Online Data Supplement

Lung volumes predict survival in patients with chronic lung allograft dysfunction

Nikolaus **Kneidinger**, Katrin **Milger**, Silke **Janitza**, Felix **Ceelen**, Gabriela **Leuschner**, Julien **Dinkel**, Melanie **Königshoff,** René **Schramm**, Thomas **Weig**, Hauke **Winter**, Jürgen **Behr**, Claus **Neurohr**

Methods

Surveillance and Assessment

After lung transplantation during hospital stay and inpatient rehabilitation lung function analysis are performed on a weekly basis. Upon discharge lung function tests are performed approximately once per month for the first postoperative year and at least once every 3 months thereafter. All lung transplant recipients are followed up in our center and every lung function test consists of a spirometry in combination with body-plethysmography. These lung function tests consist of spirometry, plethysmography (Master Screen Body, Jaeger, Wuerzburg, Germany) and blood gas analysis, and are performed according to the European Respiratory Society (ERS) Guidelines [1, 2]. The equipment was implemented in 1989 and remained unchanged over the study period. Only lung function tests obtained in our institution were used for analysis (n=9,083).

Additionally all patients are advised to use a home spirometry device on a daily basis. Furthermore, X-ray or high-resolution computed tomography (CT) of the chest,

bronchoalveolar lavage and transbronchial biopsies are routinely performed for the first postoperative year and when clinically indicated thereafter.

From 2003 on patients were treated on a regular basis with azithromycin when requested from the attending physician. Post-CLAD use of azithromycin was recorded from patient charts. In some instances montelukast was used to treat bronchiolitis obliterans syndrome (BOS). Recently, in selected case extracorporeal photopheresis and in case of suspected antibody mediated rejection rituximab, plasmapheresis and IVIG were applied. From 1990 to 1996 the immunosuppressive regimen consisted of a triple therapy with cyclosporine, azathioprine and steroids, which was then replaced by tacrolimus, mycophenolic acid, and steroids.

Radiology Review

CT-scans performed 30 days before and up to 90 days after CLAD onset were eligible for inclusion. For subjects with multiple eligible CTs, the scan performed nearest to the time of CLAD onset was selected. Scans were performed using various scanners employing different scan protocols. Standardized analysis of CT-scans was performed by a blinded radiologist (J.D.). The following findings were systematically noted and characterized as defined by the Fleischner Society [3]: ground-glass opacities, centrilobular micronodules or tree-in-bud infiltrates, bronchiectasis or bronchial wall thickening, interstitial changes (septal thickening, consolidative or reticular opacities), pleural thickening and emphysema. Furthermore, in the case of interstitial changes the area with most prominent changes was recorded (upper, middle, lower lung and diffuse distribution).

Cause of death

Cause of death was assessed and classified as CLAD-related and non-CLAD-related. In case of CLAD-related death, causes were further classified into primary infection- and non-infection associated, respectively.

Statistics

The Mann-Whitney-U test was used to test if two independent groups differed with respect to a metric variable. Differences between the groups with respect to categorical variables were tested using Fisher's exact test.

Survival estimation was done using Kaplan-Meier curves [4]. The differences between the survival curves of two groups, for example defined by R-CLAD status, were tested using the log-rank test when hazards were proportional or nearly proportional [5].

Since the present data seemed to fulfill the assumption of proportional hazards, we used Cox regression to model survival times in dependence of multiple risk factors [6]. Risk factors included the severity of CLAD, time to CLAD and baseline characteristics, such as age, sex, underlying disease, approach of thoracotomy (clamshell vs. anterolateral thoracotomy), use of azithromycin, immunosuppressive regimen (tacrolimus vs. cyclosporine) and year of transplantation. In addition to these risk factors, lung volume measurements were included as an additional covariate. These were included in form of a binary (e.g. R-CLAD status) or continuous variable (e.g. $TLC/TLC_{baseline}$).

We considered severity of CLAD, time to CLAD, age, sex, underlying disease, approach of thoracotomy, use of azithromycin, immunosuppressive regimen and year of transplantation as important potential confounding variables that should be controlled in a multiple regression when assessing the effect of R-CLAD status and AT-CLAD status,

respectively. The multiple Cox regression models included thus many confounding variables relative to the number of events observed. For this reason sensitivity analyses with fewer confounding variables were performed to assess if this has an impact on the conclusions regarding the association with R-CLAD status and AT-CLAD status [7]. Since sensitivity analyses came to very similar conclusions regarding the association with R-CLAD status and AT-CLAD status, they are not reported in the paper.

When assessing the effect of a continuous variable, in most survival models the effect of the covariate on the hazard is assumed to have a log-linear form. Nonlinear relationships are, however, common in medical applications and ignoring the presence of nonlinear effects may lead to wrong conclusions regarding the prognostic value of lung function measurements; see, e.g. [8]. To allow for the modeling of a smooth nonlinear effect, we used penalized splines implemented in the function spline of the R package survival.

Wald tests were used to assess the significance of the risk factors in the Cox models.

A significance level of 5% was used for all statistical tests. All analyses were performed using the statistical software R version 3.0.1 [9].

Results

Non-R/AT-CLAD

Of all patients with non-R/AT-CLAD, 39 patients (66.1%) had a obstructive lung function (FEV1/FVC <70%,) pattern without air trapping. Of the remaining 20 patients (33.9%) with a restrictive physiology (i.e. FEV1/FVC>70%), 12 patients (20.3%) had a concomitant loss of TLC of baseline without reaching the threshold of 90% and 6 patients had an increase of TLC compared to baseline.

R- and AT-CLAD (R+AT-CLAD)

Radiographic findings on chest CT-scan concurrent with CLAD onset were assessed. Of 6 patients with both R- and AT-CLAD the main radiographic findings were: interstitial changes (6; 100%), bronchiectasis (3, 50.0%), centrilobular micronoduley/tree in bud (2; 33.3%), ground-glass opacities (2; 33.3%), pleural thickening (2; 33.3%), emphysema (2; 33.3%).

References

1. Coates AL, Peslin R, Rodenstein D, Stocks J. Measurement of lung volumes by plethysmography. *Eur Respir J*. 1997;10:1415-27.
2. Wanger J, Clausen JL, Coates A, et al. Standardisation of the measurement of lung volumes. *Eur Respir J*. 2005;26:511-22.
3. Hansell DM, Bankier AA, MacMahon H, McLoud TC, Muller NL, Remy J. Fleischner Society: glossary of terms for thoracic imaging. *Radiology*. 2008;246:697-722.
4. Kaplan E, Meier P. Nonparametric-estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
5. Harrington D, Fleming T. A class of rank test procedures for censored survival-data. *Biometrika*. 1982;69:553-566.
6. Cox DR. Regression models and life tables (with discussion). *J R Stat Soc*. 1972;34:187-220.
7. Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. *Am J Epidemiol.* 2007;165:710-718.
8. Sleeper L, Harrington D. Regression splines in the Cox model with application to covariate effects in liver disease. *J Am Stat Assoc*. 1990;85:941-949.
9. R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *http://wwwR-project.org/*.

Table S1. Combined study endpoints and causes of death for all patients with CLAD.

	CLAD, all (53/94; 56.4%)	**Non-R/AT-CLAD** (24/59; 40.7%)	**R-CLAD** (17/20; 85.0%)	**AT-CLAD** (17/21; 81.0%)
Retransplantation, n (%)	5 (5.3%)	5 (8.5%)	0 (0%)	0 (0%)
Death, n (%)	48 (51.1%)	19 (32.2%)	17 (85.0%)	17 (81.0%)
CLAD-related, n (%)	41 (85.4%)	16 (89.5%)	14 (82.4%)	15 (88.2%)
Infectious, n (%)	18 (43.9%)	4 (25.0%)	9 (64.3%)	8 (53.3%)
Non-infectious, n (%)	23 (56.1%)	12 (75.0%)	5 (35.7%)	7 (46.7%)
Non-CLAD-related, n (%)	5 (10.4%)	2 (10.5%)	2 (11.8%)	1 (5.9%)
Unknown, n (%)	2 (4.2%)	1 (5.3%)	1 (5.6%)	1 (5.9%)

Definition of abbreviations: CLAD, chronic lung allograft dysfunction; R-CLAD, restrictive CLAD; AT-CLAD, CLAD with air trapping. As some patients are classified as R-CLAD and ~~H~~AT-CLAD at the same time, the sum of R-CLAD+AT-CLAD + non-R/AT-CLAD may be greater than CLAD-all.

Table S2. Multiple Cox regression models assessing the effect of $RV/TLC_{baseline}$ on survival

Covariate	HR [95% CI]	p-value
$RV/TLC_{baseline}$, %	-	$0.117^{\#}$, $0.351^{\#\#}$
Age	1.00 [0.97; 1.03]	0.960
Sex (reference: male)	0.70 [0.39; 1.27]	0.243
Underlying disease (reference: CF)		
COPD	1.56 [0.60; 4.03]	0.361
ILD	0.43 [0.17; 1.11]	0.080
Others	0.65 [0.25; 1.77]	0.379

Hazard ratios (HR), 95% confidence intervals (CI) and p-values from multiple regression models with outcome post-CLAD survival. – indicates that the estimated effect is non-linear and can only be described via a graph. $^{\#}$p-value for a linear trend, $^{\#\#}$p-value for nonlinearity (*). Definition of abbreviations: CLAD, chronic lung allograft dysfunction; ILD, interstitial lung disease; COPD, chronic obstructive lung disease; CF, cystic fibrosis; RV, residual volume; TLC, total lung capacity.

(*) Therneau, Terry M., and Patricia M. Grambsch. *Modeling survival data: extending the Cox model*. Springer Science & Business Media, 2000.

Table S3. Lung function parameters of patients with both R- and AT-CLAD.

Pat.	**FEV1/FEV1$_{best}$, %**	**FVC/FVC$_{baseline}$, %**	**FEV1/FVC, %**	**RV/RV$_{baseline}$, %**	**TLC/TLC$_{baseline}$, %**	**RV/TLC, %**
1	77	87	79	78	82	54
2	79	66	86	123	86	51
3	78	92	76	94	88	53
4	69	78	69	101	89	55
5	76	79	89	98	87	50
6	34	30	98	174	67	67

FEV1, forced expiratory volume in one second; FVC, forced vital capacity; ILD, interstitial lung disease; RV, residual volume; TLC, total lung capacity. R-CLAD, restrictive chronic lung allograft dysfunction; AT-CLAD, CLAD with air trapping.

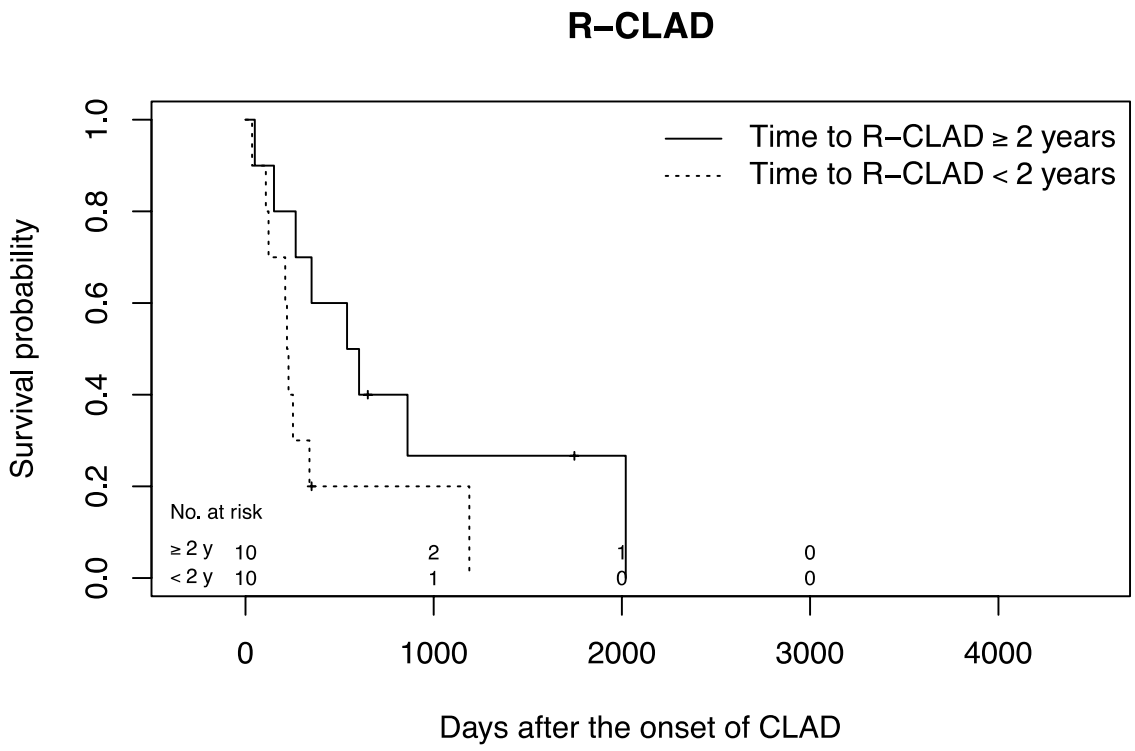

Figure S1. Survival probability of patients with R-CLAD ($TLC/TLC_{baseline}$ <90%) assessed by Kaplan Meier analysis stratified according to time to CLAD (≥2 years vs. <2 years). Definition of abbreviations: CLAD, chronic lung allograft function; R-CLAD, restrictive CLAD.

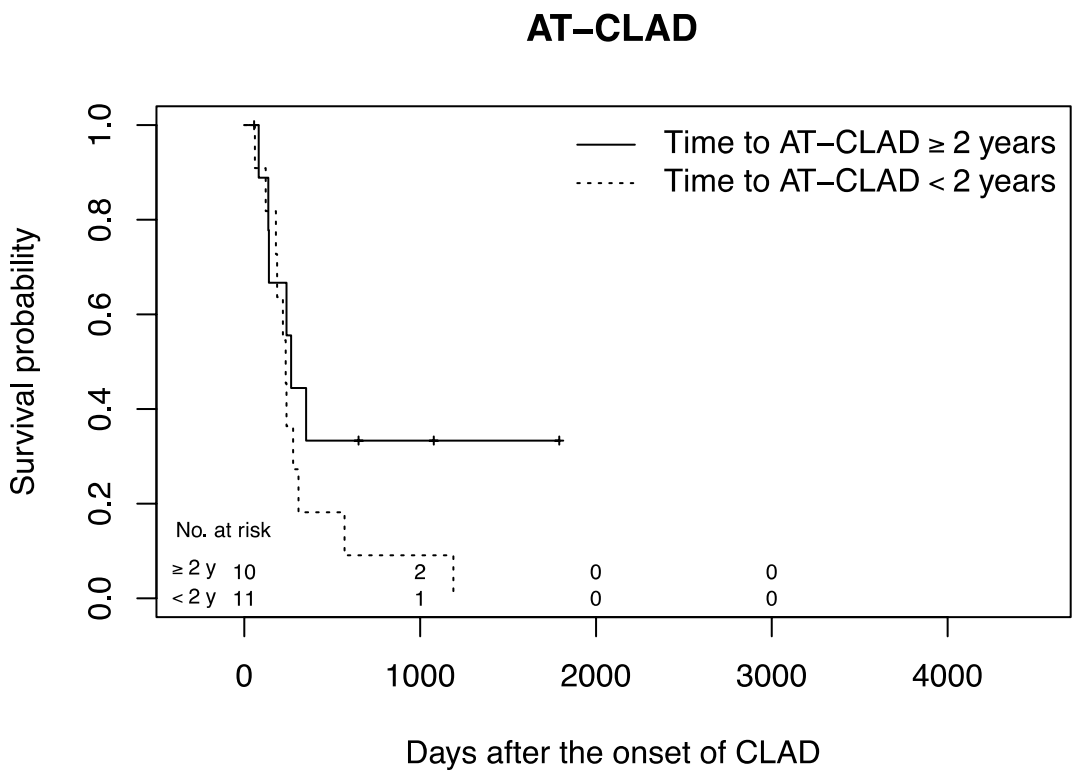

Figure S2. Survival probability of patients with AT-CLAD (RV/TLC ≥50%) assessed by Kaplan Meier analysis stratified according to time to CLAD (≥2 years vs. <2 years). Definition of abbreviations: CLAD, chronic lung allograft function; AT-CLAD, CLAD with air trapping.

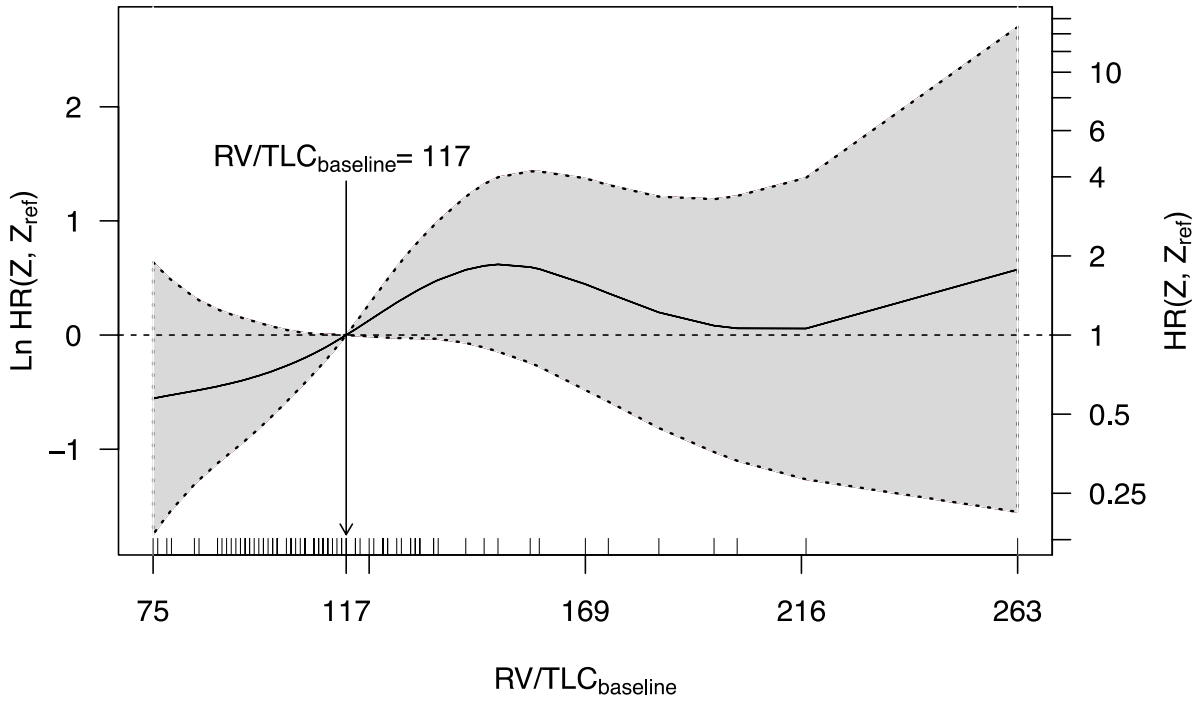

Figure S3. Effects of RV/TLC$_{baseline}$, % represented by smooth, non-linear functions. The solid curve is the estimated (log) hazard ratio as a function of RV/TLC$_{baseline}$, %. The hazard ratios are interpreted with respect to a patient with mean RV/TLC$_{baseline}$, % of 117. The gray area represents the 95% pointwise confidence intervals. Definition of abbreviations: CLAD, chronic lung allograft function; RV, residual volume; TLC, total lung capacity.

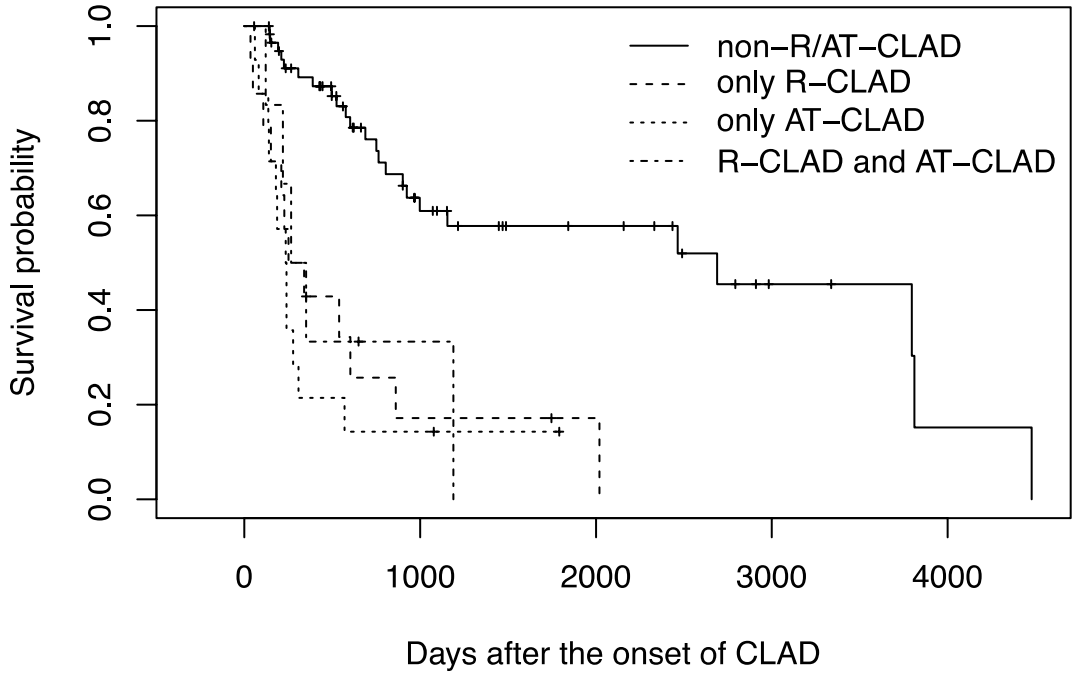

Figure S4. Survival probability of patients with neither R-CLAD nor AT-CLAD (n=59), only R-CLAD (n=14), only AT-CLAD (n=15) and both R-CLAD and AT-CLAD (n=6), respectively assessed by Kaplan Meier analysis. Definition of abbreviations: R-CLAD, restrictive chronic lung allograft function; AT-CLAD, CLAD with air trapping.

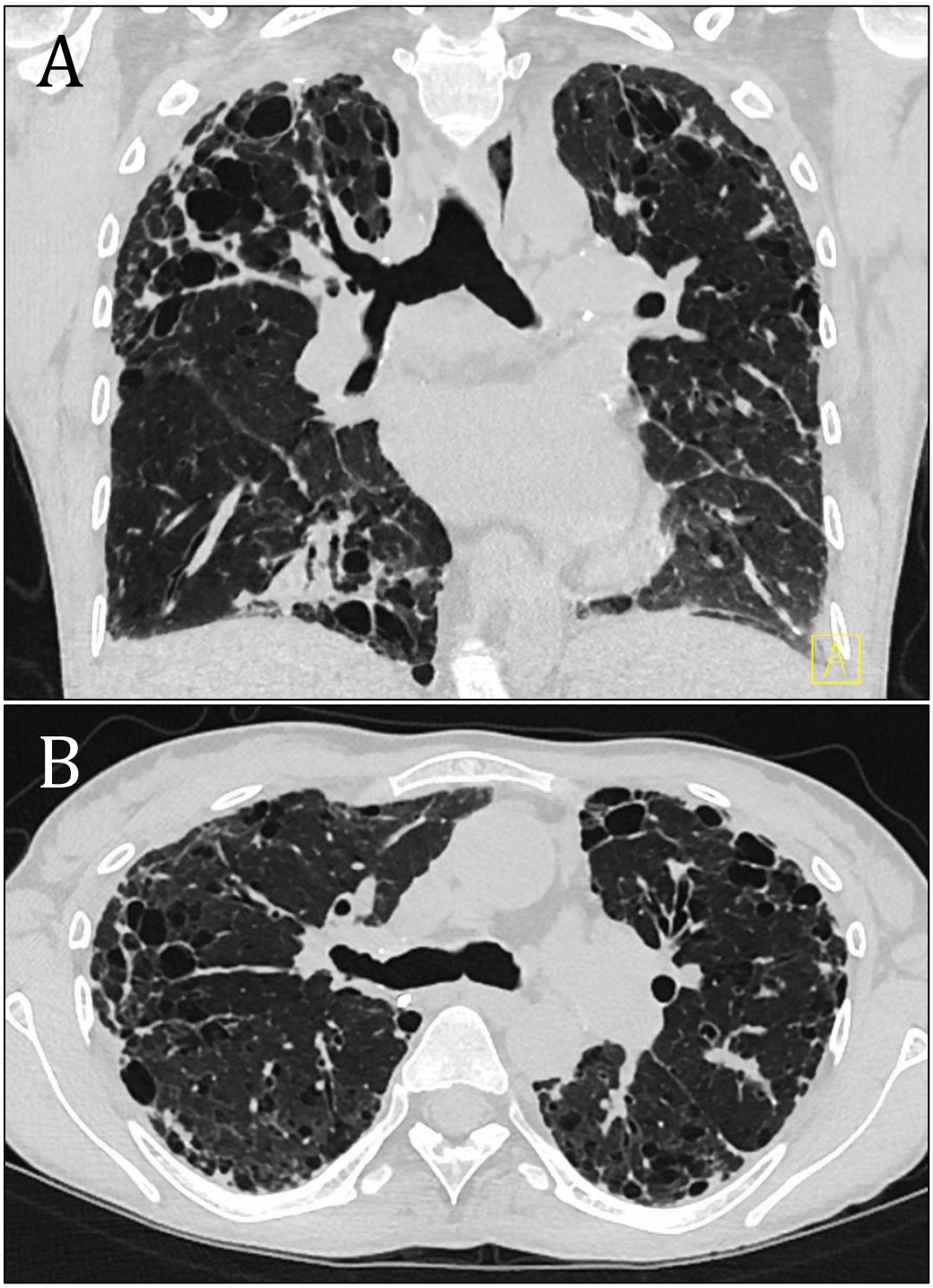

Figure S5. Representative inspiratory CT-scan from a patient with a both R- and AT-CLAD, indicative for a combined phenotype. CT-scan in frontal (A) and transverse (B) plane. Definition of appreciation: CT, computed tomography; R-CLAD, restrictive chronic lung allograft dysfunction; AT-CLAD, CLAD with air trapping.