Chapter 6

Severely reduced diffusion capacity in idiopathic pulmonary arterial hypertension – patient characteristics and treatment responses

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ABSTRACT

Background

A subgroup of patients with idiopathic pulmonary arterial hypertension (IPAH) has severely reduced diffusion capacity for carbon monoxide (DLCO) and poor prognosis. Their characteristics are currently unknown. The aim of this study is to contrast clinical characteristics and treatment responses of IPAH-patients with a severely reduced and more preserved DLCO.

Methods

Retrospectively, 166 IPAH-patients were included and grouped based on a DLCO cut-off value of 45% of predicted ($IPAH_{<45\%}$ and $IPAH_{\geq 45\%}$). Clinical characteristics, treatment responses and survival were compared.

Results

$IPAH_{<45\%}$ were older, more often male, had a more frequent history of coronary disease and a higher tobacco exposure. FEV_1, FEV_1/FVC, TLC and alveolar volume-values were slightly lower and CT scan abnormalities more prevalent in patients with a low DLCO. Age and number of pack years were independently associated with DLCO<45%. $IPAH_{<45\%}$ showed no different hemodynamic profile, yet worse exercise performance and a worse survival, which were both related to age, gender and the presence of coronary disease.

Conclusion

A severely reduced DLCO in IPAH is associated with advanced age and a greater tobacco exposure. These patients have a worse exercise performance despite a similar hemodynamic profile. We confirm the decreased survival in this patient group and now show that this poor outcome is related to age, gender and the presence of coronary disease.

INTRODUCTION

In about 75 percent of patients with idiopathic pulmonary arterial hypertension (IPAH), the diffusion capacity for carbon monoxide (DLCO) is reduced[1]. The reduction in DLCO is moderate in the majority of patients and the presence of a severely reduced DLCO during the diagnostic work-up should raise suspicion of secondary causes of pulmonary hypertension, such as connective tissue disease[2,3], pulmonary veno-occlusive disease[4], left heart failure[5], and parenchymal lung disease[6,7]. However, in a subgroup of patients no secondary causes explaining the low DLCO are found and patients are then classified as IPAH.

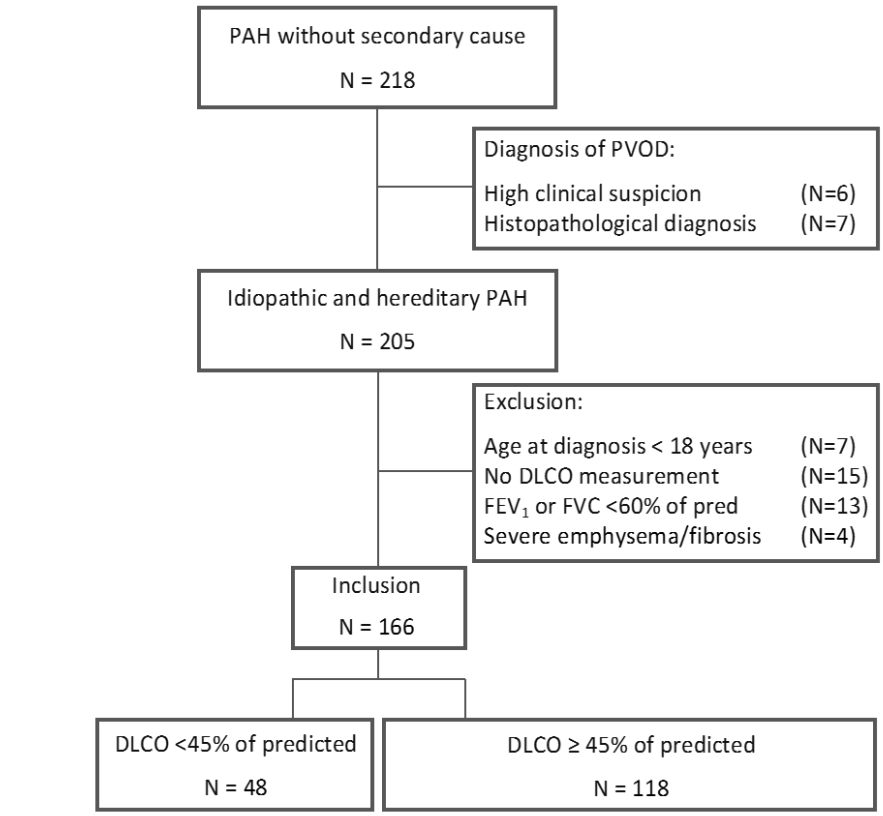
IPAH-patients with a severely reduced DLCO have a significantly worse survival[8,9]. Nevertheless, the clinical characteristics of this subgroup of IPAH-patients were never described and the factors associated with a severely reduced DLCO remain unknown. A recent cohort study in IPAH-patients provides some insight into the factors which may play a role in the reduction of DLCO. Ling et al.[10] compared two different IPAH age groups and showed that older IPAH-patients show a different IPAH phenotype with lower DLCO-values, a more frequent history of smoking, ischemic heart disease, hypertension and diabetes and a worse survival compared to younger patients. This raises the question whether age, smoking-related lung disease, or cardiovascular co-morbidities are factors playing a role in reducing DLCO.

The proportion of IPAH-patients with advanced age, lower DLCO and worse prognosis is increasing[10,11] and a more detailed study of this subgroup is needed. In this study we aimed to determine the factors which contribute to a severely reduced DLCO and contrasted the clinical characteristics of IPAH-patients with a severely reduced DLCO to those of IPAH-patients with a more preserved DLCO. In addition, we compared treatment responses and survival between the two groups.

METHODS

Study design and patient selection

We retrospectively studied IPAH- and heritable PAH (HPAH)-patients consecutively seen at the VU University Medical Center between January 1990 and November 2011. Patients were included when after clinical evaluation the multidisciplinary team had agreed upon a diagnosis of IPAH or HPAH. Clinical evaluation included echocardiography, high-resolution computed tomography (HRCT) of the


PAH without secondary cause
N = 218
Diagnosis of PVOD:
High clinical suspicion (N=6)
Histopathological diagnosis (N=7)
Idiopathic and hereditary PAH
N = 205
Exclusion:
Age at diagnosis < 18 years (N=7)
No DLCO measurement (N=15)
FEV1 or FVC <60% of pred (N=13)
Severe emphysema/fibrosis (N=4)
Inclusion
N = 166
DLCO <45% of predicted
N = 48
DLCO ≥ 45% of predicted
N = 118

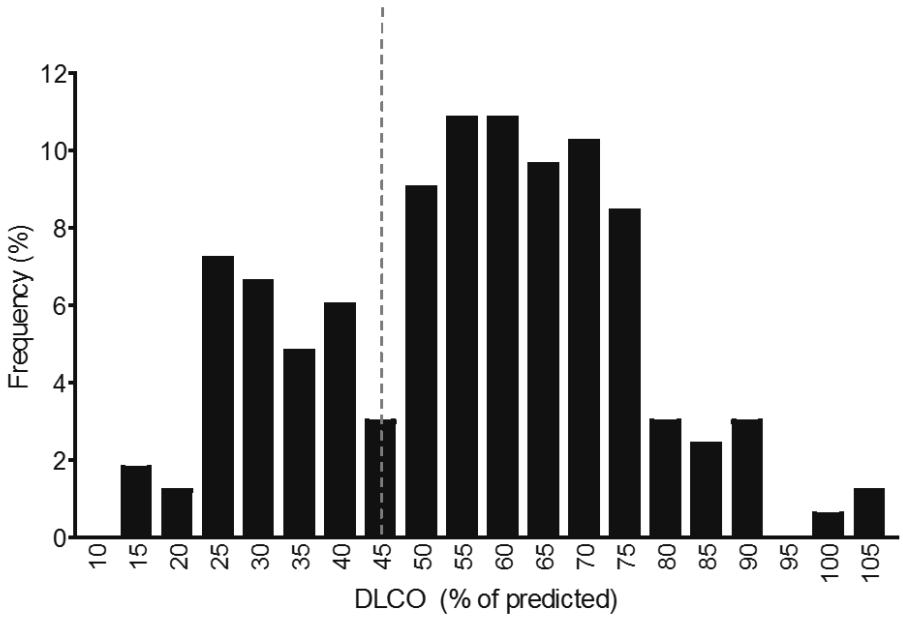

Frequency (%)
0
2
4
6
8
10
12
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
DLCO (% of predicted)

Figure 6.1. (left page) **Flowchart of the inclusion of idiopathic and hereditary PAH-patients and their DLCO frequency distribution.** A total of 218 patients with pulmonary arterial hypertension without a secondary cause were seen in our hospital. Patients diagnosed with pulmonary veno-occlusive disease (PVOD) based on either a high clinical suspicion or a pathological diagnosis of PVOD after lung biopsy or at autopsy were excluded. A high clinical suspicion of PVOD was present if a severely reduced DLCO was accompanied by two or three of the following abnormalities on HRCT: centrilobular ground-glass opacities, septal lines and mediastinal lymph node enlargement[28,29]. Thirteen patients were diagnosed with PVOD based on a high clinical suspicion (N=6) and a histopathological diagnosis (N=7). A total of 205 idiopathic or hereditary PAH-patients were enrolled in this study. Seven patients were under the age of 18 at time of diagnosis and were excluded. Idiopathic and hereditary PAH-patients with a forced expiratory lung volume after one second (FEV_1) or forced expiratory capacity (FVC) of less than 60 percent of predicted were excluded because we aimed to obtain a homogenous patient population and these patients do not meet the inclusion criteria of most randomized clinical trials on PAH targeted therapy[30]. Three IPAH patients were excluded due to the presence of severe emphysema on CT scan re-evaluation and one HPAH patient was excluded due to the presence of severe fibrosis. In 15 patients DLCO measurements were not available. The total study cohort consisted of 166 patients and were divided into a severely reduced DLCO-group ($IPAH_{<45\%}$) and a more preserved DLCO-group ($IPAH_{\geq 45\%}$) based on the frequency distribution of DLCO-values using a cut-off of 45 percent of predicted.

chest, pulmonary function testing and right heart catheterization. For the purpose of this study, a re-evaluation of the chest CT scans was performed by a radiologist blinded to the initial diagnosis. The presence of emphysema and/or fibrosis was quantified using a three-point scale. Mild emphysema was defined by subtle centrilobular emphysema in apical segments of upper lobes; moderate emphysema was defined by a cluster of centrilobular and paraseptal emphysema with a preference for upper lobes; severe emphysema was defined by generalized centrilobular and paraseptal emphysema in both upper and lower lobes. Mild fibrosis was defined by focal areas with fine reticular subpleural opacities; moderate fibrosis was defined by a continuous subpleural band of fine reticular opacities restricted to either the upper or lower lobes; severe fibrosis was defined by subpleural reticular opacities stretching along both the upper and lower part of both lungs.

IPAH and HPAH were diagnosed in patients with a mean pulmonary artery pressure (mPAP) ≥ 25 mmHg and pulmonary capillary wedge pressure (PCWP) ≤ 15 mmHg, not explained by an underlying parenchymal lung disease or chronic obstructive pulmonary disease (COPD)[12].

Not included in this study were patients diagnosed with PVOD, see inclusion flowchart (**FIGURE 6.1**) for more details. IPAH- or HPAH-patients younger than 18 years at the time of diagnosis were excluded, as were patients with a forced expiratory lung volume after one second (FEV_1) or forced expiratory capacity (FVC) of less than 60 percent of predicted (**FIGURE 6.1**). In addition, IPAH- and HPAH patients with severe emphysema and/or severe fibrosis on re-evaluation of the chest CT were excluded. The total study cohort consisted of 166 IPAH- and HPAH-patients (which will subsequently

be referred to as IPAH, unless otherwise stated) and was divided into a group with a severely reduced DLCO ($IPAH_{<45\%}$) and a group with a more preserved DLCO ($IPAH_{\geq 45\%}$). The cut-off of value of 45 percent of predicted was based on the frequency distribution of DLCO-values.

Clinical characteristics

Data on demographics, smoking history, medical history, medication use and World Health Organization-functional class (WHO-FC) were taken at the time of diagnosis. Coronary artery disease was indicated as present if the patient's history mentioned myocardial infarction, percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass surgery (CABG).

Routine laboratory test results taken within half a year from diagnosis were analyzed. Auto-immune serology and pulmonary function test results were collected at the time of diagnosis or when not present at baseline the first result during follow-up was taken (median follow-up duration 1 day, interquartile range -5 – 167 days). Spirometry, bodyplethysmography, and single-breath DLCO were measured in accordance with the European Respiratory Society guidelines[13,14]. To determine whether a change in DLCO could be observed during follow-up, subsequent pulmonary function tests were collected and compared with baseline values. Six-minute walking test results were collected within 6 months from diagnosis.

Six-minute walking test (6MWT) results included six-minute walking distance (6MWD), 6MWD as percentage of predicted, arterial oxygen saturation (SaO_2) at rest and decrease in arterial oxygen saturation during exercise.

Results from right heart catheterization were taken at baseline. Total pulmonary vascular resistance (TPVR) was calculated as mPAP divided by cardiac output. Arterial blood gas measures included pH, arterial carbon dioxide tension (pCO_2), arterial oxygen tension (pO_2), and arterial oxygen saturation (SaO_2).

Final diagnosis was either IPAH or HPAH. Heritable PAH included clinical familial cases with or without identified germline *BMPR2* gene mutations as well as clinically sporadic IPAH-patients with an identified germline *BMPR2* gene mutation[12].

Treatment response and survival

First-line treatment was given according to contemporary guidelines and consisted of either an endothelin receptor antagonist (ERA), a prostanoid, a phosphodiesterase type-5 inhibitor (PDE-5 inhibitor), a calcium channel blocker, or combination treatment. To assess differences in treatment

responses, time to clinical worsening (defined as time to add-on PAH-specific therapy, atrial balloon septostomy, lung transplantation or death) was compared between the groups.

Follow-up was until May 1 2012. Instead of overall survival we used event-free survival with lung transplantation as additional end-point. We did this because IPAH patients who receive a lung transplant have end-stage disease and an expected survival of less than four months. Therefore, by using event-free survival we minimized the bias introduced by not taking into account lung transplantation. Event-free survival was calculated from the time of diagnosis with all-cause mortality or lung transplantation as end point.

Statistical analysis

Categorical and continuous variables were compared by binary logistic regression analysis and linear regression analysis, respectively, while correcting for age. Continuous variables that are presented as percentage of predicted, and thus corrected for age, were compared by unpaired T-tests or Mann-Whitney U-tests. First-line treatment was compared by a chi-squared test. A p-value of <0.05 was considered significant.

A backward stepwise multivariate logistic regression analysis was performed to determine the characteristics which were independently associated with a severely reduced DLCO. Variables included into the analysis were age, gender, body mass index, smoking, pack years, coronary disease, FEV_1, FEV_1/FVC, total lung capacity (TLC), and alveolar volume (VA). Survival was analyzed with the Kaplan-Meier method and compared by the log-rank test. To correct the association between event-free survival and a severely reduced DLCO for age, gender, time between the DLCO measurement and diagnosis and the presence of coronary disease a cox proportional hazards regression analysis was used. The association between TTCW and a severely reduced DLCO while correcting for age was analyzed by a cox proportional hazards regression analysis.

RESULTS

Forty-eight patients had a DLCO below 45% of predicted ($IPAH_{<45\%}$) and 118 patients had a DLCO of at least 45% of predicted ($IPAH_{\geq 45\%}$). **TABLE 6.1** shows the clinical characteristics of the two DLCO-groups. $IPAH_{<45\%}$ were older, more often male and had a more frequent smoking history. The number of pack years could be acquired in 33 out of 48 $IPAH_{<45\%}$ patients and 93 out of 118 $IPAH_{\geq 45\%}$ patients. A higher

	DLCO <45% of predicted (N=48)	DLCO ≥45% of predicted (N=118)	p-value
Demographics			
Age at diagnosis (yrs)	67 (53-75)	46 (35-60)	<0.001
Gender (male)	24 (50%)	22 (19%)	0.013
BMI (kg/m^2)	26 ± 4	27 ± 6	0.035
Smoking	33 (77%)	54 (48%)	0.033
- Current smoker	8 (19%)	20 (18%)	0.765
- Former smoker	25 (58%)	34 (30%)	0.065
- Pack years	25 (0-40)	0 (0-13)	0.009
Medical history			
Coronary disease	13 (27%)	1 (1%)	0.008
Hypertension	14 (29%)	26 (22%)	0.207
Diabetes mellitus	12 (25%)	11 (9%)	0.513
Thyroid disease	4 (8%)	12 (10%)	0.701
Pulmonary disease			
- COPD (GOLD I-II)	5 (10%)	6 (5%)	0.845
- OSAS	0 (0%)	2 (2%)	0.997
- Asthma	0 (0%)	9 (8%)	0.997
Malignancy	6 (13%)	10 (9%)	0.678
Medication use			
Diuretics	20 (43%)	38 (33%)	0.730
Anticoagulants	31 (66%)	45 (39%)	0.215
Antihypertensive therapy	31 (66%)	42 (36%)	0.304
Statins	16 (34%)	10 (9%)	0.075
Nitrates	9 (19%)	4 (3%)	0.085
Bronchodilator therapy	12 (26%)	12 (10%)	0.110
Corticosteroids	10 (21%)	20 (17%)	0.567
Oxygen	5 (11%)	5 (4%)	0.517
Severity indices			
WHO functional class			
- I	0 (0%)	6 (6%)	
- II	9 (21%)	32 (29%)	0.050*
- III	27 (61%)	61 (56%)	
- IV	8 (18%)	10 (9%)	
Pulmonary function			
FEV_1 (% of predicted)	85 ± 16	91 ± 16	0.010†
FVC (% of predicted)	99 ± 16	102 ± 17	0.206†
FEV_1/FVC (%)	68 ± 9	74 ± 9	<0.001†
TLC (% of predicted)	92 ± 16	99 ± 12	0.004†
VA (% of predicted)	78 ± 14	85 ± 11	0.001†
Laboratory tests			
Hb (mmol/L)	9.5 ± 1.1	9.1 ± 1.1	0.236
Creatinine (umol/L)	99 ± 29	95 ± 22	0.757
Ureum (mmol/L)	6.9 (4.9-8.4)	5.9 (4.7-7.9)	0.127
NT-proBNP (ng/L)	999 (204-2266)	732 (214-2934)	0.765

	DLCO <45% of predicted (N=48)	DLCO ≥45% of predicted (N=118)	p-value
Arterial blood gas			
pH	7.45 ± 0.03	7.45 ± 0.04	0.368
pCO_2 (mm Hg)	31 ± 6	32 ± 6	0.015
pO_2 (mm Hg)	62 ± 16	72 ± 13	0.153
SaO_2 (%)	91 ± 5	94 ± 3	0.024
Final diagnosis			<0.001†
Non-heritable IPAH	48 (100%)	91 (77%)	
Heritable PAH	0 (0%)	27 (23%)	

TABLE 6.1. Clinical characteristics and final diagnosis according to DLCO

Values are mean ± SD, n (%), or median (25th to 75th percentile). P-values are from binary logistic or linear regression analysis corrected for age. *P-value based on WHO-FC I-II vs. III-IV. †Uncorrected p-value. DLCO, diffusion capacity for carbon monoxide; BMI, body mass index; COPD, chronic obstructive pulmonary disease; GOLD, Global initiative for Chronic Obstructive Lung Disease class; OSAS, obstructive sleep apnea syndrome; WHO, world health organization; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; TLC, total lung capacity; VA, alveolar volume; Hb, haemoglobin; NT-proBNP, N-terminal brain natriuretic peptide; pCO2, arterial carbon dioxide tension; pO2, arterial oxygen tension; SaO2, arterial oxygen saturation; IPAH, idiopathic pulmonary arterial hypertension.

number of pack years was observed in $IPAH_{<45\%}$. In $IPAH_{<45\%}$ an increased prevalence of coronary heart disease was found. No differences were observed in the prevalence of hypertension, thyroid disease, malignancy or pulmonary diseases such as mild COPD, asthma and mild OSAS.

The comparison of medication use revealed no difference in the use of anticoagulants, antihypertensive medications, statins, nitrates and bronchodilators in $IPAH_{<45\%}$ when compared to $IPAH_{\geq45\%}$. WHO-functional class at the time of diagnosis was similar in both groups. Pulmonary function testing revealed lower FEV_1, FEV_1/FVC-ratio, TLC and VA-values in $IPAH_{<45\%}$. CT-scans were available for re-analysis in 140 patients. Results are shown in **TABLE 6.4**. Abnormalities were more often seen in $IPAH_{<45\%}$ (68% vs. 33% in $IPAH_{\geq45\%}$, p 0.004). The majority of $IPAH_{<45\%}$ patients with abnormalities on CT (57%) showed mild or moderate emphysema without any signs of fibrosis. No $IPAH_{<45\%}$ patients presented with moderate emphysema in combination with moderate fibrosis. Blood test results similar hemoglobin levels in both IPAH groups, but lower arterial pCO_2 and SaO_2 in $IPAH_{<45\%}$. Arterial pCO_2 was low compared to reference values in both IPAH groups and was not associated with DLCO (r 0.17). Results from autoimmune serology testing were available in 34 $IPAH_{<45\%}$ (71%) and 81 $IPAH_{\geq45\%}$ patients (69%). The results are shown in **TABLE 6.2**. The presence of ANA antibodies tended to be increased in $IPAH_{<45\%}$. Although genetic tests were performed in 68 of the

IPAH-patients, it is of interest that a final diagnosis of heritable IPAH was only made in patients with a preserved DLCO (TABLE 6.1).

TABLE 6.3 shows the clinical characteristics associated with a severely reduced DLCO on multivariate regression analysis. Age and the number of pack years were independently associated with a severely reduced DLCO.

TABLE 6.2. Autoimmune serology according to DLCO

	DLCO <45% of predicted N=34	DLCO ≥45% of predicted N=81	p-value
Autoimmune serology			
ANA (+, %)	13 (38%)	16 (20%)	0.063
Anti-ds DNA (+, %)	0 (0%)	1 (2%)	0.998
Anti-ENA (+, %)	2 (7%)	2 (3%)	0.188
ANCA (+, %)	0 (0%)	6 (10%)	0.998

Values are n (% of total tested). P-values are from binary logistic regression analysis corrected for age. DLCO, diffusion capacity for carbon monoxide; ANA, anti-nuclear antibody; Anti-ds DNA, anti-double stranded DNA; Anti-ENA, anti-extractable nuclear antigen; and ANCA, antineutrophil cytoplasmic antibody.

TABLE 6.3. Clinical characteristics associated with a severely reduced DLCO on multivariate regression analysis

Clinical characteristic	Odds ratio	95% CI	p-value
Age	1.73	1.27-2.37	0.001
Pack years	1.35	1.07-1.71	0.016

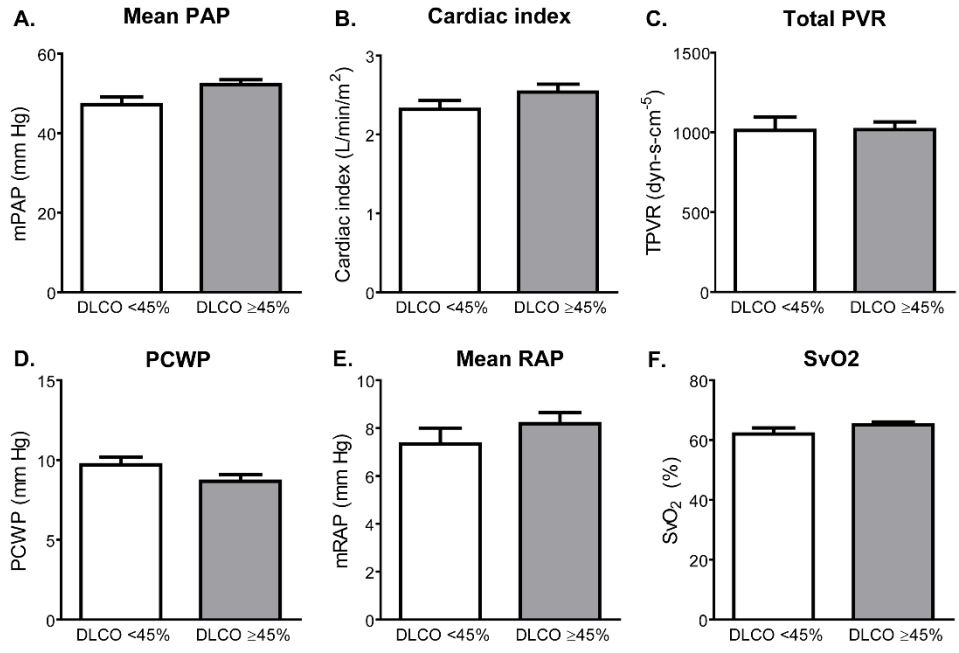
Odds ratio and 95% confidence interval (CI) are presented per 10 units.

Exercise performance & hemodynamics

FIGURE 6.2 shows baseline hemodynamic results. $IPAH_{<45\%}$ had similar mean pulmonary artery pressures when compared to $IPAH_{\geq 45\%}$. Also mean right atrial pressure (mRAP), cardiac index (CI), total pulmonary vascular resistance (TPVR), PCWP and mixed venous oxygen saturation (SvO_2) were not different between the two groups.

In 77% and 72% of $IPAH_{<45\%}$ and $IPAH_{\geq 45\%}$ patients baseline 6-minute walking test results were available. The results are shown in FIGURE 6.3. $IPAH_{<45\%}$ had a lower 6-minute walking distance compared to $IPAH_{\geq 45\%}$. Moreover, $IPAH_{<45\%}$ had a greater decrease in SaO_2 during the test.

FIGURE 6.2. Hemodynamic parameters at the time of diagnosis of pulmonary arterial hypertension according to diffusion capacity. Idiopathic PAH-patients with a severely reduced DLCO had similar mean PA pressures when corrected for age (A). No differences were observed in cardiac index (B), TPVR (C), PCWP (D), mRAP (E) or SvO2 (F). Data are presented as mean ± SEM. mPAP; mean pulmonary artery pressure, TPVR; total pulmonary vascular resistance, PWCP; pulmonary capillary wedge pressure, mRAP; mean right atrial pressure, SvO2; mixed venous oxygen saturation.



Treatment response & survival

TABLE 6.5 shows first-line treatments and treatment responses. First-line treatment was started in 99% of IPAH-patients. The remaining 2 patients died before receiving treatment. No differences in treatment choices could be observed. TTCW was similar for $IPAH_{<45\%}$ and $IPAH_{\geq 45\%}$ when corrected for age.

In 23 $IPAH_{<45\%}$ patients and 76 $IPAH_{\geq 45\%}$ patients a follow-up DLCO measurement was available. Time between the first and follow-up DLCO measurement was not different between $IPAH_{<45\%}$ (median 416 days, interquartile range 316-834 days) and $IPAH_{\geq 45\%}$ (median 447 days, interquartile range 368-1008 days, p 0.35). No difference could be observed between the first DLCO measurement ($DLCO^1$) and follow-up measurement ($DLCO^2$) in either group ($IPAH_{<45\%}$: $DLCO^1$ 33±7% and $DLCO^2$ 34±8% of predicted, p 0.42 and $IPAH_{\geq 45\%}$: $DLCO^1$ 67±13% and $DLCO^2$ 67 ± 13% of predicted, p 0.95).

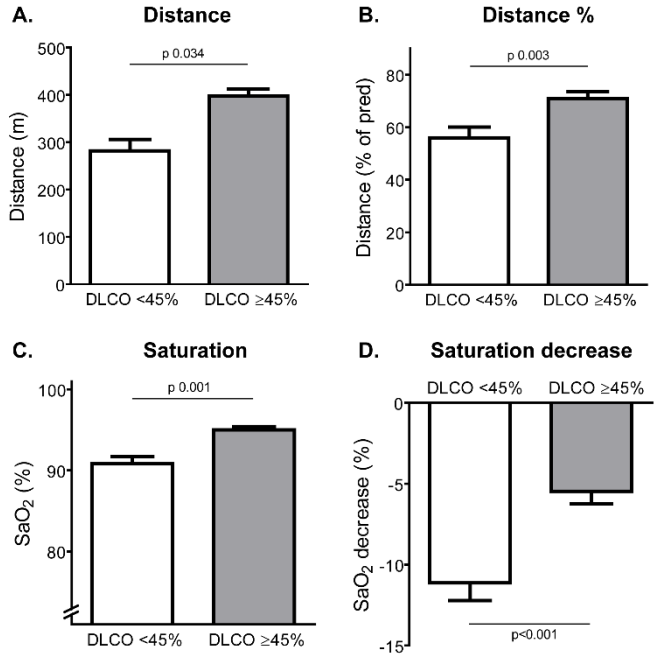


FIGURE 6.3 - Six-minute walking test results at baseline according to DLCO-groups. IPAH-patients with a severely reduced DLCO walked less far as shown by distance in meters and by distance as percentage of predicted (A,B). IPAH-patients with a severely reduced DLCO had a lower arterial oxygen saturation (SaO_2) at rest (C) and a larger decrease in saturation during exercise (D). Data are presented as mean ± SEM.

TABLE 6.4. CT findings in patients according to DLCO

		Presence of emhysema					
		DLCO < 45% of predicted N=44			DLCO ≥ 45% of predicted N=96		
		None	Mild	Moderate	None	Mild	Moderate
Presence of fibrosis	None	14 (32%)	9 (21%)	8 (18%)	64 (67%)	24 (25%)	4 (4%)
	Mild	3 (7%)	4 (9%)	4 (9%)	1 (1%)	1 (1%)	1 (1%)
	Moderate	0 (0%)	2 (5%)	0 (0%)	1 (1%)	0 (0%)	0 (0%)

Values are n (% of DLCO group). CT-scan analysis was performed by a blinded radiologist. Mild emphysema was defined by subtle centrilobular emphysema in apical segments of upper lobes; moderate emphysema was defined by a cluster of centrilobular and paraseptal emphysema with a preference for upper lobes; mild fibrosis was defined by focal areas with fine reticular subpleural opacities; moderate fibrosis was defined by a continuous subpleural band of fine reticular opacities restricted to either the upper or lower lobes.

TABLE 6.5. First-line treatment, treatment response and survival

	DLCO < 45% of predicted	DLCO ≥ 45% of predicted	p-value
First-line treatment			0.238
None	0 (0%)	2 (2%)	
Ca2+ channel blockers	3 (6%)	13 (11%)	
Endothelin receptor antagonist	23 (48%)	42 (36%)	
Prostanoids	9 (19%)	32 (27%)	
Phosphodiesterase inhibitors	11 (23%)	15 (13%)	
Combination therapy	2 (4%)	13 (11%)	0.235
Treatment response			
Time to clinical worsening (days)*	435 (153-868)	592 (315-1522)	0.539†
Survival			0.002†
1-year (%)	87	95	
3-year (%)	54	86	
5-year (%)	38	80	

Values are n (% of group tested), median (25^{th} to 75^{th} percentile). *Time to clinical worsening is defined as time to add-on therapy or an event (atrial balloon septostomy, lung transplantation or death). †p-value corrected for age.

Time of follow-up could not be acquired in 2 $IPAH_{<45\%}$ patients and 8 $IPAH_{\geq 45\%}$ patients. Median follow-up time was 4.2 years (interquartile range 1.8-7.9 years). During this period 53 events occurred. Ten patients had undergone a lung transplantation. Cause of death was retrieved in 25 patients. Eighteen patients had died due to end-stage disease. Another four patients died of RV infarction. Causes of death in the remaining three patients were multi-organ failure, left ventricular infarction and intrapulmonary bleeding. Event-free survival was lower in $IPAH_{<45\%}$ when compared to $IPAH_{\geq 45\%}$ (log-rank p<0.001). A severely reduced DLCO was associated with event-free survival univariately (HR 3.80, CI 2.20-6.58, p<0.001), but not when controlled for age at diagnosis, gender, time between the DLCO measurement and diagnosis and the presence of coronary disease (HR 1.78, CI 0.91-3.50, p 0.09).

DISCUSSION

Here, we not only confirm that the presence of a severely reduced DLCO in IPAH is associated with a poor survival[8,9], but also that in a large cohort of idiopathic and hereditary PAH-patients a low DLCO is associated with a higher age and a higher tobacco exposure, more CT abnormalities and a worse exercise performance, despite similar hemodynamic profiles. We confirm the decreased survival in

this patient group and now show that this poor outcome is related to age, gender and the presence of coronary disease.

The lack of correlation between DLCO and hemodynamic parameters such as PVR and CO confirms findings by others[1,15]. Hence, the results of this study do not suggest that a severe reduction in DLCO is the result of IPAH alone, but rather that a severe reduction in DLCO identifies a subtype of IPAH-patients in whom the disease is possibly related to smoking.

Parenchymal lung disease

Cigarette smoking is known to be a risk factor for the development of emphysema and interstitial lung disease (ILD)[16,17]. Both emphysema and ILD are known to reduce the DLCO and the presence of PH further enhances this reduction[6,13]. In our study, severe emphysema and/or evident ILD were excluded at the time of diagnosis as well as after re-evaluation of the HRCT which had been routinely performed during the diagnostic work-up. Consistent with previous studies, a substantial number of IPAH-patients had mild to moderate degrees of emphysema or interstitial abnormalities on HRCT[4,18]. As the severity of emphysema on HRCT correlates inversely with DLCO[19], mild to moderate emphysema will probably only moderately reduce the DLCO. Likewise, mild parenchymal abnormalities are known to only slightly reduce DLCO[20,21]. Smoking appears to augment this reduction in DLCO[21]. It is possible that in some IPAH-patients, additive effects of smoking, mild or moderate interstitial lung abnormalities and/or emphysema together explain a severe reduction in DLCO. This is supported by the higher prevalence of these abnormalities observed in IPAH-patients with a severely reduced DLCO compared to IPAH-patients with a more preserved DLCO.

The CT findings also indicate that in our study cohort the presence of mild forms of the syndrome of combined pulmonary fibrosis and emphysema (CFPE) could not be excluded. The typical features of CFPE such as older age, male gender, smoking history and a relatively preserved pulmonary function in the presence of a severely reduced DLCO[7] were also present in our IPAH-patients with a severely reduced DLCO. It is currently unclear whether patients with severely increased PA pressures and mild to moderate parenchymal abnormalities with preserved pulmonary function should be diagnosed and treated as IPAH-patients or as patients with lung disease associated (but 'out of proportion') PH.

Smoking-related pulmonary vasculopathy

Tobacco exposure was independently associated with a severe reduction in DLCO. Therefore, IPAH-patients with a severely reduced DLCO may represent a subgroup of PAH-patients with a smoking-related distinct pulmonary vasculopathy[22,23].

Previous studies have shown that tobacco exposure alone can cause pulmonary vascular remodeling leading to PH[22,23]. An experimental animal study also showed that the degree of PA pressure elevation caused by smoking is similar to that provoked by hypoxia, but that the underlying mechanism differs between the two types of exposure[22]. It can be hypothesized that in smoking IPAH-patients with a severely reduced DLCO, vascular lesions caused by smoking are predominantly present. It is unclear, however, whether these vascular lesions could fully explain a severe reduction in DLCO or whether additional parenchymal damage or interstitial thickening is required.

Interestingly, patients with a severe reduction in DLCO were relatively more frequently males and male gender may have interacted with the effects of smoking on the lung parenchyma or vasculature[24-26], together inducing a severe reduction in DLCO. Male smokers may be more susceptible than female smokers to develop PAH, as suggested by a case-control study which was conducted in Switzerland. In that study, the prevalence of smoking was higher in male PAH-patients than in the general population, while in women the prevalence of smoking was similar[24].

Pulmonary veno-occlusive disease

The IPAH-patients with a severely reduced DLCO in this study share some similarities with patients with PVOD. First of all, DLCO is severely reduced as is the case in PVOD[27]. Secondly, a lower arterial oxygen saturation, a similar gender distribution, increase in tobacco exposure and six-minute walking distance is found in our subgroup of IPAH-patients[27]. The amount of similarities between the two groups suggests that some IPAH-patients with a severe DLCO reduction may actually have PVOD. This is supported by histopathological review studies on IPAH-patients showing that about 10 per cent of patients with a clinical diagnosis of IPAH were found to have PVOD[28,29].

However, we consider it unlikely that all IPAH-patients with a severely reduced DLCO are actually PVOD-patients. We excluded all patients who were suspected of PVOD based on either clinical criteria[4,18] or histopathological evidence. This gives a PVOD prevalence of 7% in our IPAH population, which is comparable to the estimated prevalence of 10 per cent[28,29]. All of these patients were excluded from further analysis. Nevertheless, we cannot exclude that some PVOD-patients present with a different, non-classical profile and are therefore wrongly diagnosed as IPAH. Although non-

classical PVOD may explain a low DLCO in some patients, it is unlikely that this serves as an explanation for the entire group of low DLCO patients. Further histopathological studies are required in this specific cohort to study the nature of the pulmonary vascular lesions.

Left heart disease

A severely reduced DLCO is found in about 25% of patients with PH due to left heart failure with preserved ejection fraction[5]. Therefore, a possible explanation for a severe reduction in DLCO in IPAH may be the presence of left heart failure. Overt left heart failure is however very unlikely, as in our study cohort no patients were included with increased PCWP or evident left ventricular dysfunction on echocardiography. In addition, no differences in PCWP could be observed between the two groups, strongly indicating that there were no differences in left heart function at rest. We did not routinely perform fluid challenge or exercise during right heart catheterization as these interventions are not part of the current diagnostic algorithm. Consequently, we cannot exclude the existence of occult diastolic dysfunction. We observed an increased prevalence of coronary artery disease in IPAH-patients with a low DLCO. As this finding was not independently predictive of a low DLCO, this finding may rather reflect the increased tobacco exposure of IPAH-patients with a low DLCO. Future studies on PCWP changes after fluid challenge or exercise could identify patients with diastolic dysfunction. Whether these patients require a different treatment strategy remains to be established.

Clinical implications

IPAH is usually considered a homogenous disease, but here we show that within the IPAH population heterogeneity can be observed. We characterized a subgroup of IPAH-patients with a severely reduced DLCO and showed that this group has a distinct clinical profile with presentation at a higher age, a relative over-representation of male patients, a greater tobacco exposure, an increased prevalence of coronary disease and more often abnormalities on chest CT scan. Remarkably, not one patient with a severely reduced DLCO had heritable disease. These findings suggest that the group of IPAH-patients with a severely reduced DLCO is a mixed population and may contain patients with occult diastolic dysfunction, patients with parenchymal lung disease not severe enough to be classified in WHO-group III of the Dana-Point classification, and perhaps patients with non-classical PVOD. Our subgroup of IPAH-patients shares similarities with the older IPAH patient population of the Compera registry[11]. This registry had a predominance of elderly patients showing that the demographics of IPAH-patients are changing. With the IPAH population getting older, heterogeneity will probably

increase and our subgroup of IPAH-patients with a severely reduced DLCO will probably be of great relevance in the future. Our findings raise the question whether within the IPAH population as a whole, subgroups exist which may require a different diagnostic and/or therapeutic strategy.

Study limitations

Our study was retrospective in nature and some data were missing in individual patients. The number of missing values did not exceed 10% in the majority of parameters studied. However, in 3 parameters that were considered to be possible contributors to a severely reduced DLCO we found the number of missing values to exceed the 10%: 1) The amount of pack years, 2) cardiac output, and 3) pulmonary vascular resistance. Therefore, the lack of an association of CO or PVR with DLCO cannot be ruled out based on the present study. Because >90% of data was available for the majority of parameters studied, we consider it unlikely that the nature of the study explains the finding of a distinct clinical profile in the low DLCO group.

Pulmonary function test results used to divide the IPAH-patients into two DLCO-groups were not all measured at baseline. However, we observed that DLCO did not change over time. Indeed, only 2 patients with a severely reduced DLCO, and 4 patients with a more preserved DLCO changed DLCO so that they also changed DLCO-group. Therefore, the time of DLCO measurement presumably did not influence our results.

We used TTCW to determine treatment response. With our broad inclusion period the definition of TTCW may not be a good reflection of true worsening due to the differences in available PAH-specific drugs between early and recent diagnosed patients.

We included IPAH- and hereditary PAH-patients diagnosed between 1990 and 2011. This is a broad inclusion period and several changes in IPAH-patients characteristics have occurred over the last decades[10]. Therefore, it could be that the IPAH-patient groups do not completely represent the incident cases of IPAH and hereditary PAH seen today. However, as our subgroup of IPAH-patients with a severely reduced DLCO share many characteristics with the newly diagnosed older IPAH-patients described by both Ling et al. and Hoeper et al.[10,11], we believe our subgroup is still of relevance today and may even be of greater relevance in the future.

Conclusions

We show that a severe reduction in diffusion capacity in IPAH is associated with a higher age at presentation, a greater tobacco exposure and a poor exercise performance, despite a hemodynamic profile which is not different from other IPAH-patients. We confirm the decreased survival in this patient group and now show that this poor outcome is related to age, gender and the presence of coronary disease.

Competing interests

None.

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