to be observational and therefore waived the need for informed consent. All functional tests were routinely performed for diagnostic and therapeutic purposes.

Our analysis revealed systematic differences (i.e., a proportional error) in *D*LNO and *D*LCO z-scores between the two equations [9, 13]; see figures 1 a-d. Moreover, comparing both equations [9, 13] we noticed differential agreement of *D*LNO and *D*LCO z-scores between sexes, with much wider limits of agreement for females (figures 1b, 1d).

The magnitudes of the difference in *D*LNO and *D*LCO z-scores between the two reference equations [9, 13] are likely multifactorial, including disparities at the population level as well as technical and methodological discrepancies. However, both *D*LNO and *D*LCO were consistently different across the entire spectrum of z-scores, which points toward a systematic methodological difference. We were not able to compute z-scores for the reference equation by Aguilaniu et al. [12], because the authors did not report the residual standard deviation for their equation. Consequently, we could not compare z-scores from this equation with those from Munkholm et al. [13] to assess the relative contribution of this study in the reference equation from Zavorsky et al. [9]. Nevertheless, percent-predicted values for *D*LNO and *D*LCO were on average 30% and 24% lower comparing Aguilaniu et al. [12] with Munkholm et al. [13] equations (data not shown), and this study [12] contributed about half (54 %) of the measurements to the pooled dataset from Zavorsky et al. [9]. This suggests that the differences are not primarily due to differences in equipment (e.g., different devices and nitric oxide analysers) and/or breath-hold times, but partially due to inclusion of data from Aguilaniu et al. [12]. For their analysis, Aguilaniu et al. [12] chose the *D*LNO value from the highest *D*LCO test and not a mean value as the other authors did [10, 11, 13]. This apparently resulted in reference equations with the highest *D*LNO predicted values [13]. However, to what extent the differences in percent-predicted values between the two equations impact on z-scores cannot be determined.

The use of different analytical approaches is another reason for the discrepancies between the two reference equations [9, 13]. Munkholm et al. [13] reported sex-specific equations with residual standard errors (RSE) separately for males and females, whereas

Zavorsky et al. [9] did not apply gender-specific RSE in their equation. For example, Munkholm et al. [13] uses an RSE for *D*LNO of 11.4 for females and 16.6 for males, while Zavorsky et al. [9] uses an RSE of 20.0 for both sexes. The smaller difference in standard errors between males results in different regression slopes between sexes (figure 1a-b) with an overall better agreement between *D*LNO z-scores for male patients [9, 13]. The same observation applies for *D*LCO. Since sex is an independent explanatory variable of *D*LNO [9, 13] and a significant and independent predictor of *D*LCO [14], sex-specific reference equations for combined *D*LNO-*D*LCO [10, 13] are appropriate.

In conclusion, our data confirms considerable differences in *D*LNO and *D*LCO z-scores between different references equations [9, 13] in a large cohort of SSc patients. In an attempt to improve the validity of reference equations, pooling of available datasets [9, 13] would significantly strengthen the robustness of *D*LNO and *D*LCO equations, and facilitate interpretation of pulmonary diffusing capacity measurements for both clinicians and researchers. Future work on *D*LNO-*D*LCO reference values is required to allow for calculation of sex-specific precise lower and upper limits of normal based on a large population including people of different ethnic backgrounds - a prerequisite to ultimately evaluate and use the clinical potential of this promising technique.

Acknowledgements

We kindly thank Dr. Julia Braun and Dr. Sarah Haile (Biostatisticians at the University of Zurich) for their support with regard to statistical analyses and data interpretation.

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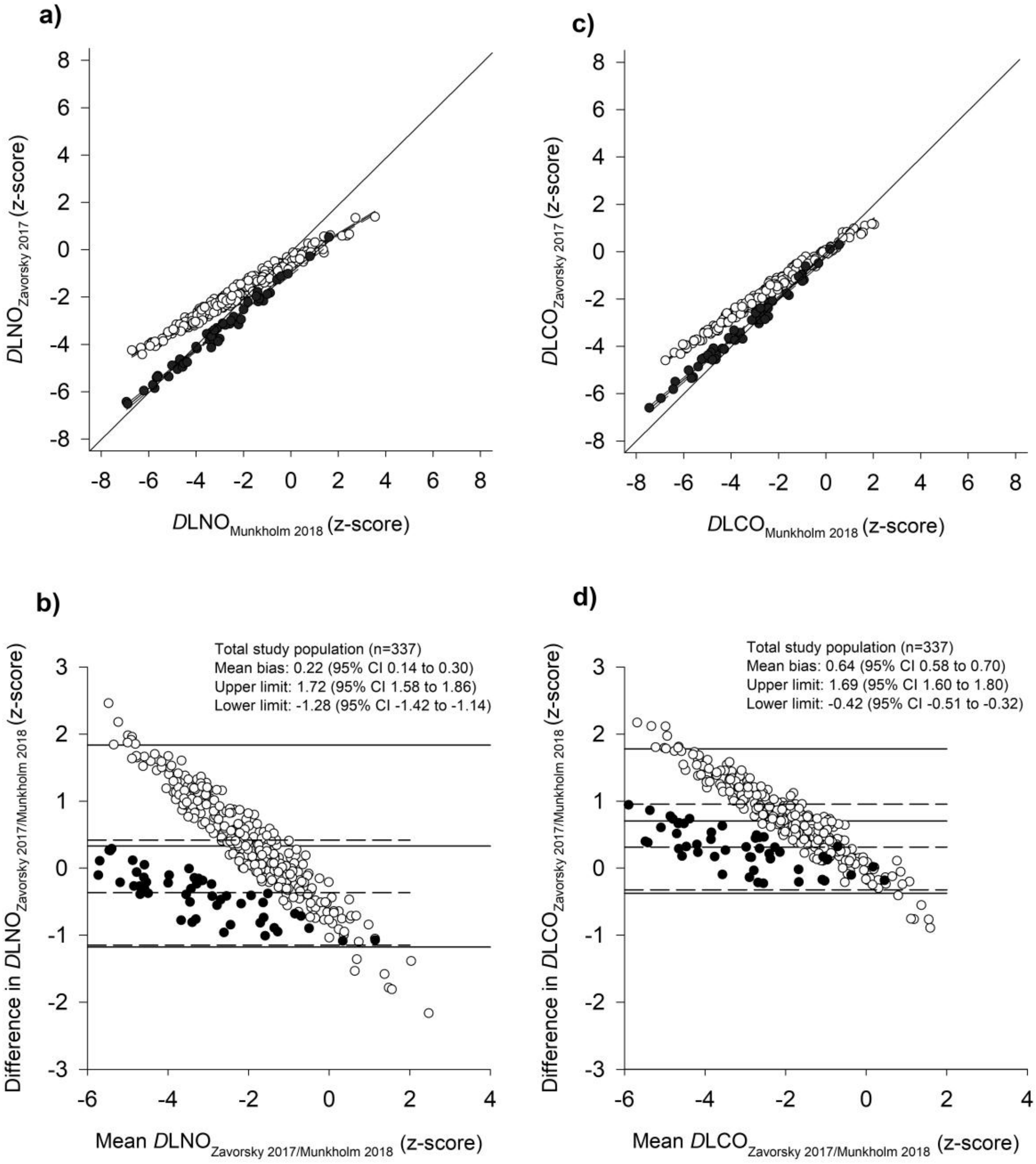
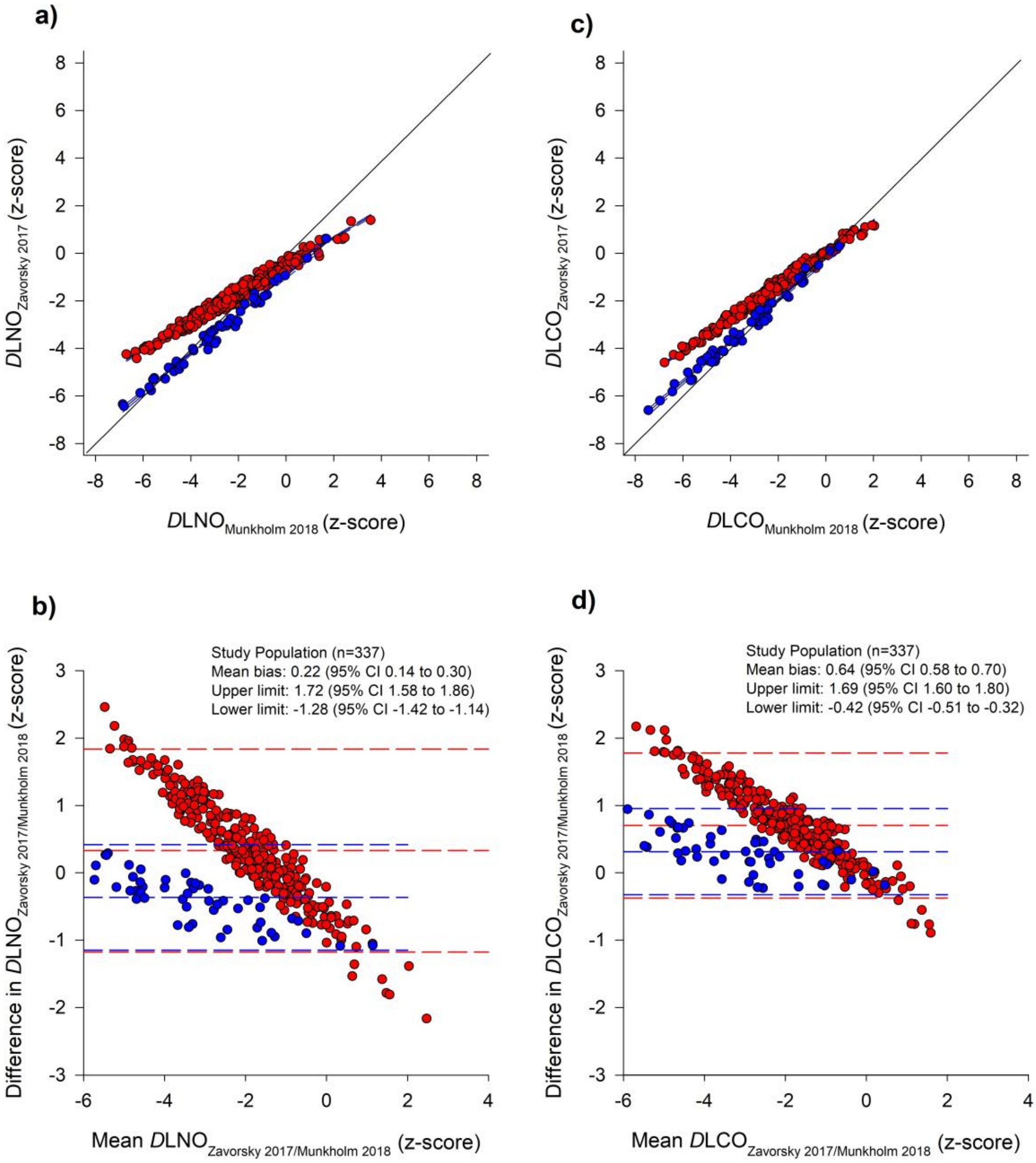
Figure legend

Figure 1. Scatter and Bland-Altman plots for comparisons between z-scores for pulmonary diffusing capacity for nitric oxide (*D*LNO, figures 1a,b) and carbon monoxide (*D*LCO, figures 1c,d) between reference equations published by Zavorsky et al. [13] and Munkholm et al. [9]. Solid black circles represent males and open white circles circles represent females. The solid lines (females) and dashed lines (males) in figures 1b and 1d represent the mean bias and upper and lower limits of agreement (mean ± 1.96 x standard deviation).



Scatter and Bland-Altman plots for comparisons between z-scores for pulmonary diffusing capacity for nitric oxide (DLNO, figures 1a,b) and carbon monoxide (DLCO, figures 1c,d) between reference equations published by Zavorsky et al. [13] and Munkholm et al. [9]. Solid black circles represent males and open white circles circles represent females. The solid lines (females) and dashed lines (males) in figures 1b and 1d represent the mean bias and upper and lower limits of agreement (mean ± 1.96 x standard deviation).



Bland-Altman plots for comparisons between z-scores for pulmonary diffusing capacity for nitric oxide (DLNO, figures 1a,b) and carbon monoxide (DLCO, figures 1c,d) between reference equations published by Zavorsky et al. [13] and Munkholm et al. [9]. Blue circles represent males and red circles represent females. The blue and red dashed lines (figures 1b and 1d) represent the mean bias and upper and lower limits of agreement (mean ± 1.96 x standard deviation).