Characteristics and survival of patients with anti-U1RNP antibodies in connective tissue disease associated pulmonary arterial hypertension

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Characteristics and survival of patients with anti-U1RNP antibodies in connective tissue disease associated pulmonary arterial hypertension

Running head: Anti-U1RNP antibodies in CTD-PAH

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ABSTRACT

Objectives: Pulmonary arterial hypertension (PAH) is a severe complication of connective tissue diseases (CTDs). This study aimed to study the clinical and hemodynamic characteristics and survival of patients with anti-U1RNP antibodies in CTD-PAH, with a focus on systemic sclerosis (SSc)-PAH.

Methods: We implemented a prospective database that included CTD-PAH patients with clinical, autoantibody and mortality data. We compared clinical and hemodynamic characteristics accordingly to anti-U1RNP antibodies status. We then assessed whether anti-U1RNP antibodies could be a prognostic factor in CTD-PAH with a focus on SSc-PAH.

Results: A total of 342 CTD-PAH patients were studied, of whom 36 (11%) were anti-U1RNP antibodies positive. Patients with anti-U1RNP antibodies were younger and less functionally impaired than anti-U1RNP negative patients in CTD- and SSc-PAH. Hemodynamic parameters were similar between anti-U1RNP positive and negative patients. In CTD-PAH, anti-U1RNP positivity was associated with a decreased mortality in univariable analysis (HR 0.34 [95% CI: 0.18-0.65]; p<0.001). In multivariable analysis, anti-U1RNP was also associated with a decreased mortality (HR 0.44 [0.20-0.97]; p=0.043), independently of age, sex, functional parameters, lung involvement and hemodynamic. In SSc-PAH, results were similar although the association between anti-U1RNP positivity and survival did not reach significance in univariable (HR 0.47 [0.22-1.02]; p=0.055) and multivariable analysis (HR 0.47 [0.20-1.11]; p=0.085).

Conclusion: Anti-U1RNP positivity was associated with distinct clinical characteristics and survival in CTD- and SSc-PAH. While hemodynamic parameters were similar between anti-U1RNP positive and negative patients, our results suggest

that anti-U1RNP positivity could be a protective factor of mortality in CTD-PAH and SSc-PAH.

Key words: anti-U1RNP antibodies – pulmonary hypertension – systemic sclerosis – systemic lupus erythematosus – mixed connective tissue disease

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a leading cause of morbidity and mortality in patients with connective tissue diseases (CTDs) (1-4). Systemic sclerosis (SSc) is the CTD with the higher prevalence of PAH (around 10%) and the worse prognosis, as a recent meta-analysis estimated the 3-yr overall survival at 56% for patients with SSc and PAH (SSc-PAH) (1,5-7). In other CTDs like systemic lupus erythematosus (SLE) or mixed connective tissue diseases (MCTD), there are less robust data on the PAH prevalence but it is very probably lower than in SSc (3,4). The prognosis of SLE/MCTD-associated PAH (SLE/MCTD-PAH) is also better than in SSc-PAH with a 3 yr-overall survival between 74-88% in SLE-PAH and 63-64% in MCTD-PAH (8,9). There is no clear explanation for this difference in survival between CTD-PAH (10,11).

Among the prognosis factors of SSc-PAH, a lot attention has been made on hemodynamics and exercice tolerance (NYHA functional class and 6 min walk test) (12). Data are much more limited concerning the potential of autoantibodies as prognostic factors in SSc-PAH. Among the few studies assessing this role, anticentromere or antitopoisomerase antibody positivity did not influence outcome (1,13). Anti-U1RNP antibodies are another important candidate as prognosis factor in SSc- and CTD-PAH. Anti-U1RNP antibodies are shared by CTDs characterized by different prevalence of PAH and prognosis. Indeed, anti-U1RNP antibodies are found in 2-14% of SSc patients, 20-40% of SLE patients and, by definition, in 100% of MCTD patients (14,15). Some studies have suggested an association between anti-U1RNP antibodies and the occurrence of pulmonary damage in SLE patients (16) and especially pulmonary hypertension (17-19). In SSc, although anti-U1RNP

antibodies are usually associated with a milder disease (15), several studies have suggested an association with PAH (20,21).

To date there are no studies focusing on the role of anti-U1RNP antibodies as prognosis factors in CTD-PAH. This study aimed to fill this gap and study the clinical and hemodynamic characteristics and survival of patients with anti-U1RNP antibodies in CTD-PAH, with a focus on SSc-PAH.

METHODS

Cohort of patients and PH diagnosis

The Royal Free Hospital (RFH) Pulmonary Hypertension (PH) database included prospectively all patients who underwent at least one right heart catheterization (RHC) between January 1st 1998 and December 31st 2012. It contains hemodynamic parameters for each RHC: right atrial pressure (RAP), pulmonary capillary wedge pressure (PCWP), mean pulmonary artery pressure (mPAP), mean aortic pressure (mAoP), cardiac index (CI), pulmonary vascular resistances (PVR), arterial oxygen saturation (SaO2) and venous oxygen saturation (SvO2).

According to the guidelines for PH diagnosis (22,23), PH was defined as a mPAP ≥ 25mmHg by RHC at rest without raised cardiac output. Post-capillary PH was defined as PH with a PCWP > 15mmHg. Patients with PH and an elevated PCWP or left ventricular end-diastolic pressure (LVEP) > 15mmHg were considered to have PH secondary to left heart disease (PH-LHD). Patients with pre-capillary PH (PCWP ≤ 15mmHg) were divided into two groups: PH-ILD (PH associated with interstitial lung disease) for patients with a forced vital capacity (FVC) less than 70% predicted and/or ILD extent above 20% on high-resolution CT-scan (HRCT) (24); and PAH (no ILD or ILD with FVC % predicted ≥ 70% and extent on HRCT ≤ 20%).

CTD diagnosis

The type of CTD was defined at the time of PH diagnosis. Patients were diagnosed with SSc if they fulfilled the American College of Rheumatology criteria (25) and/or LeRoy and Medsger criteria (26), and classified as having diffuse (dcSSc), limited cutaneous (lcSSc) or limited SSc (lSSc) form according to LeRoy and Medsger (27). SLE were diagnosed according to usual criteria (28,29). In cases of overlap between SSc and SLE, patients were entered into the SSc group. As previously described

(30), MCTD was defined in patients without full criteria for a definite CTD and fulfilling at least one of three most commonly used criteria sets of MCTD: Sharp's criteria set, Kasukawa and co-workers or Alarcón-Segovia and Villareal.

Immunological tests

Autoimmune serology was extracted from the clinical database or chart records (data were missing in 31 patients). Identification of ANA specificities (anti-topoisomerase I antibodies (ATA), anti-U1RNP, anti-SSA/Ro, anti-SSB/La and anti-Jo1 antibodies) was performed as part of routine clinical care using both specific immunofluorescence patterns on HEp-2 cells substrate (Bio-Diagnostics Ltd, Upton-upon-Severn, UK) and counter immunoelectrophoresis as previously described (31). Anti-centromere antibody (ACA) was identified by characteristic staining pattern on HEp-2 cell substrate. Anti-double stranded DNA (dsDNA) antibodies were identified by a commercially available ELISA method (Thermo Fisher Scientific, Immunodiagnostics, Uppsala, Sweden).

Other measurements

Other variables were retrospectively implemented into the PH database. Survival data were retrieved from clinical letters or United Kingdom NHS (National Health Service) database (data were censored at 1st March 2013 for analysis). Demographic data, date of disease onset (defined as age at the first non-Raynaud's symptom), pulmonary function tests (FVC % predicted value and DLCO [diffusion capacity of the lung for carbon monoxide] % predicted value), WHO functional class (FC), 6 minute walking distance (6MWD) were retrieved from letters, PH and/or SSc local databases. This study was approved by the Royal Free Hospital local ethics committee (London-Hampstead NRES Reference Number 6398).

Statistical analyses

Continuous variables were described using mean and standard deviation (SD) and compared using Kruskal-Wallis test. Categorical variables were described using number and percentage (%) and compared using Fisher exact test. Survival estimates were performed by Kaplan-Meier analyses with comparisons performed by log-rank test.

Multiple Cox proportional hazards regression models examined factors associated with survival. A first non-adjusted model was performed to study survival according to anti-U1RNP positivity. Then two adjusted models were built: (A) model adjusted on age and sex because significant differences were observed between groups; (B) model adjusted on functional parameters, lung involvement (FVC % predicted value, WHO FC) and hemodynamic parameters (RAP, PCWP, mPAP and CI). Proportional hazards hypothesis was verified for each model. Analyses were performed for the entire population of CTD-PAH (SSc-, SLE- and MCTD-PAH) and then only for SSc-PAH. For the SSc-PAH population, models were also adjusted on the cutaneous subtype (lcSSc vs. dcSSc).

Sensitivity analyses were conducted: (i) because patients with overlap between SSc and SLE were entered into the SSc group, we studied whether exclusion of these patients (n=5) modified results of the Cox regression analyses; (ii) anti-U1RNP status and the type of CTD (SSc, SLE, MCTD) were strongly associated, precluding adjustment on the later (non-convergence of Cox regression models). Consequently we assessed whether adjusting on the type of CTD (SSc, no SSc) with or without excluding MCTD modified the results.

Statistical analyses were performed using R Software version 3.1.2 (32). A p value less than 0.05 was taken as significant throughout.

RESULTS

Study population

On 2250 patients who underwent a RHC for a suspicion of pulmonary hypertension, 1013 had been diagnosed previously as having a CTD (SSc, SLE or MCTD). Pulmonary hypertension was confirmed in 626/1013 CTD patients. Among them, 342 CTD patients had pre-capillary PH of group 1 (PAH), and constituted our study population (**Figure 1A**).

As shown in **Figure 1B**, the prognosis was significantly different between CTDs. The 3- and 5-year survival rates from PAH diagnosis were 63% and 43% for SSc-, 86% and 85% for SLE-, 100% and 100% for MCTD-PAH, respectively (p<0.001).

Thirty-six out of 342 (11%) CTD-PAH patients had anti-U1RNP antibodies: 14 with SSc, 10 with SLE, 2 with an overlap SSc/SLE and 10 with MCTD (**Figure 1C**).

Anti-U1RNP antibodies in CTD-PAH

Clinical and hemodynamic characteristics

Comparisons between anti-U1RNP positive and negative CTD-PAH patients (**Table 1**) showed that anti-U1RNP positive patients were younger (45.3 ± 14.2 versus 61.9 ± 11.8 years; p<0.001) and had a lower CTD duration at PH diagnosis (9.8 ± 8.8 vs. 14.0 ± 10.3 years; p=0.040). Anti-U1RNP positive patients were less functionally impaired as shown by a larger proportion of patients in WHO FC **I-II** vs. **III-IV** (39% vs. 22%; p=0.031) and a higher 6MWD (352 ± 109 vs. 258 ± 131 meters; p=0.006). The mean DLCO was higher in the anti-U1RNP positive group (49.1 ± 9.9 vs. 42.6 ± 14.4%; p=0.004). Hemodynamic parameters were similar except for a lower mAoP (94.0 ± 19.9 vs. 101.9 ± 17.8 mmHg; p=0.025) and a higher SaO2 (95.5 ± 2.6 vs. 93.9 ± 4.0%; p=0.020) in the anti-U1RNP positive group.

Survival analysis

Kaplan-Meier analysis showed that patients with anti-U1RNP antibodies had a better survival than anti-U1RNP negative patients (**Figure 2A**). The 5-year and 10-year survival rates were 78% and 59% in the anti-U1RNP positive group versus 44% and 23% in the anti-U1RNP negative group, respectively (p=0.001).

Cox regression analyses were performed to highlight predictors of mortality in CTD-PAH (**Table 2**). In univariable analysis, anti-U1RNP positivity was associated with a better survival (hazard ratio (HR) 0.34 [95% CI: 0.18-0.65]; p<0.001). Besides anti-U1RNP positivity, sex, age at PH diagnosis, WHO FC, 6MWD, DLCO % predicted, RAP, PCWP, mPAP, CI, PVR, SaO2 and SVO2 were significantly associated with mortality. There was a trend for a negative association between FVC % predicted and mortality (p=0.054). There was no association between CTD duration at PH diagnosis or mAoP and mortality. In multivariable analysis, anti-U1RNP positivity remained negatively associated with mortality in both models: model A including anti-U1RNP positivity, age at PH diagnosis and sex (HR 0.54 [0.28-1.05]; p=0.067) and model B including anti-U1RNP positivity, age at PH diagnosis, sex, WHO FC, FVC % predicted and hemodynamic parameters (HR 0.44 [0.20-0.97]; p=0.043).

Focus on SSc-PAH

Clinical and hemodynamic characteristics

In SSc-PAH, anti-U1RNP positive patients were younger at PAH diagnosis (54.4 ± 12.8 vs. 62.7 ± 11.3 years; p=0.012), had a higher mean DLCO (48.6 ± 10.9 vs. 41.9 ± 13.9 %, p=0.031) and a higher proportion of patients in WHO FC I-II vs. III-IV (50% vs. 21%; p=0.020) than anti-U1RNP negative patients (**Table 1**). There was no difference in the proportion of dcSSc between anti-U1RNP positive and negative

patients. Hemodynamic parameters were similar, except for a trend in a lower RAP (7.6 ± 7.2 vs. 8.2 ± 4.7 mmHg; p=0.089) in anti-U1RNP positive patients.

Survival analysis

Survival analysis showed a trend for a better survival in anti-U1RNP positive patients (p=0.055; **Figure 2B**). The 5-year and 10-year survival rates were 71% and 36% in the anti-U1RNP positive group versus 41% and 20% in the anti-U1RNP negative group, respectively. Cox regression analyses were performed using a similar methodology than in CTD-PAH (**Table 3**). In univariable analysis, there was a trend towards a positive association between anti-U1RNP positivity and a better survival (HR 0.47 [0.22-1.02]; p=0.055). Besides anti-U1RNP positivity, sex, age at PH diagnosis, WHO FC, 6MWD, FVC % predicted, DLCO % predicted, RAP, PCWP, mPAP, CI, PVR, SaO2 and SVO2 were significantly associated with mortality. CTD duration at PH diagnosis and cutaneous form of SSc were not significantly associated with mortality. In multivariable analysis, anti-U1RNP positivity remained negatively associated with mortality in both models but did not reach significance: model A (HR 0.58 [0.27-1.25]; p=0.164); model B (HR 0.47 [0.20-1.11]; p=0.085).

Sensitivity analyses

Results of the Cox regression analyses in CTD-PAH yielded similar results with the same and constant trend for anti-U1RNP positivity to be associated with a better survival when we reran the models (**Figure 3**): (i) by excluding SSc/SLE overlap patients (HR for anti-U1RNP positivity in model B: 0.49 [0.22-1.08]; p=0.075); (ii) by adjusting on the type of CTD (SSc vs. non-SSc) (HR 0.52 [0.23-1.15]; p=0.107); (iii) by adjusting on the type of CTD and cutaneous form of SSc (dcSSc vs. lcSSc vs. non-SSc) (HR 0.53 [0.24-1.19]; p=0.124); (iv) by excluding the MCTD patients and

adjusting on SSc vs. SLE (HR 0.55 [0.25-1.22]; p=0.140); (v) by excluding the MCTD patients and adjusting on cutaneous form of SSc (dcSSc vs. lcSSc vs. SLE) (HR 0.56 [0.25-1.26]; p=0.160).

DISCUSSION

The main results of our study are as follows: 1) the survival was significantly different between the CTDs (SSc, SLE and MCTD) associated with PAH, in accordance with previous reports, 2) in the population of CTD-PAH, anti-U1RNP positivity was significantly associated with several clinical characteristics and a better survival in univariable and multivariable analysis, and 3) in the population of SSc-PAH, results were similar although the association between anti-U1RNP positivity and survival missed the statistical significance in univariable (p=0.055) and multivariable analysis (p=0.085).

Survival analyses showed that prognosis of SSc-PAH was poor in our population with a 3- and 5-year survival rates were of 63% and 43%, respectively. This is in keeping with the results of a recent meta-analysis of survival studies in SSc-PAH, showing a 3 year-survival of 56% (95% CI: 51-61) (1). Recent data from REVEAL (Registry to Evaluate Early and Long-term Pulmonary Arterial Hypertension Disease Management) showed a 3-year survival in CTD-PAH (SSc represented about 2/3 of the cohort) of 57% and a 5-year survival of 44% (33). Regarding SLE-PAH, we found a 3-year and 5-year survival rates of 86%. This is similar to the 3-year survival of 74% shown in a UK cohort and 88% in a Chinese cohort (8,9). In our study, survival in MCTD-PAH was 100% at 5 years. This result should be interpreted with caution, as only a small number (n=10) of patients were included. However, it confirms that prognosis in MCTD-PAH might be better than SSc-PAH. Chung et al. found a 1-year survival rate of 88% (34) and Condliffe et al. found a 3-year survival rate of 63% (8) in this population.

Among the characteristics differentiating these CTDs, the positivity of anti-U1RNP antibodies is a major element. Anti-U1RNP antibodies are shared by CTDs

characterized by different features: 2-14% of SSc patients, 20-40% of SLE patients and, by definition, in 100% of MCTD patients (14,15). Therefore we first focused on CTD-PAH and compared anti-U1RNP positive vs. negative patients. Patients with anti-U1RNP antibodies were younger and had a lower CTD duration at PH diagnosis. These differences might be due to a higher proportion of SLE or MCTD patients in the anti-U1RNP positive group. Chung et al. showed in the REVEAL cohort that SLE-PAH patients were younger than SSc-PAH (45.5 ± 11.9 vs. 61.8 ± 11.1 years; p<0.0001) (34). Condliffe et al. found similar results (42.0 ± 12.9 vs. 63.9 ± 10.5 years; p<0.001) (8). These values are comparable to what we found in anti-U1RNP positive and negative groups (45.3 ± 14.2 vs. 61.9 ± 11.8 years; p<0.001). In a cohort of 70 Japanese CTD-PAH patients, SLE or MCTD were younger than SSc patients at PH diagnosis. MCTD had the lowest time interval between CTD onset and PH diagnosis (35).

In our study, anti-U1RNP positive patients were less functionally impaired as shown by a higher proportion of patients in WHO FC I or II and a higher 6MWD and had a higher mean DLCO. Again, these differences might be due to a majority of SLE- or MCTD-PAH in the anti-U1RNP positive group. Condliffe et al. showed that SLE-PAH had higher 6MWD and mean DLCO than SSc-PAH, but there was no difference in term of WHO FC (8). Chung et al. found a higher mean DLCO in SLE-PAH than in SSc-PAH. There was no significant difference for 6MWD and WHO FC (34).

Hemodynamic parameters were similar except for a lower mAoP and a higher SaO2 in the anti-U1RNP positive group. In the studies comparing hemodynamic values between SSc- and SLE-PAH, no differences were found for RAP, mPAP, CI, PVR, SvO2 (8,34,35). Only PCWP was significantly different between SSc- and SLE-PAH in the REVEAL cohort (34). Interestingly, despite a similar hemodynamic severity,

anti-U1RNP positive patients had a better survival than those who were negative. Moreover, multivariable analyses showed that anti-U1RNP positivity were associated with survival, independently of age, sex, functional impairment and hemodynamic severity in CTD-PAH. These results highlight a possible serological homogeneity carried by anti-U1RNP antibodies between the different CTD-PAH, with an impact on disease characteristics and survival.

We then assessed whether these findings were similar inside a selected CTD. Among our SSc-PAH population, we found comparable characteristics (younger patients in anti-U1RNP positive group, less functionally impaired with a similar hemodynamic severity). Anti-U1RNP positivity remained associated with a better survival (HR were similar than in CTD-PAH group but did not reach significance). Overall these results are consistent with a unique phenotype and a different prognosis of anti-U1RNP positive patients in CTD- and SSc-PAH.

Anti-U1RNP antibodies bind to U1 small nuclear ribonucleoprotein autoantigen (U1snRNP), a complex that is involved in splicing heterogeneous nuclear RNA into mRNA (15). In SSc, anti-U1RNP antibodies are usually associated with overlap syndromes and are more frequent among lcSSc patients compared to those with dcSSc (36-38). Patients with anti-U1RNP antibodies tend to be younger at SSc diagnosis with a less severe skin involvement and uncommon renal involvement. Puffy hands, Raynaud's phenomenon, arthritis and myositis are commonly seen (15,38,39). Nevertheless, although anti-U1RNP antibodies have been classically associated with a milder disease (15), several studies have suggested an association with PAH in SSc (20,21,40,41). In SLE, anti-U1RNP antibodies, Raynaud's phenomenon and antiphospholipid antibodies have been associated with PAH (18,19,42,43). In a cluster analysis of MCTD patients, Szodoray et al. have

shown a cluster strongly associated with PH. This cluster presented a higher frequency of swollen hands, Raynaud's phenomenon, livedo reticularis and secondary anti-phospholipid syndrome (44).

The exact mechanisms of PAH in CTD remain elusive. Chow et al. have suggested that anti-U1RNP antibodies in SLE could confer a vasculopathy similar to SSc and that antiphospholipid antibodies could lead to a thromboembolic process (17). Histologic studies of pulmonary arteries in MCTD-PAH patients showed intimal hyperplasia, hypertrophic media, plexiform lesion and locally formed microthrombi. These features are similar to those found in SSc- or SLE-PAH. Vegh et al. found a higher frequence of anti-endothelial cell antibodies and higher serum thrombomodulin and von Willebrand factor antigen concentrations suggesting endothelial cell activation. Interestingly, anti-U1RNP antibodies levels were higher in MCTD patients with PAH than in those without PAH (45). Thus anti-U1RNP antibodies might be a hallmark of a distinct phenotype in CTD-PAH.

Previous studies have suggested that immunosuppressive therapy in SLE- or MCTD-PAH could improve survival in responding patients (10,11). Therefore patients with SLE- or MCTD-PAH might have received more frequently an immunosuppressive therapy than SSc-PAH in which this treatment has not proved efficacy (10). However, one of the results highlighted here is that SSc-PAH patients with anti-U1RNP are different than SSc-PAH patients without anti-U1RNP. Whether or not immunosuppressive treatment could be efficient in SSc-PAH with anti-U1RNP antibodies deserves further studies.

To the best of our knowledge, this study is the first to compare hemodynamic data and survival in a subgroup of CTD-PAH patients characterized by a serological homogeneity. However this work has some limitations. First, this was a single-center

study analysing a selected population of patients referred to a PAH referral centre. Nevertheless, we included all consecutive patients with SSc-, SLE- or MCTD-PAH referred to our centre during a 14-year period. This design allows a valuable analysis of the patients' characteristics and outcomes. Moreover, hemodynamic parameters were entered into the database at the time of the RHC resulting in a very limited number of missing data and robustness of hemodynamic variables. Second, due to the retrospective design of clinical variables implementation, we were unable to collect precise data on specific treatment for PAH (especially immunosuppressive therapies) or data on causes of death. MCTD patients can present clinical symptoms suggestive of SSc (i.e. swollen fingers, digital ulcers, oesophageal dysmotility etc.). Studying whether the existence of SSc manifestations in MCTD patients could have a role in the prognosis evaluation would have been of interest. Unfortunately, specific detailed organ involvement of CTD patients was not gathered. Finally, although this is one of the largest cohorts of CTD-PAH patients, we lacked the statistical power to confirm the association in SSc-PAH patients because of the small number of patients with anti-U1RNP antibodies positive.

In conclusion, our study confirms that survival is significantly different between SSc-, SLE- and MCTD-PAH. Anti-U1RNP antibodies positivity is associated with distinct clinical characteristics and survival in CTD- and SSc-PAH. Although hemodynamic parameters were similar between anti-U1RNP positive and negative patients, anti-U1RNP positivity was negatively and independently associated with mortality in CTD-PAH. In SSc-PAH, survival analyses suggested a negative association between anti-U1RNP antibodies and survival. These results highlight the clinical need for a better characterization of CTD-PAH phenotypes, especially in therapeutic studies.

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TABLES

Table 1: Comparison of clinical and hemodynamic characteristics between anti-U1RNP positive and negative patients.

Table 2: Predictors of mortality in CTD-PAH patients.

Table 3: Predictors of mortality in SSc-PAH patients.

Table 1: Comparison of clinical and hemodynamic characteristics between anti-U1RNP positive and negative patients.

	CTD-PAH				SSc-PAH			
	N	**Anti-U1RNP positive (n=36)**	**Anti-U1RNP negative (n=306)**	**p**	**N**	**Anti-U1RNP positive (n=16)**	**Anti-U1RNP negative (n=292)**	**p**
Age at PH diagnosis, *years*	342	45.3 ± 14.2	61.9 ± 11.8	**< 0.001**	308	54.4 ± 12.8	62.7 ± 11.3	**0.012**
Sex, *female*	342	31 (86)	261 (85)	> 0.999	308	14 (88)	248 (85)	> 0.999
Cutaneous form of SSc, *diffuse*					292	2 (13)	37 (13)	> 0.999
CTD duration at PH diagnosis, *years*	179	9.8 ± 8.8	14.0 ± 10.3	**0.040**	162	11.9 ± 10.6	14.2 ± 10.3	0.386
Follow-up, *years*	338	5.6 ± 4.1	3.7 ± 2.9	**0.010**	305	5.7 ± 4.2	3.6 ± 2.8	**0.032**
FVC, *% predicted*	277	87.8 ± 12.6	93.4 ± 17.8	0.166	255	92.7 ± 13.2	93.4 ± 17.6	0.871
DLCO, *% predicted*	272	49.1 ± 9.9	42.6 ± 14.4	**0.004**	250	48.6 ± 10.9	41.9 ± 13.9	**0.031**
WHO FC I-II	318	13 (39)	62 (22)	**0.031**	286	7 (50)	58 (21)	**0.020**
vs. III-IV		20 (61)	223 (78)			7 (50)	214 (79)	
6MWD, *meters*	92	352 ± 109	258 ± 131	**0.006**	67	321 ± 174	248 ± 130	0.428
RAP, *mmHg*	338	7.9 ± 6.0	8.1 ± 4.7	0.273	305	7.6 ± 7.2	8.2 ± 4.7	0.088
PCWP, *mmHg*	333	10.0 ± 2.4	10.3 ± 3.3	0.644	302	9.4 ± 2.1	10.2 ± 3.2	0.250

mPAP, *mmHg*	342	39.9 ± 12.2	40.3 ± 12.7	0.889	308	36.8 ± 14.2	39.9 ± 12.4	0.116
mAoP, *mmHg*	301	94.0 ± 19.9	101.9 ± 17.8	**0.025**	270	100 ± 19	102 ± 18	0.606
CI, $L.min^{-1}.m^{-2}$	308	2.6 ± 0.8	2.7 ± 0.7	0.484	277	2.7 ± 1.0	2.7 ± 0.7	0.767
PVR, $dynes.s.cm^{-5}$	323	644 ± 471	615 ± 432	0.584	292	646 ± 640	607 ± 417	0.579
SaO2, *%*	303	95.5 ± 2.6	93.9 ± 4.0	**0.020**	274	95.2 ± 3.4	93.8 ± 4.0	0.134
SvO2, *%*	314	67.7 ± 8.1	65.9 ± 9.9	0.506	283	67.9 ± 8.0	65.8 ± 9.9	0.693

Definition of abbreviations: PH: pulmonary hypertension; CTD: connective tissue disease; FVC: forced vital capacity; DLCO: diffusion capacity of the lung for carbon monoxide; WHO FC: world health organization functional class; 6MWD: 6-minute walking distance; RAP: right atrial pressure; PCWP: pulmonary capillary wedge pressure; mPAP: mean pulmonary arterial pressure; mAoP: mean aortic pressure; CI: cardiac index; PVR: pulmonary vascular resistances; SaO_2: arterial oxygen saturation; SvO_2: mixed venous oxygen saturation

Continuous variables were summarized by the mean ± standard deviation and categorical variables by frequency (percentage).

Table 2: Predictors of mortality in CTD-PAH patients.

	Univariable	Multivariable	
		Model A	Model B
Variable	HR (95% CI)	HR (95% CI)	HR (95% CI)
Sex, *male vs. female*	1.49 (1.03-2.17)*	1.96 (1.33-2.88)***	2.07 (1.31-3.26)**
Age at PH diagnosis, *per year*	1.04 (1.03-1.06)***	1.04 (1.03-1.06)***	1.05 (1.03-1.07)***
CTD duration at PH diagnosis, *per year*	1.00 (0.98-1.02)		
Anti-U1RNP, *positive vs. negative*	0.34 (0.18-0.65)***	0.54 (0.28-1.05)§	0.44 (0.20-0.97)*
FVC, *per %*	0.99 (0.98-1.00)§		0.99 (0.98-1.01)
DLCO, *per %*	0.96 (0.95-0.97)***		
WHO FC, *III-IV vs. I-II*	2.51 (1.65-3.80)***		1.44 (0.86-2.42)
6MWD, *per 100 m*	0.56 (0.41-0.78)***		
RAP, *per mmHg*	1.06 (1.03-1.09)***		1.10 (1.04-1.16)**
PCWP, *per mmHg*	0.91 (0.86-0.95)***		0.85 (0.79-0.92)***
mPAP, *per mmHg*	1.03 (1.02-1.04)***		1.00 (0.98-1.02)
mAoP, *per mmHg*	1.00 (0.99-1.01)		
CI, *per* $L.min^{-1}.m^{-2}$	0.53 (0.42-0.68)***		0.91 (0.64-1.30)
PVR, *per 100* $dynes.s.cm^{-5}$	1.12 (1.09-1.15)***		
SaO2, *per %*	0.91 (0.88-0.94)***		
SvO2, *per %*	0.94 (0.93-0.96)***		0.98 (0.95-1.00)§

§p<0.10, *p<0.05, **p<0.01, ***p<0.001

Table 3: Predictors of mortality in SSc-PAH patients.

	Univariable	Multivariable	
		Model A	Model B
Variable	HR (95% CI)	HR (95% CI)	HR (95% CI)
Sex, *male vs. female*	1.51 (1.03-2.21)*	1.95 (1.31-2.89)***	1.94 (1.23-3.08)**
Age at PH diagnosis, *per year*	1.03 (1.02-1.05)***	1.04 (1.02-1.05)***	1.04 (1.02-1.06)***
Cutaneous form of SSc, *l . diffuse*	0.72 (0.47-1.12)		
CTD duration at PH diagnosis, *per year*	0.99 (0.97-1.01)		
Anti-U1RNP, *positive vs. negative*	0.47 (0.22-1.02)§	0.58 (0.27-1.25)	0.47 (0.20-1.11)§
FVC, *per %*	0.99 (0.98-1.00)*		0.99 (0.98-1.01)
DLCO, *per %*	0.97 (0.95-0.98)***		
WHO FC, *III-IV vs. I-II*	2.50 (1.64-3.79)***		1.41 (0.84-2.37)
6MWD, *per 100 m*	0.65 (0.46-0.93)*		
RAP, *per mmHg*	1.07 (1.04-1.10)***		1.08 (1.02-1.14)**
PCWP, *per mmHg*	0.91 (0.86-0.96)***		0.86 (0.80-0.93)***
mPAP, *per mmHg*	1.04 (1.02-1.05)***		1.01 (0.99-1.03)
mAoP, *per mmHg*	1.00 (0.99-1.00)		
CI, *per* $L.min^{-1}.m^{-2}$	0.48 (0.37-0.61)***		0.89 (0.62-1.27)
PVR, *per 100* $dynes.s.cm^{-5}$	1.13 (1.10-1.17)***		
SaO2, *per %*	0.92 (0.89-0.95)***		
SvO2, *per %*	0.94 (0.93-0.96)***		0.97 (0.95-1.00)*

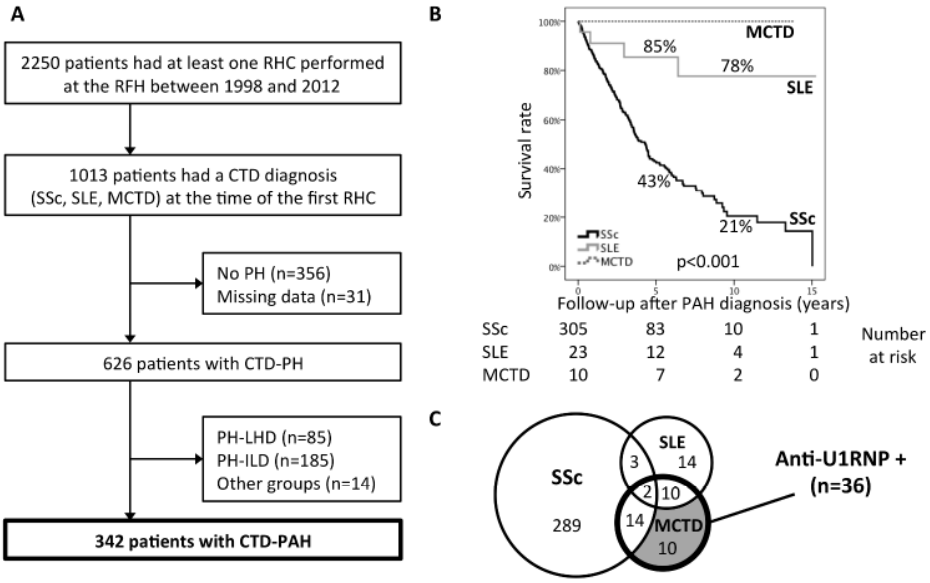
§p<0.10, *p<0.05, **p<0.01, ***p<0.001

FIGURES LEGENDS

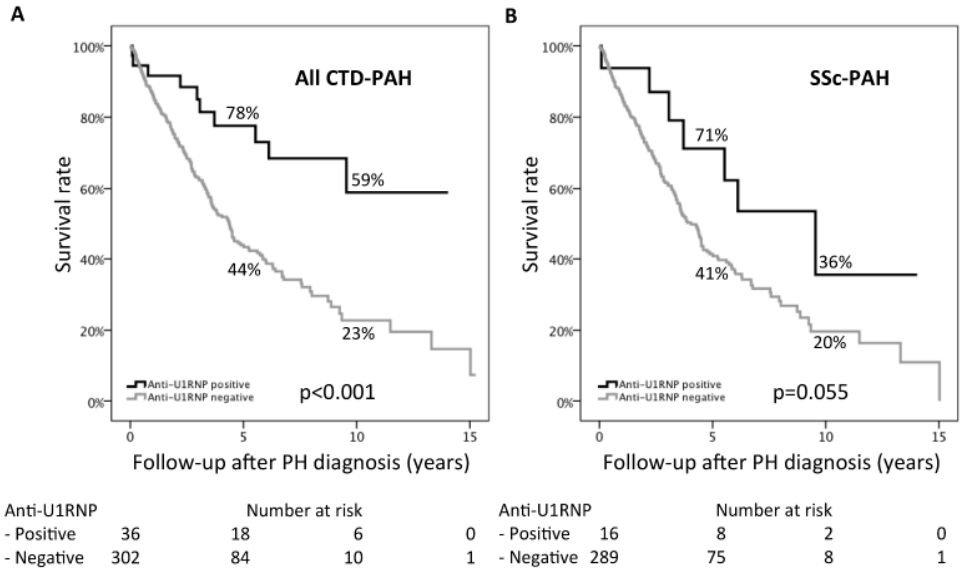
Figure 1: A. Patients included in this study. **B.** Kaplan-Meier curves of survival after PAH diagnosis. **C.** Venn diagram representing distribution of CTDs and anti-U1RNP positivity among the PAH population.

Figure 2: Kaplan-Meier curves of survival after PAH diagnosis. **A:** In all CTD-PAH patients. **B:** In SSc-PAH patients.

Figure 3: Results of sensitivity analyses: hazard ratios of survival for anti-U1RNP positivity.



A. Patients included in this study. B. Kaplan-Meier curves of survival after PAH diagnosis. C. Venn diagram representing distribution of CTDs and anti-U1RNP positivity among the PAH population.
253x154mm (300 x 300 DPI)



Anti-U1RNP	Number at risk			
- Positive	36	18	6	0
- Negative	302	84	10	1

Anti-U1RNP	Number at risk			
- Positive	16	8	2	0
- Negative	289	75	8	1

Kaplan-Meier curves of survival after PAH diagnosis. A: In all CTD-PAH patients. B: In SSc-PAH patients.
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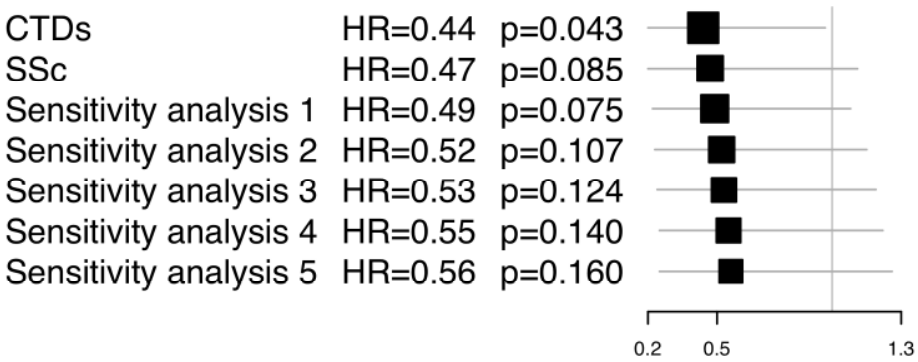

Results of sensitivity analyses: hazard ratios of survival for anti-U1RNP positivity.
173x71mm (300 x 300 DPI)