ORIGINAL ARTICLE

High-density lipoprotein cholesterol levels and pulmonary artery vasoreactivity in patients with idiopathic pulmonary arterial hypertension

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KEY WORDS

high-density lipoprotein cholesterol, inflammation, long-term responder, vascular reactivity

ABSTRACT

INTRODUCTION Metabolic dysregulation has been recognized as a prognostic marker in idiopathic pulmonary arterial hypertension (IPAH).

OBJECTIVES We aimed to investigate the association between cardiometabolic risk factors and vascular reactivity of pulmonary arteries in patients with IPAH.

PATIENTS AND METHODS Between June 2009 and January 2015, we recruited 66 consecutive patients with IPAH. We assessed main cardiometabolic risk factors, inflammatory markers, and markers of IPAH severity. Hemodynamic evaluation included pulmonary vasoreactivity testing with the use of inhaled nitric oxide. Reduced mean pulmonary artery pressure was considered a marker of acute vasoreactivity. Acute responders were treated with calcium channel blockers and classified as long-term responders if they had sustained vasoreactivity (near-normal hemodynamics and World Health Organization functional class I or II) for at least 1 year.

RESULTS Thirteen patients (19.7%) showed a positive response to acute pulmonary vasoreactivity testing; however, only 9 (13.6%) remained vasoreactive at follow-up. Machine-learning algorithms indicated 4 variables associated with acute vasoreactivity of pulmonary arteries: high-density lipoprotein cholesterol (HDL-C), right atrial pressure, cardiac index, and creatinine level, and 4 predictors of long-term vasoreactivity: HDL-C, 6-minute walking distance, creatinine level, and high-sensitive C-reactive protein level.

CONCLUSIONS HDL-C level is associated with pulmonary vasoreactivity in acute testing and predicts long-term responsiveness to calcium channel blockers in patients with IPAH.

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INTRODUCTION Idiopathic pulmonary arterial hypertension (IPAH) is a disease with high mortality and multifactorial pathobiology. Most typical vascular hallmarks of the disease were identified as vasoconstriction, enhanced pulmonary vascular cell proliferation, disrupted apoptosis, and angiogenesis.[1-3] The pathogenesis of underlying vascular malfunction and remodeling is poorly understood. Recent research on IPAH pathobiology revealed genetic predispositions, derangements in numerous cellular signaling pathways, and altered cytokine levels.[4-7] What is more, metabolic dysregulations have been recognized as a prognostic marker and potential pathogenetic factor of the disease.[8-13]

Metabolic abnormalities such as high levels of low-density lipoprotein cholesterol (LDL-C), low levels of high-density lipoprotein cholesterol (HDL-C), and hyperglycemia are the main risk factors for the development and progression of systemic vascular disease directly related to cardiovascular complications.[14,15] They initiate and promote endothelial activation characterized by decreased availability of vasodilators and increased

synthesis of vasoconstrictors, prothrombotic factors, and adhesion molecules.

Recent data suggest the presence of significant alterations in lipid and glucose metabolism also in patients with IPAH. Low levels of LDL-C,[11] HDL-C,[10] and insulin resistance[8,16] were shown to be important predictors of increased mortality in this population; however, the association between metabolic abnormalities and IPAH severity is not fully understood. We hypothesized that, similarly to systemic circulation, metabolic abnormalities can exert their effect by impacting vascular reactivity, which significantly affects prognosis in IPAH patients. Therefore, we aimed to investigate the association between cardiometabolic risk factors and vasoreactivity of pulmonary arteries in this group.

PATIENTS AND METHODS

Study population Between June 2009 and January 2015, we recruited consecutive treatment-naive patients who were newly diagnosed with IPAH in our center. Eligible patients had precapillary pulmonary hypertension (mean pulmonary arterial pressure [MPAP] ≥25 mm Hg at rest and pulmonary artery wedge pressure ≤15 mm Hg) with pulmonary vascular resistance of more than 3 Wood units in the absence of other causes of precapillary pulmonary hypertension (such as lung diseases, chronic thromboembolic pulmonary hypertension, or other rare diseases) and pulmonary arterial hypertension (PAH; such as HIV infection, portal hypertension, congenital heart disease, and connective tissue disease). The institutional ethics committee approved the study protocol, and informed consent was obtained from each patient before the study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Clinical assessment Clinical assessment, laboratory measurements, and hemodynamic evaluation were made at the first evaluation of a patient in our center. For the purpose of this study, we assessed cardiovascular risk factors and markers of IPAH severity as potential modifiers of pulmonary vasoreactivity. Clinical assessment included demographic data, patient's medical history, calculation of body mass index (BMI), measurement of blood pressure, N-terminal pro-B-type natriuretic peptide (NT-proBNP) level, and 6-minute walking distance (6-MWD) test according to current standards.[1] Medical history also included the World Health Organization functional class (WHO-FC)[1] and use of lipid-lowering drugs such as statins, niacin, fibrates, and estrogen replacement therapy. Definitions of cardiovascular risk factors used for the purpose of the present study are included in Supplementary material.

Laboratory measurements Peripheral venous blood was drawn after overnight fast in all patients on the day of diagnostic right heart catheterization (RHC). Blood tests were performed using the Cobas® 6000 analyzer (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). Plasma LDL-C, HDL-C, and triglyceride (TG) concentrations were measured directly by using the colorimetric technique with polyethylene glycol (PEG)-esterase, PEG-oxidase, and peroxidase for HDL-C and LDL-C, and with lipase and peroxidase for TG concentrations. Creatinine levels were assessed using a kinetic colorimetric assay. High-sensitivity C-reactive protein (hs-CRP) was assessed by latex assay (Roche Diagnostics International Ltd). For explanatory purposes, additional markers of systemic inflammation and endothelial function were assessed in the last 34 consecutive patients in whom we had collected plasma samples. We measured the concentrations of interleukin (IL) 1β and IL-6 (ELISA kit, FineTest, Wuhan Fine Biotech, Wuhan, China), and endothelin 1 (ET-1) (ELISA, Elabscience, Houston, Texas, United States).

Hemodynamic evaluation Hemodynamic evaluation was performed during RHC using a Swan–Ganz catheter according to current recommendations.[17] Cardiac output (CO) was assessed using the oxygen consumption method with direct measurement of oxygen consumption. Pulmonary vasoreactivity testing was performed at the time of RHC, with the use of inhaled nitric oxide (NO) for 5 minutes at 20 parts per million as recommended.[1] As a marker of vasoreactivity, we used a change in MPAP (ΔMPAP) during vasoreactivity testing, which was calculated as a difference between MPAP before and at the end of vasoreactivity testing.

A positive acute response was defined according to the current guidelines as a reduction of the MPAP by 10 mm Hg or higher to an absolute value of 40 mm Hg or lower with an increased or unchanged CO.[1]

Follow-up According to the current guidelines, all patients with positive response to acute vasoreactivity testing were prescribed calcium channel blockers (CCB), while patients with negative response were prescribed IPAH-specific therapies including phosphodiesterase-5 inhibitors, endothelin antagonists, and prostanoids. Patients referred for CCB treatment were followed 1 month after initiation of therapy and then every 6 months. Additionally, RHC was performed 1 year after initiation of therapy or in case of clinical deterioration. We aimed to achieve maximal tolerated doses of CCB. For the purpose of the present study, patients were classified as long-term responders in the case of positive acute response during vasoreactivity testing and if demonstrated sustained clinical (WHO-FC I–II) and hemodynamic improvement after at least 1 year on CCB without addition of IPAH-specific therapy. According to the guidelines of the European Society of Cardiology, hemodynamic improvement was defined as near-normalization of pulmonary hemodynamics (MPAP <30 mm Hg, normalization of cardiac index) at follow-up RHC.[1] Acute responders to NO

TABLE 1 Baseline characteristics of the study group (n = 66, unless otherwise indicated)

Variable	Value
Age, y, mean (SD)	49.4 (17)
Sex, female, n (%)	43 (65)
WHO-FC, II/III/IV, n (%)	5 (7)/46 (70)/15 (23)
MPAP, mm Hg, median (IQR)	49.5 (41.0–56.0)
RA pressure, mm Hg, median (IQR)	7.0 (4.0–11.0)
Pulmonary wedge pressure, mm Hg, median (IQR)	9.0 (6.0–12.0)
Mean aortic pressure, mm Hg, mean (SD)	93.8 (12.8)
Cardiac output, l/min, median (IQR)	3.1 (2.4–4.1)
Cardiac index, l/min/m^2, median (IQR)	1.9 (1.4–2.3)
PVR, Wood units, median (IQR)	12.4 (9.4–19.7)
NT-proBNP, pg/ml, median (IQR)	874.3 (245.0–2545.0)
6-MWD, m, mean (SD)	329.5 (118.3)
Hs-CRP, mg/l, median (IQR)	2.5 (1.3–6.7)
Endothelin 1, pg/ml (n = 34), median (IQR)	8.2 (3.1–15.9)
IL-1β, pg/ml (n = 34), median (IQR)	12.6 (7.5–32.3)
IL-6, pg/ml (n = 34), median (IQR)	11.7 (5.0–31.7)

Abbreviations: 6-MWD, six-minute walking distance; hs-CRP, high-sensitivity C-reactive protein; IL-1β, interleukin 1β; IL-6, interleukin 6; MPAP, mean pulmonary artery pressure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PVR, pulmonary vascular resistance; RA, right atrial; WHO-FC, World Health Organization functional class

who failed to improve with long-term CCB therapy were classified as nonresponders.

Statistical analysis Continuous variables were reported as mean (SD) in the case of normal distribution or as median (interquartile range [IQR]) when the distribution was other than normal. Categorical variables were reported as counts and percentages. For the comparison of continuous variables between 2 groups, we used the *t* test or Mann–Whitney test as appropriate, and for categorical variables, the χ^2 test. *P* values were adjusted for multiple comparisons by the Benjamini and Hochberg method.

The ΔMPAP values were transformed using the Cox–Box method to fit a normal distribution as measured by the Shapiro–Wilk test.

Due to small sample size and relatively large number of potential predictors of pulmonary vasoreactivity, an L1-penalized regression model (LASSO) was fitted, and a cross-validation (10- to 30-fold) was performed to extract predictors most robustly associated with ΔMPAP. We assessed the following predictors: cardiometabolic risk factors (presence of diabetes, BMI, LDL-C, TG, and HDL-C levels, and treatment with lipid-lowering drugs), markers of IPAH severity (NT-proBNP, 6-MWD, WHO-FC, right atrial pressure, and cardiac index), and hs-CRP and creatinine levels. The robustness of the LASSO feature selection method on this dataset was further tested using the conditional random forest approach. The Spearman rank correlation was used to depict coefficient values between the ΔMPAP and its most informative continuous predictors.

When analyzing predictors of long-term response to CCB to avoid overfitting, the random forest–based approach was applied instead of the classic multiple logistic regression model. Moreover, based on the classic random forest approach, we used cross-validation (a leave-one-feature-out top-down approach) to estimate the number of predictors, which minimizes the cross-validation error. To select the most informative predictors of the long-term response, we also performed a LASSO-based analysis and a 5- and 8-fold cross-validation. As the most robust predictors of long-term response, we considered those which were significant in both the LASSO-based analysis and the random forest approach. The significance level was set at an α level of 0.05.

Statistical analysis was performed with Statistica PL software (StatSoft, Inc. [2010] STATISTICA (data analysis software system; version 9.1. Tulsa, Oklahoma, United States, www.statsoft.com). The LASSO and random forest–based analyses were done in R software, using the following packages: "glmnet", "RandomForest", "AID", and "party".

RESULTS

Study population The study included 66 patients with IPAH. All patients were newly diagnosed and treatment-naive at inclusion. Diabetes was present in 11 patients (16.7%); hypertension, in 24 (36.4%); obesity, in 9 (13.6%); and overweight, in 30 (45.5%). Twenty patients (30%) were using statins, and none of the patients were on estrogen replacement therapy, niacin, or fibrates. Patients were not advised as to any specific dietary recommendations. The mean (SD) creatinine level was 94.3 (57.7) μmol/l. None of the patients had poorly controlled thyroid disease. Clinical, demographic, and hemodynamic data of the population are presented in **TABLE 1**.

Vasoreactivity testing Positive response to acute pulmonary vasoreactivity testing was observed in 13 patients (19.7%; all women); however, only 9 (13.6%) remained stable on CCB therapy for at least 1 year. The median ΔMPAP during vasoreactivity testing was 5 (IQR, 1–11) mm Hg.

The LASSO model extracted the following predictors most robustly associated with ΔMPAP: 6-MWD, diabetes, HDL-C, creatinine, right atrial pressure, and cardiac index. The coefficients for each of the selected predictors are shown in **TABLE 2**.

Subsequently, by means of the conditional random forest algorithm, we found that NT-proBNP, HDL-C, creatinine, right atrial pressure, and cardiac index were the most informative predictors of ΔMPAP. The area under the receiver operating curve–based measurements of variable importance (in the conditional random forest algorithm) are shown in Supplementary material (*Table S1*).

For comparison, the Spearman rank correlation coefficient values between the ΔMPAP and

TABLE 2 Estimates of coefficients for the predictors of change in mean pulmonary artery pressure during acute vasoreactivity testing with nonzero coefficients in the L1-penalized regression model (LASSO)

Coefficient	10 folds	14 folds	18 folds	22 folds	26 folds	30 folds
(Intercept)	4.98	5.01	4.98	4.98	4.95	5.01
6-MWD	0.0009	0.0008	0.0009	0.0009	0.0009	0.0008
Diabetes	–0.25	–0.19	–0.25	–0.25	–0.30	–0.19
HDL-C	0.02	0.02	0.02	0.02	0.02	0.02
Creatinine	–0.0007	–0.0004	–0.0007	–0.0007	–0.001	–0.0004
RA pressure	–0.05	–0.05	–0.05	–0.05	–0.05	–0.05
CI	0.61	0.6	0.61	0.61	0.61	0.6

Abbreviations: CI, cardiac index; HDL-C, high-density lipoprotein cholesterol; others, see TABLE 1

TABLE 3 Comparison of patients with idiopathic pulmonary arterial hypertension with and without long-term vasoreactivity

Variable	Nonresponders (n = 57)	Long-term responders (n = 9)	*P* value[a]
Age, y, mean (SD)	50.5 (16.8)	42.2 (17.4)	0.28
Sex, female, n (%)	34 (59.6)	9 (100)	0.04
Markers of PAH severity			
WHO-FC, mean (SD)	3.1 (0.5)	2.6 (0.5)	0.01
6MWD, m, mean (SD)	309.5 (111)	452.0 (89.3)	0.009
NT-proBNP, pg/ml, mean (SD)	2265.5 (3726.0)	547.0 (1035.1)	0.01
MPAP, mm Hg, mean (SD)	53.7 (18.9)	40.9 (8.6)	0.03
Cardiac index, l/min/m², mean (SD)	1.8 (0.6)	2.2 (0.5)	0.02
PVR, WU, mean (SD)	16.3 (9.6)	9.6 (4.6)	0.06
RA pressure, mm Hg, mean (SD)	8.5 (5.5)	4.1 (2.7)	0.03
Cardiovascular risk factors and use of LLD			
Diabetes, n (%)	10 (17.5)	1 (11.1)	1.0
Hypertension, n (%)	22 (38.6)	2 (22.2)	0.56
BMI, kg/m², mean (SD)	25.3 (4.1)	23.7 (2.1)	0.29
LDL-C, mg/dl, mean (SD)	96.8 (34.8)	100.6 (27.1)	0.65
HDL-C, mg/dl, mean (SD)	42.6 (11.6)	69.7 (11.6)	0.001
TG, mg/dl, mean (SD)	123.9 (70.8)	70.9 (26.6)	0.009
Creatinine, µmol/l, mean (SD)	98.5 (60.9)	67.3 (5.8)	0.007
Hs-CRP, mg/l, mean (SD)	5.1 (5.0)	0.8 (0.6)	0.003
LLD, n (%)	17 (29.8)	3 (33.3)	1.0

a Benjamini–Hochberg corrected

SI conversion factors: to convert HDL-C and LDL-C to mmol/l, multiply by 0.02586; triglycerides to mmol/l, by 0.0114; and hs-CRP to nmol/l, by 9.524.

Abbreviations: BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; LLD, lipid-lowering drugs; PAH, pulmonary arterial hypertension; TG, triglycerides; others, see TABLES 1 and 2

its most informative continuous predictors (predictors indicated both by the LASSO model and the random forest algorithm) are presented in Supplementary material (*Table S2*).

Comparison of long-term responders and nonresponders Patients with positive response to acute vasoreactivity testing were treated with diltiazem or amlodipine at mean (SD) maximum doses of 324 (117) mg/d and 30 (14) mg/d, respectively. Patients classified as long-term responders included women only and were characterized by higher baseline HDL-C, creatinine, TG, and hs-CRP levels than nonresponders. Nonresponders presented with more severe disease defined by higher WHO-FC, NT-proBNP level, right atrial pressure, MPAP, lower 6-MWD, and cardiac index than long-term responders. Detailed comparison is shown in TABLE 3.

In both the classic and conditional classification scheme, that is, for the classic and conditional random forest, with the indicator of the "long-term responder" state as the dependent variable, HDL-C remained the most significant predictor positively associated with the response to treatment (FIGURE 1).

The estimated number of predictors that minimizes the cross-validation error used to classify long-term responders was 5. As this was a large number of variables, given that only 9 long-term responders were present in the dataset, we also performed a LASSO-based analysis and a 5- and 8-fold cross-validation to find that 4 variables (HDL-C, hs-CRP, 6-MWD, and creatinine levels) were most robustly associated with the dependent variable (see Supplementary material, *Table S3*). Additionally, from the classic random forest–based variable importance plot (as presented in FIGURE 1), we inferred that HDL-C, NT-proBNP, hs-CRP, 6-MWD, and creatinine levels were the 5 top-scoring predictors. Therefore, we concluded that 6-MWD, HDL-C, hs-CRP, and creatinine levels best discriminated between the 2 study groups.

As long-term vasoreactivity was observed only in the female group, we performed a subgroup analysis and showed that the 4 variables: 6-MWD ($P = 0.006$), HDL-C ($P = 0.001$), hs-CRP ($P = 0.002$), and creatinine ($P = 0.04$) well discriminated between long-term responders and nonresponders in this subgroup. A comparison of 6MWD, HDL-C, hs-CRP, and creatinine levels between long-term responders and female nonresponders is shown in Supplementary material (*Figure S1*).

High-density lipoprotein-cholesterol and markers of inflammation Higher HDL-C levels were associated with lower serum IL-1β ($r = -0.41$, $P = 0.004$), IL-6 ($r = -0.32$, $P = 0.03$), and hs-CRP ($r = -0.28$, $P = 0.03$) levels. There was no association between HDL-C and ET-1 levels ($r = 0.003$, $P = 0.98$).

DISCUSSION We showed that the HDL-C level is a cardiometabolic risk factor most strongly associated with pulmonary artery reactivity as assessed by acute vasoreactivity testing in newly diagnosed patients with IPAH. We validated this observation by showing that not only acute but also long-term vasoreactivity, as measured by 1-year response to CCB treatment, significantly correlates with HDL-C levels in this population.

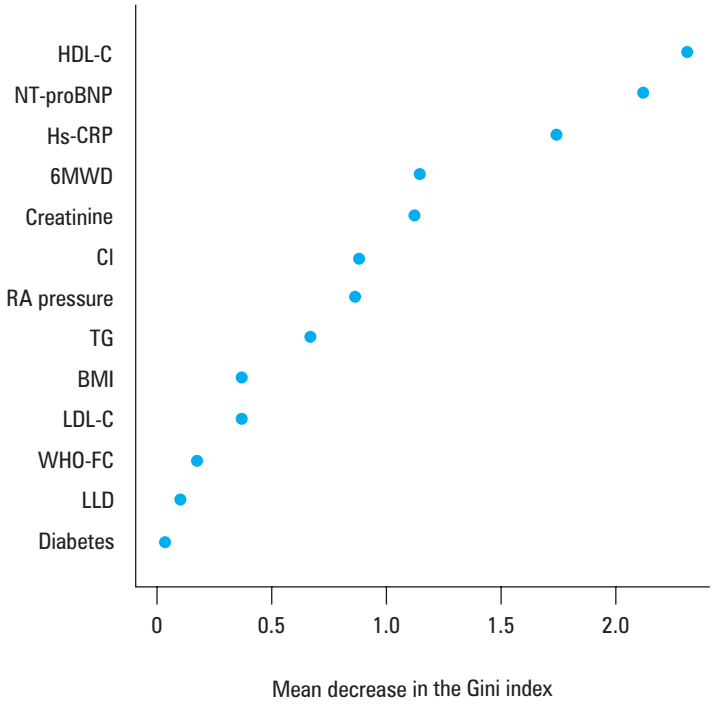

FIGURE 1 The variable importance plot based on the random forest classifier with the indicator of the event "long-term response" as the dependent variable. The features are ranked according to the mean decrease in the Gini index after removal of the given predictor.
Abbreviations: see TABLES 1, 2, and 3

Additionally, we demonstrated that higher levels of inflammatory markers are associated with lower levels of HDL-C; therefore, we speculate that chronic inflammation can mediate the relationship between HDL-C and pulmonary vascular reactivity.

Recent studies have reported the presence of metabolic alterations in patients with PAH. A higher ratio of TG to HDL-C, a surrogate for insulin resistance, has been described in women with PAH when compared with controls and was associated with worse prognosis.[16] In another study, patients with PAH more often than controls had glucose intolerance that could not be explained by differences in sex, age, or BMI, and that was associated with more advanced disease.[9] In our recent study, low LDL-C levels were also associated with worse survival in this group of patients.[11,18] Heresi et al[10] found that patients with PAH are characterized by lower HDL-C levels when compared with controls. Moreover, this phenomenon was associated with higher mortality even when adjusted for other cardiovascular risk factors, insulin resistance, and markers of disease severity.[10,19]

The absence of pulmonary vasoreactivity was shown to predict poor outcome in patients with IPAH. On the other hand, patients with preserved pulmonary vasoreactivity often respond well to long-term CCB therapy and have improved prognosis with better survival than nonresponders. In our study, 19.7% of patients with IPAH demonstrated positive response to the vasoreactivity test. The percentage of patients with preserved vasoreactivity depends on the criteria and vasodilator agent used. Most studies revealed positive vasoreactivity in 10% to 15% of patients; however, some authors reported such a finding in more than 20% of patients, with half of them achieving long-term benefits from CCB treatment.[20] This phenomenon, although not fully understood, suggests that IPAH with long-term response to CCB treatment is caused by a molecularly distinct mechanism.[21] Ideally, this distinction would be potentially detected at disease onset to ensure safe and effective treatment with CCB. However, no marker of vasoreactivity in IPAH has been established so far.[22]

HDL-C is an important cardiovascular risk marker. Numerous prospective studies have confirmed its role as a strong and independent predictor of cardiovascular events in different populations.[23-25] Both experimental and epidemiological studies have shown a vasoprotective effect of HDL-C. The best-known mechanism of HDL-C–associated vasoprotection is the ability to promote cholesterol efflux and reverse cholesterol transport. However, many other protective mechanisms that can regulate vascular reactivity in the systemic circulation have been recently identified. Several studies have indicated that HDL-C stimulates endothelial-dependent NO production, promotes endothelial repair mechanisms, and shows potent anti-inflammatory and antioxidant properties.[26-28] In our population, HDL-C was correlated with inflammatory markers. Of note, most of the known mechanisms of HDL-C action are substantial to the development of pulmonary vascular disease. Apolipoprotein A-I, which constitutes approximately 70% of the HDL protein and is present on virtually all HDL-C particles, is identical to serum prostacyclin stabilizing factor and is able to significantly prolong the prostacyclin's half-life.[29] Moreover, in patients with sickle cell disease, the lack of apolipoprotein A-I was responsible for impaired vasodilatory responses. These results implicate the possible apolipoprotein pathway in pulmonary vasculopathy associated with endothelial dysfunction.[30,31]

In this study, we demonstrated that higher levels of inflammatory markers are associated with lower HDL-C levels; therefore, we speculate that chronic inflammation can mediate the relationship between HDL-C and pulmonary vasoreactivity. Animal and human model studies indicate a significant role of systemic inflammation in the pathogenesis of IPAH.[4,32-34] Inflammation induces vascular remodeling and pulmonary hypertension,[2] and it was shown that inflammatory cells secrete mediators stimulating pulmonary arterial smooth muscle cells to proliferate.[4] In epidemiological and experimental studies, inflammation was able to decrease HDL-C levels by modification of TG lipases. It has been shown that inflammatory cytokines enhance the activity of endothelial lipase and inhibit activity of lipoprotein lipase.[35] This was supported by findings of other groups, namely, that patients with rheumatoid arthritis treated with anti–tumor necrosis factor antibodies have decreased IL-6 and increase HDL-C levels, while other lipid fractions remain unchanged.[36] Lower HDL-C levels were also shown to be associated with obesity, insulin resistance,

and metabolic syndrome.[37] Elevated plasma TG and reduced HDL-C levels were found to be related to plasma insulin concentration. Hyperinsulinemia was able to stimulate catabolism of apolipoprotein A-I, which resulted in a decrease of HDL-C levels.[38]

The cause and effect relationship between inflammation, metabolic disorders, and pulmonary vasculature function has not been well established so far; however, one of the existing hypotheses states that metabolic derangement, associated inflammation, and endothelial dysfunction cause pulmonary vascular disease in susceptible individuals.[39-42] Plasma ET-1 levels play an important role in pulmonary vascular function; therefore, we considered this particle as a potential mediator of the interaction between HDL-C and vasoreactivity. However, we did not find any evidence for the association between ET-1 and HDL-C in our patients. This may be due to several reasons. First, ET-1 is released from endothelial cells and act primarily as a paracrine mediator; therefore, plasma concentrations of ET-1 may not fully reflect its tissue action.[43] Second, the lack of correlation between HDL-C and ET-1 may be explained by the fact that these 2 particles act independently on the pulmonary vasculature. So far, the results of experimental and human studies on the association between HDL-C and ET-1 were inconsistent.[44-46]

Our study has several important strengths. First, the association between levels of HDL-C and reactivity of the pulmonary vasculature is a novel finding, which has not been previously reported. Second, prediction of long-term responsiveness to CCB therapy in IPAH is clinically important, and no predictors have been identified so far. Third, we were able to propose a possible mechanism of the observed phenomenon, as described above.

Our study has also several limitations. The main limitation is a small number of events which precluded the use of standard statistical methods to adjust the associations between HDL-C and long-term vasoreactivity for several confounding factors. However, using machine-learning algorithms, we were able to indicate the factors most strongly associated with long-term vasoreactivity. In our study, we found long-term vasoreactivity only in female patients; therefore, we cannot conclude whether HDL-C is a predictor of long-term vasoreactivity in male patients with IPAH. Taking together these limitations, we consider our findings as hypothesis generating, and we suggest that they should be confirmed in large multicenter registry studies. Second, this is an observational study and we cannot establish a cause-and-effect relationship between HDL-C and reactivity of the pulmonary vasculature. Third, our data cannot explain the exact mechanism of the observed findings. However, we were able to propose potential mechanisms based on our results and recent experimental studies.

In conclusion, our study showed that HDL-C is a marker of pulmonary artery vasoreactivity and a predictor of long-term response to CCB therapy in patients with newly diagnosed IPAH.

SUPPLEMENTARY MATERIAL Supplementary material is available with the article at www.pamw.pl.

CONTRIBUTION STATEMENT KJ, GK, and PP conceived the concept of the study. KJ, GK, and MS contributed to the design of the research and statistical analysis. KJ, WM, MW, and GK were involved in data collection. KJ, MS, and GK analyzed the data. GK coordinated the project tasks. All authors edited, revised, and approved the final version of the manuscript.

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