Serum levels of small HDL particles are negatively correlated with death or lung transplantation in an observational study of idiopathic pulmonary fibrosis

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Shareable abstract (@ERSpublications)
This is the first study showing that higher serum levels of small HDL particles correlate with a lower risk of death or lung transplantation in patients with idiopathic pulmonary fibrosis, suggesting that they may be important in IPF pathobiology https://bit.ly/2RG4UGS

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Abstract

Background Serum lipoproteins, such as high-density lipoproteins (HDL), may influence disease severity in idiopathic pulmonary fibrosis (IPF). Here, we investigated associations between serum lipids and lipoproteins and clinical end-points in IPF.

Methods Clinical data and serum lipids were analysed from a discovery cohort (59 IPF subjects, 56 healthy volunteers) and validated using an independent, multicentre cohort (207 IPF subjects) from the Pulmonary Fibrosis Foundation registry. Associations between lipids and clinical end-points (forced vital capacity, 6-min walk distance, gender age physiology (GAP) index, death or lung transplantation) were examined using Pearson's correlation and multivariable analyses.

Results Serum concentrations of small HDL particles measured using nuclear magnetic resonance spectroscopy (S-HDLP$_{NMR}$) correlated negatively with the GAP index in the discovery cohort of IPF subjects. The negative correlation of S-HDLP$_{NMR}$ with GAP index was confirmed in the validation cohort of IPF subjects. Higher levels of S-HDLP$_{NMR}$ were associated with lower odds of death or its competing outcome, lung transplantation (OR 0.9 for each 1-μmol·L^{-1} increase in S-HDLP$_{NMR}$, $p<0.05$), at 1, 2 and 3 years from study entry in a combined cohort of all IPF subjects.

Conclusions Higher serum levels of S-HDLP$_{NMR}$ are negatively correlated with the GAP index, as well as with lower observed mortality or lung transplantation in IPF subjects. These findings support the hypothesis that S-HDLP$_{NMR}$ may modify mortality risk in patients with IPF.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive, irreversible disease, characterised by fibrosis within the lung parenchyma leading to lung function decline, with an incidence of 2.8–18 per 100 000 persons per year in Europe and North America [1]. The pathogenesis of IPF reflects complex host–environment interactions with a maladaptive reparative response to alveolar epithelial injury causing fibroblast activation and proliferation, deposition of excessive extracellular matrix and remodelling of the lung interstitium. New treatments, namely nintedanib and pirfenidone slow disease progression, but despite recent advances morbidity and mortality from IPF remain higher than many malignancies.

Lipids and lipoproteins play important roles both in normal lung health and in pulmonary diseases such as acute lung injury, asthma, emphysema and pulmonary hypertension [2–4]. High-density lipoproteins

(HDL), a complex, heterogeneous group of lipoprotein particles of varying composition, size and function mediates reverse cholesterol transport out of cells, and has anti-inflammatory, anti-thrombotic and anti-oxidative properties [5]. However, modifications to HDL composition and function may influence its role in health and disease. Several studies have also suggested a role for apolipoprotein AI (APOA1), the major protein component of HDL, in modifying disease severity in IPF. Bronchoalveolar lavage fluid (BALF) proteome analysis identified that APOA1 concentrations were lower in IPF subjects than healthy controls, and correlated negatively with the percentage of foamy, lipid-laden macrophages and fibrosis scores [6]. BALF APOA1 levels were also reduced in bleomycin-challenged mice, and administration of human APOA1 abrogated both inflammation and fibrosis in a dose-dependent manner [6]. In a silica-induced model of pulmonary fibrosis, transgenic mice with inducible overexpression of human APOA1 under the control of the human surfactant protein C promoter had fewer fibrotic nodules, less soluble collagen, lower inflammatory cell counts and decreased levels of transforming growth factor (TGF) β-1, interleukin-1β and tumour necrosis factor-α than control mice [7]. Furthermore, treatment of human alveolar epithelial cells with human APOA1 or with D4F, a synthetic APOA1 mimetic peptide, inhibited TGFβ-1-mediated epithelial–mesenchymal transformation [8, 9].

These observations led us to hypothesise that circulating serum lipoproteins and lipids, and in particular HDL, may be associated with clinically important measures of disease severity in IPF. Specifically, we examined if relationships exist between serum lipids, measured by standard and nuclear magnetic resonance (NMR) spectroscopy assays and the forced vital capacity (FVC), 6-min walk distance (6MWD) and gender age physiology (GAP) index, as well as GAP predicted mortality, and the composite outcome measure of observed mortality or lung transplantation in IPF subjects.

Methods

Study population

This study was conducted in accordance with the amended Declaration of Helsinki. The National Heart, Lung, and Blood Institute institutional review board (IRB) and the Inova human research protection programme independently approved the protocols (15-H-0017 and 09.147, respectively). To be included in the study, subjects provided written informed consent, and had serum samples collected within 100 days of pulmonary function and 6-min walk tests. All IPF subjects had their diagnosis confirmed either by lung biopsy and/or radiographic imaging on high-resolution computed tomography (CT) scans of the chest and clinical features consistent with IPF upon evaluation by a multidisciplinary team specialising in care of patients with interstitial lung diseases. Patients with other primary lung diseases were excluded. Healthy controls had no history of lung disease and a normal chest radiograph at the time of sample collection. The discovery cohort consisted of 102 subjects: 56 IPF subjects were evaluated at the Advanced Lung Disease and Transplant Clinic at Inova Fairfax Hospital (Fairfax, VA, USA) between January 2010 and August 2016. 56 race- and age (±10 years)-matched, healthy volunteers and three IPF subjects were enrolled at the National Institutes of Health Clinical Center (Bethesda, MD, USA) between January 2015 and November 2016. Healthy controls were included as a comparator for the discovery cohort to assess whether relationships observed between lung function and serum lipids and lipoproteins were specific to the IPF cohort or were also present in healthy subjects. The independent validation cohort consisted of 207 randomly selected (using SAS procedure) IPF subjects enrolled in the Pulmonary Fibrosis Foundation (PFF) patient registry between 2016 and 2018 who fulfilled the study inclusion criteria and for whom clinical data and stored serum samples were available [10]. We excluded subjects from the validation cohort that were already present in the discovery cohort, since Inova Fairfax Hospital is a participating PFF site. The PFF registry protocol, consent documents and questionnaires were approved by the IRB for the coordinating centre at the University of Michigan and by each participating centre's IRB (see appendix for list of participating sites). Details about the multicentre PFF patient registry, including eligibility criteria, can be found at www.pulmonaryfibrosis.org/pff-registry and in the publication by Wang *et al.* [10].

Details about lipid measurements are provided in the supplementary material.

Statistical methods

All analyses were performed using SAS Enterprise Guide version 7.15 (SAS Institute, Cary, NC, USA). The null hypotheses were that 1) there were no significant differences in serum lipid levels by standard assay or NMR spectroscopy between the discovery IPF cohort and the healthy controls and 2) there were no significant correlations between serum lipid parameters (total cholesterol, HDL cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides and subgroups of HDL particles measured using NMR spectroscopy, as well as APOA1 and APOB concentrations) and clinical end-points (FVC, 6MWD, GAP index) in the discovery IPF cohort.

Non-normally distributed variables were log-transformed prior to analysis. Comparisons of raw means between groups were based on one-way ANOVA (Bonferroni method). GAP index and predicted mortality were calculated as described previously [11]. Correlation analyses were performed using Pearson correlation method. Linear models using the variables age, sex, race, body mass index (BMI) and C-reactive protein (CRP) levels, determined *a priori* as potential factors that might influence serum lipid levels, were fitted in multivariable regression analyses to assess relationships between serum lipid parameters and FVC, 6MWD and GAP index. Based on the results of the analyses performed on the discovery cohort, we calculated that a sample size of 194–221 subjects for the validation cohort provided 85–90% power to detect statistically significant correlations of $\geqslant$0.2 (β=0.1–0.15, α=0.05; two-tailed). Correlation and multivariable regression analyses were replicated in the validation cohort, but limited to those variables that were significant in the discovery cohort.

Based upon the results of the *a priori* statistical analysis plan, additional analyses were performed to assess whether small HDL particles measured using NMR (S-HDLP$_{NMR}$) were associated with a composite end-point of mortality or lung transplantation. The discovery and validation cohorts of IPF subjects were analysed as a single group to generate a larger cohort in order to assess the relationship between S-HDLP$_{NMR}$ and the composite end-point of observed death or lung transplantation. Since this is a cross-sectional observational study of patients at a single point in their disease course, an adjustment for disease severity at the time of study participation was required to analyse associations between lipoproteins and observed death or lung transplantation. Therefore, predicted mortality at 1, 2 and 3 years as determined by the GAP calculator was used to adjust for disease severity. The GAP models have been developed as a multidimensional prognostic staging system for IPF, and incorporate age, sex, FVC and diffusing capacity of the lung for carbon monoxide (D_{LCO}) to predict death at 1, 2 or 3 years from the time of measurement of these variables. The GAP calculator derived predicted mortality provides a more precise estimate of individual risk that could be helpful in clinical decision-making above and beyond a simple GAP score [11]. Logistic regression analyses were performed to determine whether S-HDLP$_{NMR}$ levels were associated with the composite outcome of observed death or lung transplantation, using race, GAP predicted mortality, BMI and treatment status (with anti-fibrotic and lipid-lowering medications) as additional variables. Since age, sex, and lung function (FVC and D_{LCO}) are already incorporated into the GAP calculator, these were not again included as variables in the logistic regression models. All subjects were included who had either met the composite outcome (death or lung transplantation) or had a minimum follow-up period corresponding to 1, 2 or 3 years from the time of sample collection.

Significance level was set at 0.05 for α-errors; all statistical tests were two-tailed. No adjustment was made for multiple comparisons for global hypothesis testing. The number of variables analysed in the validation cohort were limited based on the results from the discovery cohort. All multivariable analyses were performed with included variables selected *a priori*, following a backwards stepwise selection method (significance level for keeping a variable in the model was set at $p\leqslant0.2$).

Results

Demographic analysis showed that the 59 IPF subjects from the discovery cohort were significantly older, with lower FVC, D_{LCO} and 6MWD, but higher CRP levels and lipid-lowering medication use, than the 56 healthy control subjects (table 1). The validation cohort of 207 IPF subjects was similar to the discovery cohort of IPF patients, except for a lower 6MWD and higher anti-fibrotic medication use. Significantly fewer IPF patients died or had lung transplants in the validation cohort than the discovery cohort (table 1). Serum lipid and lipoprotein levels for all subjects are shown in supplementary table S1. The number of subjects with missing data are shown in supplementary table 2.

Pearson correlation analyses were performed to determine whether serum lipid and lipoprotein values were significantly associated with the clinical end-points of FVC, 6MWD and GAP index in the discovery cohort of IPF patients. Total cholesterol (TC$_{NMR}$), LDL-C$_{NMR}$ and S-HDLP$_{NMR}$, all measured using NMR spectroscopy, correlated positively with FVC and 6MWD, and negatively with GAP index (figure 1). Negative correlations were also observed between total cholesterol from the standard lipid assay, as well as NMR-derived APOB particle concentrations and the GAP index. These associations were absent in healthy controls (supplementary table S3).

Next, multivariable analyses focused on the lipid parameters (TC$_{NMR}$, LDL-C$_{NMR}$ and S-HDLP$_{NMR}$) that were significantly correlated with all three of the pre-specified clinical end-points, FVC, 6MWD and GAP. The positive correlations between TC$_{NMR}$, LDL-C$_{NMR}$ and S-HDLP$_{NMR}$ with FVC and 6MWD remained significant in the discovery cohort after adjusting for age, sex, race, BMI and CRP levels. In addition, the

TABLE 1 Demographic characteristics of healthy controls and subjects with idiopathic pulmonary fibrosis (IPF) (discovery and validation cohorts)

	Healthy controls	Discovery cohort	Validation cohort	p-value for comparison[#]	
				Healthy controls *versus* discovery cohort	Discovery cohort *versus* validation cohort
Subjects	56	59	207		
Age years	62.3±9.7	69.2±8.2	71.4±8.84	<0.0001	NS
Race white/other	52/4	53/6	195/12	NS	NS
Female/male	18/38	10/49	55/152	NS	NS
FVC % pred	106±14	66±18.3	67±17.3	<0.0001	NS
D_{LCO} % pred	82±11.2	40.2±12.6	40.5±16.6	<0.0001	NS
BMI kg·m^{-2}	27.76±4.82	28.72±4.68	28.87±4.78	NS	NS
CRP mg·L^{-1}	1.15 (0.65–2.5)	3.1 (1.3–7.1)	3.3 (1.4–6.7)	0.006	NS
6MWD m	568.7±76	419.8±134	356.6±115	<0.0001	0.003
GAP index		4.49±1.18	4.49±1.41		NS
Smokers/nonsmokers	12/44	35/24	142/65	<0.0001	NS
Lipid-lowering drug yes/no	21/35	41/18	122/85	0.0007	NS
Anti-fibrotic drug yes/no		19/40	133/74		<0.0001
Lung transplanted yes/no		11/48	14/193		0.01
Current status dead/alive[¶]	0/56	31/28	51/156		<0.0001

Data are presented as n, mean±SD or median (interquartile range), unless otherwise stated. FVC: forced vital capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; BMI: body mass index; CRP: C-reactive protein; 6MWD: 6-min walk distance; GAP: gender age physiology. [#]: two-sided p-values based on Fisher's exact tests or Satterthwaite's t-test; [¶]: of these, 19 and 27 deaths in the discovery and validation cohorts, respectively, were attributed to IPF. The cause of death was not known for 11 subjects in the discovery cohort and 17 subjects in the validation cohort.

negative correlations between the GAP index and TC_{NMR} and LDL-C_{NMR} remained significant, while that with S-$HDLP_{NMR}$ trended toward significance (p=0.09; table 2).

We then assessed whether these correlations could be replicated in the validation cohort of IPF patients and found that TC_{NMR} and LDL_{NMR} were not significantly correlated with either FVC or 6MWD, although the correlations with GAP score approached significance (p=0.06 for both by Pearson correlation; supplementary table S3). S-$HDLP_{NMR}$ levels were significantly correlated with both FVC and the GAP index (p=0.003), and the relationship with 6MWD approached significance (p=0.06; figure 2 and supplementary table S3). On multivariable regression, only the inverse relationship between S-$HDLP_{NMR}$ and GAP index remained significant in the validation cohort (estimate −0.33, 95% CI −0.65–−0.009; p=0.04). Taken collectively, analyses of two independent cohorts of IPF subjects identified a negative association between S-$HDLP_{NMR}$ and the GAP index, which is a predictor of mortality in IPF. A *post hoc* analysis showed that S-$HDLP_{NMR}$ concentrations correlated positively with D_{LCO} in both the discovery and the validation cohorts (p=0.01 for both). While D_{LCO} remained significantly associated with S-$HDLP_{NMR}$ by multivariable analysis in the discovery cohort (p=0.04), it was not so in the validation cohort (p=0.18; reduced linear models).

Subsequent analyses focused on assessing the association between S-$HDLP_{NMR}$ and mortality in the combined cohort of all IPF subjects. Logistic regression analyses showed that S-$HDLP_{NMR}$ was associated with a lower risk of the composite outcome of observed death or lung transplantation at 1, 2 and 3 years, which remained significant after adjusting for predicted mortality (as a measure of disease severity), race, and anti-fibrotic and lipid-lowering drug use (figure 3). Each 1-μmol·L^{-1} increase in S-$HDLP_{NMR}$ was associated with a decrease in the risk of death or lung transplantation by ∼10% (OR 0.9) at 1, 2 and 3 years from the time of sample collection. Although the lipid profiles of IPF subjects taking lipid-lowering medication differed from those not taking these medications as expected (supplementary table S1), treatment status did not change the relationship between S-$HDLP_{NMR}$ and observed death or lung transplantation. Furthermore, these associations remained significant even after adjusting for BMI, which could potentially influence outcomes and lipid levels in IPF patients.

Discussion

The role of lipids and lipoproteins in the pathogenesis of IPF is yet to be completely defined. Here, we found that the serum concentration of S-$HDLP_{NMR}$ correlated negatively with the GAP index in a discovery cohort of IPF subjects from a single centre, and validated this in a larger, multicentre cohort of

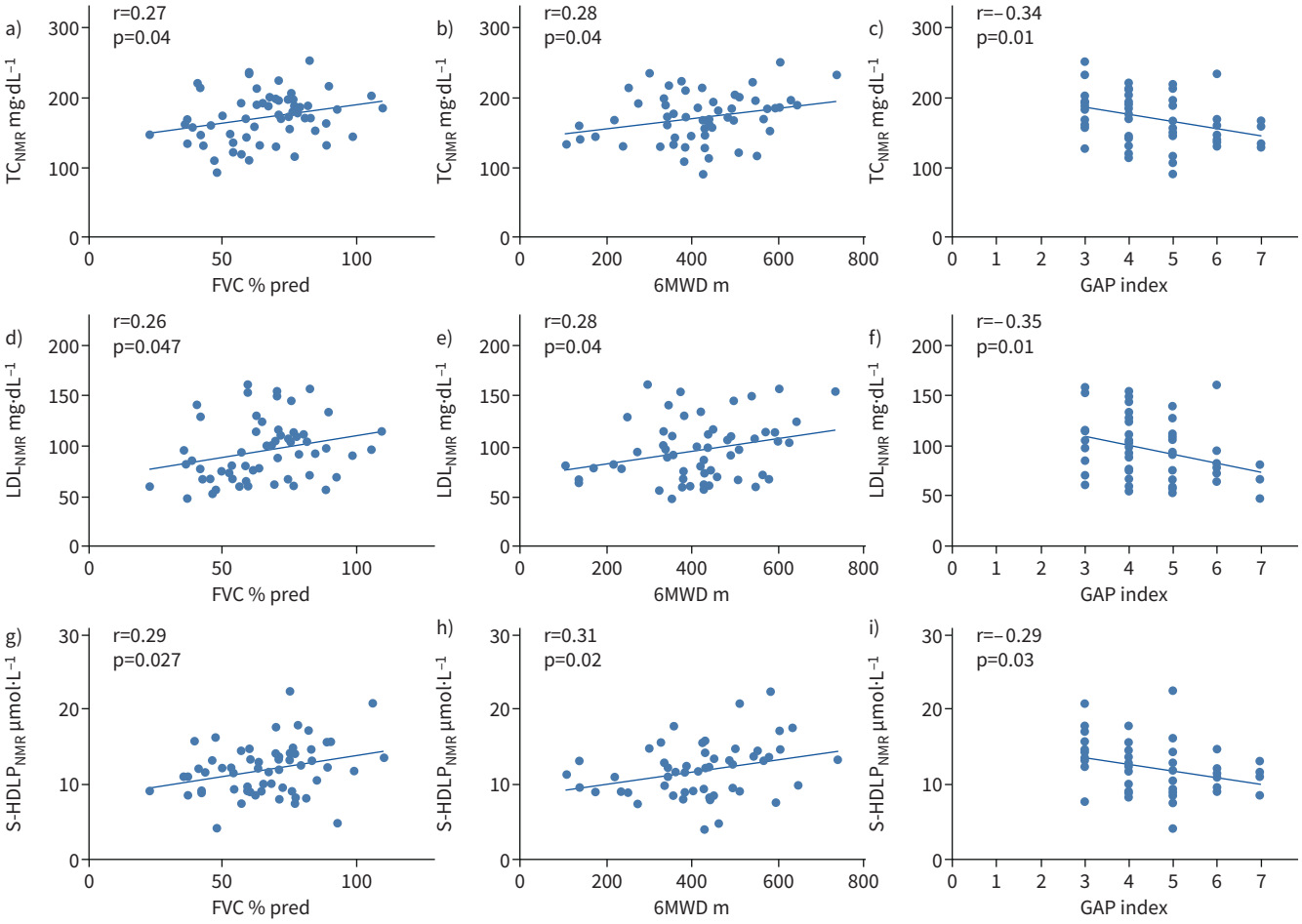

FIGURE 1 Scatter plots showing Pearson correlations between total cholesterol (TC_{NMR}), low-density lipoprotein cholesterol (LDL-C_{NMR}) and small high-density lipoprotein particles (S-$HDLP_{NMR}$) as measured by nuclear magnetic resonance spectroscopy, and the clinical end-points (forced vital capacity (FVC), 6-min walk distance (6MWD) and gender age physiology (GAP) index) in subjects with idiopathic pulmonary fibrosis in the discovery cohort.

IPF patients. These relationships remained largely significant after adjusting for BMI, sex, age, race and CRP levels. Furthermore, higher serum S-$HDLP_{NMR}$ levels were associated with a lower risk of observed death or lung transplantation, irrespective of disease severity, race, or treatment status with either anti-fibrotics or lipid-lowering medications.

TABLE 2 Multivariable analysis (reduced linear models incorporating age, sex, race, body mass index and C-reactive protein levels; SAS, Cary, NC, USA) to examine associations between lipids/lipoproteins and forced vital capacity (FVC), 6-min walk distance (6MWD) and gender age physiology (GAP) index in the discovery cohort of idiopathic pulmonary fibrosis subjects

	FVC	p-value	6MWD	p-value	GAP index	p-value
TC_{NMR}	0.59 (0.11–1.07)	**0.01**	0.07 (0.01–0.14)	**0.03**	−10 (−17.55– −2.45)	**0.008**
LDL-C_{NMR}	0.51 (0.10–0.93)	**0.01**	0.06 (0.01–0.12)	**0.03**	−8.78 (−15.19– −2.37)	**0.006**
S-$HDLP_{NMR}$	0.05 (0.003–0.10)	**0.03**	0.01 (0.00–0.01)	**0.02**	−0.65 (−1.4–0.11)	0.09

Data are presented as estimate (95% CI), unless otherwise stated. Each estimate represents the change in total cholesterol (TC_{NMR}), low-density lipoprotein cholesterol (LDL-C_{NMR}) and small high-density lipoprotein particles (S-$HDLP_{NMR}$) as measured by nuclear magnetic resonance spectroscopy associated with each unit increase in FVC, 6MWD and GAP. Thus, for example, a 1% increase in FVC is associated with a 0.59 $mg·dL^{-1}$ increase in TC_{NMR}, while an increase in GAP index by 1 point is associated with a 10 $mg·dL^{-1}$ decrease in TC_{NMR}. Bold type represents statistical significance.

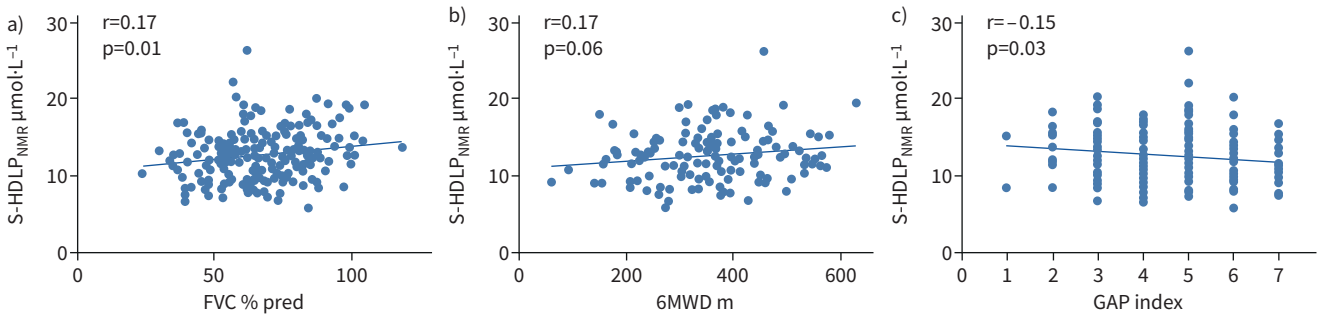

FIGURE 2 Scatter plots showing Pearson correlations between small high-density lipoprotein particles as measured by nuclear magnetic resonance spectroscopy (S-HDLP$_{NMR}$) and the clinical end-points (forced vital capacity (FVC), 6-min walk distance (6MWD) and gender age physiology (GAP) index), in subjects with idiopathic pulmonary fibrosis in the validation cohort.

Experimental murine studies have identified both harmful and protective roles for lipids and lipoproteins in IPF pathobiology [3]. For example, *Apoe*$^{-/-}$ mice fed a high-fat or Western diet develop hypercholesterolaemia with resultant systemic inflammation, oxidative stress and lipid accumulation in the lung, causing pulmonary fibrosis [3]. A paracrine lipid-signalling axis in the mouse lung has been described, linking epithelial injury with macrophage activation and pulmonary fibrosis [12]. Additionally, serum lipids and lipoproteins might modify the composition or function of pulmonary surfactant in IPF; as circulating lipids affect the content and characteristics of pulmonary surfactant both in health and disease [13–15].

HDL cholesterol was long considered protective and thus a therapeutic target for mitigating cardiovascular risk; however, simply raising HDL cholesterol levels in blood failed to deliver better cardiovascular outcomes [16]. The HDL fraction is composed of a heterogeneous group of particles varying in not only size (7.3–8.2 nm for small, 8.2–8.8 nm for medium and 8.8–13.0 nm for large HDL particles), but also in composition [17]. More than 550 proteins have been reported to be associated with HDL particles, which may vary by disease state and alter its biological function [18]. APOA1, which is the most abundant HDL-associated protein, is known to have significant anti-inflammatory and antioxidant properties. However, APOA1 did not correlate with the clinical end-points in our study, suggesting that other protein or lipid components of small HDL particles may be more important in IPF.

Small HDL particles have been shown to be associated with cardioprotection, based on their ability to accept cholesterol from foam cells by ABCA1, and have anti-oxidant, anti-inflammatory and anti-apoptotic properties [19]. Sphingosine-1-phosphate (S1P), a bioactive lipid that acts through G-protein coupled S1P

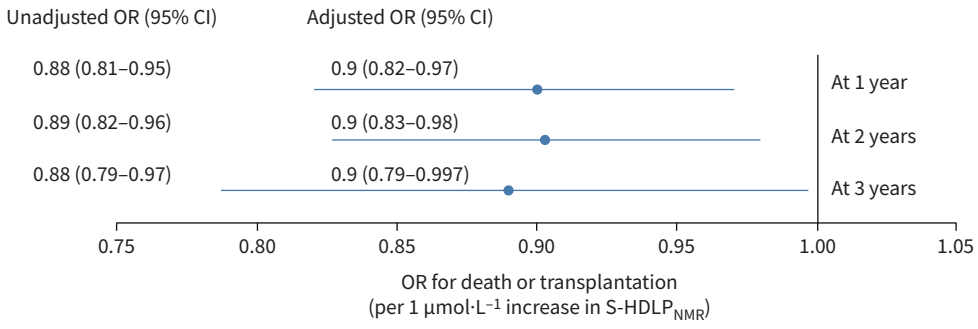

FIGURE 3 Forest plots showing the odds ratios of the composite outcome of death or lung transplantation at 1, 2 and 3 years from study entry associated with each unit increase in small high-density lipoprotein particles as measured by nuclear magnetic resonance spectroscopy (S-HDLP$_{NMR}$), from logistic regression models (SAS, Cary, NC, USA). Variables for the adjusted odds ratio models included race, treatment status, body mass index and gender age physiology (GAP)-calculated predicted mortality which also incorporates age, sex, forced vital capacity and diffusing capacity of the lung for carbon monoxide.

receptors and regulates cell proliferation, motility, apoptosis, angiogenesis, immune response and wound healing, is enriched in small dense HDL particles [20, 21]. Serum and bronchoalveolar lavage levels of S1P are higher in IPF subjects compared to healthy volunteers, and associated with epithelial to mesenchymal transition [22]. S1P has been shown to have both pro-fibrotic and anti-fibrotic effects, depending on the site of action (intracellular *versus* extracellular) [23]. Levels of serum amyloid A, which is primarily associated with HDL particles, are also increased in IPF patients as compared to healthy controls, and are negatively correlated with HDL cholesterol levels and FVC [24]. Moreover, HDL function is not static; HDL subfractions have been shown to change from being anti-inflammatory to pro-inflammatory in specific disease conditions such as diabetes or metabolic syndrome [25–28]. Therefore, further investigations to elucidate the lipid and protein components of small HDL particles and their role in IPF are indicated.

NMR spectroscopy lipid profiles are being increasingly studied regarding their association with disease risk, severity, or outcome, particularly in cardiovascular and metabolic disorders such as diabetes. In particular, assessment of HDL particle number and HDL particle profile by NMR spectroscopy has been proposed to be superior to HDL-C as a predictor of cardiovascular disease risk and mortality, which may reflect that measurement of HDL particle number by NMR is more accurate and reliable [29–31]. NMR spectroscopy lipid profiles have also been studied in lung disease. Plasma levels of the small-HDL subclass HDL-4 have been inversely related to survival in patients with pulmonary arterial hypertension [32]. A positive correlation has been found between forced expiratory volume in 1 s and HDL_{NMR} particle size, as well as the concentration of large HDL_{NMR} particles in asthmatics [33]. Similarly, the concentration of large HDL_{NMR} particles was negatively correlated with blood eosinophils, a biomarker of type 2 inflammation, while serum periostin levels were inversely associated with the concentration of total HDL_{NMR} particles in asthmatics [34]. Of note, the association between death or lung transplantation and S-$HDLP_{NMR}$ was not seen with HDL-C in our study. This may in part reflect that quantification of HDL subspecies by different methodologies are not directly comparable, as well as differences in HDL function and composition [29].

Associations between serum lipoproteins and radiographic abnormalities in the lungs were also present in subjects enrolled in the Multi-Ethnic Study of Atherosclerosis [35]. This study investigated the progression of subclinical cardiovascular disease over time in asymptomatic individuals aged 45–84 years who were not pre-selected based on lung disease. Cardiac CT images were analysed by automated quantitative CT densitometry to measure high attenuation areas (HAA) and visual inspection to detect interstitial lung abnormalities. Higher serum levels of total cholesterol, HDL-C, LDL-C, APOA1 and triglycerides were associated with lower HAA, and the association of HAA with HDL-C and APOA1 remained significant after adjusting for left ventricular function. HDL-C and APOA1 were also inversely associated with matrix metalloproteinase 7 and surfactant protein A, two potential biomarkers of lung inflammation and extracellular matrix remodelling. These observations, linking serum lipid and lipoprotein levels with disease risk, severity or outcomes support further investigations to delineate the possible role of HDL and other lipoproteins in lung disease.

One shortcoming of the current study is the relatively small sample size. In addition, the results of this cross-sectional study only characterise an association between serum lipoproteins and clinical outcomes in a cohort of IPF patients; this does not prove that a causal relationship exists between serum lipoproteins and IPF, nor does it establish S-$HDLP_{NMR}$ as a biomarker for disease severity in IPF. These findings would first have to be reproduced in well-designed, prospective studies with the specific aim of biomarker development, and the exact role of these lipoprotein particles in IPF pathobiology would have to be elucidated at a molecular level. However, to counterbalance these shortcomings, particular strengths of our study design include the well-defined clinical phenotyping of subjects and controls in the discovery cohort, the confirmation of our primary finding in the independent multicentre validation cohort, as well as the choice of outcomes analysed. To the best of our knowledge, this is the first study to show a relationship between NMR spectroscopy-derived lipid measurements with clinically important end-points, including mortality, in an IPF cohort.

Although the overall prognosis for IPF is poor, with a median survival of 2–4 years, disease course and progression varies significantly between individual patients [1, 36]. The GAP index and system of staging, and the GAP calculator of predicted mortality, were developed and validated for use in individual IPF patients with applicability to both clinical care (*i.e.* guiding decision making, such as timing of lung transplantation or hospice referral) and clinical research [11]. It must be pointed out that the GAP calculator was developed and validated prior to the era of anti-fibrotic medication use in IPF, and thus it is not known whether it accurately predicts mortality in IPF patients who are taking these medications.

However, we were able to show that S-HDLP$_{NMR}$ levels were associated with a lower risk of observed death or lung transplantation at 1, 2 and 3 years of follow up irrespective of anti-fibrotic medication use, which was included as a variable in the logistic regression models.

In conclusion, we show that serum concentrations of S-HDLP$_{NMR}$ were inversely correlated to disease severity as represented by GAP index as well as observed mortality, or its competing outcome, lung transplantation, in subjects with IPF. This finding that circulating lipids are associated with important clinical indicators of disease severity and mortality in IPF adds new evidence supporting further investigations to evaluate the role of lipids and lipoproteins in IPF pathobiology.

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