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Liver fibrosis marker, 7S domain of collagen type IV, in patients with pre-capillary pulmonary hypertension

Akiomi Yoshihisa *[,1], Yusuke Kimishima [1], Takatoyo Kiko [1], Yu Sato [1], Shunsuke Watanabe [1], Yuki Kanno [1], Satoshi Abe [1], Makiko Miyata-Tatsumi [1], Takamasa Sato [1], Satoshi Suzuki [1], Masayoshi Oikawa [1], Atsushi Kobayashi [1], Takayoshi Yamaki [1], Koichi Sugimoto [1], Hiroyuki Kunii [1], Kazuhiko Nakazato [1], Hitoshi Suzuki [1], Takafumi Ishida [1], Yasuchika Takeishi [1]

Department of Cardiovascular Medicine, Fukushima Medical University, Fukushima, Japan

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ABSTRACT

Background: Pulmonary hypertension (PH) causes right ventricular dysfunction and central venous congestion, and may lead to congestive hepatopathy. The serum 7S domain of collagen type IV (P4NP 7S) is an established marker of liver fibrosis in chronic liver disease. We aimed to determine whether P4NP 7S is related to hemodynamic parameters, and assessed the potential values of P4NP 7S to predict mortality.
Methods: Consecutive 76 pre-capillary PH patients were divided into tertiles based on their serum P4NP 7S levels. We compared right-heart catheterization, echocardiographic findings, and mortality among the tertiles, and compared P4NP 7S with other known biomarkers of mortality.
Results: Cardiac index, mean pulmonary arterial pressure, pulmonary vascular resistance, and right ventricular fractional area change did not differ among the three groups. In contrast, compared to 1st and 2nd tertiles, the 3rd tertile had higher levels of right atrial pressure, right atrial area, and right ventricular area ($P < 0.05$, respectively). In the Kaplan-Meier analysis, mortality progressively increased from the 1st to 2nd and 3rd tertiles (log-rank, $P = 0.002$). In the Cox proportional hazard analysis, P4NP 7S was a predictor of mortality. ROC analysis demonstrated that a P4NP 7S concentration of 4.75 ng/ml predicted mortality (AUC 0.85, 95% CI 0.75–0.94; $P < 0.001$), and that the prognostic value of P4NP 7S was comparable or superior to that of other biomarkers (total bilirubin, creatinine, uric acid, C-reactive protein, B-type natriuretic peptide, and troponin I).
Conclusions: Serum P4NP 7S is associated with higher central venous pressure, right-sided volume overload, and mortality in PH patients.

1. Introduction

Pulmonary hypertension (PH) is characterized by elevated pulmonary arterial pressure due to vasoconstriction and remodeling of the pulmonary microvasculature, which leads to right ventricular (RV) failure and death [1]. In patients with heart failure (HF), it has been reported that systemic venous congestion caused by decreased cardiac output and increased ventricular filling pressure is a major pathophysiology of liver dysfunction and fibrosis [2–5], and is associated with adverse prognosis [6–8]. These conditions are related to RV dysfunction with elevated central venous pressure caused by higher pulmonary artery pressure (PAP), higher central venous pressure [9,10], increased neurohumoral responses, and impaired gastrointestinal function [4]. However, the pathophysiology and prognostic impact of concurrent liver dysfunction and fibrosis in PH patients remain unclear.

The serum 7S domain of collagen type IV (P4NP 7S) is a fragment of collagen type IV, that is abundantly expressed in the basement membrane of the hepatic extracellular matrix, which is an established biochemical marker of liver fibrosis in chronic liver disease [11–13]. We hypothesized that circulating P4NP 7S might be associated with higher central venous pressure, collagen turnover, and liver fibrosis in PH patients.

Although uric acid, [14] bilirubin [15,16], creatinine [17], C-reactive protein [18], natriuretic peptide [17,19], and cardiac troponins [20] have been reported to be prognostic biomarkers, there is no completely established biomarker in PH patients [1,21].

Thus, we aimed to determine whether serum P4NP 7S is related to hemodynamic and echocardiographic parameters in PH patients.

* Corresponding author at: Department of Cardiology and Hematology, Fukushima Medical University, 1 Hikarigaoka, Fukushima 960-1295, Japan.
E-mail address: yoshihis@fmu.ac.jp (A. Yoshihisa).
[1] These authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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We also assessed the potential significance of serum P4NP 7S levels in predicting mortality in PH patients, and compared serum P4NP 7S with other known biomarkers.

2. Methods

2.1. Subjects and study protocol

The current study was a prospective observational one that enrolled consecutive pre-capillary PH patients (PAP ≥ 25 mm Hg and pulmonary artery wedge pressure [PAWP] ≤ 15 mm Hg based on right-heart catheterization) [1] who had been admitted to Fukushima Medical University Hospital for diagnosis and treatment between 2009 and 2015. The groups in which the PH patients (mean pulmonary arterial pressure 43.5 ± 14.4 mm Hg) were divided into were: those with pulmonary arterial hypertension (Group 1, 35 patients); those with pulmonary hypertension due to lung disease (Group 3, three patients); those with chronic thromboembolic pulmonary hypertension (Group 4, 31 patients); and those with multifactorial mechanisms (Group 5, seven patients) [1]. Blood samples and echocardiography were performed within three days of right-heart catheterization. All patients underwent testing for hepatitis B surface antigen and hepatitis C antibody, and their medical histories were checked for chronic liver disease (cirrhosis, hepatic tumors, bile duct disease, etc.). Patients with distinct liver diseases (n = 1), port-pulmonary hypertension (n = 1), and/or were undergoing dialysis (n = 1) were excluded. There were no patients who had undergone pulmonary endarterectomy and/or lung transplantation. The patients (n = 76) were finally divided into tertiles based on their serum P4NP 7S levels: 1st (P4NP 7S < 3.90 ng/ml, n = 27), 2nd (3.90 ≤ P4NP 7S < 5.10, n = 24), and 3rd tertiles (5.10 ≤ P4NP 7S, n = 25).

Firstly, we compared the clinical features and results from right-heart catheterization, laboratory tests, and echocardiography among the three groups. In addition, we performed multiple regression analysis, allowing for interaction between serum levels of P4NP 7S and the patients backgrounds, as well as right-heart catheterization parameters. Secondly, we followed up these patients until 2017 March for all-cause mortality as the outcome of our study. We were able to follow up on all patients. Status and date of death were obtained from the patients' medical records or their referring physicians. Survival time was calculated from the date of hospitalization until the date of death or last follow-up. Those who administered the survey were blind to the analyses, and written informed consent was obtained from all study subjects. The study protocol was approved by the Ethics Committee of Fukushima Medical University, and was carried out in accordance with the principles outlined in the Declaration of Helsinki. Reporting of the study conforms to STROBE along with references to STROBE and the broader EQUATOR guidelines [22].

2.2. Right-heart catheterizations and hemodynamic measurements

All right-heart catheterizations were performed with the patients in a stable condition, in a resting supine position under fluoroscopic guidance, at room air, and at rest for >30 min after catheter placement. PAP, PAWP, mean right atrial pressure, and cardiac output were measured using a 7F Swan-Ganz catheter (Edwards Lifesciences, Irvine, CA, USA). Cardiac output and pulmonary vascular resistance were calculated based on the direct Fick method.

2.3. Measurement of P4NP 7S, procollagen type III peptide, and hyaluronic acid

Serum P4NP 7S was measured by radioimmunoassay (Type IV collagen 7S kit, SCETI MEDICAL LABO K.K., Tokyo, Japan). Serum procollagen type III peptide was measured by immunoradiometric assay (RIA-gnost PIIIP c.t., CISbioBioassays, Codolet, France), and serum hyaluronic acid was measured by latex agglutination-turbidimetric immunoassay (LPIA Ace HA., LSI Medience Co., Tokyo, Japan). These assays were blindly performed by an outside company (LSI Medience Co., Tokyo, Japan). These markers have been determined to be indicators of liver fibrosis [11–13].

2.4. Echocardiography

Echocardiography was performed blindly by experienced echocardiographers using standard techniques. The echocardiographic parameters included left ventricular ejection fraction, left atrial volume, the early transmitral flow velocity to mitral annular velocity ratio (mitral valve E/e′), right atrium and ventricle dimensions and areas, right ventricular fractional area change (RV-FAC), inferior vena cava diameter, tricuspid regurgitation pressure gradient (TRPG), tissue Doppler-derived tricuspid lateral annular systolic velocity (tricuspid valve S′), and the ratio of the peak transtricuspid velocity during early diastole to peak tricuspid valve annular velocity during early diastole (tricuspid valve E/e′) [23]. The left ventricular ejection fraction was calculated using Simpson's method. The RV-FAC, defined as (end diastolic area - end systolic area)/end diastolic area × 100, was a measure of right ventricular systolic function [23]. Tricuspid valve E/e′ was calculated by transtricuspid Doppler flow and tissue Doppler imaging [23]. All measurements were performed using ultrasound systems (ACUSON Sequoia, Siemens Medical Solutions USA, Inc., Mountain View, CA, USA).

2.5. Statistical analysis

Normally distributed data are presented as mean ± SD, and non-normally distributed data were log transformed (e.g. C-reactive protein, B-type natriuretic peptide [BNP], troponin I, and hyaluronic acid). The categorical variables are expressed as numbers and percentages, and the chi-square test was used for their comparisons. We used the analysis of variance followed by Bonferroni's post-hoc test. Multivariable regression analysis was used to determine the relationship between serum P4NP 7S and two possible confounding factors: demographic data and right-heart catheterization data. Correlations between serum P4NP 7S and both echocardiographic and laboratory data were assessed using Pearson's correlation analysis. Kaplan-Meier analysis was used with a log-rank test to assess mortality. The prognostic value was tested by univariate and stepwise multivariate Cox regression analysis, and the best-fitting model for mortality was provided. The proportional hazards assumption for the model was checked by examining log minus-log transformed data. The Kaplan-Meier estimates of the survival curves for the three groups were plotted against the time to follow-up period. These curves help in identifying non-proportionality patterns in hazard functions such as convergence (difference in risk among the three groups decreases with time), divergence, or crossing of the curves. In addition, the Schoenfeld test for violation of proportional hazards, which assesses the correlation between scaled residuals and time, was also conducted. To assess the serum P4NP 7S level that could predict mortality, we also estimated the area under the curve (AUC) of the receiver operating curve (ROC) to compare P4NP 7S with other known biomarkers using the DeLong test. A value of $P < 0.05$ was considered statistically significant for all comparisons. These analyses were performed using a statistical software package (SPSS ver. 24.0, IBM, Armonk, NY, USA).

3. Results

The clinical features of the present study's subjects are summarized in Table 1. Age, gender, body mass index, vital signs, WHO functional class, PH classification, and pharmacotherapy did not differ among the groups. Regarding the catheterization parameters, right atrial pressure was significantly higher in the 3rd tertile than in the 1st and 2nd tertiles. In contrast, PAP, PAWP, cardiac output and index, and pulmonary vascular resistance and index did not differ among the groups. Comparisons of the laboratory data and echocardiographic parameters are shown in Table 2. The 3rd tertile had the highest levels of P4NP 7S, total bilirubin, direct bilirubin, alkaline phosphatase, gamma-glutamyl transferase, C-reactive protein, BNP, procollagen type III peptide, and hyaluronic acid. Moreover, the 3rd tertile had the lowest levels of platelet count, albumin, and cholinesterase. In contrast, total protein, creatinine, and uric acid did not differ among the three groups. With regard to the echocardiographic parameters, although left and right ventricular systolic function, mitral valve E/e′, and TRPG did not differ among the three groups, both the diameter and volume of the right atrium and ventricle were highest in the 3rd tertile. In the multiple regression analysis to determine serum P4NP 7S confounding factors (Supplementary Table 1), WHO functional class (β coefficient 0.223, $P = 0.032$) and right atrial pressure (β coefficient 0.540, $P < 0.001$) were independent predictors. In addition, there were significant correlations between serum P4NP 7S and both the diameter and volume of the right atrium and ventricle, as well as other markers of liver function testing (Supplementary Table 2).

During the follow up period (mean 1027 ± 723 days), 12 patients died of complications related to PH. As shown in Fig. 1, all-cause mortality progressively increased from the 1st to 2nd and 3rd tertiles (log-rank, $P = 0.002$). In the univariate analysis (Supplementary Table 3), male gender, WHO functional class, body mass index, PAWP, BNP, creatinine, uric acid, and P4NP 7S were predictors of mortality. In the stepwise multivariate analysis (Supplementary Table 4), given the interdependence between P4NP 7S and clinical background, two bivariate models were analyzed: Model 1 (demographic parameters; age, male gender, WHO functional class, body mass index, systolic blood pressure, and heart rate) and Model 2 (biomarkers; total bilirubin, creatinine, uric acid, C-reactive protein, BNP, and troponin I). According to the stepwise multivariate analysis, P4NP 7S was an independent predictor of mortality in both models (Supplementary Table 4). ROC analysis (Fig. 2) demonstrated that a P4NP 7S cut-off value of 4.75 ng/ml predicated mortality with a sensitivity of 92% and a specificity of 70% (AUC 0.85, 95% confidence interval, 0.75–0.94; $P < 0.001$), and that the

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