Plasma Levels of High-Density Lipoprotein Cholesterol and Outcomes in Pulmonary Arterial Hypertension

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Rationale: High-density lipoprotein cholesterol (HDL-C) promotes healthy vascular function, and it is decreased in insulin resistance. Insulin resistance predisposes to pulmonary vascular disease.

Objectives: We hypothesized that HDL-C is associated with clinical outcomes in pulmonary arterial hypertension (PAH).

Methods: Plasma HDL-C concentrations were measured in 69 patients with PAH (age, 46.7 ± 12.9 yr; female, 90%) and 229 control subjects (age, 57 ± 13 yr; female, 48%). Clinical outcomes of interest included hospitalization for PAH, lung transplantation, and all-cause mortality. Survival and time to clinical worsening curves were derived by the Kaplan-Meier method. Cox regression modeling of outcome versus HDL-C with individual covariate adjustments was performed.

Measurement and Main Results: HDL-C was low in subjects with PAH compared with control subjects (median, interquartile range: PAH: 36, 29–40 mg/dl; control subjects: 49, 40–60 mg/dl; $P < 0.001$). An HDL-C level of 35 mg/dl discriminated survivors from nonsurvivors, with a sensitivity of 100% and specificity of 60%. After a median follow-up of 592 days, high HDL-C was associated with decreased mortality (hazard ratio for every 5-mg/dl increase in HDL-C, 0.643; 95% confidence interval, 0.504–0.822; $P = 0.001$) and less clinical worsening (hazard ratio for every 5-mg/dl increase in HDL-C, 0.798; 95% confidence interval, 0.663–0.960; $P = 0.02$). HDL-C remained a significant predictor of survival after adjusting for cardiovascular risk factors, C-reactive protein, indices of insulin resistance, and severity of PAH (all $P < 0.05$).

Conclusions: Low plasma HDL-C is associated with higher mortality and clinical worsening in PAH. This association does not appear to be explained by underlying cardiovascular risk factors, insulin resistance, or the severity of PAH.

Keywords: hypertension; pulmonary; cholesterol; high-density lipoprotein; mortality

Pulmonary arterial hypertension (PAH) is a severe disease characterized by a progressive increase in pulmonary vascular resistance that leads to right ventricular failure and premature death (1). Impressive progress in the understanding of its pathobiology over the past two decades has made new therapies available that target specific PAH pathways (2). However, none of these therapies are curative, prompting the need for discovery of new pathways and therapeutic targets. Furthermore, determining prognosis is challenging in PAH, and noninvasive biomarkers of the disease are highly desirable (3).

High-density lipoprotein cholesterol (HDL-C) is associated with a lower risk of coronary heart disease (4, 5). The underlying

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

High density lipoprotein-cholesterol (HDL-C) promotes healthy vascular function and is low in insulin resistance. Insulin resistance predisposes to pulmonary vascular disease.

What This Study Adds to the Field

HDL-C levels are significantly depressed in pulmonary arterial hypertension, and this is associated with worse clinical outcomes independent from cardiovascular risk factors, insulin resistance and the severity of pulmonary arterial hypertension.

mechanisms behind this protective effect include stimulation of reverse cholesterol transport, antioxidant and antiinflammatory properties (6, 7), attenuation of endothelial dysfunction (8, 9), anticoagulant effects (10, 11), and enhancement of prostacyclin half-life (12–14). Interestingly, except for reverse cholesterol transport, derangements in all of these mechanisms have been implicated in the pathobiology of PAH. Lower levels of apolipoprotein A-I (Apo A-I), the major protein component of HDL-C, have been reported in patients with sickle cell disease and pulmonary hypertension, and these lower levels correlate with increased endothelial dysfunction (15).

A common reason for low circulating HDL-C levels is the clustering of risk factors for cardiovascular disease and diabetes mellitus called the metabolic syndrome (16), and its purported underlying pathogenic mechanism, insulin resistance. An emerging body of evidence suggests that the metabolic syndrome and insulin resistance may predispose to pulmonary vascular disease (17–20). An apolipoprotein E-deficient mouse model on a high-fat diet developed insulin resistance, pulmonary vascular remodeling, and right ventricular hypertrophy, changes that were reversed by rosiglitazone, an insulin-sensitizer drug (17). Insulin resistance defined as a triglyceride/HDL-C ratio greater than 3 has been found to be more common in female patients with PAH than in the general population (18).

On the basis of these observations, we hypothesized that circulating levels of HDL-C are associated with clinical outcomes in PAH. Preliminary results of this study have been previously reported in abstract form (21).

METHODS

Study Population

Patients were recruited from the Cleveland Clinic (Cleveland, OH) Pulmonary Vascular Program. Eligible subjects included patients with PAH diagnostic category 1 according to the updated Dana Point clinical classification (22). We excluded other types of pulmonary hypertension, as well as patients with active infections and unstable coronary syndromes. As control subjects, we used a set of subjects

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referred to the Preventive Cardiology Section from the Cleveland Clinic, who had been referred for assessment of their cardiovascular risk given the presence of risk factors. As a positive disease control we included subjects with connective tissue disease (CTD) without pulmonary hypertension, defined as the absence of clinical suspicion for PAH, estimated right ventricular systolic pressure (RVSP) less than 35 mm Hg, and normal right ventricle size and function on transthoracic Doppler echocardiography.

Laboratory Measurements

After informed consent was obtained, peripheral venous blood was drawn into heparin tubes. HDL-C and triglyceride (TG) concentrations were measured on the Abbott ARCHITECT platform (Abbott Laboratories, Inc., Abbott Park, IL) as described (23, 24). In the PAH population and a subset of control subjects, glucose, insulin levels, and high-sensitivity C-reactive protein (CRP) were also measured with the Abbott ARCHITECT platform. We calculated the homeostatic model assessment of insulin resistance (HOMA-IR) from simultaneously measured glucose and insulin concentrations, using the following formula: (glucose [mg/dl] $\times$ insulin [μ U/ml])/405 (25, 26). In a subset of patients with PAH, we measured the following soluble markers, using commercially available enzyme-linked immunosorbent assay kits (all from R&D Systems, Minneapolis, MN): interleukin 17A (IL-17A, cat. no. D1700), tumor necrosis factor- α (TNF- α , cat. no. DTA00C), intercellular adhesion molecule-1 (ICAM-1, cat. no. DY720), and CC-chemokine ligand-2 (CCL-2, previously known as monocyte chemoattractant protein-1 [MCP-1], cat. no. DCP00).

Clinical Data Collection

Data collection was prospective for 49 patients, and retrospective for the remaining 20. Clinical outcomes of interest were hospitalization for PAH, lung transplantation, and all-cause mortality, and were recorded by review of medical records, and supplemented by phone calls and the Social Security Death Index. Clinical worsening was defined as a composite end point of hospitalization for PAH, lung transplantation, or death. Other data collected included demographic information, body mass index (BMI), diabetes mellitus, systemic arterial hypertension, coronary artery disease, statin therapy, smoking status, right heart catheterization data, Doppler echocardiography measurements, 6-minute walk distance (6MWD), functional class assessed on the basis of the New York Heart Association (NYHA) classification, and the use of PAH-targeted therapies.

Statistical Analysis

Demographic, clinical, and laboratory data were summarized as frequency (%), median and interquartile range (IQR), and mean $\pm$ standard deviation (SD), and compared by means of the chi-square and Wilcoxon rank sum tests as appropriate. For the comparison of HDL-C, TG, and TG/HDL-C ratio between patients and control subjects, we used the Wilcoxon rank sum test and analysis of covariance adjusting for age, sex, smoking status, coronary artery disease, systemic hypertension, statin use, and diabetes. When modeling TG and TG/HDL ratio, their log-transformed values were used. For the comparison of HDL-C among patients with PAH, control subjects, and CTD subjects without pulmonary hypertension we used a *t* test with the Bonferroni correction for multiple comparisons.

The associations between time to death and time to clinical worsening and HDL-C were assessed using log-rank tests, with Kaplan-Meier estimation used to describe likelihood of the outcomes during the follow-up period. A receiver operating characteristic (ROC) curve analysis was used to select a cutoff value for HDL-C that maximized sensitivity and specificity. HDL-C was also summarized in its continuous form. Cox regression models were used to provide estimates of hazard ratios (HRs) and 95% confidence intervals (CIs) for outcomes with respect to their associations with HDL-C. For the analysis of continuous HDL-C, the HR corresponds to a 5-mg/dl change in HDL-C. Cox regression was also used in a multivariable fashion to explore the covariate-adjusted associations between the outcomes and HDL-C. We adjusted for selected covariates individually because the sample size and number of events did not allow for building models with multiple covariates. Results for covariate-adjusted associations were summarized using likelihood ratio *P* values

TABLE 1. BASELINE DEMOGRAPHIC FEATURES AND CARDIOVASCULAR RISK FACTORS

	Patients with PAH (<i>n</i> = 69)	Control Subjects (<i>n</i> = 229)	<i>P</i> Value
Age, yr	46.7 $\pm$ 12.9	56.7 $\pm$ 12.8	<0.001
Female	62 (89.9)	109 (47.6)	<0.001
Smoker	10 (14.5)	22 (9.6)	0.25
CAD	3 (4.3)	85 (37.1)	<0.001
HTN	15 (21.7)	123 (53.7)	<0.001
DM	0 (0)	45 (19.7)	<0.001
Statin use	8 (11.6)	106 (46.3)	<0.001

Definition of abbreviations: CAD = coronary artery disease; HTN = systemic hypertension; PAH = pulmonary arterial hypertension.

Data are presented as means $\pm$ SD and as no. (%).

and covariate-adjusted HR estimates. In addition to the outcomes analyses, patient groups defined by HDL-C levels were compared with respect to categorical study variables, using chi-square and Fisher's exact test, and with respect to quantitative and ordinal variables using Wilcoxon rank sum tests. Analyses were performed with R version 2.8.1 (27).

RESULTS

Study Population

We studied 69 patients with PAH and 229 control subjects. PAH categories were as follows: 41 idiopathic PAH, 1 heritable PAH (hereditary hemorrhagic telangiectasia), and 27 associated PAH (20 connective tissue disease, 4 congenital heart disease, 1 portopulmonary, 1 chronic hemolytic anemia, and 1 myelofibrosis). At the time of venous sampling, 18 patients (26%) were not receiving PAH-targeted therapies. Table 1 shows the baseline demographics and cardiovascular profiles of patients with PAH and control subjects. As expected, the control population was older and male dominated, and had more coronary disease, systemic hypertension, diabetes, and statin use. We also studied 21 subjects with CTD without pulmonary hypertension (age, 48 $\pm$ 14 yr; female, 76%; 10 scleroderma spectrum of disease, 7 lupus, and 2 each with dermatomyositis and mixed connective tissue disease).

Figure 1. Lower plasma high-density lipoprotein cholesterol (HDL-C) levels in 69 patients with pulmonary arterial hypertension (PAH) (median, interquartile range [IQR]: 35.3, 29.2–39 mg/dl) compared with 229 control subjects with several cardiovascular risk factors (median, IQR: 49, 40–60 mg/dl) (*P* < 0.001).

TABLE 2. HIGH-DENSITY LIPOPROTEIN CHOLESTEROL, TRIGLYCERIDES, AND INDICES OF INSULIN RESISTANCE

	Patients with PAH (n = 69)	Control Subjects (n = 229)	P Value*
HDL-C	35.3 (29.2–39)	49 (40–60)	<0.0001
TG	109 (85–140)	131 (87–208)	0.11
TG/HDL-C ratio	3.44 (2.22–4.58)	2.8 (1.57–4.53)	0.003
HOMA-IR†	1.91 (0.85–3.28)	2.08 (1.08–3.92)	0.61

Definition of abbreviations: CAD = coronary artery disease; HDL-C = high-density lipoprotein cholesterol; HOMA-IR = homeostatic model assessment-insulin resistance; HTN = systemic hypertension; PAH = pulmonary arterial hypertension; TG = triglycerides.

Data are presented as medians (interquartile range).

* P value from a linear regression model adjusting for age, sex, smoking status, CAD, HTN, diabetes, and statin use.

† HOMA-IR was calculated according to the following formula: (glucose [mg/dl] × insulin [μU/ml])/405. Data are reported on 68 patients with PAH and 29 control subjects without diabetes, hypertension, or coronary artery disease (age, 35 ± 6 yr; female, 75%).

Baseline HDL-C and Indices of Insulin Resistance

Compared with control subjects, subjects with PAH had significantly lower plasma HDL-C concentrations (Figure 1), similar TG levels, and higher TG/HDL-C ratio after adjusting for age, sex, smoking status, coronary artery disease, systemic hypertension, diabetes, and statin use (Table 2). HOMA-IR was similar in patients with PAH and a set of 29 control subjects without known cardiovascular risk factors (age, 35 ± 6 yr; female, 75%; see Table 2). Subjects with CTD without pulmonary hypertension had HDL-C levels (mean ± SD, 42.9 ± 15.6 mg/dl) in between those of control subjects (52.9 ± 19.7 mg/dl, $P = 0.0161$ vs. CTD) and patients with PAH (35.24 ± 11.49, $P < 0.001$ vs. CTD).

Clinical Outcomes

ROC curve analysis indicated that an HDL-C value of 35 mg/dl discriminated survivors from nonsurvivors with a sensitivity of 100% and specificity of 60% (area under the ROC curve, 0.82; see Figure 2A). The number and incidence of clinical events above and below an HDL-C value of 35 mg/dl are shown in Table 3. Fifty-three percent of patients with lower HDL-C had one or more of the three defined clinical events, compared with 14% of those with HDL-C higher than 35 mg/dl ($P < 0.001$). There were 11 deaths in subjects with low HDL-C compared with none in the other group (Table 3). Seven deaths were directly related to right ventricular failure, and three deaths were due to sepsis compounded by RV failure. We were unable to ascertain the cause of death in one case. Although the number of hospitalizations for worsening PAH was higher in

TABLE 3. CLINICAL EVENTS ACCORDING TO HIGH-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS

	HDL-C <35 mg/dl (n = 34)	HDL-C >35 mg/dl (n = 35)	P Value
Hospitalization	9 (26.5)	5 (14.3)	0.21
Lung transplantation	0	0	NA
Death	11 (32.4)	0	<0.001
Composite end point	18 (52.9)	5 (14.3)	0.001

Definition of abbreviation: HDL-C = high-density lipoprotein cholesterol; NA = not applicable.

Data are presented as no. (%).

patients with low HDL-C, this difference did not reach statistical significance.

Figure 3 shows the survival (Figure 3A) and clinical worsening (Figure 3B) time curves. After a median follow-up of 592 days (range, 1 to 1,608 d) patients with PAH with HDL-C above 35 mg/dl had no mortality (HR for every 5-mg/dl increase in HDL-C, 0.643; 95% CI, 0.504–0.822; $P = 0.001$) and a longer time to clinical worsening (HR for clinical worsening for every 5-mg/dl increase in HDL-C, 0.798; 95% CI, 0.663–0.960; $P = 0.02$).

The TG/HDL-C ratio was also predictive of mortality, albeit much less strongly than HDL-C: best cutoff, 3.19; area under the ROC curve, 0.66; sensitivity, 91%; specificity, 48% [Figure 2B]; HR, 8.378; 95% CI, 1.072–65.493; $P = 0.015$). In contrast, HOMA-IR had no association with mortality: best cutoff, 1.92; area under the ROC curve, 0.61; sensitivity, 73%; specificity, 53% [Figure 2C]; HR, 0.572; 95% CI, 0.167–1.956; $P = 0.37$). Neither index of insulin resistance was predictive of clinical worsening (TG/HDL-C ratio: HR, 1.965; 95% CI, 0.802–4.813; $P = 0.13$; HOMA-IR: HR, 0.502; 95% CI, 0.211–1.195; $P = 0.11$).

Clinical Features according to HDL-C Levels

We divided the PAH population into two groups, those with HDL-C above and below 35 mg/dl, and compared them regarding their baseline standard cardiovascular risk factors and insulin resistance (Table 4), as well as the usual parameters of PAH severity (Table 5). Patients with PAH with HDL-C below 35 mg/dl had a similar age and BMI as those with higher HDL-C. CRP levels and the TG/HDL-C ratio were higher in the low-HDL-C group, whereas the HOMA-IR was similar in both groups (see Table 4). In terms of PAH severity, patients with low HDL-C had more severe functional limitation, as evidenced by a higher proportion of them being in NYHA class III or IV, and by a more limited 6MWD. They also had higher right atrial pressure (RAP) and B-type natriuretic peptide (BNP) levels. Pulmonary capillary wedge pressure (PCWP) was the same in

Figure 2. Receiver operating characteristic curves showing the ability of (A) high-density lipoprotein cholesterol (HDL-C), (B) triglyceride (TG)/HDL-C ratio, (C) and homeostatic model assessment of insulin resistance (HOMA-IR) to predict mortality. The areas under the curve were 0.82, 0.66, and 0.61, respectively.

Figure 3. (A) Survival and (B) time to clinical worsening in 69 patients with pulmonary arterial hypertension according to high-density lipoprotein cholesterol (HDL-C) levels.

both groups, as were other parameters of PAH severity (Table 5). HDL-C had significant correlations with NYHA functional class ($r = -0.42$, $P < 0.001$), 6MWD ($r = 0.37$, $P = 0.003$), RAP ($r = -0.41$, $P = 0.001$), and BNP levels ($r = -0.45$, $P < 0.001$).

When adjusting individually for baseline cardiovascular risk factors, HDL-C remained statistically associated with mortality (Table 6). Similarly, HDL-C remained a significant predictor of death when adjusted individually for type of PAH, NYHA functional class, the 6MWD, the presence of a pericardial effusion, RAP, cardiac index, BNP levels and the use of parenteral prostanoid therapy (see Table 7).

HDL-C and Inflammatory Markers

Patients with low HDL-C had elevated CRP levels (see Table 4), and there was a significant inverse correlation between HDL-C and CRP ($r = -0.38$, $P = 0.001$). High CRP levels were associated with more clinical worsening (see Figure 4B: HR, 2.563; 95% CI, 1.052–6.246; $P = 0.032$), but not with mortality (Figure 4A: HR, 2.736; 95% CI, 0.724–10.332; $P = 0.12$). HDL-C remained predictive of both clinical outcomes when accounting for CRP levels (adjusted HR for mortality for every 5-mg/dl increase in HDL-C, 0.656; 95% CI, 0.501–0.858; $P = 0.003$). Patients with low HDL-C had higher levels of TNF- α , IL-17A, CCL-2, and ICAM-1 (Table 8).

DISCUSSION

Our study shows that plasma HDL-C is low in PAH, and that this is associated with higher mortality and increased clinical worsening. This association does not appear to be explained by underlying cardiovascular risk factors, insulin resistance, or the severity of PAH.

We demonstrate significantly depressed circulating levels of HDL-C in subjects with PAH, even when compared with a population that was older and had a higher frequency of males, coronary artery disease, systemic hypertension, and diabetes, all conditions associated with lower HDL-C concentrations. Although it is known that HDL-C is low in chronic inflammatory conditions, such as scleroderma (28), we show intermediate HDL-C levels in subjects with CTD but without pulmonary hypertension. HDL-C in this group was higher than in subjects with PAH but lower than in control subjects.

There is no consensus on how to diagnose insulin resistance, and “gold standard” tests such as the euglycemic hyperinsulinemic clamp and insulin suppression tests are laborious and expensive. Proposed surrogate markers are elevated TG/HDL-C (sensitivity, 64%; specificity, 68%) (29) and high HOMA-IR (correlation coefficient between -0.69 and -0.88) (25, 26). Our PAH cohort was insulin resistant by the former, but HOMA-IR

Figure 4. (A) Survival and time to (B) clinical worsening in 69 patients with pulmonary arterial hypertension according to C-reactive protein (CRP) levels.

was similar to apparently healthy control subjects. These could suggest that the observed low HDL-C levels are not related to insulin resistance. However, as we did not use the gold standard for the diagnosis of insulin resistance, our data do not exclude its role in PAH.

Zamanian and colleagues have reported similarly low HDL-C levels in 81 females with PAH (43.3 ± 1.58 mg/dl), compared with age- and sex-matched National Health and Nutrition Examination Surveys control subjects (60.5 ± 0.54 mg/dl) (18). Whereas our findings confirm their observation that the TG/HDL-C ratio is associated with event-free survival (18), another index of insulin resistance, HOMA-IR, had no association with clinical outcomes. HDL-C had a much stronger association with mortality and clinical worsening than either index, and remained significant when adjusted for both HOMA-IR and TG/HDL-C ratio. Many studies have shown that insulin resistance and the metabolic syndrome carry a higher risk of systemic cardiovascular events (30–34), but this is not universal (35–39). In one study (32) cardiovascular events were more closely dependent on insulin resistance than HDL-C levels, whereas another study suggested that HDL-C was the better predictor (37). Certainly, more clinical research is needed to clarify these issues regarding pulmonary vascular disease.

Our data do not rule out a role for insulin resistance, but they do suggest that HDL-C may have prognostic significance in pulmonary vascular disease above and beyond insulin resistance. Possible explanations include the fact that HDL-C has antioxidant and antiinflammatory properties (6, 7), attenuates endothelial dysfunction (8, 9), has anticoagulant effects (10, 11), and enhances the half-life of prostacyclin (12–14). The antiinflammatory properties of HDL-C may be particularly relevant here, because we observed systemic inflammation and endothelial activation in patients with PAH with low HDL-C levels (Table 8).

Patients with lower HDL-C were not older or more obese than subjects with higher HDL-C, suggesting that the association between HDL-C and mortality was not driven by obesity or older age. Furthermore, HDL-C remained associated with mortality when adjusting individually for established cardiovascular risk factors, the presence of coronary artery disease, statin use, and CRP levels. Elevated CRP levels are associated with the metabolic syndrome and cardiovascular mortality (40), and have been reported to be predictive of outcomes in PAH (41). We confirmed the latter finding, but HDL-C predicted mortality independently of CRP levels. Patients with PAH with low HDL-C had more severe functional limitation, higher BNP

TABLE 4. CARDIOVASCULAR RISK FACTORS AND INDICES OF INSULIN RESISTANCE ACCORDING TO HIGH-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS

	HDL-C <35 mg/dl (n = 34)	HDL-C >35 mg/dl (n = 35)	P Value
Age, yr	48.21 ± 11.86	45.20 ± 13.60	0.37
Female	30 (88.2)	32 (91.4)	0.71
BMI	32.25 ± 7.86	30.70 ± 8.33	0.36
HTN	6 (17.6)	9 (25.7)	0.42
MAP, mm Hg	93.44 ± 12.33	92.16 ± 10.20	0.9
CAD	1 (2.9)	2 (5.7)	1
Smoker	6 (17.6)	4 (11.4)	0.51
Statin	4 (11.8)	4 (11.4)	1
CRP, mg/L	17.02 ± 25.88	8.37 ± 13.06	0.017
TG, mg/dl	105 (90.25–134.5)	102 (83.5–142.5)	0.7
TG/HDL-C	3.99 (3.25–5.6)	2.57 (1.92–3.75)	<0.001
HOMA-IR*	1.96 (0.92–2.93)	1.52 (0.86–4.8)	0.86

Definition of abbreviations: BMI = body mass index; CAD = coronary artery disease; CRP = C-reactive protein; HDL-C = high-density lipoprotein cholesterol; HOMA-IR = homeostatic model assessment-insulin resistance; HTN = systemic hypertension; MAP = mean arterial pressure at the time of right heart catheterization; TG = triglycerides.

Continuous data are presented as means ± SD and as medians (interquartile range); categorical data are presented as no. (%).

* HOMA-IR was calculated according to the following formula: (glucose [mg/dl] × insulin [μU/ml])/405.

levels, and more elevated right atrial pressures, suggesting more severe pulmonary vascular disease. Nevertheless, the association between HDL-C and mortality does not appear to be explained by the severity of PAH. Importantly, PCWP was identical in patients with high and low HDL-C, arguing against the presence of more diastolic dysfunction in subjects with lower levels.

As this is an observational study, we cannot establish a cause-and-effect relationship between HDL-C and clinical outcomes in PAH. It is possible that patients with more severe PAH are more functionally limited, hence more sedentary and obese, and thus lower HDL-C is simply a consequence of this and is not related to mortality. However, our data show that patients with lower HDL-C were not more obese or older. Strikingly, patients with PAH had lower HDL-C levels compared with a large population

TABLE 5. CLINICAL CHARACTERIZATION OF PULMONARY ARTERIAL HYPERTENSION ACCORDING TO HIGH-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS

	HDL-C <35 mg/dl (n = 34)	HDL-C >35 mg/dl (n = 35)	P Value
IPAH/APAH	18 (52.9)/16 (47.1)	23 (65.7)/12 (34.3)	0.28
NYHA III or IV	28 (82.4)	17 (48.6)	0.005
6MWD, m	318.51 ± 115.24	407.92 ± 115.66	0.009
Pericardial effusion	13 (38.2%)	9 (26.5%)	0.3
RAP, mm Hg	13.44 ± 5.87	8.06 ± 5.69	<0.001
mPAP, mm Hg	52.41 ± 12.95	51.43 ± 14.58	0.52
PCWP, mm Hg	10.74 ± 4.06	10.34 ± 5.02	0.43
CI, L/min/m ²	2.36 ± 1.06	2.43 ± 0.85	0.49
PVR, Wood units	11.97 ± 6.41	10.85 ± 8.22	0.23
BNP, pg/ml	344.25 ± 360.65	107.15 ± 206.90	0.001
Prostanoid therapy	20 (58.8)	14 (40)	0.12

Definition of abbreviations: 6MWD = 6-minute walk distance; APAH = associated pulmonary arterial hypertension; BNP = B-type natriuretic peptide; CI = cardiac index; HDL-C = high-density lipoprotein cholesterol; IPAH = idiopathic pulmonary arterial hypertension; mPAP = mean pulmonary artery pressure; NYHA = New York Heart Association functional class; PCWP = pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; RAP = mean right atrial pressure.

Continuous data are presented as means ± SD and categorical data are presented as no. (%).

TABLE 6. ASSOCIATION BETWEEN HIGH-DENSITY LIPOPROTEIN CHOLESTEROL AND MORTALITY WHEN ADJUSTED INDIVIDUALLY FOR BASELINE CARDIOVASCULAR RISK FACTORS AND INDICES OF INSULIN RESISTANCE

Variable	HDL-C Adjusted HR (95% CI)*	P Value
Age	0.525 (0.371–0.744)	<0.001
BMI	0.670 (0.466–0.963)	0.03
HTN	0.591 (0.447–0.783)	<0.001
CAD	0.637 (0.497–0.817)	0.001
Smoking status	0.592 (0.441–0.794)	<0.001
Statin use	0.645 (0.503–0.827)	0.001
CRP, mg/dl	0.656 (0.501–0.858)	0.003
TG, mg/dl	0.639 (0.497–0.822)	<0.001
TG/HDL-C	0.651 (0.493–0.860)	0.003
HOMA-IR	0.658 (0.511–0.847)	0.001

Definition of abbreviations: BMI = body mass index; CAD = coronary artery disease; CI = confidence interval; CRP = C-reactive protein; HDL-C = high-density lipoprotein cholesterol; HOMA-IR = homeostatic model assessment-insulin resistance; HR = hazard ratio; HTN = systemic hypertension; MAP = mean arterial pressure at the time of right heart catheterization; TG = triglycerides.

* HR for every 5-mg/dl increase in HDL-C.

with more cardiovascular risk factors. Furthermore, HDL-C remained predictive of important clinical outcomes when adjusted for age and BMI, several cardiovascular risk factors, functional class, and the 6MWD. Another potential limitation is the lack of data on low-density lipoprotein cholesterol (LDL-C), as it is an important predictor of death and cardiovascular risk. However, the Framingham Study (4) and more recent studies (5, 42) have also shown that HDL-C is a protective factor for cardiovascular events, independent of the levels of LDL-C.

In summary, we report low plasma levels of HDL-C in patients with PAH, which were associated with worse clinical outcomes. This association seems to be independent of other cardiovascular risk factors, insulin resistance, and the severity of PAH. Our novel findings of the association between HDL-C and clinical outcomes in PAH suggest that HDL-C may play an important role in the initiation and/or progression of pulmonary vascular disease.

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TABLE 7. ASSOCIATION BETWEEN HIGH-DENSITY LIPOPROTEIN CHOLESTEROL AND MORTALITY WHEN ADJUSTED INDIVIDUALLY FOR SEVERITY OF PULMONARY ARTERIAL HYPERTENSION

Variable	HDL-C Adjusted HR (95% CI)*	P Value
IPAH vs. APAH	0.669 (0.520–0.861)	0.002
NYHA class	0.657 (0.510–0.848)	0.002
6MWD, m	0.637 (0.462–0.877)	0.008
Pericardial effusion	0.714 (0.549–0.928)	0.01
RAP, mm Hg	0.664 (0.500–0.881)	0.006
Cardiac index, L/min/m ²	0.666 (0.515–0.861)	0.002
BNP, pg/ml	0.719 (0.525–0.984)	0.04
Prostanoid therapy	0.601 (0.450–0.804)	<0.001

Definition of abbreviations: 6MWD = 6-minute walk distance; APAH = associated pulmonary arterial hypertension; BNP = B-type natriuretic peptide; CI = confidence interval; HDL-C = high-density lipoprotein cholesterol; HR = hazard ratio; IPAH = idiopathic pulmonary arterial hypertension; NYHA = New York Heart Association functional class; RAP = mean right atrial pressure.

* HR for every 5-mg/dl increase in HDL-C.

TABLE 8. INFLAMMATORY MARKERS ACCORDING TO HIGH-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS

	HDL-C <35 mg/dl	HDL-C >35 mg/dl	P Value
TNF- α , pg/ml	13.44 $\pm$ 5.78 (n = 15)	9.06 $\pm$ 1.53 (n = 9)	0.009
IL-17A, pg/ml	23.14 $\pm$ 5.37 (n = 27)	19.27 $\pm$ 4.68 (n = 19)	0.009
CCL-2, pg/ml	376.49 $\pm$ 129.62 (n = 14)	266.94 $\pm$ 108.50 (n = 9)	0.038
ICAM-1, ng/ml	23.84 $\pm$ 8.3 (n = 10)	15.49 $\pm$ 2.93 (n = 6)	0.03

Definition of abbreviations: CCL-2 = CC-chemokine ligand-2; HDL-C = high-density lipoprotein cholesterol; ICAM-1 = intercellular adhesion molecule-1; TNF = tumor necrosis factor.

Data are presented as means $\pm$ SD.

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