Author's response to reviews

Title: Lung diffusing capacity for nitric oxide and carbon monoxide in relation to morphological changes as assessed by computed tomography in patients with cystic fibrosis

Authors:

Holger Dressel (Holger.Dressel@med.uni-muenchen.de)
Laura Filser (Laura.Filser@gmx.de)
Rainald Fischer (Rainald.Fischer@med.uni-muenchen.de)
Katharina Marten (Marten@roentgen.uni-wuerzburg.de)
Ullrich Müller-Lisse (Ullrich.Mueller-Lisse@med.uni-muenchen.de)
Dorothea de la Motte (Dorothea.de.la.Motte@med.uni-muenchen.de)
Dennis Nowak (Dennis.Nowak@med.uni-muenchen.de)
Rudolf M Huber (Huber@med.uni-muenchen.de)
Rudolf A Jörres (Rudolf.Joerres@med.uni-muenchen.de)

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Author's response to reviews: see over
Dear Referees

Enclosed please find our revised manuscript entitled "Lung diffusing capacity for nitric oxide and carbon monoxide in relation to morphological changes as assessed by computed tomography in patients with cystic fibrosis", which we would like to resubmit for publication in BMC Pulmonary Medicine.

We are very grateful for all of your critical and helpful comments and have tried to incorporate all specific suggestions into the revised version. Please find our point-by-point response to your concerns on the following pages.

Sincerely yours,

H. Dressel
on behalf of all authors
Response to the comments of Referee 1

Thank you very much for your critical and helpful comments.

This article reflects the disharmony that exists in pulmonary medicine between imaging on the one hand and functional testing on the other hand. These two different pathophysiological aspects are coming together in this manuscript: function meets imaging. Therefore, the main question of this small but fine study is challenging.

Because NO is not influenced by capillary blood volume, the DLNO is a true measure for the diffusive properties of the alveolo capillary membrane. In fact, CO is much less classified for this measure. The fact that DLNO is not widely accepted, is pure technical: only since short time DLNO equipment is available. It is logical to search for correlations between this new measure (DLNO) with well known measures like the CT for parenchymal destruction evaluation.

Thank you very much for appreciating our research question.

This leads directly to one of the weaker points of this study: the Brody score consists mainly of scoring airway disease. Only parenchymal destruction will influence the DLNO. The reason that a strong correlation was found between the DLNO and Kno and the Brody CT score, could be based on the fact that the parenchymal disease is correlated with the Brody score, but not based on loss of surface of the membrane, but on other factors. In that case, the good correlation between DLNO and Brody score should not be causal connected. This possibility should be explored in the discussion section.

We agree that it is not possible to establish a causal connection between the changes in the Brody CT score and of DLNO or KNO. We included your arguments in the discussion section (page 10, last paragraph, line 4-7 – page 11, first paragraph, lines 1-2) and point out that we only describe correlations between a CF severity marker mainly comprising airway disease (CT score) and DLNO/KNO as markers of parenchyma destruction.

In the methods section, no mention is made that [Hb] is measured, I trust that were is stated that "dlico is corrected to the standard Hb", indeed Hb of the subjects is measured. This is of course essential. Please more detailed description here.

We now state explicitly that Hb was measured (page 5, lines 1-2).

Table 4 showes a 5 * 16 matrix of correlation coefficients. This leads to 80 parameters, in which no one will surprised that some significant relation will be found. Furthermore, many parameters on the vertical axis are intercorrelated, for example Raw and FEV1. Some parameters like DLNO and Dm have of course exactly the same correlation coefficients, therefore this table has too abundant data in it, to my opinion. If data is shown, odd correlations need to be explained: eg. how can anyone explain the significant correlation between Vcap and peribronchial thickening. I can't. Therefore, leave some irrelevant data out please.

We reduced table 4 (now table 3) substantially. As you suggested we combined DLNO and Dm in one line. We omitted several parameters who are similar to other measures, do not contain important additional information or show only weak correlations: VA, RAW, ITGV, TLC, and the difference TLC-VA. We would like to keep both SRAW as a measure of obstruction obtained by bodyplethysmography and FEV1 obtained by spirometry.

In conclusion, the main results that the DLNO has significant correlation with the Brody score in CF patients is worthy of publication. Whether this proves that DLNO in this study reflects the parenchymal destruction is open for discussion. This subtle distinction should be more specified in the discussion section.
As stated above we included your suggestions in the discussion. Thank you again for your help.
Response to the comments of Referee 2

It is ok basically.

Thank you for appreciating our study.
Response to the comments of Referee 3

Because I would like all my comments addressed, my comments are thus labeled “major compulsory revisions” to everything below:

Thank you very much, indeed, for the helpful comments and suggestions. We have tried to incorporate all suggestions into the revised version.

Major comments
The authors correlated DLNO, DLCO and other lung function parameters to the extent of cystic fibrosis damage from CT scanning. They proposed that DLNO would be superior to DLCO for the quantification of structural changes in the lung. Since thin section CT is the method of choice for looking at morphological changes in CF patients, they used CT scanning to correlate with various lung function parameters. They showed that that DLNO and DLCO were correlated well with the CT score. I like this study!

Thank you very much for appreciating our study.

I however would not agree with the statement that DLNO seemed more sensitive to detect CF compared to the DLCO. I would suggest deleting the second sentence in the conclusion section of the abstract. An r² or 0.66 versus 0.56 is not statistically different from each other. The 95 % CI interval for the R² (see http://www.danielsoper.com/statcalc/calc28.aspx) for both DLCO and DLNO overlap with each other so the DLNO and DLCO relationship with CT score are similar to one another.

We agree that the difference is not statistically significant. As you suggested we deleted the second sentence in the conclusion section of the abstract.

Methods
Why was there an exclusion criterion of an interval of more than 3 yrs between CT and PFT testing? Why not 1 yr? or 2 yrs? Is there any data showing that CT or PFT results are stable only up to 3 yrs? I think the authors should research the stability of CT and PFT testing over the long term in CF patients and include a paragraph or two in their manuscript. Page 10 in their discussion where they refer to reference 6 is a good start. They should rewrite their first sentence on page 5 in their “CT examination and scoring” section of the manuscript about this to make it more clear. The authors should report the mean, SD and range of the time between the CT scan and the pulmonary function testing. Page 10 in your discussion where you refer to reference 6 is a good start.

We now state that this time interval was chosen as CF patients in this clinic are usually monitored by CT every 3 years (page 5, paragraph 3, lines 1-2). Additionally to the references about the annual decline of CT changes in CF [6,22] we now also report the corresponding declines in lung function from those articles (page 11, second paragraph, lines 12-15). We now provide mean, SD and range of the time interval between CT and PFT.

What was the NO analyser used? The authors should report this.

We now report that this commercially available device uses electrochemical sensors (page 4, 3rd paragraph, lines 2-3).

Why did the authors express Dm as DLNO / 1.97 when more recent data (see their reference 18, page 77, second column and also in Table 6 from reference 18) show that Dm is better reflected by DLNO/2.42?

We are aware of the ongoing debate whether Dm is better calculated as DLNO/1.97 or DLNO/2.42 and very much appreciate your important contributions to this discussion. As a large number of studies used 1.97 we adhered to this for comparability reasons. Moreover, the correlations which are the focus of our work, remain the same irrespec-
tive of the factor used. However, we now discuss this issue in more detail (page 9, 1st paragraph, lines 4-6).

As well reference 18 predicts DLNO based on a patient’s VA. It would be interesting to have a percent predicted DLNO based on the patient’s VA. Then that percent predicted should be compared to the percent predicted based on the patient’s height (Table 4 from reference 18). Compare the two percent predicteds using a paired t-test to see if there is a difference. Therefore these authors can discern whether the reduction in DLNO from CF patients is mostly based on gas exchange abnormalities or lung volume problems or both (see discussion on page 75, second column of Reference 18). Then this could be included in the discussion section as you touch upon the topic on page 9.

We appreciate very much your recent work on DLNO reference values. We now included your approach of using reference equations including either VA or height (page 10, lines 5-13). Following your reasoning from reference 18, both abnormal gas exchange and reduced lung volume contributed to the reduction in predicted DLNO found in our sample.

DLCO was corrected to the standard Hb concentration. Is there evidence to support that patients with CF have normal Hb values? Can these authors find a reference for this and then include it?

We now state explicitly that the actual Hb was measured and that DLCO was corrected according to international guidelines (page 5, lines 1-2).

Results
What was the predicted VA in these 21 subjects? I think they should report % predicted VA. And then add this finding to your discussion section.

We now included VA expressed as percent of predicted in table 2 using the formula applied in reference [18] and added a sentence to the discussion stating that the slightly reduced VA may explain partially the reduced DL$_{NO}$ (page 10, lines 12-13).

How many CF patients were below the lower limit of normal (LLN) for DLCO and DLNO? (See Reference 18). I think the authors should report that.

We now report that by applying the equation from reference 18, 15 subjects (71%) were below the 95% Lower Limit of Normal (LLN) concerning DLNO compared to only 9 subjects (43%) concerning DLCO (page 7, lines 5-7).

I also think the authors should report the DLNO to DLCO ratio in Table 2.

DLNO/DLCO is now included in table 2.

Page 6 last 4 lines on the page, please round the percent predicted to a whole number. Decimals are not needed for % predicted. Are the % predicted from references 16 to 18 all based on gender, age and height?

The percent predicted were now rounded to a whole number and we state explicitly that all predictions are based on gender, age and height (page 7, line 1).

Discussion
Page 8, 4th line from the bottom, starting with “Remarkably…” as mentioned earlier, I would like this sentence deleted. An r2 or 0.66 versus 0.56 is not statistically different from each other. The 95% CI interval for the R2 (see http://www.danielsoper.com/statcalc/calc28.aspx) for both DLCO and DLNO overlap with each other so the DLNO and DLCO relationship with CT score are similar to one another.
We deleted this sentence.

Conclusion
Page 10 second line, remove “most”
Page 11, change to read ...challenging question of whether the....

We changed the text accordingly.

Tables
SD should be used instead of the SEM. Please change.

SD are used in table 2.

Table 2 the % predicted values should be placed in the table. Maybe three different columns should be made for the % predicted values given that there are three different DLNO prediction equations

We included DLNO %pred according to reference [18] and DLCO %pred according to reference [15] in the table.

Table 2, for DLNO DLCO and Dm, one decimal place should be used only. For age and Vc round to the nearest whole number.

We changed accordingly.

Table 3 should be combined with Table 2 to make one table.

We combined both tables.

The percent predicted should be rounded to the nearest whole number.

We changed accordingly.

Figure and figure legend
Also I rather that the authors report the “r2” rather than the “r”. As well the 95% CI interval for the r2 should be reported for both Figure 1 Panel A and B and Figure 2 panel A and B. A web-link is provided to help the authors with this: http://www.danielsoper.com/statcalc/calc28.aspx
The authors should report the regression equation for each figure.

For all correlations with the CT score we calculated Spearman’s rank correlation coefficient r_s as we did not want to assume a priori a strict linear correlation with the diffusing capacities. As a score is not a strict metric scale we did not apply the Pearson correlation coefficient that shows linear correlations but used a non-parametric approach independent of the distributions of variables. The calculation of r^2 with the corresponding confidence intervals is usually performed for the Pearson correlation coefficient. As we don’t want to imply a strict linear relationship we would like to keep the Spearman correlation coefficient and also would prefer not to report the regression equations. To further underline this point we now also omitted the regression lines from our figures.

Overall assessment
My overall assessment of this manuscript is “accept with revisions” provided all my comments are adhered to.

Thank you for proposing to accept the revised manuscript, we tried to incorporate all of your helpful suggestions.
Response to the comments of Referee 4

Thank you very much for the helpful comments and suggestions.

The authors aim to study the relationship between Dlno, DLco vs CT

General comments
The rational to identify a new diffusion marker for CF lung disease is interesting. The major aim of the lung is diffusion and thus it makes sense to monitor this capacity.
This is a cross sectional study in an adult population of CF patients. Inclusion criteria were when a CT was available not older than 3 years. Hence, there was a substantial time interval between CT and PFT.

Thank you for appreciating our research question.

Major comments
What were the indications of the CT. Is it done routinely? Or were some of the CTs taken during exacerbations. This needs to be specified.

These CTs were done routinely. Usually CF patients are monitored by CT every 3 years in this outpatient clinic. We now specify this (page 5, 3rd paragraph, lines 1-2).

A major weakness of the study is its retrospective nature. Up to three years time interval is allowed between CT and PFTs. In addition it is not well defined how patients were selected.
The major disadvantage of tests that aim to measure diffusion is that they become problematic when there is obstruction. This is considered an important reason for the lack of correlation for Dlco. The same accounts for Dlno. The fact that a correlation is found in this study does not exclude this problem. This should be discussed

Thank you for raising this point which we now included in the discussion (page 8, 3rd paragraph, lines 2-4). We agree that diffusion measurements are influenced by a variety of functional or structural alterations in CF, e.g. the obstruction typically found in those patients, and thus may be difficult to interpret.

In CF everything correlates with everything in a cross sectional setting. There is a wide range of PFT values and CT scores. Hence it is likely that a patient with a normal CT has (near) normal PFTs for a patient with severely abnormal CT has substantial abnormal PFTs. Why an extra test is needed? Does it tell something we do not know. In the discussion possible advantages on the use of DLno over other tests should be discussed.

As DLNO is a measurement that recently has become commercially available and is proposed to be a good measure of membrane diffusion the primary aim of this manuscript was to compare this measure with the classic DLCO and look for correlations with standard CF monitoring parameters. We now added some sentences concerning a possible additional value of DLNO compared to other techniques (page 9, lines 10-19). However, we stress that prospective longitudinal studies are needed to decide whether the measurement of DLNO may provide additional value to the existing standard monitoring parameters.

P4, para2
It is stated that patients with a history of recent exacerbations or marked functional deteriorations within the time interval between CT and PFT were excluded from the study. Please specify in more detail the criteria of in and exclusions. How many of the patients had an RTE between CT and PFT's. Please define in detail what is meant by ‘marked functional deteriorations’
P6, para 1
We now explain in more detail the clinical exclusion criteria (page 4, 2nd paragraph, lines 5-8). During the time between the CT and the PFT the clinical status of the patients had been regularly monitored by specialists from the Cystic Fibrosis Outpatient unit, usually with 3 months intervals between visits. Patients were excluded if severe exacerbations, resulting in hospitalisation, were recorded.

To my knowledge the max Brody score is 180 when air trapping is excluded. The maximal score of 6x36=216 can not be obtained since some combinations can not occur. Please specify.

We applied the Brody score as described in reference [13] (Brody et al. J Thorac Imaging 2006;21:14-21) and as displayed in our table 1. In reference [13] the possible range for the overall score is 0-40.5. The ranges for the subscores we used are 0-12 for bronchiectasis, 0-6 for mucous plugging, 0-9 for peribronchial thickening, and 0-9 for parenchymal changes. A subscore representing the whole lung was defined as the average of this subscore assessed in each of 6 lobes. Thus the range for our overall score omitting air trapping was 0-36.

Furthermore to do the correlations were the scores normalized on a 0-100% scale. Please do so since it will allow comparisons with other studies.

Thank you for raising this issue. However, when normalizing the scores the correlation coefficients will remain the same. As we only focus on correlations in this work and only use one score we would prefer to display the Brody score in its original form as described in reference [13].

Page 3, last sentence 1st para  
It is stated that ‘Dlco plays only a minor role’. More correct is no role. To my knowledge there are no data to support its use  
Page 3, last sentence 2nd para  
However; ‘its’ rate. Please change to; the reactivity of CO with red...............

We changed the first sentence accordingly. However, we kept the second as the rate of reaction with the red blood cell is much higher for NO than for CO, see reference [9].

P5, final para  
Please define explicitly whether the order in which the CTs were scored was randomized and specify which randomization system was used.

Now we state explicitly that the order in which CTs were scored was not randomized (page 6, line 5).

Discussion page8, 2nd para  
DLco is a useful marker. Pelase change to DLco is not a useful marker.

We kept the sentence as there are indeed a few references [3, 20] that proposed DLCO as a useful marker in CF.

For all comparisons it is best to make CT the independent variable and the PFts the dependent variable. This was done so in figure 1 and 2. Please change to this perspective throughout the manuscript.

We did not intend to perform regression analysis in this manuscript, but only present non-parametric correlations using the Spearman rank correlation coefficient. The regression lines in figure 1 and 2 were only inserted for illustrative purpose. We now omitted the regression lines from the figures to stress this focus on correlations. Thus the correlations between each two variables are completely symmetric.

P9, 2nd para  
Thickness of the pulmonary... please change to the volume of blood. The thickness is not of importance.
We included a new reference [21] in this sentence. Glenet et al. describe in their physiologic work the thickening of the blood sheet when experimentally deflating the lung. Thus we would like to keep the sentence.

**Strikingly, however a high correlation**...... Delete the ‘in the range of 0.8’

We deleted the whole sentence.

P10, 2nd para

'Tfairly stable course of their disease’. As discussed before it is a major weakness of this study that there was a very large window between CT and PFT. What the authors mean with stability be clearly defined

Please see our answer above concerning this point.

Thank you very much, indeed, for your comments and for helping to clarify several important issues concerning our manuscript.